FEDERATION OF STEM CELL ASSOCIATIONS

StemNet

BOOK OF ABSTRACTS

FIRST INTERNATIONAL STEMNET MEETING

Padua, September 22 - 24, 2021

MUSME, the Museum of History of Medicine in Padua

GISM Gruppo Italiano Staminali Mesenchimali

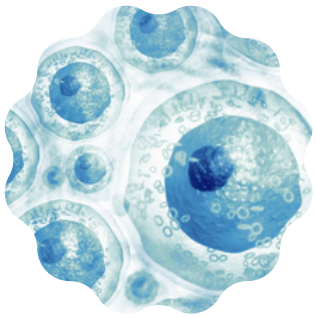
International Placenta Stem Cell Society

IPLASS

FIRST

SCR ITALY

STEM CELL RESEARCH ITALY



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Presentation

*Welcome to the **First International StemNet Meeting**, taking place at the Museum of the History of Medicine in Padua on September 22-24, 2021.*

StemNet is a federation of the four main associations of stem cell researchers (GISM, IPLASS, SCR- Italy, FIRST), established to share the different experiences enhancing both the quality and the impact of research in this rapidly advancing field. This meeting is the first tangible event evidencing the constructive relationship among our associations. StemNet also aims to become a reference subject for the Institutions and Regulatory Authorities, to improve the exchange of truthful information in the field of cell therapy.

Accordingly, the program of our first meeting is focused on relevant methodological advancements in stem cell research and particularly in translational clinical applications, but it also includes discussion of relevant ethical, social and regulatory issues. A faculty of qualified speakers that will undoubtedly stimulate exciting and inspiring debates supports such a challenging scientific program.

The meeting is housed in the historical 15th-century building of the St. Francisco's hospital, which was one of the most evolved healthcare institutions of the modern age. The hospital was one of the first places of application and experimentation of medical science in the university field, devoted in particular to the hospitalization and care of poor people.

The city of Padua has just been awarded the World Heritage Status by the United Nations culture organization UNESCO due to its unique 14th-century frescos. The 'Padova Urbs Picta' site encompasses eight buildings hosting fresco cycles painted by various artists between 1302 and 1397, including Giotto's Scrovegni Chapel, considered as a revolutionary development in the history of mural painting.

Welcoming you to Padua, we wish you a pleasant stay and a fruitful conference.

LOCAL ORGANIZING COMMITTEE

Maurizio Muraca,
Michela Pozzobon

StemNet

Federation of Stem Cell Associations

First International StemNet Meeting

Padua, September 22- 24, 2021

Wednesday, September 22, 2021

13.00 REGISTRATION

14.00 OPENING CEREMONY

SESSION 1: ADVANCES AND PITFALLS OF CELL THERAPY AT BEDSIDE: WAS THE PROMISE FULFILLED? 14.30 - 16.00
CHAIR: Antonella Viola (Padua), Massimo Dominici (Modena)

14.30-15.00
Genetically-engineered somatic cells to cure genetic disorders and cancer: a critical assessment
Franco Locatelli (Rome)

15.00-15.30
From cell therapy to genes: regenerating the field of cardiac regeneration
Mauro Giacca (London, UK)

15.30-16.00
Discussion

SESSION 2: LE ASPETTATIVE DEL PAZIENTE TRA BIOETICA, REGOLAMENTAZIONE E COMUNICAZIONE 16.00 - 17.00
CHAIR: **Augusto Pessina (Milan), Maurizio Muraca (Padua)**

16.00-16.20
Turismo medico, cliniche offshore e cure compassionevoli: il punto di vista degli enti regolatori
Daniela Melchiorri (Rome)

16.20-16.40
La bioetica tra vecchie e nuove sfide
Renzo Pegoraro (Rome)

16.40-17.00
Comunicare la scienza
Giovanni Carrada (Roma)

17.00-18.30
Dibattito – Tavola Rotonda
Coordinator: Francesco Jori (Padua)
Franco Locatelli, Mauro Giacca, Renzo Pegoraro, Giovanni Carrada, Daniela Melchiorri, Gino Gerosa, Eugenio Baraldi

18.30-19.30 WELCOME COCKTAIL

Thursday, September 23, 2021

SESSION 3: BASIC AND APPLIED RESEARCH ON FOETAL AND ADULT STEM CELLS 09.00 - 10.45
CHAIR: Michela Pozzobon (Padua), Ornella Parolini (Rome)

09.00-09.25
Modelling Amniotic Membrane healing effect on chronic and diabetic foot ulcers into an in vitro cell system to unravel the molecular mechanisms behind its therapeutic effect
Francisco J. Nicolás (Murcia, Spain)

09.25-09.50
Mesenchymal Stromal Cells in Crohn's disease
Rachele Ciccocioppo (Verona)

09.50-10.15
Where gene therapy meets cell therapy
Alessandra Biffi (Padua)

10.15-10.30
Clinical grade mesenchymal stromal cell-derived extracellular vesicles promote human cartilage recovery in vitro
Elisabetta Palamà (Genova)

10.30-10.45
Amniotic mesenchymal stromal cells inhibit CD8 T lymphocyte activation and memory commitment via mTOR signaling pathway
Andrea Papait (Brescia)

10.45-11.15 TECHNOLOGICAL SESSION
CHAIR: Alessandra Pelagalli (Naples)

10.45-10.55
Automated, closed manufacturing and sorting of PSCs and their derivatives
Claudia Maldini (Miltenyi Biotec, Bologna)

10.55-11.05
Isoplexis: the proteomics suite that brings secretomics at a single-cell level
Giulia Anselmi (Alfatest, Cinisello Balsamo)

11.05-11.15
Bioinks and bioprinting technologies to tailor cell microenvironment
Pierre-Alexandre Laurent (Cellink, Gothenburg, Sweden)

11.15- 11.30 COFFEE BREAK

SESSION 4: MESENCHYMAL STROMAL CELLS AND THE NERVOUS SYSTEM 11.30 - 13.00
CHAIR: Maria Harmati (Szeged, Hungary), Umberto Galderisi (Naples)

11.30- 11.55
DNA damage response of neural stem and progenitor cells and neurodevelopmental pathologies
François Boussin (Paris-Saclay, France)

11.55-12.20
Challenge to medical innovation by non-tumorigenic endogenous reparative Muse cells
Mari Dezawa (Sendai, Japan)

12.20-12.35
Role of amniotic membrane on endothelial cells from umbilical cord of women affected by gestational diabetes: new insights into the treatment of non-healing diabetic foot ulcers.
Caterina Pipino (Chieti)

12.35-12.50
Characterization of senescent vascular mesenchymal stromal cells and their role in the development of the abdominal aorta aneuruysm
Gabriella Teti (Bologna)

12.50-13.05
Equine freeze-dried secretome for joint regeneration in veterinary medicine: production process and in vitro efficacy
Priscilla Berni (Parma)

13.05-14.00 LUNCH TECHNOLOGICAL SESSION
CHAIR: Francesca Paino (Milan)

13.00-13.10
Celector the "cell chromatograph" to separate, characterize and collect living stem cells
Silvia Zia (Stem Sel srl, Bologna)

13.10-13.20
New technologies for aseptic production allow a faster and reliable access to in house ATMP manufacturing
Marco Fadda (Comecer, Castelbolognese RA)

13.20-13.30
Standardized Single Particle Measurement of Number, Size and Charge is required for Confidence in Nanomedicine Engineering and Development
Stephane Mazlan (Izon Science LTD, France)

13.30-13.40
Cytoflex New Generation Flow Cytometry Analysis of Circulating Microvesicles and Exosomes
Claudia Maria Radu (Padua)

13.50-14.00
3D, Label-Free, Live Cell Imaging for Cell Morphology and Characterisation
Daniel Ghete, Tomocube Inc. (Daejeon, South Corea)

14.00-15.00 POSTER SESSION

SESSION 5: CANCER STEM CELLS AND TUMOR MICROENVIRONMENT
CHAIR: Krisztina Buzas (Szeged, Hungary), Enrico Lucarelli (Bologna)
15.00 - 16.30

15.00-15.25
Cancer stem cells and tumor microenvironment: a target for chemoprevention?
Adriana Albini (Milan)

15.25-15.50
An antitumor cellular viroimmunotherapy using mesenchymal progenitor cells
Javier-Garcia Castro (Madrid, Spagna)

15.50-16.05
Angiogenic properties of pericytes isolated from micro-fragmented fat tissue
Ekta Manocha (Brescia)

16.05-16.20
In-vivo evaluation of bimodal nanoparticle-loaded mesenchymal stromal cell for osteoarcoma treatment
Enrico Lucarelli (Bologna)

16.20-16.35
Primary glioblastoma gliospheres incorporate mesenchymal stem cells
Anna Andrzjewska (Warsaw, Poland)

16.35-17.00 COFFEE BREAK

SESSION 6: CELL FREE THERAPY
CHAIR: Barbara Lukomska (Warsaw, Poland), Maria Luisa Torre (Pavia)
17.00 - 18.30

17.00-17.25
First clinal experiences with MSC derived extracellular vesicles
Eva Rohde (Salzburg, Austria)

17.25-17.50
MSC-derived extracellular vesicles: perspectives in oncology and regenerative medicine
Benedetta Bussolati (Turin)

17.50-18.15
Functional heterogeneity of cellular and extracellular vesicle-based therapeutics: implications for the development of potency assays
Bernd Giebel (Essen, Germany)

18.15-18.30
Systemically but not locally injected Mesenchymal Stem Cell derived Extracellular Vesicles induce muscle recovery in a murine model of Volumetric muscle loss.
Fabio Magarotto (Padua)

18.30-20.00 SOCIETY ASSEMBLY

Friday, September 24, 2021

SESSION 7: ORGANOIDS
CHAIR: Lorenza Lazzari (Milan), Roberta Piva (Ferrara)

09.00 - 11.00

09.00-09.25
Organoids culture systems: unique tools for studying biological processes and creating the medicine of the future
Salvatore Simmini (Cambridge, UK)

09.25-09.50
Engineering organoids: four-dimensional control of the organoid environment
Nicola Elvassore (Padua)

09.50-10.15
Organoids in cancer research
Giulia Della Chiara (Milan)

10.15-10.30
3D Bioprinted Scaffolds Containing Mesenchymal Stem/Stromal Lyosecretome: Next Generation Controlled Release Device for Bone Regenerative Medicine
Franca Scocozza (Pavia)

10.30-10.45
3D bioprinted silk-gelatin ink system for cartilage regeneration
Gina Lisignoli (Bologna)

10.45-11.00
Treating induced pluripotent stem cells-derived β cells with monoclonal antibody Brentuximab reduces the risk of teratoma upon transplantation
Valentina Zamarian (Milano)

11.00-11.30 COFFEE BREAK

SESSION 8: OPPORTUNITIES FOR YOUNG RESEARCHER
CHAIR: Francesco Alviano (Bologna), Assunta Pandolfi (Chieti)

11.30 - 12.30

11.30-11.45
Opportunities in 2021-2022 for short-term fellowships in Europe
Filippo Piccinini (Meldola)

11.45-12.30
YOUNG INVESTIGATOR AWARDS AND CLOSING REMARK

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Maurizio Muraca, Michela Pozzobon

FACULTIES

Albini Adriana – Scientific Director MultiMedica Onlus, Milan.
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Biffi Alessandra – Department of Women and Children Health, Director of Pediatric Oncohematology, University of Padua.
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Bussolati, Benedetta – Department of molecular Biotechnology and Health Sciences, University of Torino.
Buzas Krisztina – Faculty of Medicine, University of Szeged Szeged, Hungary.
Carrada Giovanni – Comunicatore della scienza e autore Superquark, Roma.
Ciccocioppo Rachele – Gastroenterology Unit, Department of Medicine, University of Verona.
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Dezawa Mari – Department of Stem Cell Biology and Histology, Tohoku University, Graduate School of Medicine, Japan.
Elvassore Nicola – Department of Industrial Engineering, University of Padova.
Garcia Castro Javer – Head of Cellular Biotechnology Unit, Instituto de Salud Carlos II, Spain.
Gerosa Gino – Functional Department Artificial “organs and tissue regeneration”, Azienda Ospedaliera, University of Padua.
Giacca Mauro – Cardiovascular Department, Sciences in the School of Cardiovascular Medicine & Sciences, Kings College, London, UK.
Giebel Bernd – Institute for Transfusion Medicine, University Hospital Essen, Germany.
Jori Francesco – Journalist, Padua.
Harmati Maria – Biological Research Centre, Hungary.
Locatelli Franco – President of Consiglio Superiore di Sanità, Department of Oncohematology, Cell and gene therapy Department, IRCCS Pediatric Hospital Bambino Gesù, Rome.
Lukomska Barbara – NeuroRepair Department, Mossakowski Medical Research Centre, Warsaw, Poland.
Melchiorri Daniela – Department of Pharmacology, University of La Sapienza, Rome.
Nicolás Francisco J. – Laboratorio de Regeneración, Hospital Universitario Virgen de la Arrixaca, Murcia, Spain.
Pandolfi Assunta – Department of Medical, Oral and Biotechnological Sciences, University D’Annunzio, Chieti.
Pegoraro Renzo – Chancellor of Pontifical Academy for Life, Rome.
Piccinini Filippo – Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy.
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Torre Maria Luisa – Department of Drug Sciences, University of Pavia.
Viola Antonella – Director of Foundation Institute of Pediatric Research Città della Speranza, Padua and Department of Biomedical Sciences, University of Padua.

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FIRST INTERNATIONAL STEMNET MEETING

o r a l c o m m u n i c a t i o n s

YIA: Young Investigator Award

NOTES ✎

CARDIAC REGENERATION AFTER MYOCARDIAL INFARCTION, AN APPROACHABLE GOAL

Author: Mauro Giacca

Affiliation: King's College London, British Heart Foundation Centre of Research Excellence, School of Cardiovascular Medicine & Sciences, London UK; Department of Medical, Surgical and Health Sciences, University of Trieste and ICGEB, Trieste, Italy

There is an impelling need to develop new therapeutics for myocardial infarction and heart failure, as pharmacological treatment for these conditions has not evolved significantly since the mid-'90s. A main reason underlying the prevalence of heart failure relates to the incapacity of the heart to regenerate after an insult. Myocardial infarction can kill as many as 25% cardiomyocytes in the left ventricle. In addition, these cells die during several chronic conditions, ranging from inherited cardiomyopathies to drug-induced cardiac toxicity. This contrasts with the rate of cardiomyocyte renewal in adult life, which is estimated to be less than 1% new cell generation per year, far too low to provide clinical benefit.

My laboratory aims to develop new biotherapeutics for cardiac protection and cardiac repair. Over the last years, we have pursued the concept that cardiac regeneration can be achieved through the stimulation of endogenous cardiomyocyte proliferation and have identified a series of microRNAs that stimulate this process in both small and large animals. A challenge for the translation of these findings to patients is to identify a mode for non-coding RNA delivery that is suitable to clinical application. Expression of pro-proliferative small RNAs in cardiomyocytes using AAV vectors is highly efficient. However, transgene expression cannot be controlled and becomes deleterious over time. Transient administration of synthetic RNAs using cationic lipid-based lipoplexes is sufficient to restore cardiac function, but these molecules show an unacceptable safety profile due to their pro-inflammatory properties. Lipid nanoparticles (LNPs) based on the Stable Nucleic Acid Lipid Particles (SNALPs) technology have already reached clinical approval for siRNA administration with patisiran in 2018 and have shown remarkable efficacy in the COVID-19 vaccines by Pfizer-BioNTech and Moderna for mRNA delivery recently. Different LNP formulation carrying the pro-regenerative miRNA-199a-3p in both mice and pigs are effective at stimulating cardiomyocyte proliferation, are maintained in the heart for over 10 days in an active form and are sufficient to stimulate functional cardiac repair. Further improvements can be achieved by including chemical modifications in the miRNA backbone to extend stability over time. One of the tested miR-199-SNALPs (LNP199) is our current lead formulation for clinical development.

BIOETHICS BETWEEN OLD AND NEW CHALLENGES - STEM CELLS

Author: Renzo Pegoraro, MD, STL

Affiliation: Pontifical Academy for Life
Email: cancelliere@pav.va

The issue of stem cells is at the center of a debate in which several exemplary dimensions of bioethical reflection intersect. First of all, they raise ethical questions about the sources from which they can be obtained, which present various scientific and ethical problems: embryos, reprogramming of adult cells (iPS), cloning (SCNT), fetal or adult. The still incomplete knowledge of the biology of these cells calls for particular caution in the phase of medical research and clinical trials. Indeed, the high expectations placed on regenerative medicine may entail the risk of overestimating the benefits and underestimating the risks (therapeutic misconception). This alters the conditions, which enable patients who are candidates for enrolment to make an informed and balanced assessment of their participation in experimental protocols. The communication component is of considerable importance here, both in the specific circumstances in which the patients find themselves and in relation to the wider public: it requires us to proceed responsibly and realistically.

It is precisely the high expectations placed on regenerative medicine that also raise more radical questions about the purpose of medical intervention and the conception of clinical practice itself. The possibility of modifying the course of the spontaneous processes of ageing and tissue degeneration tends to change the vision of the tasks of medicine. Its role could become that of not limiting itself to prevention and treatment, but of extending and enhancing biological functions, intervening on the hereditary characteristics of individuals and species. These perspectives involve not only biological aspects of the organism, but also the understanding that human beings have of themselves, as regards the beginning of life, its molecular bases, relations between generations and with other living organisms populating the planet.

The socio-political impact of these forms of therapy, which require advanced and costly technologies, must also be mentioned: it is necessary to assess how a fair distribution of risks and benefits can be guaranteed, and the medium- and long-term socio-economic consequences. The tendency to produce a gap in availability of and access to treatment, which undermines justice and the right (to protection) of health, must be fought.

THE RATIONALIST DELUSION

Author: Giovanni Carrada

Public communication on “hot” biomedical topics is more complex than most doctors, scientists and public health officials think. Perhaps the most widespread misconception is that conveying the facts through a rational argument, using a simple language, is just about all it takes to get the message across. Several prominent failures, including the current difficulty in reducing Covid-19 vaccine hesitancy, however, should have already warned us that most of the times facts alone don’t persuade people. Feelings do. As recent developments in cognitive psychology suggest, human rationality has not evolved to seek the truth, but to defend decisions that have already been taken by the emotional mind, and especially criticize other people’s arguments. Purely rational arguments only work in science because it is a collective, rule-based enterprise in which any claim is checked by peers. Outside the scientific community, any communication aiming at persuading people should therefore be much more sophisticated. From Aristotle’s rules of rhetoric to the most recent findings on cognitive biases and moral intuitions, several tools can help to craft messages that are not just factually correct, but whose interpretation by ordinary people is also correct. Recent examples of successful science communication show how powerful messages can be when they are based on a better understanding of how human minds really work.

MODELLING AMNIOTIC MEMBRANE HEALING EFFECT ON CHRONIC AND DIABETIC FOOT ULCERS USING IN VITRO CELL SYSTEMS TO UNRAVEL THE MOLECULAR MECHANISMS BEHIND ITS THERAPEUTIC EFFECT

Authors: Ángel Bernabé-García[1], Sergio Liarte[1], Mónica Rodríguez-Valiente[1,2], Caterina Pipino[3], Javier Stelling-Férez[1,4], José M. Moraleda[5], Assunta Pandolfi[3], Gregorio Castellanos[2], Francisco J. Nicolás[1]

Affiliation: [1]Regeneration, Molecular Oncology and TGFß. IMIB-Arrixaca, Murcia, Spain. [2]Chronic Wounds and Diabetic Foot Ulcer unit, Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain. [3]Department of Medical, Oral and Biotechnological Sciences, University G. d'Annunzio CHIETI-PESCARA, Italy. [4]Department of Nutrition and Food Technology, UCAM, Guadalupe, Murcia, Spain. [5]Hematology, Haematopoietic Transplantation and Cellular Therapy, University of Murcia, Murcia, Spain

OBJECTIVE

During wound healing, the migration of keratinocytes onto newly restored extracellular matrix aims to finalize the healing process. Transforming growth factor (TGF)-ß plays a very important role at the wound healing process. Alteration of its expression may end up in a chronification of the wound, impeding its natural healing. The application of Amniotic Membrane (AM) at wounds stalled at the wound healing process (e.g., chronification, diabetic foot ulcer) has proven very successful at restarting wound healing, in particular re-epithelialization.

MATERIALS AND METHODS

We used cryopreserved AM obtained from elective cesareans on a cell model (HaCaT) to investigate its effect. Additionally, we used wound healing scratch assay to test the effect of AM on cell migration and we measured by cytoimmunofluorescence staining the expression of important proteins required for cell migration at the wound edge of scratch assays. Also, we used long term transforming growth factor (TGF)-ß treated HaCaT cells that mimic the chronic wound environment and Human Umbilical Vein Endothelial Cells (HUVEC) from gestational diabetic mothers that recapitulate diabetes affected vessel's endothelium. We also studied patient's chronic wound tissue sections.

RESULTS

When applied on epithelial cells, AM activates several important signaling pathways required for migration and proliferation. Importantly, we have seen that AM exert a delicate control on TGFß pathway. The management of TGFß signaling on cell proliferation together with stimulation of migration confers to AM the ideal control on different parameters that must be controlled for the keratinocytes successfully re-epithelialize. Moreover, an important role of AM in the cytoskeleton and focal adhesions restructuring has been envisaged. Additionally, we have assayed the effect of AM on HUVEC coming from gestational diabetes mothers. The analysis of several parameters, upon application of AM, suggest an improvement of features compatible with vascularization improvement. Finally, we have used AM in the treatment of TGFßchronified keratinocytes. In this cell system, the presence of AM produces a clear improvement of several parameters related with the resetting of a successful wound healing. The comparison to patient´s chronic wounds offered interesting similarities.

CONCLUSIONS

Amniotic membrane is a powerful agent at healing complicated wounds. The precise knowledge of the molecular mechanisms involved on the phenomena will allow us to improve its application and to look for successful AM based ameliorated strategies for future therapeutic application.

MESENCHYMAL STROMAL CELLS IN CROHN'S DISEASE

Authors: **Rachele Ciccocioppo**, Valeria Zuliani, Luca Frulloni

Affiliation: Gastroenterology Unit and Laboratory of Gastroenterology, Department of Medicine; A.O.U.I. Policlinico G.B. Rossi & University of Verona - Italy

Crohn's disease (CD) is a lifelong segmental inflammatory enteropathy caused by a dysregulated immune response towards bacterial antigens that develops in genetically susceptible individuals. Three behaviors are recognized, i.e. inflammatory, structuring and penetrating, with the last one being the most invalidating. The advent of cellular therapies, mainly based on the use of mesenchymal stromal cells (MSCs) represents a great step forward thanks to their immune-evasiveness, immunomodulatory effects and high safety profile. Recently, a number of Phase 1-2 studies were carried out, where the use of autologous or allogeneic systemic infusions of bone marrow- and placenta-derived MSCs for treatment-resistant inflammatory CD was tested. These studies showed that this therapeutic approach is feasible and safe, as well as significantly effective since disease remission was achieved in half the patients with a follow-up ranging from 6 weeks to 24 months despite only refractory cases were enrolled. Remarkably, the best outcome was obtained when serial infusions were performed. This opens up the question of the half-life or at least the duration of the therapeutic effects of MSCs, a crucial point in establishing the right timing for infusions. This prompted the Gastrointestinal Committee of the International Society for Cell & Gene Therapy to establish a Consensus in an effort to design informative and consistent clinical trials for the intravenous use of MSCs in this condition. Clearer and more unambiguous results were obtained when using MSC local injections for fistulising refractory Crohn's disease. Notably, the results of the first phase III multicentre trial, where 212 patients were enrolled and randomly assigned to receive a single local injection of an industrial preparation of allogeneic adipose tissue derived-MSCs (Darvadstrocel) or placebo, showed that MSCs performed better than placebo to achieve remission at week 24 within a shorter period of time. Moreover, fistula healing was maintained in most cases at one year, although further studies showed that the proportion of patients relapsing upon a longer follow-up increased over time. An *in vitro* study showed the ability of MSCs to induce apoptosis of mucosal T-cells isolated from CD patients and to decrease pro-inflammatory cytokines, thus interrupting the magnification of inflammation, while a sustained increase of regulatory T-cells was observed *in vivo*. Finally, MSCs were also successfully applied in an experimental model of colonic fibrosis where the ability to inhibit the accumulation of fibrotic tissue, the expression of fibrotic molecules and the epithelial-to-mesenchymal transition was clearly evident. Should these results be confirmed in clinical trials, the chance of safely and efficaciously treating this dreadful condition will become a real prospect.

HSC GENE THERAPY FOR THE TREATMENT OF NEUROMETABOLIC AND NEURODEGENERATIVE CONDITIONS

Author: Alessandra Biffi, MD

Affiliation: Haematology, Oncology and Stem Cell Transplant Division, Padua University Hospital, Padua, Italy

Hematopoietic stem and progenitor cell (HSPCs) have the ability to engraft in the central nervous system (CNS) upon systemic or local transplantation in myeloablated recipients and give rise to a microglia-like progeny. Prior to transplantation, HSPCs can be genetically modified so that their CNS progeny could i) deliver therapeutic molecules across the blood brain barrier and ii) modulate neuroinflammation and other local neurodegenerative processes. We will discuss recent results obtained by optimization of genetically modified HSPC transplant approaches for the treatment of neurometabolic and neurodegenerative conditions.

CLINICAL GRADE MESENCHYMAL STROMAL CELL-DERIVED EXTRACELLULAR VESICLES PROMOTE HUMAN CARTILAGE RECOVERY *IN VITRO*

Authors: Maria Elisabetta Federica Palamà[1], Simona Coco[2], Georgina Shaw[3], Daniele Reverberi[2], Dario Pisignano[4,5], Katia Cortese[6], Frank Barry[2], Mary Murphy[2], Chiara Gentili[1]

Affiliation: [1]Department of Experimental Medicine (DIMES), University of Genoa, Italy. [2]IRCCS Policlinico San Martino, Genoa, Italy. [3]Regenerative Medicine Institute (REMEDI), National University of Ireland Galway (NUI Galway), Galway, Ireland. [4]Nanoscience Institute CNR-NANO (NEST), Pisa, Italy. [5]Department of Physics, University of Pisa, Pisa, Italy. [6]DIMES, Department of Experimental Medicine, Human Anatomy, University of Genoa, Italy

OBJECTIVE

Osteoarthritis (OA) is a disabling joint disorder causing articular cartilage degeneration. Currently, treatments mainly aimed to pain and symptoms relief, rather than disease amelioration. Human bone marrow stromal cells (hBMSCs) have emerged as a promising paracrine mechanism-based approach for the treatment of OA. Here, we investigate the therapeutic potential of extracellular vesicles (EVs) isolated from hBMSC in an in vitro model of OA.

MATERIALS AND METHODS

We developed a "donor-to-patient" closed, scalable and automated system for aseptic therapeutic cell manufacturing using a a xeno-free culture system (XFS). We characterized extracellular vesicles (EVs) derived from hBMSCs, grown in XFS compared to a conventional fetal bovine serum (FBS) culture system, in normoxic and hypoxic culture setting. We investigated also the therapeutic potential of EVs in an in vitro model of OA, on IL-1α-treated human articular chondrocytes (hACs). We characterized the miRNA content of EVs in different culture setting to select putative miRNA that could be involved in a biological function.

RESULTS

The biological effects of XFS- and FBS-cultured hBMSCs was tested on IL-1α treated hACs in an experiment designed to mimic the OA environment. We observed that under inflammatory conditions hACs are able to recruit and internalize more MSC-derived EVs, especially those derived from cells cultured in our XFS system and in hypoxic condition. Treatment with hBMSC-EVs inhibited IL-1α-induced expression of IL-6, IL-8, and COX-2 by hACs. Furthermore, we observed that hBMSCs grown in XFS produced a higher amount of EVs compared to FBS-culture. Analysis of miRNA content showed the upregulation in XFS-hBMSC-derived EVs of miRNA known to have a chondroprotective role, such as miR-17, miR-140, miR-145, miR-30a, miR-29a, miR-130a, miR-199a. Interestingly, most of the miRNA found in our preparations seem to be involved in cartilage homeostasis and they affect TGF-beta signaling.

CONCLUSIONS

In conclusion, the XFS medium was found to be suitable for isolation and expansion of hBMSCs with increased production of EVs. The application of cells cultured exclusively in XFS overcomes issues of safety associated with serum-containing media and makes ready-to-use clinical therapies more accessible.

AMNIOTIC MESENCHYMAL STROMAL CELLS INHIBIT CD8 T LYMPHOCYTE ACTIVATION AND MEMORY COMMITMENT VIA MTOR SIGNALING PATHWAY

Authors: Andrea Papait[1,2], Elsa Vertua[1], Patrizia Bonassi Signoroni[1], Antonietta Silini[1], Ornella Parolini[2,3]

Affiliation: [1]Centro di Ricerca E. Menni, Fondazione Poliambulanza, Brescia, Italy. [2]Department of Life Sciences and Public Health, Università Cattolica del Sacro Cuore, Rome, Italy. [3]Policlinico Universitario Fondazione Agostino Gemelli

OBJECTIVES

CD8 T lymphocytes are responsible for the formation of immunological memory. CD8 lymphocytes also play a fundamental role in rejection mechanisms due to their ability to recognize HLA class I mismatching. Their activation leads to the formation of two cell subsets involved in the prompt response and in the establishment of immunological memory: short-lived effector cells (SLEC) and memory precursor effector cells (MPEC). Over the years, several mesenchymal stromal cells (MSC) have been shown to modulate the activation of T lymphocytes, in particular CD4, inhibiting their differentiation towards inflammatory subsets and triggering instead the polarization towards the Treg subset. However, little is known about the effects of MSC on CD8. We previously demonstrated that amniotic mesenchymal stromal cells (hAMSC) inhibit the proliferation of CD8 lymphocytes and the expression of cytotoxicity markers. However, their impact on memory cell differentiation remains completely unexplored. Here, we analyzed in detail the effects of hAMSC on the formation of memory subsets.

MATERIALS AND METHODS

hAMSC were co-cultured with allogeneic PBMCs derived from healthy donors and stimulated with anti-CD3 monoclonal antibody. Activation and proliferation of CD8 T lymphocytes, as well as differentiation toward different Naïve/Memory, SLEC, and MPEC subsets, were assessed at day 3 and day 7. In addition, CD8 T lymphocyte phosphorylation and transcriptional profiles were evaluated.

RESULTS

Flow cytometry analysis revealed that hAMSC inhibited the proliferation of both naïve and memory CD8 lymphocytes, thus affecting SLEC and MPEC subset differentiation. In addition, hAMSC reducted phosphorylation of AKT and mTOR, two key factors of the PI3K pathway, that drive T lymphocyte activation and differentiation. Furthermore, hAMSC also affected the expression of the T box transcription factors Tbet and Eomesodermin (Eomes) that play a key role in orchestrating SLEC or MPEC differentiation.

CONCLUSIONS

Although much is known about the ability of MSC to modulate the immune response, some aspects remain unexplored. Here we provide new insights into the effects of hAMSC on CD8 lymphocyte activation and differentiation, and also on the mechanism of action involved.

DNA DAMAGE RESPONSE OF NEURAL PROGENITORS AND NEURODEVELOPMENTAL PATHOLOGIES

Author: O Etienne[1], C Granotier-Beckers[1], S Mokrani[1], T Kortulewski[1], JP de Villartay[2], **FD Boussin**[1]

Affiliation: [1]LRP UMR SGCSR, Inserm, Université de Paris, Université Paris-Saclay, CEA, 92265 Fontenay-aux Roses, France. [2]Genome Dynamics in the Immune System Laboratory, Inserm, UMR 1163, Institut Imagine, Université Paris-Descartes, Sorbonne Paris-Cité, France

OBJECTIVE

Neural progenitor (NP) have to deal with DNA double-strand breaks (DSBs) that may arise spontaneously from various endogenous sources in the developing brain. High fidelity of genetic transmission has been long time considered to be crucial for brain development and homeostasis. However, recent studies have identified recurrent DSB clusters in dividing NP that may underlie the diversity of neuronal cell types, raising the need in understanding how NP sense and repair DSBs. To this end, we have investigated the consequences for mouse NP *in vitro* and *in vivo* of the deficiency of XLF/Cernunnos which is involved in cNHEJ, a major DNA DSB repair pathway.

MATERIAL AND METHODS

The effects of acute (γ-radiation) and chronic (incorporation of tritiated thymidine —^{3}H-T— into DNA) genotoxic stress were tested on cultures of NP and on proliferating non-stem cells (murine embryonic fibroblasts, MEF) harvested from *wt* and *Xlf-/-* C57Bl/6N embryos at E14.5 days. The consequences *Xlf* deficiency on the mouse brain development were then appreciated by various histologic approaches.

RESULTS

Xlf-/- cells highlighted that NP have higher capacity than MEF to maintain their chromosome stability in response to acute and chronic genotoxic stress *in vitro.* This was related to a higher DNA repair activity, further enhanced by an adaptive response to genotoxic stress, and also to the elimination of too damaged cells by apoptosis.

We evidenced a low increase of apoptosis in the brain of *Xlf-/-* mouse embryos, but also, strikingly, an early shift of neural progenitors from proliferative to neurogenic divisions. This premature neurogenesis resulted from an increase in chromatid breaks affecting mitotic spindle orientation of *Xlf-/-* neural progenitors and appears to play an important role in the neurodevelopmental pathologies observed in *Xlf-/-* mice.

CONCLUSIONS

Our study unveils some particular features of the DNA damage response of NP in direct connection with their specific generative/regenerative functions, which requires a fully functional cNHEJ pathway. These features may allow them both to preserve their genome stability and to insure their newly described possible function in creating neuronal genetic diversity. Impairments of NP are key events in neurodevelopmental disorders, particularly those resulting from exposure to environmental genotoxic stress. A full characterization of how NSP sense and repair DNA damage is a critical step for the elucidation of the mechanisms involved.

REPARATIVE MEDICINE; CHALLENGE TO MEDICAL INNOVATION BY NON-TUMORIGENIC PLURIPOTENT MUSE CELLS

Author: Mari Dezawa, MD, PhD

Affiliation: [1]Department of Stem Cell Biology and Histology, Tohoku University Graduate School of Medicine, Japan

Multilineage-differentiation stress enduring (Muse) cells are naturally existing, non-tumorigenic reparative endogenous stem cells identified by SSEA-3(+), a surface marker for pluripotent stem cells. They are able to differentiate into endodermal-, mesodermal- and ectodermal-lineage cells in the body, according to 'the logic of the site' where they integrated. Normally, they are mobilized from the bone marrow to the peripheral blood and distribute to the connective tissue of every organ, where they daily replace damaged/lost cells by spontaneous differentiation into tissue-constituent cells and contribute to tissue homeostasis. Circulating Muse cells express receptor for damage signal, sphingosine-1-phosphate, and are thus able to specifically home to damaged site to regenerate tissue. When the number of endogenous Muse cells is not sufficient, however, administration of exogenous Muse cells by intravenous drip is expected to deliver robust functional recovery, as demonstrated in animal models of liver cirrhosis, stroke, chronic kidney disease, myocardial infarction and diabetes mellitus.

Muse cells do not need to be "induced" or genetically manipulated to exhibit pluripotency or to differentiate into target cell type for clinical use. Intravenous drip is the main method of administration, making surgical operation unnecessary. Furthermore, because Muse cells have an immunomodulatory system similar to the placenta, donor-derived Muse cells can be directly administered to patients without HLA-matching or long term-immunosuppression therapy. Allogeneic Muse cells remain in the host tissue as differentiated functional cells for more than half a year. Clinical trials for the treatment of myocardial infarction, stroke, epidermolysis bullosa, spinal cord injury and cerebral palsy with intravenous drip of donor-derived Muse cells are currently being conducted by the Life Science Institute Inc., a member of Mitsubishi Chemical Holdings Corporation.

Muse cells may safely provide clinically relevant effects compatible with the 'body's natural repair systems' by a simple cost-effective strategy; collection, expansion and intravenous drip, thus may deliver medical care innovation.

ROLE OF AMNIOTIC MEMBRANE ON ENDOTHELIAL CELLS FROM UMBILICAL CORD OF WOMEN AFFECTED BY GESTATIONAL DIABETES: NEW INSIGHTS INTO THE TREATMENT OF NON-HEALING DIABETIC FOOT ULCERS

Authors: Caterina Pipino[1], Ángel Bernabé-García[2], Ilaria Cappellacci[1], Javier Stelling-Férez[2,3], Pamela Di Tomo[1], Carlos Navalón[2], Manuela Santalucia[1], Nadia Di Pietrantonio[1], Natalia Di Pietro[1], Assunta Pandolfi[1], Francisco José Nicolás[2]

Affiliation: [1]Center for Advanced Studies and Technology-CAST (ex CeSI-MeT), Department of Medical, Oral and Biotechnological Sciences, University G. d'Annunzio Chieti-Pescara, StemTeCh group, Chieti, Italy. [2]Regeneration, Molecular Oncology and TGFß, IMIB-Arrixaca, Murcia, Spain. [3]Department of Nutrition and Food Technology, UCAM, Murcia, Spain

OBJECTIVE

Diabetic foot ulcer (DFU) is a severe diabetes complication which affects 15% of patients with advanced stages of the disease. Despite improvements in the treatment, DFU remains the main cause of lower limb amputation. One of the most relevant diabetic wounds healing issues is the reduced peripheral blood flow, the diminished neovascularization and chronic inflammation. Amniotic membrane (AM) has shown promising results in the treatment of DFU. Here, the potential role of AM on endothelial cells isolated from umbilical cords of gestational diabetes affected women (GD-HUVECs) has been investigated. Indeed, GD-HUVECs have shown a pro-inflammatory phenotype and reduced migration capacity as well as decreased vessels formation on Matrigel compared to control HUVECs, representing a useful model for studying the role of AM in the neovascularization of chronic non-healing wounds.

MATERIALS AND METHODS

For GD-HUVECs cultured under different conditions we evaluated: monocyte-endothelium interaction (adhesion assay); Vascular and Intercellular adhesion molecules 1 (VCAM-1, ICAM-1), E-selectin (SELE) and chemokine CC ligand-2 (CCL2) protein and gene expression (flow cytometry and qPCR); VCAM-1, ICAM-1 membrane exposure (flow cytometry); NO/cGMP (nitric oxide/cyclic guanosine monophosphate) levels (Elisa); NF-kB nuclear translocation (Immunofluorescence); vessel formation (Matrigel tube formation assay).

RESULTS

AM treatment notably improved cell migration and vessel formation in GD-HUVECs. Moreover, it significantly reduced TNF-α stimulated monocyte-endothelium interaction and the expression of VCAM-1, ICAM-1, SELE and CCL2 in C- and GD-HUVECs. That was also reflected on a reduction on membrane exposure of VCAM-1 and ICAM-1 in both cell types upon AM stimulation. This was associated with a significant decrease of NF-kB cytoplasm-nucleus translocation. Finally, AM-increased NO bioavailability was affected by eNOS inhibitor L-NAME, suggesting that AM activation of eNOS could be a possible way of regulating this phenomenon.

CONCLUSIONS

Overall, our results indicate that AM is effective in reducing TNF-α-induced inflammation and improving vasculogenesis, possibly through the modulation of NO bioavailability, which plays a key role in the vascular homeostasis balance. This study suggests that AM improves chronic wound healing by both enhancing angiogenesis and decreasing inflammation, thus reinforcing its clinical application for diabetic foot ulcers.

CHARACTERIZATION OF SENESCENT VASCULAR MESENCHYMAL STROMAL CELLS AND THEIR ROLE IN THE DEVELOPMENT OF THE ABDOMINAL AORTA ANEURYSM

Authors: Gabriella Teti[1], Maria Carla Mazzotti[2], Laura Ingrà[1], Valentina Gatta[1], Francesca Chiarini[3,4], Mirella Falconi[1]

Affiliation: [1]Department of Biomedical and Neuromotor Sciences, University di Bologna, 40126, Bologna, Italy. [2]Department of Medical and Surgical Sciences, University of Bologna. [3]CNR-National Research Council of Italy, Institute of Molecular Genetics "Luigi Luca Cavalli-Sforza", Unit of Bologna, 40136 Bologna, Italy. [4]IRCCS Istituto Ortopedico Rizzoli, 40136 Bologna, Italy

OBJECTIVE

Aging is defined as a time dependent functional decline affecting multiple organs possibly caused by an increase of the number of damaged cells over time. Cellular senescence is considered one of the main hallmarks of aging. It is a consistent hyporeplicative state which has beneficial roles either in physiological and pathological conditions. However, the accumulation of senescent cells during aging has adverse consequences and it is believed to have a key role in the onset and development of several age-related diseases. Abdominal aorta aneurysm (AAA) is an age-related vascular disorder characterized by the dilation of the abdominal aorta, which could cause vascular rupture over time. Reparative abilities of vascular mesenchymal stromal cells (MSCs) have a key role in vascular remodeling. However, unbalanced activity of vascular MSCs support AAA pathogenesis. Thus, our study was aimed to investigate, in cells isolated from segments of abdominal aorta, the presence of vascular MSCs with a senescent phenotype, which could be responsible of the impaired reparative abilities of MSCs in vascular remodeling.

MATERIALS AND METHODS

Vascular MSCs were isolated from pathological and healthy segments of abdominal aorta. Proliferation rate, ROS production, cell surface area, expression of the senescent markers $p21^{CIP1}$ and $p16^{INK4a}$, activation of the DNA damage response, autophagy and vascular differentiation ability were investigated in MSCs isolated from AAA samples and compared to MSCs isolated from vascular healthy segments.

RESULTS

All the data from AAA samples compared to the data of healthy ones clearly demonstrated a reduced proliferation ability, an increase of ROS levels, the positive expression of the senescent markers $p21^{CIP1}$ and $p16^{INK4a}$, a dysregulated autophagy and a strongly impaired ability in differentiating toward an endothelial phenotype.

CONCLUSIONS

All these results indicate the presence of senescent vascular MSCs in the wall of AAA and strongly support the hypothesis that an accumulation of senescent vascular MSCs could have a pivotal role in the onset and development of AAA.

(YIA) EQUINE FREEZE-DRIED SECRETOME FOR JOINT REGENERATION IN VETERINARY MEDICINE: PRODUCTION PROCESS AND IN VITRO EFFICACY

Authors: Priscilla Berni[1], Michela Mocchi[2], Stefano Grolli[1], Silvia Dotti[3], Dario Di Silvestre[4], Riccardo Villa[3], Virna Conti[1], Giulia Passignani[4], Francesca Brambilla[4], Maurizio Del Bue[5], Laura Catenacci[2], Milena Sorrenti[2], Lorena Segale[6], Elia Bari[2], Pierluigi Mauri[4], Maria Luisa Torre[2,7], Sara Perteghella[2,7]

Affiliation: [1]Department of Veterinary Medical Science, University of Parma, Parma, Italy. [2]Department of Drug Sciences, University of Pavia, Pavia, Italy. [3]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Brescia, Italy. [4]Institute of Biomedical Technologies, Milano, Italy. [5]Freelance Veterinary Medical Doctor, Parma, Italy. [6]Departemt of Pharmaceuticals Sciences, University of Piemonte Orientale, Novara, Italy. [7]PharmaExceed S.r.l., Pavia, Italy

OBJECTIVE

To produce and characterize a freeze-dried secretome (Lyo-secretome) from equine Mesenchymal Stromal Cells (MSCs) to be applied in the treatment of equine musculoskeletal diseases. The Lyo-secretome has been tested in-vitro on equine tenocytes, chondrocytes, synovial fluid (SF) and adipose-MSCs (AD-MSCs) to assess its cytocompatibility and efficacy to maintain cell proliferation.

MATERIALS AND METHODS

The product was characterized in terms of total protein and lipid content, Nanoparticle Tracking Analysis, scanning electron microscopy, Fourier Transform Infrared Spectroscopy (FTIR), Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC). A proteomic investigation based on nano Liquid Chromatography and high-resolution mass spectrometry was performed. The ability of Lyo-secretome (dosage 0-20 mg/mL, corresponding to 0-200x10^3 cell equivalents/mL) to improve the metabolic activity of AD-MSCs, SF-MSCs, tenocytes and chondrocytes was evaluated by MTT test. The FU quality control test for injectable pharmaceutical forms were also performed to evaluate sterility, and bacterial endotoxin and mycoplasma content of Lyo-secretome.

RESULTS

Equine Lyo-secretome contained a mean protein amount of 9.4 ± 0.66 µg/mg and lipid amount of 1.16 ± 0.16 µg/mg (mean values ± SD, n=3). EVs mean diameter resulted 157.8 ± 6.4 nm. TGA revealed a residual water content of 0.4% w/w; DSC analysis indicated that the lyophilization process was concluded successfully, and FTIR spectra analysis showed bands corresponding to the presence of both lipids and proteins (bands at 1738 cm^{-1} due to the presence of carbonyl bonds, at 1457 cm^{-1} and 1374 cm^{-1} related to CH_2 and CH_3 groups). MTT assay showed a dose-response increase in cell metabolic activity on tenocytes, chondrocytes, SF-MSCs and AD-MSCs, reaching almost 80% at higher concentrations of Lyo-secretome, considering 10% FBS as the positive control (100%). Lyo-secretome resulted suitable for injectable pharmaceutical dosage forms. Furthermore, the proteomic analysis demonstrated the presence of proteins endowed with antibacterial activity, and proteins involved in the modulation of inflammatory process, tissue homeostasis and cartilage biology.

CONCLUSION This work demonstrates the feasibility of the preparation of a bioactive Lyo-secretome from equine AD-MSCs. Lyo-secretome exerts a direct activity on tenocytes/chondrocyte as well as on tissue resident MSCs. The work paves the way for the preclinical use of Lyo-secretome aimed to establish its safety and efficacy in equine musculoskeletal disease (tendonitis, osteoarthritis).

STANDARDIZED SINGLE PARTICLE MEASUREMENT OF NUMBER, SIZE AND CHARGE IS REQUIRED FOR CONFIDENCE IN NANOMEDICINE ENGINEERING AND DEVELOPMENT

Authors: Stephane Mazlan, PhD

Affiliation: Izon Science

Primarily thought to be recycled cellular waste biomolecules, extracellular vesicles (EVs) have now emerged as a new class of therapeutics for regenerative nanomedicine. Therefore, efficient isolation and accurate quantification and size determination are imperative in nanomedicine studies involving nano-sized EVs. The progression from bench to the bedside, a hallmark of translational research and application, renders both quality and discipline of EV isolation and measurement increasingly critical. Indeed, advancement in isolation and measurement technology has evolved to meet this need. Accurate measurements can now be done efficiently, rapidly and easily.

Ultracentrifugation has been deemed the gold standard for EV isolation. However, this often results in a reduced yield due to damaged or lost EVs, urging researchers to balance between yield and purity. EVs isolated by Size Exclusion Chromatography (SEC) columns, however, have been shown to not only preserve EV morphology but also provide higher EV purity attested by a higher level of EV marker detection.

In terms of EV measurement, high resolution is an important aspect. It has been shown that dynamic light scattering (DLS) for instance, lacks the resolution necessary to for accurate and precise nanoparticle measurements especially when it comes to multimodal samples. This is highly important especially in measuring complex nano biological particles such as EVs where size heterogeneity is an aspect. The level of detail and certainty that Tunable Resistive Pulse Sensing (TRPS) offers is indeed beneficial in the nanomedicine field. This technology has demonstrated precision in size, concentration and zeta potential determination where subpopulations in multimodal samples can be accurately portrayed and distinguished. The current TRPS instrument provides the accuracy and resolution of EV measurements and is even capable of measuring EVs as small as 30 nm.

The nano-medicine research community is beginning to make advances in developing EVs as a new class of biological therapeutics. The downstream translation applications range from gene therapy to drug delivery systems. The use of mesenchymal stem cell-derived EVs, for example, are demonstrated to elicit beneficial effects therapeutically and eliminate stem cell transplantation risks. As the potential for EV therapeutics grows, so does the ever-evolving need for precise isolation and measurement of these complex nano biological particles – a challenge that Izon strives to achieve.

3D, LABEL-FREE, LIVE CELL IMAGING FOR CELL MORPHOLOGY AND PHENOTYPING

Author: Daniel Ghete

Holotomography (HT) is a technology that allows researchers to image the cell morphology in 3D using no labels. HT uses laser interferometry to measure 3D refractive index (RI) distribution. Thus, 3D images of live cells without any molecular labelling, such as dye staining or DNA transfection, can be obtained with high spatial resolution (resolved to 110 nm). Furthermore, HT images can be analysed to provide quantitative information -cell volume, dry mass and protein concentration of a single cell.

Due to the extremely low amount of light needed to acquire HT images, long term Live Cell Imaging is possible as there is no phototoxicity to the cell.

The technology itself aids a precise measurement of Refractive Index (RI) at a nanoscale, granting the visualization of sub-cellular organelles. Also, RI quantification permits a precise estimation of protein and lipid concentration in the cells and their changes over time.

The Tomocube system uses a Digital Micro-mirror Device (DMD) to enable the illumination beam rotation. We developed our proprietary technology to precisely control the intensity and angle of the beam reflected from a DMD. The patented technology behind the beam rotation provides unique advantages over other methods. Highly stable, fast and reliable electronic control of the light path through the DMD eliminates moving parts for better stability and improved image resolution.

The cell membrane fluctuation can be assessed as the capture speed of the data sets is very fast: 3D images can be captured at 2FPS while 2D images can be acquired at over 150FPS.

The new Tomocube HT-2 microscope is a Fast, Quantitative, and Label Free instrument with the capability to acquire images that combines 3D HT and 3D Fluorescence (FL) to allow correlative imaging with specificity. The acquisition of HT and FL are decoupled to allow independent strategies, which minimize the phototoxicity from FL imaging. The use of FL imaging with HT imaging allows for accurate description of cell morphology and phenotype.

CANCER STEM CELLS AND TUMOR MICROENVIRONMENT: A TARGET FOR CHEMOPREVENTION?

Author: Adriana Albini

Affiliation: Laboratory of Vascular Biology and Angiogenesis, IRCCS MultiMedica, Milan, Italy

The Tumor Microenvironment (TUMIC) consists of extracellular matrix components as well as cellular players among which endothelial, stromal and immune cells. The modulation of Cancer Stem Cells or Cancer Initiating Cells (CSC/CIC) activities by the tumor microenvironment (TUMIC) is crucial for tumor heterogeneity. CSC/CIC may interact with the TUMIC depending on the cells or proteins encountered, providing and responding to signals to/ from the CSC/CIC. The interplay contributes to mechanisms through which CSC/CIC may reside in a dormant state or giving rise to tumor recurrence and metastasis. Connective tissue and TUMIC cells, can differentially activate CIC/CSC in different areas of a tumor and contribute to the generation of cancer heterogeneity.
Chemoprevention represent a novel therapeutic option that could target both CSC/CIC and the microenvironment to elude resistance mechanisms activated by CSC/CIC, and “prime” the CSC to respond to Chemotherapy.
We will present examples of chemopreventive on repurposed drugs (i.e. metformin) effects on CSC.

AN ANTITUMORAL CELLULAR VIROIMMUNOTHERAPY USING MESENCHYMAL PROGENITOR CELLS

Author: Javier Garcia-Castro, PhD

Affiliation: Cellular Biotechnology Unit. National Institute of Health Carlos III, Madrid-Spain

OBJECTIVE

The main aim is the improved of a new cellular virotherapy consisting in an oncolytic adenovirus carried by mesenchymal progenitor cells. This therapy, called Celyvir, has been tested in pre-clinical murine models, a veterinary clinical trial and, finally, a human clinical trial.

MATERIALS AND METHODS

Ex vivo infection of murine, canine and human mesenchymal progenitors cells with murine, canine and human oncolytic adenovirus. Weekly intravenous administration of infected mesenchymal progenitors cells in murine inmunodeficient and immunocompetent mice, in canine patients with spontaneous tumors and in human oncologic pediatric patients.

RESULTS

Antitumoral effects detected in mice models, 75% of clinical response in veterinary trial, including a 15% of complete response and, clinical benefits in human patients –including two complete remissions– with a remarkable absence of side effects. Our studies also indicates that the antitumor efficacy is not only derived from the lytic cycle of the virus, but also from the capacity of the treatment to switch immunologically cold tumors into pro-inflammatory hot tumors.

CONCLUSIONS

Delivered by mesenchymal progenitor cells, oncolytic virotherapies may add value to the intrinsic viral oncolytic capacities and immunotherapeutic effects. Several characteristics of the mesenchymal progenitor cells, related to their tumor-homing and immune-modulation capacities, would contribute to the success of this cellular viroimmunotherapy.

(YIA) ANGIOGENIC PROPERTIES OF PERICYTES ISOLATED FROM MICRO-FRAGMENTED FAT TISSUE

Authors: **Ekta Manocha**[1], Emilio Ciusani[2], Moris Cadei[3], Arnaldo Caruso[1], Giulio Alessandri[1]

Affiliation: [1]Section of Microbiology, Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy. [2]Laboratory of Clinical Pathology and Neurogenetic Medicine, Fondazione IRCCS Neurological Institute C. Besta, Milan, Italy. [3]Section of Pathological Anatomy, Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy

OBJECTIVE

Pericytes (PCs) are mesenchymal stromal cells (MSCs) that act as a support to endothelial cells for vascular stability. Adipose MSCs isolated from liposuction of fat tissue has gained interest due to their potential regenerative capacity and treatment of a variety of pathological conditions like wound healing, stroke, or brain injury. The fragmented tissue has been observed to secrete a plethora of factors which may have a role in their potential angiogenic activity towards endothelial cells. Our aim is to identify the interaction and function of PCs isolated from micro-fragmented adipose tissue (MFAT) in relation to their activity on human endothelial cells (ECs).

MATERIAL AND METHODS

PCs were selected on the basis of CD146 marker-based cell sorting. Adhesion assays were performed to study the interactive properties with HUVECs whereas collagen-based migration assay was performed to study the response of PCs motility in the presence of different growth factors. Matrigel assay and 3-D spheroid assay were performed to identify the angiogenic properties of PCs. Conditioned medium (CM) from CD146-selected cells and fat tissue was used for proliferation assay.

RESULTS

Here, we found that CD146-selected pericytes from adipose tissue induced the proliferation, migration and angiogenic activity of endothelial cells (HUVECs). In addition, we also found that the two different populations (CD146-/CD146+) adhere differently to ECs and therefore may dictate their stage of maturity in regulating vessel stability. We speculate the expression of adhesion molecules on the surface of CD146+ PCs differently from CD146- PCs hence making CD146 an indispensable marker for pericyte selection.

CONCLUSION

We investigate the interactive properties of different population of pericytes from micro-fragmented adipose tissue (MFAT) with ECs defining the maturity stages of MFAT-derived CD146+ or CD146- pericyte population in deciphering their range of function from vascular remodeling to angiogenic activity.

IN-VIVO EVALUATION OF BIMODAL NANOPARTICLE-LOADED MESENCHYMAL STROMAL CELL FOR OSTEOARCOMA TREATMENT

Authors: Enrico Lucarelli[1], Elisa Martella[2], Barbara Dozza[1], Claudia Ferroni[2], Chiara Bellotti[1], Andrea Guerrini[2], Marta Columbaro[3], Giovanna Sotgiu[2], Lucia Martini[4], Milena Fini[4], Luca Cevolani[5], Davide Maria Donati [1,5,6], Toni Ibrahim [7], Greta Varchi[2], Serena Duchi[2]

Affiliation: [1]Unit of Orthopaedic Pathology and Osteoarticular Tissue Regeneration, 3rd Orthopaedic and Traumatologic Clinic prevalently Oncologic, IRCCS Istituto Ortopedico Rizzoli, Via di Barbiano 1/10, 40136 Bologna, Italy. [2]Institute of Organic Synthesis and Photoreactivity (ISOF), National Research Council (CNR), Via Gobetti, 101, 40129 Bologna, Italy. [3]Scientific Direction, IRCCS Istituto Ortopedico Rizzoli, Via di Barbiano 1/10, 40136 Bologna, Italy. [4]Complex Structure of Surgical Sciences and Technologies, IRCCS Rizzoli Orthopaedic Institute, Bologna, Italy. [5]3rd Orthopaedic and Traumatologic Clinic prevalently Oncologic, IRCCS Istituto Ortopedico Rizzoli, Via di Barbiano 1/10, 40136 Bologna, Italy. [6]Department of Biomedical and Neuromotor Sciences (DIBINEM), Alma Mater Studiorum University of Bologna, Via Ugo Foscolo 9, 40123 Bologna, Italy. [7]Osteoncology, Bone and Soft Tissue Sarcomas and Innovative Therapies Unit, IRCCS Istituto Ortopedico Rizzoli, Via di Barbiano 1/10, 40136 Bologna, Italy

OBJECTIVE

Mesenchymal stromal cells (MSC) have the intrinsic property to migrate and infiltrate the tumour stroma. Thus, the use of MSC as carrier for anticancer drugs to enhance their cytotoxicity while decreasing systemic side effects is widely investigated. Osteosarcoma (OS) is an aggressive tumour that has the highest incidence among children and young adults. In our project we combined chemotherapy with photodynamic therapy (PDT), an approach where a photosensitizing agent, activated by light, produces intracellular ROS inducing cell death. To deliver both drugs, keratin-based nanoparticles (NP) were developed. Our objective was to test the efficacy of the photoactivation of NP-loaded MSC in a mouse orthotopic OS model.

MATERIAL AND METHODS

Human MSC were isolated from bone marrow of 5 different donors. Complete characterization in terms of proliferation rate (growth curve), fibroblast-colony forming unit (CFU) assay, immunophenotypic profile, and trilineage-differentiation potential of each MSC line was performed. NP were composed of hydrosoluble high molecular weight keratin chosen as carrier of a chemotherapeutic drug, paclitaxel (PTX), and a photosensitizer, chlorin e6 (Ce6). NP were obtained by a drug-induced self-aggregation method.
Based on very promising *in vitro* data, NP-loaded MSC and NP were tested *in vivo*. Orthotopic OS was induced by intra-tibia injection of SaoS-2 cells in six-week-old male immunocompromised mice (BALB/c). Five weeks after OS cells injection, mice received a peritumoral injection of NP-loaded MSC or NP and half mice were exposed to light irradiation (LED light at 668±3 nm) for 15 min. The protocol was repeated the subsequent week and mice were euthanized one week after the last treatment.

RESULTS

Results show that the combination of NP and PDT induces a massive death of tumour cells and an important immune response inside the tumour mass and in all surrounding tissues. On the contrary, histochemical analyses reveals that NP-loaded MSC and PDT do not induce tumor death. Adverse effects in term of edematous tissue and difficulty to move the leg were observed only in NP/PDT treated mice.

CONCLUSION

Taken together these results support the efficacy of these innovative NP, although MSC decrease the efficacy of the system. Further studies are needed to evaluate whether the efficacy of NP-loaded MSC could be improved modifying treatment parameters such as MSC dosage or injection numbers.

(YIA) PRIMARY GLIOBLASTOMA GLIOSPHERES INCORPORATE MESENCHYMAL STEM CELLS

Authors: **Anna Andrzejewska**[1], Blazej Nowak[2,3], Piotr Rogujski[1], Miroslaw Janowski[4,5], Barbara Lukomska[1]

Affiliation: [1]NeuroRepair Department, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland. [2]Department of Neurosurgery, Central Clinical Hospital of Ministry of the Interior and Administration, Warsaw, Poland. [3]Neurosurgery Department, John Paul II Western Hospital, Grodzisk Mazowiecki, Poland. [4]Center for Advanced Imaging Research, Department of Diagnostic Radiology and Nuclear Medicine, University of Maryland Marlene and Stewart Greenebaum Comprehensive Cancer Center, University of Maryland, Baltimore, MD, USA. [5]Tumor Immunology and Immunotherapy Program, University of Maryland Marlene and Stewart Greenebaum Comprehensive Cancer Center, University of Maryland, Baltimore, MD, USA

OBJECTIVE

Mesenchymal stem cells (MSCs) draw the attention of a wide range of scientists and clinicians. MSCs have been used in experimental attempts of central nervous system (CNS) diseases treatment. In neuro-oncology, a lot of controversies have arisen regarding the effect of MSCs on tumor development. Numerous studies indicated that MSCs transplantation could inhibit or promote growth of glioblastoma (GBM), the most aggressive cancer of CNS. However, the cause for such duality of MSC action is unknown. Our study tests the hypothesis that the impact of MSCs on GBM cells varies by molecular subtype of GBM (i.e., classical, proneural, or mesenchymal).

MATERIALS AND METHODS

The subject of our study was human GBM cells isolated from tumor samples obtained during surgical resection. Initially, we established the primary culture of GBM cells from 7 patients. Next, the subtype of GBM was determined by analyzing selected antigen expression (CD44; EGFR; MERKT; p53; Olig2) using flow cytometry. Finally, GBM cells were co-cultured with human bone marrow MSCs for 7 days.

RESULTS

We established the method of obtaining a repetitive pattern of GBM cell growth. During *in vitro* culture, GBM cells formed gliospheres that reached a diameter of 100 μm at 7 days *in vitro* (DIV) and 250 μm at 14 DIV. We have confirmed that ~100% of cells in gliospheres expressed cancer stem cell marker Nestin, indicating that our GBM cell population contains glioblastoma stem cells (GSCs). Analysis of the expression of selected antigens in GSCs revealed that so far, we have obtained 2 cultures of classical GBM subtype (with high EGFR and low p53 expression) and 5 cultures of mesenchymal GBM subtype (with high MERKT and CD44 expression). During GBM and MSCs co-culture, we observed that GBM gliospheres incorporated MSCs and grew in suspension. The assessment of the impact of MSCs on primary GBM gliospheres growth is in progress.

CONCLUSION

We established the method for the formation of primary GBM gliospheres. Our studies revealed that the determination of GBM subtypes using flow cytometry is feasible, and in the future, could translate into a viable clinical diagnostic tool. In addition, we observed the mutual interactions between MSCs and GBM cells in co-culture; however, deciphering the true nature of these interactions requires further analysis.

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CATEGORY: BEYOND STEM CELLS: THERAPEUTIC USE OF EXTRACELLULAR VESICLES
FIRST CLINICAL EXPERIENCES WITH MSC DERIVED EXTRACELLULAR VESICLES

Authors: Athanasia Warnecke[1,§,] Nils Prenzler[1], Jennifer Harre[1], Ulrike Köhl[2], Lutz Gärtner[1], Thomas Lenarz[1], Sandra Laner-Plamberger[3], Georg Wietzorrek[4], Hinrich Staecker[5], Teresa Lassacher[6,7], Julia Hollerweger[6,7], Mario Gimona[3,6,7,8], **Eva Rohde**[3,6,8 §]

Affiliation: [1]Department of Otorhinolaryngology, Head and Neck Surgery, Hannover Medical School, Carl Neuberg-Str. 1, 30625 Hannover, Germany. [2]Institute for Cellular Therapeutics, Hannover Medical School, Carl Neuberg-Str. 1, 30625 Hannover and Institute of Clinical Immunology, University of Leipzig as well as Fraunhofer Institute for Cell Therapy and Immunology (IZI), Leipzig, Germany. [3]Department of Transfusion Medicine, University Hospital, Salzburger Landeskliniken GesmbH (SALK) and Paracelsus Medical University (PMU), Strubergasse 21, 5020 Salzburg, Austria. [4]Institute of Molecular and Cellular Pharmacology, Medical University of Innsbruck, Innsbruck, Austria. [5]Department of Otolaryngology Head and Neck Surgery, University of Kansas School of Medicine, Kansas City, USA. [6]GMP Unit, Spinal Cord Injury & Tissue Regeneration Centre Salzburg (SCI-TReCS), Paracelsus Medical University (PMU), Salzburg, Austria. [7]Research Program Nanovesicular Therapeutics, Paracelsus Medical University (PMU), Salzburg, Austria. [8]Research and Transfer Centre for Extracellular Vesicle Theralytic Technologies, EV-TT, Salzburg, Austria

OBJECTIVES

Among particles and soluble factors, extracellular vesicles (EVs) are secreted by many human cell types. EVs participate in (patho-)physiological processes and mediate intercellular communication as cell-derived extracellular "shuttle-service". Consequently, EVs of defined cells may serve for various therapeutic approaches, including drug-delivery, pathogen-vaccination, anti-infectious, anti-tumour, immune-modulatory or regenerative therapies. EVs derived from the secretome of human mesenchymal stromal cells (MSC) contain numerous factors that are known to exert anti-inflammatory effects. MSC-EVs may serve as promising cell-based therapeutics for the inner ear to attenuate inflammation-based side effects from cochlear implantation, which represents an unmet clinical need.

MATERIALS AND METHODS

In an individual treatment performed on a 'named patient basis', we surgically applied allogeneic umbilical cord-derived MSC-EVs (UC-MSC-EVs) produced according to good manufacturing practice (GMP).

RESULTS

A 55-year-old patient suffering from Menière's disease received intracochlear delivery of EVs prior to the insertion of an electrode cochlear implant. Short-term safety as well as long-term safety over a 24 months period revealed no adverse events.

CONCLUSIONS

Translating EV-based therapeutics into clinical evaluation requires the focus on efficacy, safety and a tight control of manufacturing and quality assurance. This first-in-human use of UC-MSC-EVs demonstrates the feasibility of this novel adjuvant therapeutic approach. The safety and efficacy of intracochlear EV-application to attenuate side effects of cochlea implants have to be determined in controlled clinical trials.

MSC-DERIVED EXTRACELLULAR VESICLES: PERSPECTIVES IN ONCOLOGY AND REGENERATIVE MEDICINE

Author: Benedetta Bussolati

Affiliation: Department of Molecular Biotechnology and Health Sciences, University of Torino

Extracellular vesicles released by mesenchymal stromal cells (MSC EVs) are a promising resource for regenerative medicine. In particular, small MSC EVs represent the active EV fraction for therapeutic applications. Single quantitative EV analysis of small MSC-EVs single-vesicle using super-resolution microscopy and ExoView analysis provides a deeper understanding of MSC-EV heterogeneity, to set the bases for their clinical application. Robust preclinical data support the therapeutic effect of exogenous EV administration for tissue repair, in acute and chronic diseases, as well as in organ perfusion before transplant. On the other hand, in oncology, several groups approached the possible use of stem cell-derived EVs to inhibit tumor growth and progression, with variable results. In particular, MSC EVs have been reported to mainly act on tumor endothelium, either promoting or inhibiting tumor angiogenesis, whereas other stem cell derived EVs also appear to act on tumor cells. Strategies to engineer EVs to further increase the therapeutic activity will be finally presented.

FUNCTIONAL HETEROGENEITY OF CELLULAR AND EXTRACELLULAR VESICLE-BASED THERAPEUTICS: IMPLICATIONS FOR THE DEVELOPMENT OF POTENCY ASSAYS

Authors: Fabiola Nardi Bauer[1], Michel Bremer[1], Verena Börger[1], Bernd Giebel[1]

Affiliation: [1]Institute for Transfusion Medicine, University Hospital Essen, University Duisburg-Essen, Virchowstraβe 179, 45147 Essen, Germany
Email: fabiola.nardi-bauer@uk-essen.de; bernd.giebel@uk-essen.de
www.uk essen.de/transfusionsmedizin/AGGiebel

OBJECTIVE

Extracellular vesicles (EVs) derived from mesenchymal stromal cells (MSCs) are promising agents in regenerative medicine and immunotherapy. Considering that connected to the heterogeneity of MSCs independent MSC-EV preparations differ in their therapeutic function, we are comparing the biological activity of independent MSC-EV preparations in various assays *in vitro* and *in vivo*. Indeed, independent MSC-EV preparations with comparable physical and molecular features were found to differ in their functional activities significantly. Aiming to translate MSC-EVs preparations into the clinics, we searched for a functional assay predicting the *in vivo* potency of respective MSC-EV preparations.

MATERIALS AND METHODS

Different functional assays were set up to monitor different biological activities of given MSC-EV preparations *in vitro*. Promising results are obtained from a novel type of a mixed lymphocyte reaction (MLR) assay. Here, peripheral blood-derived mononuclear cells (PBMCs) from up to 12 different healthy donors are pooled resulting in a high allogeneic cross-reactivity, even following an optimized freezing and thawing procedure. After thawing, mixed PBMCs are cultured for 5 days in the absence or presence of given MSC-EVs preparations. Thereafter, cell morphologies are documented and cells are phenotypically characterized by flow cytometry.

RESULTS

By analyzing the expression of a collection of different antigenes, we observed that some of the MSC-EV preparations modulate the content of CD4 and CD8 T cells expressing activation markers, while others failed to recognizably affect cells within the assay. So far, the results correlate with *in vivo* data obtained in a Graft-*versus*-Host disease model, but only partially with results obtained in an ischemic stroke model. Results of the other functional assays tested, did not correlate with the outcome in the *in vivo* models at all.

CONCLUSIONS

Due to the functional heterogeneity among independent MSC-EV preparations, it is mandatory that MSC-EV preparations intended to be used within the clinics are tested in appropriate functional assays upfront. Although the novel MLR assay seems to provide promising results, the usage of primary human cells challenges its qualification as an appropriate potency assay, which still is lacking. Consequently, it remains a major challenge in the MSC-EV field to identify and qualify appropriate potency assays for clinical MSC-EV preparations.

(YIA): SYSTEMICALLY BUT NOT LOCALLY INJECTED MESENCHYMAL STEM CELL DERIVED EXTRACELLULAR VESICLES INDUCE MUSCLE RECOVERY IN A MURINE MODEL OF VOLUMETRIC MUSCLE LOSS

Authors: Fabio Magarotto[1,2], Alberto Sgrò[2], Agner Henrique Dorigo Hochuli[3], Marina Andreetta[2], Michele Grassi[2], Mattia Saggioro[1,2], Leonardo Nogara[4,9], Anna Maria Tolomeo[2,10], Riccardo Francescato[1], Federica Collino[11], Giuseppe Germano[5], Federico Caicci[6], Edoardo Maghin[2,7], Martina Piccoli[7], Marcin Jurga[8], Bert Blaauw [4,9], Piergiorgio Gamba[2], Maurizio Muraca[2,5,10], Michela Pozzobon[1,2]

Affiliations: [1]Stem Cells and Regenerative Medicine Lab, Institute of Pediatric Research Città Della Speranza, Padova, Italy. [2]Department of Women and Children Health, University of Padova, Italy. [3]Pontificial University of Paranà, Brazil. [4]Biomedical Sciences Department, University of Padova, Italy. [5]Institute of Pediatric Research Città Della Speranza, Padova, Italy. [6]Department of Biology, University of Padua, Italy. [7]Tissue Engineering Lab, Institute of Pediatric Research Città Della Speranza, Padova, Italy. [8]The Cell Factory BVBA (Esperite NV), Italy. [9]Venetian Institute of Molecular Medicine (VIMM), Padova, Italy. [10]L.i.f.e.L.a.b. Program, Consorzio per La Ricerca Sanitaria (CORIS), Veneto Region, Padova, Italy. [11]Laboratory of Translational Research in Paediatric Nephrourology, Fondazione Ca' Granada IRCCS Ospedale Maggiore Policlinico, Milano, Italy

OBJECTIVE

It is known that decellularized muscle tissues are the best support to improve muscle recovery when a serious muscle mass loss occurs. However, at long term, the development of fibrosis jeopardizes the muscle functional recovery. Recently Extracellular Vesicles (EVs) derived from Mesenchymal Stromal Cells (MSC) are being studied in regenerative medicine for their usefulness in treating multiple pathologies. This work aims at evaluating the muscle regeneration effect of Mesenchymal Stromal Cells Extracellular vesicles (MSC EVs) when used as stimuli in an *in vivo* murine model of Volumetric Muscle Loss (VML).

MATERIALS AND METHODS

Healthy mouse muscles were decellularized using a detergent enzymatic treatment and used as scaffold in the damaged muscle. Human MSC EVs isolated from Wharton Jelly cells were characterized by Macsplex cytofluorimetric analysis. An in vitro 3D decellularized muscle model engineered with muscle cells and damaged with cardiotoxin was set to analyze MSC EVs pro-regenerative effect. An *in vivo* immunocompetent murine model was created after ablation of tibialis anterior muscle (TA). As muscle reconstruction treatment, the decellularized scaffold was inserted in the site of damage and at first, embedded with MSC EVs. Secondly, a new administration of MSC EVs was performed 72h after damage in two separated groups: 20 mice underwent local injection and 20 mice systemic injection (intraperitoneal). The control group was treated with PBS alone. After 7 and 30 days from the surgical procedure, animals were euthanized, and TA recovered for multiple analysis. The effect of MSC EVs was evaluated analyzing (1) the macrophage compartment, (2) the angiogenesis, (3) the myogenic and the fibrosis processes by immunofluorescence, qRT-PCR and cytofluorimetric analysis from freshly isolated cells. 30 days after damage, functional analysis was performed only in mice after systemic treatment.

RESULTS

Local injection of MSC EV did not give any promising results. Mice that underwent MSC EV systemic injection, increased the pro-regenerative macrophages population, angiogenesis and myogenesis in respect to the animals PBS treated. . In the same MSC EV treated group, fibrosis decreased at later time point and force recovery was ameliorated.

CONCLUSIONS

We demonstrated that human MSC EVs are not rejected and act in the neural-muscle compartment improving not only muscle regeneration but also strength functional recovery.

APICAL-OUT ORGANOIDS: A NEW PLATFORM FOR INFECTIOUS DISEASE STUDIES AND ANTIVIRAL DRUG SCREENING

Authors: Georgios Stroulios[1], Philipp Kramer[2], Ryan K. Conder[2], Allen Eaves[1,2,3], Sharon Louis[2], Wing Chang[1], **Salvatore Simmini**[1]

Affiliation: [1]STEMCELL Technologies UK Ltd., Cambridge, UK. [2]STEMCELL Technologies Inc., Vancouver BC, Canada. [3]Terry Fox Laboratory, BC Cancer, Vancouver BC, Canada

OBJECTIVE

In vivo, the lining of human epithelia is characterized by the presence of specialized cells that expose their apical side to the lumen and respond to external cues including pathogens. Culturing epithelial cells as organoids recapitulates the architecture and some key functions of the in vivo tissues. However, the organoid structure in itself, is limited by the accessibility of the apical side of the luminal cells to study host-pathogen interactions. Here, we describe novel methods to generate both apical-out intestinal and airway organoids and their potential application in high-throughput antiviral drug screening.

MATERIALS AND METHODS

Intestinal organoids were maintained in Matrigel® domes and in the presence of IntestiCult™ Organoid Growth Medium (Human) (IOGMH). Organoids reached a size of 150-250 µm at 3-5 days after seeding. For polarity inversion, organoids were incubated with Gentle Cell Dissociation Reagent with agitation at 2-8° C to remove Matrigel®, then organoids were replated in Matrigel®-free suspension culture with IOGMH.

Human bronchial epithelial cells were cultured in PneumaCult™ Ex-Plus, dissociated to single cells, then forced to aggregate in an AggreWell™ 24-well plate in PneumaCult™ Apical-Out Airway Organoid Medium for 1 to 6 days. Next, aggregates were resuspended in the same culture medium and transferred to a new 24-well tissue culture plate for differentiation to apical-out organoids in ECM-free suspension. Organoids were infected with viruses targeting the human airways.

RESULTS

Intestinal organoids require the complete removal of Matrigel® in order to obtain efficient polarity inversion of the cultures, which takes about 24 hours to occur. Apical-out intestinal organoids show a distinct localization of apical markers, such as VILLIN and ZO-1, at the outer side of the epithelium (n=10). Apical-out airway organoids are generated within 15 days, and are composed of ciliated cells with beating cilia and KRT5-expressing basal cells (n=10). Apical-out airway organoids are susceptible to infections by a variety of viruses and are responsive to two antiviral drugs for enterovirus D68 such as rupintrivir and itraconazole (n>100).

CONCLUSIONS

Tissue specific epithelial derived organoids are recognized as powerful platforms that can be used to model viral infection of epithelial cells. Easy access to the apical surface of the epithelium and scaled-up production of organoids described here, offer an alternative and powerful in vitro model to study host-pathogen interactions in high-throughput settings.

ENGINEERING ORGANOIDS: FOUR-DIMENSIONAL CONTROL OF THE ORGANOID ENVIRONMENT

Authors: Anna Urciuolo[1], Giovanni Giobbe[2], Cecilia Laterza[3], Onelia Gagliano[3], **Nicola Elvassore**[1,3]

Affiliation: [1]Department of Molecular Medicine, University of Padova. [2]Developmental Biology & Cancer Dept, UCL GOS Institute of Child Health, University College London. [3]Department of Industrial Engineering, University of Padova

Hydrogel-based geometrical constrains, mechanical and biochemical signaling are used to control the self-organization of three-dimensional (3D) organ-like cultures. This control is restricted to the initial culture conditions and cannot be adapted to the dynamic morphological changes of complex 3D cultures during their developmental trajectory. Here, we developed a method that overcome this spatiotemporal limit. 3D hydrogel structures can be printed within pre-existing hydrogel-based organoid or ex-vivo organotypic cultures according to the spatiotemporal requirements to control the self-organization of the 3D multicellular cultures.

With this technique we could fabricate natural or synthetic hydrogels that scale from sub-organoid (10 micrometer) to supra-organoid (millimeter) sizes with desired 3D shape and tunable mechanical properties able to guide and control cell behavior of 3D organ-like cultures.

Our results show that it is possible to control the biochemical composition, mechanical properties and geometrical constrains within 3D organ-like cultures. In addition, we envision to adopt bioengineering-inspired approaches to integrate microfluidics within a 3D hydrogel system to improve the traceability, reproducibility, and physiologic relevance of in vitro organ-like morphogenetic patterning.

ORGANOIDS IN CANCER RESEARCH

Authors: **Giulia Della Chiara**[1,2], Michaela Fakiola[1], Claudia D'Oria[1,2], Lorenzo Drufuca[1], Ramona Bason[1,2], Raoul Jean Pierre Bonnal[1], Grazisa Rossetti[1], Stefano Piccolo[1,3*], Massimiliano Pagani[1,2 *]

Affiliation: [1]IFOM, the FIRC Institute of Molecular Oncology, Milan, 20139, Italy. [2]Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan, 20129, Italy. [3]Department of Molecular Medicine, University of Padua, Padua, 35121, Italy

OBJECTIVE

Cancer is characterized by pervasive epigenetic alterations with enhancer dysfunction orchestrating the aberrant cancer transcriptional programs and transcriptional dependencies. The three-dimensional, self-organizing organoid cultures represent such powerful ex vivo models that, contrary to cell lines, recapitulate the overall architecture and functional features of the tissues from which they originate. Here, we harness this organoid technology to epigenetically characterize human colorectal cancer (CRC) using de novo chromatin state discovery on a library of different patient-derived organoids.

MATERIALS AND METHODS

We collected human CRC patients' samples and used them to generate 3D organoids biobank, then we performed histopathological and molecular characterization of the organoids, validating them as surrogates of the primary tumors from which they derive, and established their genome-wide epigenetic landscape.

RESULTS

By exploring this 3D organoids resource from the epigenomic perspective, we uncover a tumor-specific deregulated enhancerome that is cancer cell-intrinsic and independent of interpatient heterogeneity. We also show that the transcriptional coactivators YAP/TAZ act as key regulators of the conserved CRC gained enhancers. The same YAP/TAZ-bound enhancers display active chromatin profiles across diverse human tumors, highlighting a pan-cancer epigenetic rewiring crucial for tumor maintenance. At single cell level, this rewiring occurs across diverse functional states of CRC malignant cells, separating them from normal-like populations.

CONCLUSIONS

Despite the considerable genetic and clinical heterogeneity in cancer, we showed that the usage of 3D organoids technology is key to unveil tumor intrinsic properties and cancer cell state and can be exploited for the development of new therapeutic avenues.

3D BIOPRINTED SCAFFOLDS CONTAINING MESENCHYMAL STEM/STROMAL LYOSECRETOME: NEXT GENERATION CONTROLLED RELEASE DEVICE FOR BONE REGENERATIVE MEDICINE

Authors: Elia Bari[1,†], Franca Scocozza[2,5,†], Sara Perteghella[1,3], Marzio Sorlini[3,4], Ferdinando Auricchio[2,5], Maria Luisa 1 Department of Drug Sciences, University of Pavia, Viale Taramelli 12, 27100 Pavia, Italy

Affiliation: [1] University of Pavia, Department of Drug Sciences. [2] University of Pavia, Department of Civil Engineering and Architecture. [3] PharmaExceed S.r.l., Pavia. [4] University of Applied Sciences and Arts of Southern Switzerland, SUPSI, Department of Innovative Technologies. [5] P4P S.r.l., Pavia. † Authors equally contributed to the work.

OBJECTIVE

The present study proposes the design and manufacturing of 3D-printed polycaprolactone (PCL) scaffolds enriched with lyosecretome, intended for the in vivo controlled release of Mesenchymal Stem Cells (MSC) paracrine factors in bone regenerative medicine.

MATERIALS AND METHODS

The present paper combines 3D-printed PCL scaffolds with lyosecretome, a freeze-dried formulation of MSC secretome containing proteins and extracellular vesicles. Two different scaffold manufacturing strategies have been proposed: (A) printing of the PCL scaffold and subsequent loading of lyosecretome by adsorption and (B) coprinting of PCL with an alginate hydrogel containing lyosecretome at two alginate concentrations (6% and 10% w/v). The first loading mode aims at providing a fast release, while the second one targets a slower release. Finally, scaffolds with different geometries have been designed and characterized in the morphology and release kinetics of secretome proteins and lipids.

RESULTS

A fast release of proteins and EVs (a burst of 75% after 30 min) was observed from scaffolds obtained by absorption loading, while coprinting of PCL and hydrogel, encapsulating lyosecretome allowed a homogeneous loading of protein and EVs and a controlled slow-release. For both loading modes, protein and EV release was governed by diffusion, as revealed by the kinetic release study. The secretome's diffusion is influenced by alginate, its concentration, or its cross-linking modes with protamine due to the higher steric hindrance of the polymer chains. Moreover, it is possible to further slow down protein and EV release by changing the scaffold shape from parallelepiped to cylindrical. In conclusion, it is possible to control the release kinetics of proteins and EVs by changing the composition of the alginate hydrogel, the scaffold's shape, and hydrogel cross-linking. Such scaffold prototypes for bone regenerative medicine are now available for further testing of safety and efficacy.

CONCLUSION

In conclusion, the proof of concept for manufacturing a cutting-edge controlled-release 3D-bioprinted scaffold containing lyosecretome has been provided. By changing the composition of the alginate hydrogel, the shape of the scaffold, and cross-linking with protamine, it is possible to control the release kinetics of proteins and EVs. Prototypes are now available for bone regenerative medicine safety and efficacy tests.

3D BIOPRINTED SILK-GELATIN INK SYSTEM FOR CARTILAGE REGENERATION

Authors: Cristina Manferdini[1], Shikha Chawla[2], Giovanna Desando[3], Elena Gabusi[1], Aarushi Sharma[2], Diego Trucco[1,4,5], Juhi Chakraborty[2], Mauro Petretta[3], Sourabh Ghosh[2], **Gina Lisignoli**[1]

Affiliation: [1]SC Laboratorio di Immunoreumatologia e Rigenerazione Tissutale, IRCCS Istituto Ortopedico Rizzoli, Bologna,Italy. [2]Regenerative Engineering Laboratory, Department of Textile Technology, Indian Institute of Technology, New Delhi, India. [3]Laboratorio RAMSES, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy. [4] The BioRobotics Institute, Scuola Superiore Sant'Anna, Pisa, Italy; [5]Department of Excellence in Robotics & AI, Scuola Superiore Sant'Anna, Pisa, Italy

OBJECTIVE

Cartilage lesions have become a major health problem worldwide because of the limited regenerative capacity of articular cartilage following trauma or chronic joint disorders like osteoarthritis (OA)1. Despite multiple efforts in addressing this clinical need, there are no efficient and long-lasting therapies available for promoting cartilage regeneration2. Tissue engineering approaches, combining a range of biomaterials, cells, and growth factors, could provide prospecting alternatives3–5. Several unanswered questions and aspects of this technology remain to be elucidated to expand its clinical application. There is still a lack of understanding of the role of specific biomaterials in modulating molecular signaling pathways during *in vitro* chondrogenic differentiation. Previously we have reported silk fibroin-gelatin (SF-G) bioink using progenitor cells or mature articular chondrocytes to make phenotypically stable engineered articular cartilage. Therefore, we aim to identify the role of SF-G bioink in modulating in vitro chondrogenic signaling pathways in human bone marrow-derived stromal cells (hMSCs) using detailed proteomics analysis.

MATERIALS AND METHODS

Silk gelatin-based 3D bioprinted constructs, composed of 5% bombyx mori silk fibroin (SF) and gelatin (G) were encapsulated with hMSCs through the bioprinting platform (3D Discovery, RegenHU). SF-G constructs were cultured with D-MEM high glucose with and without chondrogenic factors (GFs). Cell viability assessment and chondrogenic differentiation through histology, gene expression analyses by Real Time PCR and protein by immunohistochemestry and PANTHER and DAVID analyses were carried out. Chondrogenic differentiation was monitored at 1,14 and 28 days.

RESULTS

SF-G 3D bioprinted constructs resulted biocompatible ensuring high percentage of cell viability. Gene expression analyses displayed a high expression of COL2, SOX9, COMP, ACAN and low expression of COL1 and COL10 in chondrogenic 3D bioprinted SF-G-compared to control. The interaction between SF-G bioink and hMSCs augmented several chondrogenic pathways, including Wnt, HIF-1, and Notch as explored with PANTHER and DAVID analysis. We elucidated the debatable role of TGF-β signaling *in vitro*, by assessing the differential protein expression by hMSCs-laden bioprinted constructs in the presence and absence of TGF-β3. We revealed a direct role of TGF-β3 in generating stable chondrogenic differentiation. hMSCs-laden bioprinted constructs contained a large percentage of collagen type II and Filamin B, typical to the native articular cartilage. Hypertrophic markers were not identified following TGF-β3 addition.

CONCLUSIONS

This is the first study reporting such extensive proteome profiling of the *in vitro* silk based bioprinted constructs. The SF-G bioink positively modulated the expression of cartilage-specific ECM components by emphasizing the regenerative potential of this 3D bioprinted SF-G bioink system for cartilage regeneration.

This work was jointly funded by the Department of Science and Technology, India, and the Italian Ministry of Foreign Afairs and International Cooperation.

(YIA) TREATING INDUCED PLURIPOTENT STEM CELLS-DERIVED B CELLS WITH MONOCLONAL ANTIBODY BRENTUXIMAB REDUCES THE RISK OF TERATOMA UPON TRANSPLANTATION

Authors: Valentina Zamarian[1], Silvia Pellegrini[1], Elisa Landi[1], Fabio Manenti[1], Marco Schiavolena[2], Antonio Citro[1], Lorenzo Piemonti[1,3], Valeria Sordi[1]

Affiliation: [1]Diabetes Research Institute, IRCCS San Raffaele Hospital, Milan, Italy. [2]Department of Pathology, IRCCS San Raffaele Hospital, Milan, Italy. [3]Vita-Salute San Raffaele University, Milan, Italy

OBJECTIVE

Induced pluripotent stem cells (iPSC) are promising candidate cells for β cell replacement in type 1 diabetes. However, teratoma formation originating from undifferentiated iPSCs contaminating the graft is a critical concern for clinical application. Undifferentiated iPSC express CD30 surface marker at high levels. Here, we hypothesized that Brentuximab vedotin, which selectively targets CD30 and has already demonstrated its efficacy in a model of cardiac regeneration, induces apoptosis in undifferentiated iPSC while sparing the stem cell-derived beta cells. The final aim is thus to increase the safety of iPSC therapy for diabetes and to prevent teratoma formation upon transplantation.

MATERIALS AND METHODS

The presence of the protein CD30, was tested on seven different iPSC lines at different steps of differentiation. The effect of monoclonal antibody anti-CD30 Brentuximab vedotin was tested on both, undifferentiated iPSC and iPSC-derived beta cells (iβ) before transplantation to assess the efficiency in CD30-positive cells depletion and in preventing teratoma formation, respectively. Four weeks after transplantation into the kidney capsule of NOD-SCID mice, kidney was explanted and the presence of teratoma was evaluated by gross pathology and histological analysis.

RESULTS

Flow cytometry analysis confirmed that CD30 expression is consistent in undifferentiated human iPSCs clones, drastically reduced at definitive endoderm and primitive foregut stages and almost absent at the final stages of endocrine pancreatic cells and iβ. Addition of Brentuximab vedotin *in vitro* for 24 hours efficiently induced cell death in human iPSCs. The same treatment with the drug on iβ did not kill iβ nor had an impact on iβ identity and function. Brentuximab vedotin treated iβ maintained unchanged morphology, marker expression and capacity to secrete insulin in response to stimuli. Transplantation of no-treated human iβ into NOD-SCID mice consistently induced teratoma formation, with the appearance of teratoma in the graft at 4 weeks post-transplant, whereas iβ treated with Brentuximab vedotin prior to the transplantation did not form teratoma.

CONCLUSIONS

These findings suggest that *in vitro* treatment with Brentuximab vedotin, targeting the CD30-positive iPSC residual fraction, reduced the tumorigenicity in human iPSC-derived β cells, potentially providing enhanced safety for iPSC-based β cell replacement therapy in clinical scenarios.

OPPORTUNITIES IN 2021-2022 FOR SHORT-TERM FELLOWSHIPS IN EUROPE

Author: Filippo Piccinini

Affiliation: IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Via Piero Maroncelli 40, 47014 Meldola (FC), Italy

OBJECTIVE

Research should have no walls and today several organizations/societies help young researchers reaching the best laboratory to perform experiments, learn new skills, and increase the chance of success. However, there is no website or reference collecting the different opportunities today available for a young scientist looking for grant support for a short-term fellowship abroad. Here, I will introduce several opportunities for research fellowships of 1-3 months in Europe, also describing several examples of winning cases.
In this presentation we will discuss the Author's Guidelines and examples of winning applications for:
1- European Association for Cancer Research (EACR), Travel Fellowships;
2- Federation of European Biochemical Societies (FEBS), Short-Term Fellowships;
3- European Molecular Biology Organization (EMBO), Short-Term Fellowships;
4- Boehringer Ingelheim Fonds (BIF), Travel Grants;
5- Union for International Cancer Control (UICC), Technical Fellowships;
6- Union for International Cancer Control (UICC), YY Study Grants;
7- Network of European Bioimage Analysts (NEUBIAS), Short Term Scientific Missions;
8- Italian Society of Biochemistry and Molecular Biology (SIB), Short-Term Fellowships.
Furthermore, we will also introduce important freely available services/opportunities (e.g. FigShare, bioRxiv), helping the researchers to increase the visibility of the projects and the chance of success of the grant applications.

CONCLUSION

This presentation provides a wide overview of grants supporting research stays abroad. It is a unique opportunity for young researchers interested in a short-term stay in an institution, laboratory or industry in Europe. Remember, "Today's science is tomorrow's medicine".

FEDERATION OF STEM CELL ASSOCIATIONS

StemNet

FIRST INTERNATIONAL STEMNET MEETING

poster presentations

YIA: Young Investigator Award

1. (YIA) EVALUATION OF FBXW11 EXPRESSION DURING OSTEOGENIC DIFFERENTIATION

Authors: Arianna Minoia[1], Luca Dalle Carbonare[1], Jessica Bertacco[1,2], Veronica Li Vigni[1], Silvia Orsi[1], Alberto Gandini[3], Franco Antoniazzi[3], Giovanni Malerba[2], Monica Mottes[2], Maria Teresa Valenti[1]

Affiliation: [1]Department of Medicine, University of Verona, University of Verona, 37100 Verona, Italy. [2]Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, 37100 Verona, Italy. [3]Department of Surgery, Dentistry, Pediatrics and Gynecology, University of Verona, 37100 Verona, Italy

OBJECTIVE

Osteogenesis during skeletal development is driven by a succession of well-coordinated events involving epithelial-mesenchymal interaction, aggregation and differentiation. A crucial role is carried out by WNT signaling pathways and Bone morphogenetic proteins (BMP). The canonical WNT pathway is represented by WNT / β-catenin signaling which acts by inhibiting or inducing the formation of osteoblasts. Particularly, the expression of the osteogenic master genes RUNX2 is essential for Mesenchymal Stem Cells (MSCs) commitment to osteogenic differentiation. In addition, it has been shown that FBXW11, that encodes an F-box protein, part of the Skp1-cullin-F-box (SCF) ubiquitin ligase complex, negatively regulates the WNT / β-catenin -catenin pathway. As WNT / β-catenin signaling plays a central role in stem cells differentiation, we aimed to analyse the modulation of FBXW11 during osteogenic commitment and differentiation.

MATERIALS AND METHODS

We investigated the modulation of FBXW11 in vitro, by using MSCs, in vivo, by using zebrafish model, and in cleidocranial dysplasia (CCD) patients. In particular, we performed transfection experiments, RT Real Time PCR and Western Blotting analyses.

RESULTS: During osteogenic differentiation the expression of FBXW11 was opposite to that of the transcription factor RUNX2. In fact, the expression of FBXW11 increased in the late phase of differentiation (1.5 fold of expression compared to the early phase) while the expression of the transcription factor RUNX2 decreased in the late phase (0.6 fold of expression compared to early phase). Analysing the expression of RUNX2 and FBXW11 in circulating progenitor cells obtained from paediatric patients we still observed an opposite trend of the expression of RUNX2 and FBXW11. However, by analysing the expression of FBXW11 and RUNX2 in circulating progenitors obtained from paediatric patients with cleidocranial dysplasia (CCD), we observed that the levels of FBXW11 increased with increasing RUNX2. Forced expression of RUNX2, induced by transfecting MSCs with anti-miR204 during osteogenic differentiation, induced increased FBXW11 levels.

CONCLUSIONS

Our preliminary data suggest that FBXW11 regulates RUNX2 expression and that FBXW11 expression levels are stimulated by RUNX2 dysregulation during osteogenic differentiation.

2. (YIA) RADIAL MECHANICAL STIMULATION FOR 3D DIAPHRAGMATIC CONSTRUCTS: ADVANCED TISSUE-ENGINEERED SKELETAL MUSCLE FOR CONGENITAL MALFORMATIONS

Authors: Eugenia Carraro[1,2], Edoardo Maghin[1], Daniele Boso[1], Piero Pavan[1,3], Martina Piccoli[1]

Affiliation: [1]Istituto di Ricerca Pediatrica Città della Speranza, Padova, Italy. [2]Department of Biomedical Sciences, University of Padova, Padova, Italy. [3]Department of Industrial Engineering, University of Padova, Padova, Italy

OBJECTIVE

Congenital diaphragmatic hernia is a neonatal malformation in which the diaphragm does not develop properly, leaving a defect in the muscle from which the abdominal organs can raise into the thoracic cavity, impeding lung development. An alternative solution to the gold standard treatments is the use of a portion of decellularized diaphragm extracellular matrix (dECM) to close the large muscle defects. We recently showed that the use of a tissue-specific dECM in substitution of synthetic materials allows new blood vessel growth, long-term muscle regeneration and re-innervation, improving diaphragm performance and functional recovery in mouse. However, due to a persistent imbalance between *in vivo* dECM remodeling and skeletal muscle regeneration, dECM patches did not activate a complete defect regeneration. We demonstrated that dECM is a suitable scaffold for the growth and differentiation *in vitro* of human primary skeletal muscle cells (hSkMCs). In this environment hSkMCs, together with fibroblasts, allowed the generation of a functioning 3D skeletal muscle structure. Moreover, to overcome the random muscle fiber alignment due to static culture setting, we focused our attention on the production and validation of a in house developed bioreactor for the radial mechanical strain of 3D diaphragmatic constructs.

MATERIALS AND METHODS

We designed and manufactured a bioreactor platform exploiting numerical modelling to reproduce *in vitro* the physiological mechanical stimulation occurring *in vivo*. Recellularized diaphragmatic constructs were cultivated in static vs dynamic conditions, and then analyzed for the expression of myogenic markers and myofiber disposition.

RESULTS

When mechanically stimulated, hSkMCs were able to align following the axes of strain, demonstrating an oriented myotubes' formation in respect to the static culture. The recellularized diaphragm constructs, cultivated for two weeks in dynamic condition**,** maintained a proliferating cell pool, but also increased myotubes alignment and maturation in respect to the standard static culture. In addition, dynamic constructs presented cells expressing mature myogenic markers and phenotype.

CONCLUSIONS

Our bioreactor system is a tunable platform to improve muscle cell homing, alignment and maturation for the *in vitro* regeneration of a 3D diaphragmatic-like construct for future advanced clinical applications.

3. TREATMENT OF A CASE WITH COVID-19 SEVERE PNEUMONIA WITH MESENCHYMAL STROMAL CELLS: A FRUITFUL INTERACTION BETWEEN A PUBLIC CELL FACTORY AND A CLINIC DURING PANDEMIC

Authors: Giuseppe Astori[1], Stefano Ugel[2], Davide Gibellini[3], Martina Bernardi[1,4], Angela Bozza[1,4], Katia Chieregato[1,4], Francesca Elice[5], Simone Caligola[2], Francesco De Sanctis[2], Stefania Canè[2], Alessandra Fiore[2], Rosalinda Trovato[2], Antonio Vella[2], Varvara Petrova[2], Giuseppe Amodeo[6], Monica Santimaria[7], Annarita Mazzariol[3], Luca Frulloni[6], Marco Ruggeri[5], Enrico Polati[8], Vincenzo Bronte[2], Rachele Ciccocioppo[6]

Affiliation: [1]Laboratory of Advanced Cellular Therapies (LTCA), Hematology Unit, Vicenza Hospital, Italy. [2]Immunology Unit, Department of Medicine, A.O.U.I. Policlinico G.B. Rossi & University of Verona, Italy. [3]Microbiology Section, Department of Diagnostics and Public Health, University of Verona, Italy. [4]CORIS, Consorzio per la Ricerca Sanitaria, Padova, Italy. [5]Hematology Unit, Vicenza Hospital, Italy. [6]Gastroenterology Unit, Department of Medicine, A.O.U.I. Policlinico G.B. Rossi & University of Verona, Italy. [7]Nuclear Medicine Unit, Vicenza Hospital, Italy. [8]Intensive Care Unit, Department of Surgery, Dentistry, Maternity and Infant, A.O.U.I. Ospedale Maggiore & University of Verona, Italy

OBJECTIVE

The hyper-inflammatory response occurring during coronavirus disease (COVID)-19 is the main cause of the acute respiratory distress syndrome (ARDS). Its hallmarks include lymphopenia, activated monocytes/macrophages and elevated levels of inflammatory cytokines. A number of therapeutic approaches are under investigation, although none has proved successful to substantially modify the patients' outcome. In this scenario, mesenchymal stromal cells (MSCs) seems a valid option thanks to their actions having the ultimate effect of dampening inflammation while favoring tissue healing. We offered MSC therapy to a patient admitted to the Intensive Care Unit of the AOUI (Verona, Italy) because of severe COVID-19 pneumonia.

MATERIALS AND METHODS

MSC treatment was proposed under the hospital exemption rule. After approval by the Agenzia Italiana del Farmaco, the patient received two intravenous infusions of umbilical cord-derived MSCs one week apart. MSCs were produced at the Laboratory for Advanced Cellular Therapies (Vicenza Hospital, Italy) according to Good Manufacturing Practice. After collection, the umbilical cord was minced in clean room, and seeded on T-flasks in culture medium containing platelet lysate. On day 26th, cells were harvested and stored in liquid nitrogen. MSCs were shipped to the clinic on dry ice. Upon receiving, the bags were thawed, diluted in a solution consisting of saline, albumin, and infused within 30 minutes.

RESULTS

No adverse events were registered. MSC therapy produced an amelioration of respiratory, inflammatory and pro-thrombotic indexes, together with normalization of renal function parameters. Remarkably, the high levels of IL-1β IL-6, IL-8 IL-10, TNF-α decreased after MSC therapy, whilst an increase of IFN-γ, IL-2, IL-15 became evident, together with an increase of the total number of $CD4^+$ and $CD8^+$ T cells paralleled by a reduction of activated neutrophils and classical monocytes. Unfortunately, upon the isolation of Klebsiella pneumoniae non responsive to a cycle of antibiotics the patient passed away after 4 weeks from MSC therapy.

CONCLUSIONS

This experience showed the feasibility of this therapeutic strategy even in the context of pandemic. The immunological results highlight the multifaceted immunomodulatory effects of MSCs in COVID-19.

4. SUCCESSFUL MUSCLE REGENERATION BY A HOMOLOGOUS MICRO-PERFORATED SCAFFOLD SEEDED WITH AUTOLOGOUS MESENCHYMAL STROMAL CELLS IN A PORCINE ESOPHAGEAL SUBSTITUTION MODEL

Authors: Maurizio Marzaro[1], Mattia Algeri[2], Luigi Tomao[2], Stefano Tedesco[3], Tamara Caldaro[2], Valerio Balassone[2], Anna Chiara Contini[2], Luciano Guerra[2], Giovanni Federici D'Abriola[2], Paola Francalanci[2], Maria Emiliana Caristo[4], Lorenzo Lupoi[4], Ivo Boskoski[5], Angela Bozza[6,7], Giuseppe Astori[6], Gianantonio Pozzato[8], Alessandro Pozzato[8], Guido Costamagna[5], Luigi Dall'Oglio[2]

Affiliation: [1]Pediatric Surgery Department, Ospedale Ca' Foncello, Treviso, Italy. [2]Ospedale Pediatrico Bambino Gesù, Roma, Italy. [3]Telea Biotech, Vicenza, Italy. [4] Università Cattolica del Sacro Cuore, Cen. Ri.S. [5]Digestive Endoscopy Unit, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy. [6]Laboratory of Advanced Cellular Therapies (LTCA), Hematology Unit, Vicenza Hospital, Italy. [7]Consorzio per la ricerca sanitaria (CORIS), Padova, Italy. [8]Telea Electronic Engineering, Vicenza, Italy.

OBJECTIVE

Congenital and acquired esophageal diseases often require esophageal substitutions. Tissue-engineered products are an interesting therapeutic alternative for esophageal replacement, as they could mimic organ structure and architecture. Natural scaffolds obtained from decellularization of native matrices showed promising results. However, these scaffolds present some limits in terms of cell adhesion and homing.
We developed a porcine esophageal substitute constituted of a decellularized scaffold seeded with bone marrow mesenchymal stromal cells (MSCs). Scaffolds were micro-perforated by Quantum Molecular Resonance (QMR) technology in order to improve cell seeding and distribution throughout the matrix.

MATERIALS AND METHODS

Esophagus were collected from donor pigs (*Sus scrofa domesticus*), cut longitudinally and the *tunica mucosa* and *submucosa* were removed, leaving the muscular layer only. Four cm-length scaffolds were decellularized, evaluating protocol efficiency by DNA quantification. Scaffolds were then subjected to QMR perforative treatment prior to autologous MSCs static seeding.
Scaffolds were implanted in recipient animals: 4 pigs received a non-seeded scaffolds (NSS); 8 pigs a seeded scaffolds (SS), substituting only the muscle layer in a mucosal sparing technique.

RESULTS

QMR perforative treatment did not cause tissue damages or burns; and the extracellular matrix (ECM) architecture was preserved. The treatment created a microporous network of around 1200 pores/cm^2 with a diameter of 80-100µm. After seeding cells colonized both outer and inner layers of the scaffolds, migrating through channels and pores. After 3 months from surgery, all the animals exhibited a normal weight increase, except one case in the SS group. Interestingly, muscle regeneration occurred in SS group and not in NSS group, as shown by actin and desmin staining of the post-implant scaffolds. An esophageal substenosis was observed in 2/4 NSS and in 6/8 SS pigs and a non-practicable stricture in 1/4 NSS and 2/8 SS pigs.

CONCLUSIONS

We developed a homologous acellular esophageal scaffold valuable for esophageal replacement approaches. QMR perforative treatment resulted a promising technology for enhancing cell adhesion and full colonization of natural scaffolds, creating a multiporous network without affecting native ECM structure and architecture. After three-months follow up from implant, scaffolds seeded with MSCs resulted in better outcomes, as evidenced by muscle regeneration.

5. (YIA) IN VITRO RESTORATION OF LIPID MEMBRANE COMPOSITION AMELIORATES IN VITRO BIOLOGICAL FEATURES OF HUMAN AMNIOTIC EPITHELIAL CELLS

Authors: Valeria Pizzuti[1], Provvidenza Maria Abruzzo[1], Alexandros Chatgilialoglu[2], Pasquale Marrazzo[1], Giovannamaria Petrocelli[1], Ilaria Longo[1], Paola Poggi[2], Federica Facchin[1], Silvia Canaider[1], Laura Bonsi[1], Francesco Alviano[1]

Affiliation: [1]Department of Experimental, Diagnostic and Specialty Medicine-Unit of Histology, Embryology and Applied Biology-University of Bologna, Italy. [2]Remembrane Srl, Imola, Italy

OBJECTIVE

In this study we focused on human Amniotic Epithelial Cells (hAECs) and on how *in vitro* maintenance influences some biological features, particularly hAECs membrane lipid profile and fatty acid (FA) species. We analyze the ability of a customized lipid supplement namely Refeed® to physiologically restore the membrane lipidomic signature and how the membrane lipidomic retuning affects hAEC morphology, senescence characteristics, migration ability and immunomodulatory properties.

MATERIAL AND METHODS

hAECs were isolated from amniotic membrane by enzymatic digestion. Cell membranes were purified at isolation and in hAECs cultured until passage 4. The membrane FA signature was assessed by gas chromatography, both in cells cultivated with standard culture medium and supplemented with Refeed®. Cell area analysis and β-Galactosidase assay were performed in order to assess the morphological and senescence features of hAECs. Wound healing assay was performed to study migratory capacity, analyzing scratched open area and the time for its closure. For the immunomodulatory capacity, hAECs were co-cultured with Peripheral Blood Mononuclear Cells (PBMCs) and BrdU assay was performed.

RESULTS

The analysis of membrane lipidome in hAECs cultured in basal medium showed a decrease in membrane polyunsaturated FA(PUFA) during passages, especially Omega-6 (ω-6), in comparison with freshly isolated hAECs. The addition of Refeed® lipid supplement in the medium restored a physiological PUFAs level, by increasing ω-6 amount. Treatment with Refeed® was associated with a lower cell size in all passages and β-Galactosidase assay showed a lower percentage of senescent cells in later culture passages. Refeed® addition resulted in an early closure of the scratched area in the first two passages. The immunomodulatory capacity of hAECs was improved by Refeed® treatment at passages 1 and 2.

CONCLUSIONS

hAEC FA profile is altered by *in vitro* culture. Refeed® supplement restored a membrane composition closer to *in vivo* one. A delay in senescence onset was observed in hAECs cultured with Refeed®, along with an increase in their migratory and immunomodulatory properties, especially during early cell passaging. Understanding the changes in membrane lipidome could led to useful implications in the development of optimized *in vitro* models. In parallel, by reducing the gap between *in vivo* complexity and *in vitro* expansion, Refeed® treatment could improve future cell therapy protocols.

6. EFFECTS OF FOCUSED VIBRATIONS ON HUMAN SATELLITE CELLS

Authors: Mariangela Basile[1], Sara Nobilio[1], Silvia Sancilio[1], Antonio Ruggiero[1], Ester Sara Di Filippo[2], Gianmarco Stati[1], Stefania Fulle[2], Raoul Saggini[3], Roberta Di Pietro[1]

Affiliation: [1]Department of Medicine and Ageing Sciences, "G. d'Annunzio" University, Chieti-Pescara, Italy. [2]Department of Neuroscience Imaging and Clinical Sciences, "G. d'Annunzio" University, Chieti-Pescara, Italy. [3]Department of Medical and Oral Sciences and Biotechnologies, "G. d'Annunzio" University, Chieti-Pescara, Italy

OBJECTIVE

Skeletal muscle is a dynamic tissue in constant change where satellite cells provide for the generation, maintenance and regeneration of skeletal muscle. Satellite cells ageing determines the reduced ability of muscle regenerative potential and a progressive atrophy of skeletal muscle. This condition, named sarcopenia, has important health care implications, as it contributes to frailty, functional loss and premature death. The sarcopenia treatment is usually based on physical exercise and nutritional plans, possibly associated with rehabilitation programs, such as vibratory stimulation. High-frequency vibratory stimulation has emerged over the last decades as a promising non-invasive treatment to significantly stimulate muscle strength. However, the possible direct effect of vibration on satellite cells is still unclear. Based on these observations, the main objective of the present study was to evaluate the effects of focused mechano-acoustic vibration on an *in vitro* culture of human satellite cells.

MATERIALS AND METHODS

Primary human satellite cells were isolated from *Vastus Lateralis* of at least 3 young and 3 aged subjects and cultured *in vitro*. Focused mechano-acoustic vibrations were administered by means of *Vibration Sound System*® (ViSS) at increasing time intervals (10, 20, and 30 min). After 72 h, cell proliferation was evaluated with MTT assays, cell morphology and cell differentiation were analyzed with light microscopy and immunofluorescence, apoptosis was assessed with terminal deoxynucleotidyl transferase (TdT)-mediated nick-end labelling (TUNEL) technique and myotubes formation was documented by time laps videos.

RESULTS

After vibratory stimuli we found in both age groups reduced percentages of apoptotic cells, increased cell size and percentages of aligned cells, mitotic events, and activated cells, with stronger effects on aged samples treated for 20-30 min. We also found an increased number of cells per field only in young samples. Our results highlight for the first time the presence of direct effects of mechanical vibrations on human satellite cells. Interestingly, these effects seem to be age-dependent, due to the mainly proliferative response of young satellite cells *vs* the mainly differentiative response of aged satellite cells.

CONCLUSION

All in all, our results show that the treatment with focused mechano-acoustic vibration could be an appropriate approach to counteract sarcopenia in old subjects.

7. AMNION-DERIVED EPITHELIAL CELLS AND MOLECULES MEDIATE IMMUNE ACCEPTANCE IN ALLOGENIC SETTINGS,AVERTINGPHARMACOLOGICAL IMMUNOSUPPRESSION

Authors: Roberto Gramignoli

Affiliation: KarolinskaInstitutet, Stockholm, Sweden

Placenta is a non-controversial and readily available source of stem cells for regenerative medicine. We previously reported that human amnion epithelial cells (hAEC) from term placenta are not tumorigenic, and once transplanted differentiate into functional hepatocyte-like cells. In preclinical studies with immune-competent mice, hAEC engrafted and survived without administration of immunosuppressive drugs, resulting in correction of r inborn errors of metabolism and reversal of acute liver failure, particularly where a deranged immune response is present. In pre-clinical and clinical settings allogenic hAEC have been transplanted without immunosuppression. However, little or null was known about immunomodulatory amnion cells.

OBJECTIVE
During the past years, our group studied and profiled molecular pathways responsible for modulating host immune response.

MATERIALS AND METHODS
We recently reported as such critical anti-inflammatory and modulatory capacities are mediated by intact hAEC and secreted vesicles (hAEV). We performed a complete surface screening of hAEC, and purified hAEV to evaluate surface expression.

RESULTS
Amnion mediators characteristically lacks HLA class 2 expression and expresses both class 1a and non-polymorphic class 1b (critical for maternal immune toleration of the fetus). We quantified the level of expression of HLA-G and HLA-E molecules as membrane-bound both on hAEC and hAEV, as well as released soluble forms. Notably, purinergic mediators, hydrolyzed by classical and alternative nucleotidase pathways, have been identified to regulate immune cell response. Surprisingly, hAEC and hAEV constitutively express all known ecto-enzymes, playing critical role in modulating immune effector cells (T-, B- and NK-cells). Additional soluble mediators have been effectively induced macrophage switch from M1 to M2 phenotype.

CONCLUSIONS
Primary hAEC are characterized by immunological tolerance and long-term acceptance upon transplantation. The ability to treat the most common (liver) diseases with one stem cell therapy without the administration of immunosuppressive drugs could be a “game changer” and will greatly expand the number of patients who could receive cellular therapy. Based on their safety and the successful preclinical studies, approval was granted to perform allogenic hAEC transplants on 10 patients with liver disease without immunosuppression in support.

8. ADAPTATION OF CELL LINE TO "TOTALLY ANIMAL-FREE" CULTURE SYSTEMS AND EVALUATION OF GROWTH CURVES WITH XCELLIGENCE

Authors: Martina Angela Checco[1], Daniela Romano[1], Mara Fusi[2], Andrea Franzò[1], Silvia Dotti[1]

Affiliation: [1]Centro di Referenza Nazionale per i Metodi Alternativi, Benessere e Cura degli Animali da Laboratorio. Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Brescia, Italy. [2]Doctor in Veterinary Medicine (DVM)

OBJECTIVE

Routine cell culture demands the use of animal-derived products, mainly fetal bovine serum (FBS) and porcine trypsin. The use of FBS involves various technical and ethical concerns, such as the collection and production of serum. According to 3Rs principle and Guidance Document on Good *In Vitro* Method Practices (GIVIMP), the replacement with animal-free products is recommended for *in vitro* methods and is demanded for tissue engineering and stem cell based therapies. The aim of this study is the evaluation of a cell culture adaptation protocol to totally animal-free culture systems. To verify cell growth the xCELLigence instrument was used. It measures the differences in electrical impedance, indicated by the Cell Index (CI), based on the number, adhesion and morphology of cells.

MATERIALS AND METHODS

Hep-2 and LLC-MK2 (IZSLER Biobanking facility) were subcultured with four protocols. The first employed animal trypsin and MEM+10% FBS (MEM+FBS). In the other three methods, trypsin was replaced with a recombinant enzyme (TrypLE, Gibco), while media were respectively: serum-free complete medium (VP-SFM, Thermofisher), MEM+5% PL (Human Platelet Lysate XF, LSP), or MEM+FBS. To compare the xCELLigence CI among different culture conditions, a statistic analysis with ANOVA and T-test was conducted.

RESULTS

Hep-2 cell line was sequentially adapted to 99% VP-SFM, while LLC-MK2 were directly adapted to 100% VP-SFM. The CI at 24 h of HEP-2 cells cultured with VP-SFM 99% and MEM+FBS+TrypLE grew less than MEM+FBS cells ($p<0.05$), while MEM+5% PL cells had the same growth ($p>0.05$). After 48 h VP-SFM 99% cultured cells grew less than MEM+FBS cells ($p<0.05$). Growth of cells cultivated in MEM+FBS+TrypLE and MEM+5% PL was similar to MEM+FBS ($p>0.05$). About LLC-MK2 cells, at 24 h VP-SFM 100% cells grew more paired to MEM+FBS cells ($p<0.05$), while MEM+5% PL grew less ($p<0.05$) and MEM+FBS+TrypLE cells show the same growth ($p>0.05$). At 48 h VP-SFM 100% and MEM+FBS+TrypLE cells growth was the same ($p>0.05$), MEM+5% PL cells grew less than MEM+FBS cells ($p<0.05$).

CONCLUSIONS

The results show that synthetic trypsin can substitute porcine trypsin. Replacing the use of FBS with VP-SFM or MEM +5% PL seems promising. Their efficacy depends on the cell lines' ability to adapt to the new culture medium. The future goal will be to adapt new cell lines both to serum-free media or to PL. Adapted cell lines will be stocked in IZSLER Biobank and made available for future applications.

9. COMPARATIVE ANALYSIS OF CA-DEPOSITIONS EVOLUTION IN HUMAN BONE-MARROW MESENCHYMAL STEM CELLS AND SAOS-2 OSTEOSARCOMA CELLS

Authors: Giovanna Picone[1], Concettina Cappadone[1], Giovanna Farruggia[1], Francesca Rossi[1], Emil Malucelli[1], Andrea Sorrentino[2], Stefano Iotti[1]

Affiliation: [1]Dept of Pharmacy and Biotechnology, Univ. of Bologna, Bologna, Italy. [2]ALBA Synchrotron Light Source, Cerdanyola del Vallès, Barcelona 08290, Spain

OBJECTIVE

The aim of this study is to characterize the mineral depositions at early phase of bone biomineralization, in human bone-marrow mesenchymal stem cells (hbMScs) and in SaOS-2 osteosarcoma cell line. To investigate the evolution of the Ca- depositions during the differentiation, we used synchrotron-based cryo-soft-X-rays transmission microscopy techniques. We compared the biomineralization process occurring in hbMSCs induced to osteoblastic lineage with that occurring in differentiating SaOS-2. A deeper knowledge of the early phase of bone formation and of the mineral nanostructure could clarify the relationship between osteoblastic differentiation and osteosarcoma (OS), possibly leading to novel treatments for this challenging neoplasm.

MATERIALS AND METHODS

We investigated the quality of minerals produced by hbMSCs as healthy control, and Saos-2, osteoblastic-like cells with high mineralizing activity. Both hbMSC and SaOS-2 cells were subjected to osteogenic medium according to Pasini et al.. The localization and characterization of crystalline phase, and Ca concentration in the depositions were performed in frozen-hydrated cells at 4 and 10 days after osteoblastic induction, using cryo-XANES microscopy at the Ca L2,3-edges and cryo-soft-X-ray tomography, respectively for 2D mapping of Ca chemical states, and for 3D reconstruction. The tomography allowed us to analyze intracellular Ca accumulation and extracellular spot deposition.

RESULTS

The acquired spectra and tomography showed that in hbMSCs, the calcite was present after 4 days and evolved into HA crystals after 10 days of osteogenic induction. Surprisingly, the crystalline calcite was already present in the undifferentiated SaOS-2 cells at early time. In differentiating SaOS-2, parallel to the increase of micro-vesicles, the number and size of mineral depositions grew over time and their crystalline structure changed. These Ca-depositions were in fact more similar to Ca-phosphate and HA.

CONCLUSIONS

The results suggested crystalline calcite as a precursor of HA depositions within hbMSCs at the early phase of bone biomineralization. In undifferentiated SaOS-2, the presence of calcite crystal confirms an alteration during differentiation, as previously reported. Interestingly, their evolution into Ca-phosphate and HA after osteogenic induction could restores the physiological biomineralization process, inducing OS cells towards a less aggressive phenotype.

10. VALIDATION OF AN AUTOMATED CELL COUNTING METHOD FOR THE MANUFACTURING OF HUMAN INDUCED PLURIPOTENT STEM CELLS FOLLOWING CURRENT GOOD MANUFACTURING PRACTICES

Authors: Paolo Manzini[1], Valeria Peli[1], Araceli Rivera-Ordaz[1], Silvia Budelli[1], Francesco Rusconi[1], Mario Barilani[1], Lorenza Lazzari[1]

Affiliation: [1]Laboratory of Regenerative Medicine – Cell Factory, Department of Transfusion Medicine and Hematology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via F. Sforza 35, 20122 Milano, Italy

OBJECTIVE

Human induced pluripotent stem cells (hiPSCs) are manufactured as advanced therapy medicinal products (ATMPs) for tissue replacement applications. A crucial step in the hiPSC production under current Good Manufacturing Practice (cGMP) is their expansion that must be monitored via a fast and reliable cell counting method. The conventional manual cell counting by the hemocytometer method is dependent on the operator's expertise and is time-consuming. Our aim was to validate the use of an automated cell count method to improve precision and rapidity for the hiPSCs manufacturing under cGMP requirements.

MATERIALS AND METHODS

Following the guidelines contained in IHC Q2(R1), the validation of the automated counting method by NucleoCounter NC-100 (Chemometec) was performed assessing its characteristics in the following order: specificity, linearity, range, accuracy and precision. Different hiPSC batches were evaluated to account for biological variability and three runs of analysis were performed for each batch.

RESULTS

For specificity, D-PBS was analyzed as the matrix of the hiPSC samples, and no contaminating particles/unspecific events were detected. To assess linearity across the entire range of measurement two different starting hiPSC concentrations were serially diluted and measured values were directly proportional to estimated values in both experimental conditions for all hiPSC batches (R^2>0.98). Then, a range of 0.19-5.06 million cells/mL was defined based on linearity data. Furthermore, the automated method showed a suitable accuracy in the performed runs, compared to a Bürker hemocytometer manual count as reference method (CV<10%). Finally, precision was evaluated as repeatability by the same operator and intermediate precision by two operators, and both analyses met the defined acceptance criteria (CV<5%).

CONCLUSIONS

We validated the use of the automated cell counting system for hiPSCs, manufactured following GMP protocols. Our results proved that the automated method can be used effectively and with higher precision than the manual one for hiPSC cell counting, underlining that it is possible to fully validate analytical methods also for an innovative ATMP such as hiPSCs.

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11. YIA A SINGLE CELL SEQUENCING APPROACH FOR THE CHARACTERIZATION OF HUMAN PANCREATIC ORGANOIDS

Authors: Francesco Rusconi[1], Alessandro Cherubini[1], Stefano Nardone[2], Roberta Piras[1], Marta Dossena[1], Valeria Sordi[3], Cecilia Mei[1], Mario Barilani[1], Valeria Peli[1], Paolo Manzini[1], Clelia Pistoni[1], Kaja Nicole Wächtershäuser[4], Antonio Zito[5], Silvia Pellegrini[3], Lotta Hof[4], Francesco Pampaloni[4], Mariacristina Crosti[6], Marilena Mancino[6,7], Vincenza Dolo[8], Lorenzo Piemonti[3,9], Lorenza Lazzari[1]

Affiliation: [1]Laboratory of Regenerative Medicine – Cell Factory, Fondazione IRCCS Ca' GrandaOspedale Maggiore Policlinico, Milan, Italy. [2]Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA [3]IRCCS Ospedale San Raffaele, San Raffaele Diabetes Research Institute, Milan, Italy. [4]Physical Biology Group, Buchmann Institute for Molecular Life Sciences (BMLS), Goethe Universität Frankfurt am Main, Frankfurt am Main, Germany. [5]Department of Twin Research & Genetic Epidemiology, King's College London, London, SE1 7EH, UK. [6]Istituto Nazionale Genetica Molecolare, Padiglione Romeo ed Enrica Invernizzi, IRCCS Ospedale Maggiore Policlinico, Milan, Italy. [7]Department of Clinical Science and Community, University of Milan, Milan, Italy. [8]Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy. [9]Vita-Salute San Raffaele University, Milan, Italy

OBJECTIVE

Pancreas is an organ composed by two morphologically and functionally distinct compartments: the exocrine tissue (ductal and acinar cells) and the endocrine islets (α-, β-, δ-, ε- and PP-cells). Many diseases affect the exocrine and endocrine pancreas. However, in this context, there is a slow development in regenerative medicine and drug screening due to the lack of an appropriate *in vitro* model for understanding mechanisms associated with initiation and progression of pancreatic disorders. The aim of our work is to deeply characterize human healthy pancreatic organoids (hPO) to generate an *in vitro* model that can be crucial to investigate the initiation and progression of pancreatic diseases.

MATERIALS AND METHODS

First, we described hPO focusing on their morphology and long-term culture molecular aspects (gene expression and protein levels of pancreatic markers). Then, through a single-cell RNA sequencing analysis followed by a pseudotemporal trajectory investigation, we defined all cell types present in hPO and their heritage.

RESULTS

hPO could be expanded in long-term culture maintaining their genetic stability and molecular phenotype. The single-cell RNA sequencing analysis revealed that our organoids recapitulated the exocrine compartment of organ of origin, due to the presence of ductal and acinar-like clusters. Moreover, we identified the presence of pancreatic duct gland (PDG) cells, that brought the multipotent exocrine progenitor. The pseudotemporal trajectory underlined the potential of PDG clusters to developed into two distinct branches, the first one was associated to the ductal compartment and the other was linked to the acinar-like field.

CONCLUSIONS

This study overcomes the conventional theory of univocal ductal nature of human pancreatic organoids due to the existence of both ductal and acinar-like compartments. All these results contribute to well characterize the hPO at cellular and molecular level and pave the way for future study in the pancreatic field.

12. (YIA) LIQUID FLOW IN NATURAL BONE MICROCHANNELS: EXPERIMENTAL OBSERVATIONS AND BIOLOGICAL OUTCOME

Authors: Orlandi G.[1], Salerno E.[2,4], Ongaro C.[3], Mantovani G.[1], d'Adamo A.[3], Carnevale G.[1], Zardin B.[3], Bertacchini J.[1], Angeli D.[4]

Affiliation: [1]Department of Surgery, Medicine, Dentistry and Morphological Sciences with Interest in Transplant, Oncology and Regenerative Medicine, University of Modena and Reggio Emilia, 41125 Modena, Italy. [2]CNR-NANO S3 Research Center on nanoStructures and bioSystems at Surfaces, 41125 Modena, Italy. [3]DIEF- Engineering Department "Enzo Ferrari", 41125 Modena, Italy. [4]Department of Sciences and Methods for Engineering, University of Modena and Reggio Emilia, 42122 Reggio Emilia, Italy

OBJECTIVE

To study the morphological and biological effects of static (zero-flow) and dynamic fluid shear stress (FSS) conditions on osteoblast precursor cells (MC3T3) cultured inside a microfluidic channel-based scaffold (B-HA). FSS conditions, linked to scaffold morphological features, were applied by integrating the cellularized B-HA in an microfluidic bioreactor.

MATERIALS AND METHODS

For morphological analysis, 9 cellularized B-HA were tested (CTRL-zero flow, Low/ High FSS). In order to visualize the attached cells, B-HA were fixed with 4% (w/v) paraformaldehyde for 15 min and washed with PBS 1× for 5 min. To analyze cells through fluorescence microscopy, before the fixation procedure, cells were stained with a dye (5 μM) for 30 min. For FEG-SEM analysis, B-HA were dehydrated with ethanol, placed on aluminum stubs and coated with a thin layer of gold. Levels of mRNA were quantitatively determined on a QuantStudioTM 3 Real-Time PCR System using the QuantiFast SYBR Green PCR Kit. The biological assay was performed on other 9 B-HA scaffolds (CTRL, Low/High FSS), in order to have three technical replicates for each experiment. Relative gene quantification was performed using the comparative threshold method, where relative gene expression level equals $2^{-\Delta\Delta Ct}$. The fold changes in gene expression were normalized to the internal control gene GAPDH. The data were processed by the statistical student T-test and expressed as the mean ± SD from three independent experiments. For all tested group, the statistical significance was set up at p-value < 0.05.

RESULTS

FSS, in combination with the B-HA scaffold, promoted an early stage of cytoskeletal rearrangement, as confirmed by fluorescence and scanning electron microscopy investigation. Moreover, combined growing osteogenic precursors with biomaterial under mechanical FSS increased RUNX2, ALPL and PTN gene expression than to those cells subjected to zero flow conditions. These data confirmed the ability of B-HA bioactive scaffolds to promote the differentiation process along osteogenic lineage.

CONCLUSIONS

B-HA scaffolds can be effectively coupled to fluidic devices for studying the morphological and biological events of osteoblasts exposed to FSS dynamic conditions. The effects of FSS showed a powerful ability to induce an early stage of cytoskeletal rearrangement to promote osteogenic differentiation. This strategy may be considered as a helpful for treating musculoskeletal degenerative diseases.

13. (YIA) PREPARATION AND CHARACTERIZATION OF POLYPHENOLS-LOADED SILK SERICIN SELF-ASSEMBLING NANOSYSTEMS

Authors: Orlandi G.[1], Bari E.[1], Catenacci L.[1], Sorrenti M. L.[1], Segale L.[2], Perteghella S.[1], Torre M.L.[1]

Affiliation: [1]Department of Drug Sciences, University of Pavia, 27100 Pavia, Italy. [2]Department of Pharmaceutical Sciences, University of Piemonte Orientale, 28100 Novara, Italy

OBJECTIVE

We prepared and characterized silk sericin nanoparticles (SNPs) loaded with natural polyphenols for tissue regenerative purposes.

MATERIALS AND METHODS

SNPs were prepared by self-assembly using Lutrol F127 as a stabilizer. SNPs were loaded with proanthocyanidins (P), quercetin (Q) and epigallocatechin gallate (E) as bioactive molecules. Each formulation of SNPs was characterized in terms of morphology (SEM), particle size distribution (NTA), *in vitro* drug loading capacity (spectrophotometric analysis)/encapsulation efficiency (EE) and *in vitro* drug release (dialysis technique). Finally, *in vitro* antioxidant, anti-elastase, and anti-tyrosinase activities of SNPs were evaluated and compared with free drugs.

RESULTS

SNPs showed a round shape and smooth surface; NTA confirmed that nanoparticles loaded with E (SNP-E) showed larger average size (201.4±15.15 nm) than SNP-P and SNP-Q (141.2±15.15 and 137.4±12.37 nm, respectively) (mean value±SE, n=5). SNPs loaded more effectively hydrophilic P (2.6±0.37) and E (1.3±0.17) than lipophilic Q (0.7±0.21) with EE% values of 82.2±11.58, 41.5±5.25 and 20.5±6.59 for P, E and Q, respectively, due to the different drug-core compatibility (mean value±SD, n=9). Regarding the drug release, in 8 h SNP-Q released up to 25% of Q, while SNP-P and SNP-E released only 3.5% of P and 6.0% of E. A plateau, corresponding to about 50% of the drug released, was observed after 48 h for all the formulations.

All SNPs presented antioxidant activity above 85% for all tested concentrations, with except for unloaded SNPs ($p<0.05$, n=3). Overall, the anti-elastase and anti-tyrosinase activities of P, E, Q were lower after the encapsulation into SNPs ($p<0.05$, n=3). In particular, at SNP concentration of 15 mg/mL, the anti-elastase activity % was 56.7±3.92 for SNP-P and 46.81±5.42 for SNP-E, contrary to that of free drugs (80.7±3.14 for P and 57.8±4.77 for E). Only for Q, a synergic anti-elastase effect was observed with an inhibiting activity % raised from 1.3 ± 0.17 to 87.7 ± 5.67 after the loading. Finally, P and E showed a dose-dependent anti-tyrosinase activity % which decreased after encapsulation for P from 63.3±3.46 to 5.89±1.436 and for Q from 59.4±3.01 to 1.34±0.89 (mean value±SD).

CONCLUSIONS

SNPs loaded more effectively hydrophilic drugs, releasing them in a controlled mode. Overall, the encapsulation process preserved the biological properties of P, E, and Q allowing their employment in the tissue regeneration.

14. (YIA) HUMAN STROMAL LENTICULE AS A BIO-SCAFFOLD FOR MESENCHYMAL STEM CELLS AND EXTRACELLULAR VESICLES: POTENTIAL INNOVATIVE MODEL FOR POSTERIOR OCULAR DISEASE TREATMENT

Authors: Letizia Pelusi[1,2], Domitilla Mandatori[1,2], Mario Nubile[3], Manuela Santalucia[1,2], Valeria Schiavone[1,2], Simone De Fabritiis[2,3], Pasquale Simeone[2,3], Erminia D'Ugo[3], Luca Agnifili[3], Leonardo Mastropasqua[3], Assunta Pandolfi[1,2]

Affiliation: [1]Department of Medical, Oral and Biotechnological Sciences, University G. D'Annunzio Chieti-Pescara, StemTeCh group, Chieti, Italy.[2]Center for Advanced Studies and Technology-CAST (ex CeSI-MeT). [3]Department of Medicine and Aging Sciences, University G. d'Annunzio Chieti-Pescara, Chieti, Italy

OBJECTIVE

Posterior ocular diseases, such as glaucoma, are one of the main causes of blindness worldwide. However, efficacy therapeutic approaches are not available so far. Recently, stromal lenticule extracted during refracting surgery, was proposed as a potential bio-scaffold for tissue engineering. Until now, innovative studies have demonstrated a therapeutic potential of Mesenchymal Stem Cells (MSCs) and their released Extracellular Vesicles (EVs) in posterior ocular disease treatment. Combining the advantages of tissue engineering and cell therapy approaches, the aim of this study was to demonstrate the feasibility of using cell-free lenticules as scaffolds to be re-cellularized and engineered with Wharton Jelly-MSCs (WJMSCs) and their derived EVs.

MATERIAL AND METHODS

Human stromal lenticules (thickness 100-120 μm) were collected from healthy donors undergoing refractive surgery. Following 0.1% SDS (Sodium dodecyl sulfate) treatment, cell-free lenticules were re-cellularized (72 h incubation at 37 °C) with WJMSCs, isolated through spontaneous migration from human umbilical cords. Subsequently, cell-free lenticules were engineered with EVs isolated from WJMSCs (confluence 80%) conditioned medium (24 h) through polyethylene glycol (PEG) precipitation and sorting for CD90+ expression (flow cytometry sorter). Particularly, isolated EVs (labeled with PKH26 dye and CD90+ antibody) were incubated with cell-free lenticules for 5 h at 200 rpm. Later, the successful lenticule engineering has been confirmed by immunofluorescence and confocal microscopy.
The release of EVs from engineered lenticules and their human keratocytes potential uptake were evaluated following 24, 48, 96 h incubation.

RESULTS

We demonstrated that SDS treatment efficiently removed cellular component of human stromal lenticules without making alterations in extracellular matrix organization. In these conditions, WJMCs were able to re-cellularize this bio-scaffold, both in the upper and lower surface. Additionally, we generated cell-free lenticules functionalized with EVs-derived WJMSCs, which were released from the scaffold and internalized by keratocytes up to 96 h, with a greatest release and internalization at 48 h.

CONCLUSION

Our results indicated that human corneal stromal lenticules can be decellularized and engineered by MSCs and their derived EVs, thus representing a natural, biocompatible, non-immunogenic scaffold potentially useful for posterior ocular diseases treatment.

15. VALIDATION OF A CLINICAL-GRADE LONG-TERM CRYOPRESERVATION METHOD TO MAINTAIN ADIPOSE TISSUE STEMNESS

Authors: Francesca Favaretto[1,2], Chiara Compagnin[2], Elisa Cogliati[1], Francesco Dell'Antonia[3], Giorgio Berna[3], Roberto Vettor[2], Gabriella Milan[2], Diletta Trojan[1]

Affiliation: [1] Fondazione Banca dei Tessuti di Treviso Onlus, FBTV, 31100 Treviso. [2] Clinica Medica 3, DIMED, UNIPD, 35128 Padova. [3] Chirurgia Plastica, ULSS2 La Marca, 31100 Treviso

OBJECTIVE

Autologous fresh adipose tissue (AT) transplantation is commonly used in plastic surgery to treat multiple diseases. Several trials involving the use of adipose derived stem cells (ASCs) are actually approved worldwide but in Italy only few Cell Factories are allowed by AIFA to manipulate cells under the EMEA regulation of the advanced therapy medicinal products (ATMPs). Thus, for chronic diseases, the use and cryopreservation of the whole AT could represent an alternative approach, improving the quality of life of patients given that it could be obtained in one step procedure and then stored in allowed Tissue Banks that meet the national criteria for donation, procurement, processing, storage and distribution of human tissues. The aim our study was to identify a clinical-grade method for the AT cryopreservation retaining its stemness properties during a long-term storage.

MATERIALS AND METHODS

Human AT was procured and manipulated in grade A with background B premises, evaluating microbiological contaminations during each step. Two different cryopreservation methods (Trehalose and DMSO-based) were compared to identify the best strategy to ensure the highest viability of ASCs. Next, we used microscopy and cytometry to investigate if the storage on liquid nitrogen vapors (from 1 month to 3 years) affected cell viability (by Cell Viability Imaging and Annexin V staining), composition (phenotyping by multiparametric cytometry) and adipogenic potential. Statistical analyses were performed using SigmaPlot Software.

RESULTS

The protocol we set up to manipulate AT is devoided by microbiological contamination. The cryopreservation with DMSO allowed the recovery of the highest number of viable cells. Cryopreserved AT displayed a slight decrease in total cell content in comparison with fresh ($p>0.5$). The percentage of live, apoptotic and necrotic cells together with adipogenic (CD34+CD90+CD31-CD45-) and endothelial (CD34+CD31+CD45-) precursors and endothelial mature cells (CD34-CD31+CD45-) were unaffected by the duration of the storage. We also found that progenitor cells are able to maintain their adipogenic potential in vitro.

CONCLUSIONS

Clinical-grade cryopreserved AT could potentially be used to treat chronic diseases, retaining also vital and functional ASCs. Our results led us to begin a clinical study to evaluate its application in the correction of the alterations of the prosthetic post-mastectomy reconstruction in patients affected by breast cancer.

16. YIA MESENCHYMAL STROMAL CELL-DERIVED EXTRACELLULAR VESICLES COUNTERACT THE DEVELOPMENT OF FIBROSIS IN A RAT MODEL OF BRONCHOPULMONARY DYSPLASIA

Authors: Paola Bisaccia[1,2], Fabio Magarotto[1,2], Stefania D'Agostino[1,2], Marcin Jurga[3], Michela Pozzobon[1,2], Maurizio Muraca[2,4,5]

Affiliation: [1]Stem Cells and Regenerative Lab, Institute of Pediatric Research Città della Speranza, Padova, Italy. [2]Department of Women and Children Health, Università di Padova, Italy. [3]Exobiologics (NV), Belgium. [4]Institute of Pediatric Research Città della Speranza, Padova, Italy. [5]Lifelab Program, Consorzio per la Ricerca Sanitaria (CORIS), Veneto Region, Padova, Italy

OBJECTIVE

Bronchopulmonary Dysplasia (BPD) is a life-threatening disorder affecting premature newborns, for which new therapeutic approaches are needed. Both mesenchymal stromal cells (MSCs) and their secreted extracellular vesicles (EVs) proved to be effective in preventing the development of BPD in animal models, showing promising results in preliminary clinical trials, but their mechanism of action is largely unknown. The role of hyperoxia in the pathogenesis of bronchopulmonary dysplasia (BPD) is well known. In fact, in the lung sublethal O_2 concentrations increase the proliferation of fibroblasts, with deposition of collagen into the interstitium resulting in fibrosis and in secondary pulmonary hypertension. Here, we evaluated if the MSC EVs treatment affects the development of fibrosis both in vivo and in vitro.

MATERIALS AND METHODS

BPD rat pups were divided in 3 groups: normoxia + PBS vehicle n=10 (control group), hyperoxia with PBS n=10 (untreated), hyperoxia with MSC EVs n=10 (treated). Both PBS and EVs were injected intratracheally (IT) at day3, 7 and 10. We evaluated the expression of the genes involved in fibrosis pathways such as TGFβ1, SMAD3, 7, COL1A1, αSMA. To evaluate epithelial secretory function, we analyzed the expression of glicosaminoglycans (Alcian blue staining) and of surfactant protein C (SFTPC) with and without MSC EV treatment by immunohistochemistry and immunofluorescence. We also set up an in vitro model of fibrogenesis and evaluated the effect of MSC EVs on αSMA expression in macrophages.

RESULTS

Pups under hyperoxia exhibited an increased expression of pro-fibrotic genes. These genes were significantly less (P=0.05) expressed in pups injected with MSC EVs.

The area of lung tissue expressing glycosaminoglycans was significantly (P<0.001) increased in MSC EV-treated rat pups in respect to untreated (17±2,35% vs. 13±1,98 % of tissue area). In addition, cells expressing the surfactant protein SFTPC were markedly more present (P<0.001) in MSC EV treated pups with respect to the untreated group (8 ± 1,63% versus 5±1,67% of cells/field, respectively). In vitro, MSC EVs suppressed the induction of αSMA expression in macrophages.

CONCLUSIONS

These results show that MSC EVs can help preventing the development of pulmonary fibrosis following hyperoxic injury.

17. DECELLULARIZATION TREATMENT PROVOKES COLLAPSE OF THE EXTRACELLULAR MATRIX: HERE THE TOOL TO PREVENT SAMPLE LOST.

Authors: Francesca Costabile[1,2,3], Marta Nardini[1], Patrizio Castagnola[2], Daniela Verzola[4], Maria Rosaria Gangemi[2], Francesca Ferrera[1], Gilberto Filaci[12], Maddalena Mstrogiacomo[1]

Affiliation: [1]Department of Internal Medicine, University of Genoa, Genoa, Italy. [2]Bioterapy Unit IRCCS Ospedale Policlinico San Martino-IST, Genoa, Italy. [3]Department of Radiation Oncology and Center for Cellular Immunotherapies, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA. [4]Division of Nephrology, Dialysis and Transplantation, Department of Internal Medicine, University of Genoa and IRCCS Ospedale Policlinico San Martino-IST, Genoa, Italy

OBJECTIVE

Tumor extracellular matrix is a complex system modulating cells' function and growth. Among *in vitro* 3D models, decellularized tissue represents a suitable scaffold providing the original biological features based on the content of a variety of proteins and glycosaminoglycans. However, these techniques still need to be standardized since often these scaffolds do not provide a proper recellularization. To date, the reason of lacking cells infiltration is still unknown and here we elucidate the problem.

MATERIALS AND METHODS

Murine B16f10 derived tumors were processed for decellularization with an extensively used protocol in literature and the generated scaffolds were studied to verify their efficacy as 3D culture system. To have a better understanding of the ECM structure, decellularized and fresh tumor tissue were analysed for matrix density and collapse measurement, respectively by detection of collagen type VI through Z-stacking method (immunofluorescence) and Scanning Electron Microscopy (SEM).

RESULTS

The decellularization process removed all the cells from the tissue and was not detrimental to the extracellular fibres, as confirmed by trichrome staining and collagen type VI detection. To test the efficacy of the decellularized tissue as scaffolds for 3D culture, we executed different recellularization experiments: with single cell line (melanoma or fibroblast), with coculture of both, with *ex vivo* cells recovered from murine melanoma tumor. As expected, after 7 days of culture in any case the cell attachment was limited just to de border of the tissue. However, recellularization was possible with a polyurethane, demonstrating that cells were not able to pass through the ECM of the scaffold that we generated. Structural analysis showed an increase of ECM in the decellularized tissue compared to the not decellularized one, encouraging our hypothesis of matrix collapse following decellularization process. Through SEM images, we were able to quantify the collapse of the ECM comparing fresh and decellularized mouse pulmonary parenchyma, where structural modifications are easy to be detected due to the geometric structure of the tissue (p value 0.013).

CONCLUSIONS

Our results explain the reason of the lacking cells penetration in decellularized tissue scaffolds. Here, we also provide for the first time a method to verify and quantify the damage that the decellularization process provokes on samples. In this way, the damage of precious specimen is prevented.

18. (YIA) CANINE MESENCHYMAL STEM CELL LYO-SECRETOME: IN VITRO POTENCY EVALUATION AND IN VIVO PRELIMINARY APPLICATION IN DOGS WITH NATURALLY OCCURRING OSTEOARTHRITIS

Authors: Priscilla Berni[1], Michela Mocchi[2], Elia Bari[2], Silvia Dotti[3], Riccardo Villa[3], Virna Conti[1], Maurizio Del Bue[4], Gian Paolo Squassino[5], Lorena Segale[6], Roberto Ramoni[1], Maria Luisa Torre[2,7], Sara Perteghella[2,7], Stefano Grolli[1]

Affiliation: [1]Department of Veterinary Medical Science, University of Parma, Italy. [2]Department of Drug Sciences, University of Pavia, Italy. [3]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Brescia, Italy. [4]Freelance Veterinary Medical Doctor, Parma, Italy. [5]Veterinary Practitioner, Studio Tecnico Veterinario, Asti, Italy. [6]Department of Pharmaceutical Sciences, University of Piemonte Orientale, Novara, Italy. [7]PharmaExceed S.r.l., Pavia, Italy

OBJECTIVE

Despite promising results obtained in experimental and clinical studies, Mesenchymal Stem Cells (MSCs) therapeutic applications are seriously influenced by the in-vivo implantation of an active and proliferating cell population. In this study, we focused on the MSCs-derived Lyo-secretome, a new freeze-dried, cell-free product with enriched with bioactive molecules secreted by MSCs, showing practical advantages in terms of storage, handling and administration. At first, we aimed to evaluate the in-vitro biological effects of canine Lyo-secretome on three different cell populations: canine adipose-derived MSCs, tenocyte and chondrocytes. Subsequently, dogs with naturally occurring bilateral osteoarthritis (elbow or knee) were enrolled in an in-vivo double-blinded study and treated with a double intra-articular injection of Lyo-secretome at 40-days interval to assess preliminary safety data.

MATERIALS AND METHODS

The cytocompatibility and proliferation ability of Lyo-secretome at increasing doses (50,000, 100,000 and 200,000 cell equivalents) were evaluated by the MTT test. The anti-elastase activity was investigated (at 2, 5, 10, 20 mg mL-1). Sterility and microbiological control tests were performed. The animals enrolled were treated with intra-articular injection of Lyo-secretome in the right joint, and placebo in the left, both resuspended in hyaluronic acid, repeated after 40 days. Lameness, pain, functional disability, and range of motion were evaluated (Day 0-7-20-40) and a questionnaire containing the Helsinki Chronic Pain Index was submitted to the owners (Day 0-2-4-7-20-40). The data were elaborated with multifactorial ANOVA analysis of variance.

RESULTS The Lyo-secretome in-vitro treatment stimulated cell proliferation and metabolic activity in a dose-dependent manner for all the cell types tested, reaching 85% for the higher Lyo-secretome concentration compared to the 10%FBS positive control (100%). Similarly, the anti-elastase activity showed a dose-dependent trend (reaching 85% at 20 mg/mL). Finally, the clinical data about the in-vivo treatment suggest that intra-articular injections of allogeneic Lyo-secretome are safe and do not induce a clinically significant local or systemic adverse response.

CONCLUSIONS This preliminary study provides the basis for the in-vivo application of Lyo-secretome derived from canine adipose tissue-derived MSCs, assessing the short and long-term efficacy in animals with naturally occurring joint diseases, where such treatments can represent an innovative regenerative therapy.

19. MULTILINEAGE-DIFFERENTIATING STRESS-ENDURING (MUSE) CELLS AS IN VITRO MODEL FOR NEUROLOGICAL DISEASES

Authors: Domenico Aprile[1], Sura Hilal Ahmed Al-Sammarraie[1], Deanira Patrone[1], Nicola Alessio[1], Tiziana Squillaro[1], Giovanni Di Bernardo[1], Umberto Galderisi[1]

Affiliation: [1]Department of Experimental Medicine, "Luigi Vanvitelli" Campania University, Naples, Italy

OBJECTIVE

IQSEC2 is a genetic disorder that causes intellectual disability and also other physical, neurological, and psychiatric symptoms. This condition is caused by mutations in the IQSEC2 gene, located on chromosome X. The study of the early stages in the functional development of neurons and neuronal networks in neurodevelopmental diseases such as the IQSEC2 syndrome is difficult because there are a limited number of neural stem cells (NSCs) in the brain and spinal cord, which are both difficult areas to access. To overcome these challenges, the discovery of induced pluripotent stem cells (iPSCs) has provided an option as *in vitro* neural model in recent years. iPSCs are adult somatic cells manipulated into dedifferentiated embryonic stem cells (ESCs). For this reason, we propose the use of endogenous Multilineage-differentiating stress-enduring (Muse) cells, which are stress-tolerant stem cells that reside in the stromal tissue and are capable of ecto-endo-mesodermal differentiation both *in vitro* and *in vivo*.

MATERIALS AND METHODS

IQSEC2 fibroblasts and normal fibroblasts (nFIB) were obtained from punch-skin biopsies and the cells were cultured *in vitro*. At passage 3 (P3) we isolated the population of Muse cells by MACS sorting for the surface marker, SSEA3. After 10 days, Muse cells were collected and plated to evaluate biological parameters and neural differentiation capacity.

RESULTS

IQSEC2-Muse cells showed an increase in the G1 phase and a decrease in the S phase of the cell cycle compared to the nFIB-Muse. These data are in accordance with the onset of senescence as demonstrated by an increase in beta-galactosidase positive cells. Also for the apoptosis process, we observed an increase in IQSEC2-Muse concern to nFIB-Muse. Of note, in IQSEC2-Muse after neural differentiation induction for 21 days, there was a bias to generate astrocytes respect to neurons compared to nFIB-Muse.

CONCLUSIONS

Our study showed the onset of senescence in the IQSEC2-Muse cells that could affect the properties of the stem cells, leading to a deficit in differentiation properties. Neural differentiation induction evidenced a trend to astrocyte differentiation in the IQSEC2-Muse.

We will aim to study the cellular population generate after IQSEC2-Muse astrocyte differentiation by specific protocol and characterize the obtained astrocytes by specific markers. Furthermore, our goal will be to support Muse cells as a new model for the study of other diseases.

20. (YIA) HUMAN AMNIOTIC FLUID STEM CELL SECRETOME FORMULATIONS: BIOACTIVE AGENTS FOR CARDIAC REGENERATION

Authors: Ambra Costa[1], Antonella De Palma[2], Davide Ceresa[3], Carolina Balbi[4], Sara Santamaria[1], Katia Cortese[1], Domenico Coviello[5], De Biasio Pierangela[3], Dario Paladini[5], Lucio Barile[6], Paolo Malatesta[1,3], Pierluigi Mauri[2], Sveva Bollini[1]

Affiliation: [1]Department of Experimental Medicine (DIMES), University of Genova, Genova, Italy. [2]Proteomics and Metabolomics Unit, Institute for Biomedical Technologies, Milan, Italy. [3] IRCCS Ospedale Policlinico San Martino, Genova, Italy. [4]Cellular and Molecular Cardiology, Cardiocentro Ticino Foundation, Lugano, Switzerland. [5]IRCCS Istituto na Gaslini, Genova, Italy. [6]Cardiovascular Theranostic, Cardiocentro Ticino Foundation, Lugano, Switzerland

OBJECTIVE

We previously showed that II trimester human amniotic fluid stem cells (fetal hAFS) possess relevant cardio-protective paracrine potential in preclinical models of myocardial infarction (MI) and drug-induced cardiotoxicity. Here, we analyse whether fetal hAFS secretome formulations, as cell-conditioned medium (CM) over extracellular vesicles (EVs), have superior cardio-active effect over III trimester cell (perinatal hAFS) secretome to trigger cardiomyocyte renewal for heart regeneration.

MATERIALS AND METHODS

Fetal hAFS (f-hAFS) were obtained by leftover samples of amniocentesis; perinatal hAFS (p-hAFS) were isolated from clinical-waste scheduled C-section samples. c-KIT+ hAFS were primed under 1%O_2 preconditioning for 24h to collect hAFS-CM and hAFS-EVs further separated by serial ultracentrifugation from it. hAFS-EVs were profiled by transmission electron microscopy, nanoparticle tracking analysis, proteomics and miRNA sequencing (miRNAseq). Neonatal mouse ventricular cardiomyocytes (mNVCM) were enzymatically isolated from R26pFUCCI2 transgenic pups endowed with fluorescent tagging of cell-cycle stages by nuclear signal (G1-phase by mCherry; S-G2-M-phase by mVenus). mNVCM were treated by hAFS-CM over hAFS-EVs from f- and p-hAFS for 48h to evaluate the most cardio-active formulation by immunostaining for further analysis in a R26pFUCCI2 4-day-old mouse pup model of MI.

RESULTS

Gestational stage doesn't affect f- and p-hAFS secretome formulation yield; f-hAFS-EVs and p-hAFS-EVs present similar size distribution and cytokine and chemokine profiling. miRNAseq reveals a stable miRNA core of EVs cargo, while proteomics reports significant enrichment of Agrin in f-hAFS-EVs (****$p<0.0001$), an important mediator for heart regeneration. f-hAFS-EVs only sustain in vitro cardiomyocyte cell-cycle transition from S-M-phase by 2.3-fold (*$p<0.05$) and increase cytokinesis by 4.5-fold (*$p<0.05$) compared to untreated cells. f-hAFS-EVs also decrease Cofilin-2 gene expression by 0.6-fold (***$p<0.001$), suggesting cardiomyocyte de-differentiation, required for cell-cycle re-entry. f-hAFS-EVs confirm in vivo cardiomyocyte S-M phase transition by 2.0-fold (*$p<0.05$) in the remote zone of myocardium after 3 days from MI.

CONCLUSIONS

Our preliminary findings suggest immature f-hAFS-EVs as more appealing bioactive agents for cardiomyocyte renewal over perinatal ones. Further potency assays are ongoing to define f-hAFS-EVs mechanism of action with Agrin as molecular candidate.

21. EFFECTS OF MESENCHYMAL STROMAL CELLS ON GROWTH OF HUMAN MALIGNANT PLEURAL MESOTHELIOMA MODELS

Authors: Silvia La Monica[1], Mara Bonelli[1], Roberta Alfieri[1], Valentina Coccè[2], Daniela Lisini[3], Francesca Paino[2], Giulio Alessandri[4], Francesco Petrella[2,5,6], Augusto Pessina[2]

Affiliation: Department of Medicine and Surgery, University of Parma, 43126 Parma, Italy. [2] CRC StaMeTec, Department of Biomedical, Surgical and Dental Sciences, University of Milan, 20122 Milan, Italy. [3]Cell Therapy Production Unit-UPTC, Fondazione IRCCS Istituto Neurologico Carlo Besta, 20133 Milan, Italy; [4] University of Brescia, 25100 Brescia, Italy. [5]Department of Thoracic Surgery, IRCCS European Institute of Oncology, 20139 Milan, Italy. [6]Department of Oncology and Hemato-Oncology, University of Milan, 20122 Milan, Italy

OBJECTIVE

Malignant Pleural Mesothelioma (MPM) is an aggressive tumor mainly caused by asbestos exposure and there are still no effective therapies with the consequent poor prognosis for patients. The aim of this study was to investigate the efficacy of human adipose tissue-derived mesenchymal stromal cells (AT-MSCs) on the proliferation of human mesothelioma cell lines. The role of mesenchymal stromal cells (MSCs) in cancer is controversial due to their opposite effects on tumor growth and still now only few data are reported for MSCs in MPM.

MATERIALS AND METHODS

The anti-proliferative and pro-apoptotic efficacy of AT-MSCs, their lysates and MSC-conditioned medium (secretome) was evaluated in MSTO-211H, NCI-H2452 and NCI-H2052 MPM cell lines. After large-scale MSCs production in a bioreactor, their efficacy was also evaluated on a human MPM xenograft in mice.

RESULTS

MSCs, their lysate and secretome induced cell cycle arrest and inhibition of proliferation of MPM cell lines. Furthermore, MSC lysate induced apoptotic cell death. The efficacy of MSC was confirmed *in vivo* by a significant inhibition of tumor growth, similar to that produced by the systemic administration of paclitaxel. Interestingly, when the treatments were stopped no tumor progression was observed in the animals treated with MSC indicating a long-lasting effect. By contrast, tumors of the paclitaxel group started to regrow.

CONCLUSIONS

These data demonstrated that MSCs, both through paracrine and cell-to-cell interaction mechanisms, induced a significant inhibition of tumor growth *in vitro* and *in vivo* models of MPM. Since the prognosis for MPM patients is poor and the therapeutic options are limited, cell therapy based on MSCs could provide a potential new therapeutic strategy for this malignancy.

22. ADVANCES IN CRI DU CHAT SYNDROME UNDERSTANDING: IN VITRO NEURONAL MODEL STARTING FROM INDUCED PLURIPOTENT STEM CELLS

Authors: Giovanna Piovani[1], Rosalba Monica Ferraro[2,1], Silvia Clara Giliani[1,2]

[1]*Affiliation:* Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy. [2]A. Nocivelli Institute for Molecular Medicine, Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy

OBJECTIVES

In the last decade, induced pluripotent stem (iPS) cells have revolutionized the utility of human in vitro models of diseases associated with genetic defect. This study performs a previously undocumented functional and molecular analysis of Cri-du-Chat hiPSC-differentiated cells (UNIBSi004-A iPSC line) in order to shed light on the syndrome. We have set out to address our findings to create an in vitro induced CdC neuronal model.

Cri-du-chat syndrome (CdCs), also referred to as 5p deletion syndrome, is a syndrome caused by total or partial deletion in the short arm of chromosome 5. The syndrome is named by high-pitched cat-like cry. The features of CdCs are facial dysmorphism, developmental motor delay, including difficulties in mobility, dexterity, and verbal communication.

Loss of several genes in the 5p region contributes to the phenotype. *TERT, SEMA5A, MARCH6, CTNND2, NPR3, TPPP, SLC6A3, CDH18, CDH12, CDH10,* and *CDH6* are known to be expressed in the brain, and to play a role in neuronal migration and in the development of the nervous system. It is critical to understand how their interplay might affect the development and presentation of the disease.

MATERIALS AND METHODS

We performed the study with our CdC iPSC lines and with a human iPSCs line (BJ) as a control. Neuronal cells were differentiated in vitro, their phenotype studied to modeling the human CdC tissue. The neuronal differentiation process consisted of an initial neural induction of iPSCs, mediated by appropriate concentrations and gradients of several morphogenetic factors that are normally expressed in the developing brain. The fate specification and differentiation of the different subtypes of neurons were determined by the specific combinations of transcription factors responsible for their functional diversity.

RESULTS

We have obtained to date induced neurons from BJ and CdC IPSCs lines with 28 days of growth. iPSC-derived neurons were used for immunofluorescence, immunocytochemistry, and morphological analysis starting from day 14. In addition, to estimate the neuronal culture purity, gene expression of astrocytes and oligodendrocytes markers is also evaluated.

CONCLUSIONS

The research has create an in vitro CdCS neuronal model, starting from our CdC iPSCs, to have a new translational framework to characterize the pathological morphology of induced neuronal cells, to evaluate the role of genes involved in the deletion characteristic of the syndrome.

23. HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED MESENCHYMAL STEM CELLS: ALTERNATIVE STRATEGY TO STUDY THESE CELLS IN RARE GENETIC DISEASE

Authors: Giovanna Piovani[1], Rosalba Monica Ferraro[2,1], Silvia Clara Giliani[1,2]

Affiliation: [1]Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy. [2]A. Nocivelli Institute for Molecular Medicine, Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy

OBJECTIVES

The discovery of induced pluripotent stem cells (iPSCs) has revolutionized disease modeling, drug discovery, and regenerative medicine over the past decade. iPSCs can be reprogrammed from adult cells back into the stem cell-like stage with unlimited proliferation and differentiation abilities. Conventionally, mesenchymal stromal cells (MSC) are isolated from various tissues but in vitro they have limited proliferation potentials and lose biological functions after prolonged expansion. To overcome limitations of tissue-derivated MSCs, we have differentiated IPSCs, theoretically unlimited expansion potential, into mesenchymal ones in order to better study this tissue type in patient with rare disease from whom it would be difficult or problematic to recover them. Cri-du-chat syndrome (CdCs), also known as 5p deletion syndrome, is a syndrome caused by a total or partial deletion in the short arm of chromosome 5. Deleted genes play a role in brain development and premature ageing. No information on the microenvironment and MSCs obtained from CdC patients is available in the literature. The induction of CdC iPSCs in fibroblast-like cells that phenotypically and functionally resemble MSCs is an interesting approach to study pathological homeostasis of CdC tissues, premature ageing and senescence suffered by CdC patients. We focused our attention on their characteristics compared to iPSC-MSCs obtained from normal cell line.

MATERIALS AND METHODS

We have reprogrammed to pluripotency peripheral blood mononuclear cells derived from CdC patient and a control line. Mesenchymal differentiation was induced in a monolayer with dedicated and standardized protocols.

RESULT

We have obtained induced MSCs that respect the three key criteria. At first thay are plastic adherent, ≥95%; MSC-like cells were analyzed by flow cytometry to check for the expression of a full panel of MSC-associated markers, for an absence of the expression of pluripotency markers as well as for an absence of hematopoietic markers. Finally, the iPSC-MSC like are able to differentiate to osteoblasts, adipocytes, and chondrocytes under standard *in vitro* differentiation protocols.

CONCLUSION

We obtained iPSC-differentiated fibroblast-like cells that phenotypically and functionally resemble adult mesenchymal stromal cells (MSC) in a patient with a rare disease. The results suggest excellent reproducibility of the method with the possibility to carry out further studies using this cell type.

24. DOLPHIN UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS: POTENTIAL SOURCE FOR CELL-BASED THERAPY AND CLINICAL APPLICATIONS

Authors: Annalisa Altigeri[1], Cristiano Cocumelli[2], Ettore Galvano[1], Luca Polito[1], Alessia Zepparoni[1], Flavio Maggi[3], Annalisa Duri[3], Paola Ghisellini[4,5], Cristina Rando[4,5], Roberto Eggenhöffner[4,5], Katia Barbaro[1,5]

Affiliation: [1]Virology, Istituto Zooprofilattico Sperimentale Lazio e Toscana M. Aleandri, Rome, Italy. [2]General Diagnostic, Istituto Zooprofilattico Sperimentale Lazio e Toscana M. Aleandri, Rome, Italy. [3]Zoomarine Italia SPA, Rome, Italy. [4]Department of Surgical Sciences and Integrated Diagnostics (DISC), University of Genoa, Italy. [5]INBB, Biostructures and Biosystems National Institute, Rome, Italy

OBJECTIVE

Mesenchymal stem cells (MSCs) have become a promising tool in cellular therapy. Here, we obtained mesenchymal stem cells from dolphin's umbelical cord (DOC-MSC; Tursiops truncatus, female; Zoomarine, Rome) as a renewable and effective source of mesenchymal cells to promote tissue repair and immune response.

MATERIALS AND METHODS

Umbilical cord (UC) was obtained from a female bottlenose dolphin at the end of gestation and collected in phosphate-buffered saline (PBS) under a sterile conditions. In the laboratory, UC was washed with PBS and blood cells were quickly removed. Sample was scraped in a sterile petri dish and obtained fragments were submitted to enzymatic digestion (IA collagenase· Sigma-Aldrich). Following centrifugation, the pellet was resuspended in growth medium (DMEM + 10% FBS; 100 U/ml penicillin and 100 μg/ml streptomycin) and cells were then seeded (37 °C with 5% CO2 atmosphere). After reaching about 70–80% confluence, specific differentiation media were added for two weeks. Evaluation of osteogenic, chondrogenic and adipogenic differentiation was performed by cytochemical staining to confirm presence of calcium production, intra- and extracellular glycosaminoglycan residues and intracellular accumulation of lipid-richvacuoles. Cell viability was confirmed also after freezing/thawing in liquid nitrogen.

RESULTS

Cells isolated from the dolphins's umbelical cord can proliferate and generate homogeneous colonies as well as prove to meet all the criteria for stem cells: cells are plastic adherent, they have a spindle-shaped morphology and can differentiate into adipogenic, chondrogenic and osteogenic cell lineages.

CONCLUSIONS

The DOC-MSC cell provides a novel *in vitro* animal model for cell and gene therapy, cloning and biotechnological applications. DOC-MSC can constitute the basis for biological treatments in wound healing and tissue regeneration in dolphins. In addition, in view of the great interest in mortality of aquatic mammals due to viral agents, these cells could represent the ideal substrate for in vitro virus isolation. Indeed, DOC-MSC could be also used as a fundamental tool for toxicology tests, as an alternative to animal models, to identify the citotoxicity levels caused by chronic exposure to environmental pollutants (organochlorines and heavy metals), providing valuable information regarding conservation and protection of marine mammals.

25. FULL CHARACTERIZATION OF MICROGRAGMENTED ADIPOSE TISSUE: TISSUE ARCHITECTURE, MESENCHYMAL STEM CELL CONTENT AND RELEASE OF PARACRINE MEDIATORS

Authors: Marco Viganò[1], Enrico Ragni[1], Enrica Torretta[1,2], Cecilia Gelfi[2], Laura de Girolamo[1]

Affiliation: [1]IRCCS Istituto Ortopedico Galeazzi. [2]Università degli Studi di Milano

OBJECTIVE

The use of microfragmented adipose tissue (mFAT) in different branches of medicine is gaining popularity following the positive results reported in recent case series and clinical trials. The purpose of this study is to characterize mFAT in terms of structure, cell content and secreted factors, to understand the possible mechanisms of action in the view of regenerative medicine.

MATERIALS AND METHODS

Tissue samples (mFAT and lipoaspirate, LA) were collected from 7 donors. Each sample was tested for:

- Tissue structure by hematoxylin and eosin staining; expression of CD31, CD90, CD146 by immunohistochemistry.
- Expression of CD235a, CD45, CD271, CD105 and percentage of senescent cells by flow cytometry (after enzymatic digestion).
- Release of paracrine mediators by proteomics and miRNomics analyses (after culture for 24 hours in serum free culture medium).
- Tissue composition by proteomics analysis

RESULTS

Tissue structure was maintained after microfragmentation; the reduction of blood elements was evident from both histologic and flow cytometry tests, with a -76% of erythrocytes and -79% lymphocytes observed in the processed samples compared to LA. Senescence was limited in all samples (<5%). Profile of released miRNAs was similar between mFAT and LA, where 381 and 376 different elements were identified, respectively. Significant differences in terms of content were observed for 217 proteins between mFAT and LA conditioned media, with a reduction of protein related to the acute phase in the former. Tissue proteomic analyses showed a reduction of extracellular matrix and blood components in mFAT compared to LA.

CONCLUSIONS

Taken together, these results suggest that processing of LA into mFAT allow for removal of blood elements, in terms of red blood cells, lymphocytes, acute phase and complement system proteins, and for the reduction of extracellular matrix components. Otherwise, tissue structure and content of Mesenchymal Stem Cells were not influenced by tissue processing. Then, microfragmentation process represents a safe and efficient method for the application of adipose tissue derived products in Regenerative Medicine, allowing for the maintenance of all the effector elements for tissue regeneration while removing possible detrimental agents such as inflammatory mediators.

26. (YIA) ROLE OF PD-L1 IN LICENSING IMMUNOREGULATORY FUNCTION OF DENTAL PULP MESENCHYMAL STEM CELLS

Authors: Rosanna Di Tinco[1], Giulia Bertani[1], Alessandra Pisciotta[1], Laura Bertoni[1], Elisa Pignatti[1], Jessika Bertacchini[1], Paola Sena[1], Stefania Croci[2], Martina Bonacini[2], Carlo Salvarani[1,3], Gianluca Carnevale[1]

Affiliation: [1]Department of Surgery, Medicine Dentistry and Morphological Sciences with Interest in Transplant, University of Modena and Reggio Emilia, Modena, Italy. [2]Clinical Immunology, Allergy and Advanced Biotechnologies Unit, Azienda Unità Sanitaria Locale-IRCCS di Reggio Emilia, Reggio Emilia, Italy. [3]Rheumatology Unit, Azienda Unità Sanitaria Locale-IRCCS di Reggio Emilia, Reggio Emilia, Italy

OBJECTIVE

Mesenchymal stem cells have been attracting a constantly increasing interest due to their well characterized multipotency and immunomodulatory abilities, which make them a valuable candidate for the treatment of autoimmune/inflammatory diseases. Dental pulp stem cells (DPSCs) are a mesenchymal stem cell population owning peculiar properties due to their embryological origin from neural crest, their low immunogenicity and their capability to modulate the immune response through different mechanisms, including Fas/FasL pathway. To this regard, the present study aimed to investigate a new immune-regulatory pathway, i.e., PD1/PD-L1, exploited by DPSCs when exposed to an inflammatory microenvironment.

MATERIALS AND METHODS

Inflammatory microenvironment was created *in vitro* by the activation of peripheral blood mononuclear cells (PBMCs) isolated from healthy donors and rheumatoid arthritis (RA) patients with anti-CD3 and anti-CD28 antibodies. Direct and indirect co-cultures between DPSCs and PBMCs were carried out to evaluate the activation of immunomodulatory checkpoints in DPSCs and the inflammatory pattern in PBMCs through Real-Time PCR, Western Blot, Immunofluorescence and Immunohistochemistry analyses.

RESULTS

Our data revealed that the inflammatory stimuli trigger DPSCs immunoregulatory functions that can be exerted by both direct and indirect contact, as shown by the increased expression of PD-L1 in DPSCs after co-culture with pre-activated PBMCs. This ability seems to be strictly related to the stemness status of DPSCs as confirmed by the lost capability to express PD-L1 in differentiated DPSCs co-cultured with pre-activated PBMCs. Moreover, as demonstrated by using a selective PD-L1 inhibitor, DPSCs were able to activate compensatory pathways (i.e., Fas/FasL) targeting to orchestrate the inflammatory process through the modulation of pro-inflammatory cytokines in pre-activated T lymphocytes. The involvement of PD-L1 mechanism was also observed in autologous inflammatory status (pulpitis) and after direct exposure to pre-activated PBMCs isolated from RA patients.

CONCLUSIONS

Our results demonstrated that DPSCs can modulate the inflammatory microenvironment by the activation of PD1/PD-L1 pathway synergistically cooperating with other immune-regulatory pathways including Fas/FasL. In light of this evidence, these properties might be functional in controlling the inflammatory milieu typical of autoimmune diseases, such as rheumatoid arthritis.

27. IDENTIFICATION OF STABLE FEATURES AND KEY INGREDIENTS OF A CELL-FREE THERAPEUTIC DERIVED FROM MESENCHYMAL STEM/ STROMAL CELL

Authors: Chiara Giannasi[1,2], Stefania Niada[2], Elena Della Morte[2], Sara Casati[1], Alice Gualerzi[3], Anna Teresa Brini[1,2]

Affiliation: [1]Department of Biomedical, Surgical and Dental Sciences, Università degli Studi di Milano, Milan, Italy. [2]IRCCS Istituto Ortopedico Galeazzi, Milan, Italy. [3]IRCCS Fondazione Don Carlo Gnocchi ONLUS, Milano, Italy

OBJECTIVE

Over the years, the transplantation of Mesenchymal Stem/Stromal Cells (MSCs) has paved the way to the successful clinical management of a variety of diseases. The paradigm shift acknowledging MSC paracrine action as the main effector of cell therapy has addressed the scientific focus towards the investigation of the therapeutic potential of their conditioned medium (CM). Nowadays more than 10 trials are investigating the clinical use of MSC-CM (either as a complete formula or as isolated subcomponents) in counteracting different pathologies. Nevertheless, the regulatory framework for cell-free therapeutics still needs to be defined and the pipeline for their preparation lacks effective and shared guidelines. Prior MSC-CM use in clinics, appropriate quality controls are needed to assess its reproducibility. Here we investigate several CM features that can be harnessed for its standardization.

MATERIALS AND METHODS

Adipose-derived MSCs (ASCs) were isolated from the adipose tissue of donors differing for sex, age and type of surgery. For CM production, 90% confluent ASCs were grown in starving conditions (no FBS) for 72h. Supernatants were collected, centrifuged at 2500g to remove debris and large apoptotic bodies, and concentrated about 60 times using centrifugal filter devices with a 3kDa molecular weight cut-off. CM characteristics, including general features and precise protein and lipid concentrations, were assessed by Nanoparticle Tracking Analysis (NTA), Raman Spectroscopy (RS), ELISA and Mass Spectrometry.

RESULTS

NTA demonstrated a homogeneous and relevant number of extracellular vesicles ($>10^9$ particles per million donor ASCs), with a mean size <200nm. RS provided an overlook of ASC-CM composition in terms of proteins, lipids and nucleic acids, further confirming a high homogeneity among the samples. The quantification of a panel of factors involved in relevant biological pathways revealed the presence of 104 proteins and 7 lipids in all the specimens. Of these, 26 proteins (among which MCP-4, DKK-1, RAGE, TFN RI and PDGF-AA) and 2 lipids (SEA and PGE2) resulted highly conserved (coefficient of variation<33%) among the batches.

CONCLUSIONS

We assessed that ASC-CM samples present stable features and we identified key ingredients that may entail a therapeutic action. In addition, their consistent levels among batches may represent a quality control step in the perspective of a future clinical translation.

28. (YIA) THE ROLE OF SHEAR STRESS IN THE MODULATION OF STEMNESS PROPERTIES IN HUMAN DENTAL PULP STEM CELLS

Authors: Giulia Bertani[1], Rosanna Di Tinco[1], Alessandra Pisciotta[1], Laura Bertoni[1], Giulia Orlandi[1], Paola Sena[1], Michele Signore[2], Ruggero De Maria[3], Jessika Bertacchini[1], Gianluca Carnevale[1]

Affiliation: [1]Department of Surgery, Medicine Dentistry and Morphological Sciences with Interest in Transplant, University of Modena and Reggio Emilia, Modena, Italy. [2]RPPA Unit, Proteomics Area, Core Facilities, Istituto Superiore di Sanità, Rome, Italy. [3]Institute of General Pathology, Universita Cattolica del Sacro Cuore Facolta di Medicina e Chirurgia, Roma, Italy

OBJECTIVE

Human dental pulp stem cells (DPSCs) represent a mesenchymal stem cells niche localized in the perivascular area of dental pulp and characterized by low immunogenicity and immunomodulatory/anti-inflammatory properties. Our previous findings demonstrated the ability of DPSCs to support tissue vascularization *in vivo*. It is well established that inflammation and biomechanical forces including dynamic changes determined by blood flow, vessel geometry and blood pressure play a pivotal role in tissue regenerative process. This fluid shear stress should influence the angiogenic potential of DPSCs. To this purpose, the present study aimed to evaluate how the angiogenic potential of DPSCs might be influenced by shear stress.

MATERIALS AND METHODS

DPSCs were cultured under both static and dynamic conditions with or without peripheral blood mononuclear cells activated with anti-CD3 and anti-CD28 antibodies (aPBMCs). Laminar shear stress was generated by using a specific peristaltic pump. The evaluation of the angiogenesis process and inflammatory properties in DPSCs was performed by Reverse-Phase protein microarrays (RPPA), immunofluorescence and western blot analyses.

RESULTS

Our data showed that DPSCs expressed typical mesenchymal stem cells markers (i.e., CD73, CD90, CD105) and PDGFRβ. Our results highlight that DPSCs cultured in endothelial differentiation medium are able to express typical angiogenic markers (i.e., VEGF, Tie2, angiopoietin 1, CD34) even after 72 hours of induction through the activation of Akt/eNOS pathway. At the same time, DPSCs showed the ability to form tube structures. Then, the influence of fluid shear stress on DPSCs angiogenic potential was also investigated. RPPA analyses revealed that laminar shear stress modulates the phosphorylation of proteins involved in angiogenesis and increase DPSCs pro-inflammatory phenotype when compared to stem cells cultured in static conditions. The inflammatory properties of DPSCs were investigated by co-culture with aPBMCs under static and dynamic conditions. Our data revealed that DPSCs co-cultured with aPBMCs in dynamic conditions up-regulated pro-inflammatory pathways.

CONCLUSIONS

Our results pointed out that different culture conditions, such as the application of a fluid shear stress and the presence of a pro-inflammatory microenvironment are able to modulate the biological properties of DPSCs supporting the immunomodulatory plasticity of DPSCs.

29. (YIA) CYTOCHALASIN B MODULATES NANOMECHANICAL PATTERNING AND FATE IN HUMAN ADIPOSE-DERIVED STEM CELLS

Authors: Giovannamaria Petrocelli[1], Eva Bianconi[2], Riccardo Tassinari[3], Andrea Alessandrini[4,5], Gregorio Ragazzini[4,5], Claudia Cavallini[3], Provvidenza Maria Abruzzo[1], Luca Pampanella[1], Silvia Canaider[1,3], Federica Facchin[1,3], Carlo Ventura[1,3]

Affiliation: [1]Department of Experimental, Diagnostic and Specialty Medicine-Unit of Histology, Embryology and Applied Biology-University of Bologna, Italy. [2]Laboratory of Cardiovascular Biology, IRCCS Ospedale Policlinico San Martino, Viale Rosanna Benzi 10, 16132 Genova, Italy. [3]National Laboratory of Molecular Biology and Stem Cell Bioengineering of the National Institute of Biostructures and Biosystems (NIBB) – Eldor Lab, at the Innovation Accelerator, CNR, Via Piero Gobetti 101, 40129 Bologna, Italy. [4]Department of Physics, Informatics and Mathematics, University of Modena and Reggio Emilia, Via Campi 213/A, 41125 Modena, Italy. [5]CNR-Nanoscience Institute-S3, Via Campi 213/A, 41125 Modena, Italy

OBJECTIVE

Cytochalasin B (CB), a cyto-permeable mycotoxin, is able to inhibit the formation of actin microfilaments with direct effects on cell biological properties. In this study, we first assessed the effect of CB on human adipose-derived stem cells (hASCs), investigating the dose-dependency of cellular responses, as well as the hASC capability to retrieve the morphology and proliferative activity upon toxin withdrawal. In addition, we investigated the changes in stem cell mechanical properties that could affect hASC commitment.

MATERIAL AND METHODS

hASCs were obtained from 3 samples of lipoaspirate. Several properties of hASCs were investigated after a preconditioning incubation of 24h with CB and after a 24h-recovery time from the removal of this toxin. The morphological analysis was performed under optical microscope and with the live-cell imaging method. hASC metabolism was investigated by using the colorimetric assays at the end of the CB treatment and during a 72h-prolonged administration. Cell viability and proliferation were evaluated with cell count by using Erythrosin B dye. The cytoskeletal markers were evaluated by immunofluorescence. The adipogenic differentiation was analyzed with Oil Red O (ORO) staining. CB role on mechanical properties of hASCs was investigated by atomic force microscopy (AFM) using the Hertz-Sneddon and Ting's models.

RESULTS

The treatment with CB influenced hASC morphology, metabolism, number and proliferation in a dose and time-dependent manner, in association with a progressive disorganization of actin microfilaments. Furthermore, the removal of CB highlighted the ability of hASCs to restore their cytoskeletal organization. Finally, AFM revealed that CB remarkably modulated the viscoelastic properties of hASCs, by increasing their viscosity, thus affecting adipogenic fate.

CONCLUSIONS

Cytoskeletal proteins provide architectural and signaling cues within the cells. They are able to reorganize themselves in response to mechanical forces, thereby promoting specific cellular responses. Here, we show that CB acted at the nanomechanical level to modulate hASC stiffness and viscosity, affording a dose and time-dependent modulation of stem cell properties. These observations set the basis for using CB as a potential tool for further elucidating, and potentially control the mechano-sensing/-transduction, and the bioelectric properties of human stem cells favouring its use in therapeutic approach.

30. CONSEQUENCES OF LOW DOSE IRRADIATION ON STEM CELLS AND ITS CONTRIBUTION TO SENESCENCE: IMPLICATION FOR AGING

Authors: Ibrahim H. Demirsoy,[1] Nicola Alessio,[1] Tiziana Squillaro,[1] Giovanni Di Bernardo,[1] Giovanni Galano[5], Roberto De Rosa[5], Gianfranco Peluso,[3] Umberto Galderisi[1,2,3]*

Affiliation: [1]Department of Experimental Medicine, Biotechnology and Molecular Biology Section, University of Campania "Luigi Vanvitelli," Naples, Italy. [2]Sbarro Institute for Cancer Research and Molecular Medicine, Center for Biotechnology, Temple University, Philadelphia, PA, USA. [3]Institute of Agri-Environmental Biology and Forestry (IBAF), CNR, Naples, Italy. [4]Department of Medical, Surgical, Neurological, Metabolic Sciences, and Aging, 2nd Division of Neurology, Center for Rare Diseases and InterUniversity Center for Research in Neurosciences, University of Campania "Luigi Vanvitelli," Naples, Italy. [5]ASL Napoli 1 Centro P.S.I. Napoli Est - Barra, Naples, Italy. *Corresponding author: Center for Biotechnology, Temple University, 1900 N. 12th St., Philadelphia, PA 19107-6799

OBJECTIVE

Senescent cells secrete several factors, which are collectively named SASP (senescence-associated secretory phenotype). The released SASP induces senescence of nearby cells by paracrine actions or of distant cells through bystander effect. Genetics stressors that induce senescence, such as low dose radiation (LDR), impair cell functions, lead to senescence and contribute to aging and age-related disorders. We focused on the effect of LDR on mesenchymal stromal cell (MSCs) that reside in the bone marrow, the adipose tissue and in the stroma of other tissues. Previously, we showed that MSCs treated with LDR become senescent and changed their secretome profiles. MSCs reside close to vessels in the stroma and their secretome may easily reach blood circulation. Specifically, senescent MSCs can contribute to increase in the levels of circulating pro-aging factors, such as the insulin growth factor (IGFs) and IGF binding proteins (IGFBP4, IGFBP5 and IGFBP7). Our study assessed the levels of circulating IGFBPs in the sera of mice following LDR. We also evaluated the IGFBPs and IGFs in the sera of patients following medical exposure to LDR.

MATERIAL AND METHODS

We irradiated mice with 100 mGy X-ray and sera of the mice were collected before irradiation and 48 hours later. In human subjects, patient's blood samples were collected before computer tomography (CT) and 48 hours later.

RESULTS

The irradiated mice showed a significant increase in the level of circulating IGFBP4, IGFBP5 and IGFBP7. These results prompted us to evaluate the IGFBPs levels in the patients undergoing CT scan. A preliminary analysis on 26 patients, evidenced that 48 hours following CT scan, at least one of the different IGFBPs was increased in the serum of the great majority of patients. In addition, we found that the level of IGF- II proteins were significantly elevated in patients 48 hours following CT scan.

CONCLUSIONS

In vivo experiments suggested that MSCs underwent senescence by LDR and release in circulatory flow pro-aging factors, which may trigger senescence of distant cells. This study will contribute to better understanding of senescence and its underlying molecular mechanisms and will contribute to development of more effective senolytic drugs.

31. SENSITIVITY OF HUMAN GLIOBLASTOMA TO A NEW MONOFUNCTIONAL PT-II COMPLEX BASED ON 8-AMINOQUINOLINE

Authors: Isabella Rimoldi[1], Giorgio Facchetti[1], Valentina Coccè [2], Francesca Paino [2], Emilio Ciusani[3], Giulio Alessandri[4], Lucia Signorini[5], Francesca Sisto[2], Aldo Giannì[2,6] Augusto Pessina[2]

Affiliation: [1]Department of Pharmaceutical Science, University of Milan, Milan, Italy; [2]CRC StaMeTec, Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy; [3]Laboratory of Clinical Pathology and Neurogenetic Medicine, Fondazione IRCCS Neurological Institute Carlo Besta, Milan, Italy; [4]Department of Molecular and Translational Medicine, Section of Microbiology and Virology, Medical School, University of Brescia, Brescia; [5]Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy; [6]Maxillo-Facial and Dental Unit, Fondazione Ca' Granda IRCCS Ospedale Maggiore Policlinico, Milan, Italy

OBJECTIVE

Platinum (II) complexes play an important role in cancer treatment. We evaluated the stability and the activity of a new platinum (II)-complex (hereafter called Pt-8AQ) based on 8-aminoquinoline (8-AQ), against four human cancer cell lines. Further studies have been addressed to the human glioblastoma U87-MG, in which Pt-8AQ revealed a significant antineoplastic activity with a mechanism of cytotoxicity different from that reported for cisplatin (CisPt) used as control.

MATERIALS AND METHODS

The *in vitro* antiproliferative activity of Pt-8AQ and CisPt was tested against four tumor cell lines: glioblastoma U87-MG, pancreatic adenocarcinoma CFPAC-1, adenocarcinoma MCF-7 and bi-phasic mesothelioma MSTO-211H by an MTT assay.Their stability was assessed *in vitro* with U87-MG through cytotoxicity assay after 24 h of treatment with fresh drugs or drugs incubated for 24 h at 37°C. The ability of the Pt-8AQ to induce growth arrest was evaluated through cell-cycle analysis and Annexin-V/PI assays by flow cytometry and fluorescent microscope. Real-time PCR or colorimetric assays for specific markers regulating cell proliferation or apoptosis were checked.

RESULTS

The *in vitro* antiproliferative activity highlighted that CisPt activity is always significantly higher with the only exception for U87-MG in which Pt-8AQ showed a higher activity (IC_{50}= 3.68 ± 0.69 μM, $p<0.01$) than CisPt (IC_{50}= 7.27+1.80 μM). The stability of drugs after incubation (37°C, 24 hours) showed that Pt-8AQ still retained a significant pharmacological activity (IC_{50} 8.39 ± 0.79 μM). The effect of drugs (24 h, 10 μM) on cell cycle of U87-MG showed a decrease of the cells in G0/G1 phase and an increase of cells in S phase with CisPt, while Pt-8AQ did not significantly affect the cell cycle pattern. Finally, real-time PCR analysis showed that Pt-8AQ increased the expression of p53.

CONCLUSIONS

Our data reported that Pt-8AQ is a platinum complex endowed with a selectively higher cytotoxic activity than CisPt against U87-MG cancer cell line. The pharmacological activity of this monofunctional platinum complex is related to its ability to trigger apoptosis through a p53 dependent pathway. By considering the prominent stability of Pt-8AQ complex after 24 h incubation at 37°C and the Mesenchymal Stromal Cells (MSCs) resistance to this drug, experiments are in progress to obtain MSCs loaded with Pt-8AQ to consider for advanced cell therapy based on a selective cells' mediated drug delivery.

32. OSTEOARTHRITIS SYNOVIAL FLUID CONFERS CARTILAGE PROTECTIVE AND IMMUNOMODULATORY FEATURES TO ADIPOSE-DERIVED MESENCHYMAL STEM CELLS SECRETED FACTORS AND EXTRACELLULAR VESICLES-EMBEDDED miRNAs

Authors: Enrico Ragni[1], Carlotta Perucca Orfei[1], Laura de Girolamo[1]

Affiliation: [1]IRCCS Istituto Ortopedico Galeazzi, Laboratorio di Biotecnologie Applicate all'Ortopedia, Milano, Italy

OBJECTIVE

Intra-articular administration of adipose-derived mesenchymal stromal cells (ASCs) gained popularity as innovative regenerative medicine approach for osteoarthritis (OA) treatment. ASCs stimulate tissue repair and immunomodulation through paracrine factors (secretome), both soluble and extracellular vesicles (EV) embedded. Interaction with degenerative/inflamed environment is a crucial factor in understanding the finely tuned molecular message but, to date, the majority of reports have described ASC-secretome features in resting conditions or under chemical stimuli far from the *in vivo* environment. In this study, secretory profile of ASCs treated with naïve synovial fluid (SF) from OA patients was evaluated.

MATERIALS AND METHODS

SF was collected from 6 OA (Kellgren/Lawrence III–IV grade) patients and 12 cytokines (IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-17A, TNFα and GM-CSF) involved in OA quantified by ELISA. ASCs were obtained from adipose waste material from 3 female donors (54 ± 8 years old) and characterized by flow cytometry. ASCs were exposed to culture medium supplemented with 50% pooled OA-SF for 48 h and, afterwards, secreted factors and EVs released for 48h in serum-free medium collected. ELISA was used to determine the concentration of 200 soluble factors. EVs were characterized by Nanoparticle Tracking Analysis, electron microscopy and flow cytometry. EV-embedded miRNAs were detected by real-time RT-PCR analysis covering 754 human miRNAs. To test secretome, chondrocytes were obtained from 3 OA (KL III–IV grade) patients undergoing total hip arthroplasty and treated with 25 pg/mL IL-1β for 3 days before and during secretome treatment. Chondrocyte response to inflammation and treatment was evaluated by flow cytometry scoring VCAM1 and ICAM1.

RESULTS

Fifty-eight factors and 223 EV-miRNAs were identified, and discussed in the frame of cartilage and immune cell homeostasis. Bioinformatics gave a molecular basis for M2 macrophage polarization, T cell proliferation inhibition and T reg expansion enhancement, as well as cartilage protection, further confirmed in the *in vitro* model of OA chondrocytes. Moreover, a strong influence on immune cell chemotaxis emerged.

CONCLUSIONS

In conclusion, obtained molecular data support the regenerative and immunomodulatory properties of ASCs when interacting with osteoarthritic joint environment.

33. ENGINEERED EVS FOR OXIDATIVE STRESS PROTECTION

Authors: Anna Maria Tolomeo[1,2], Santina Quarta[2,3,*], Alessandra Biasiolo[2,3], Mariagrazia Ruvoletto[2,3], Michela Pozzobon[1,4], Giada De Lazzari[1,2], Ricardo Malvicini[1,2,5], Cristian Turato[6], Giorgio Arrigoni[7,8], Patrizia Pontisso[2,3], Maurizio Muraca[1,2,4]

Affiliation: [1]Department of Women's and Children's Health, University of Padova, Via Giustiniani, 35128 Padova, Italy. [2]L.i.f.e.L.a.b. Program, Consorzio per la Ricerca Sanitaria (CORIS), Veneto Region, Via Giustiniani, 35128 Padova, Italy. [3]Department of Medicine, University of Padova, Via Giustiniani, 35128 Padova, Italy. [4]Institute of Pediatric Research "Città della Speranza", Corso Stati Uniti, 35127 Padova, Italy. [5]Instituto de Medicina Traslacional, Trasplante y Bioingeniería (IMeTTyB-CONICET), Universidad Favaloro-CONICET, Solís 453, Buenos Aires C1078AA, Argentina. [6]Department of Molecular Medicine, University of Pavia, 9 Via A Ferrata, 27100 Pavia, Italy. [7]Department of Biomedical Sciences, University of Padova, 35121 Padova, Italy. [8]Proteomic Center of Padova, University of Padova, 35131 Padova, Italy

OBJECTIVE

Extracellular vesicles (EVs) are increasingly studied as vectors for drug delivery because they can transfer a variety of molecules across biological barriers. SerpinB3 is a serine protease inhibitor that has shown a protective anti-apoptotic function in a variety of stressful conditions. The aim of this study was to evaluate protection from oxidative stress-induced damage, using extracellular vesicles that overexpress SerpinB3 (EVs-SB3) in order to enhance the effect of extracellular vesicles on cellular homeostasis.

MATERIALS AND METHODS

EVs-SB3s were obtained from HepG2 cells engineered to overexpress SerpinB3 and they revealed significant proteomic changes, mostly characterized by a reduced expression of other proteins compared with EVs from non-engineered cells.

RESULTS

These EV preparations showed a significantly higher protection from H2O2 induced oxidative stress in both the hepatoma cell line and in primary cardiomyocytes, compared to cells treated with naïve EVs or SerpinB3 alone, used at the same concentration.

CONCLUSIONS

In conclusion, the induction of SerpinB3 transgene expression results in the secretion of EVs enriched with the protein product that exhibits enhanced cytoprotective activity, compared with naïve EVs or the nude SerpinB3 protein.

34. DEVELOPMENT AND VALIDATION OF A SCALE-UP PROCESS FOR THE AUTOMATED PRODUCTION OF MESENCHYMAL STROMAL CELLS LOADED WITH PACLITAXEL SUITABLE FOR CELL THERAPY USE IN PATIENTS WITH MALIGNANT PLEURAL MESOTHELIOMA

Authors: Angela Marcianti[1], Sara Nava[1], Simona Frigerio[1], Simona Pogliani[1], Giulia Maria Stella[2], Anna Bersano[1], Angelo Guido Corsico[2], Eugenio Agostino Parati[3], Augusto Pessina[4], Daniela Lisini[1]

Affiliation: [1]Cell Therapy Production Unit-UPTC, Cerebrovascular Diseases Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, 20133 Milan, Italy. [2]Department of Medical Sciences and Infective Diseases, Unit of Respiratory Diseases, IRCCS Policlinico San Matteo Foundation and University of Pavia Medical School, 27100, Pavia, Italy. [3]IRCCS Istituti Clinici Scientifici Maugeri, 20138 Milan, Italy. [4]CRC StaMeTec, Department of Biomedical, Surgical and Dental Sciences, University of Milan, 20100 Milan, Italy

OBJECTIVE

The development of an Advanced Therapy Medicinal Product (ATMP) is a complex process and must comply with the Good Manufacturing Practice (GMP) rules. The aim of this project was the validation of the production process of Mesenchymal Stromal Cells loaded with Paclitaxel (MSC-PTX), using a close and automatic bioreactor, through: a) large-scale preparation of MSC-PTX; b) safety and efficacy evaluation of drug product (DP); c) process validation by Media Fill. The local administration of MSC-PTX, used as ATMP, will be proposed as a new strategy for the treatment of patients affected by Malignant Pleural Mesothelioma (MPM). This approach should provide several advantages over the systemically administered conventional drug, mainly related to the increase of drug concentration in the tumor site reducing systemic chemo-toxicity. MPM is characterized by rapid and diffuse local growth whereas metastases rarely appear only in advanced disease phases. Thus, the pleural space represents an ideal space for the local approaches against MPM.

MATERIALS AND METHODS

MSCs were isolated from 12 lipoaspirate samples, expanded and loaded with PTX. DP were cryopreserved and characterized in terms of number/viability, drug content by HPLC, identity by flow cytometry analysis, potency via inhibition of cell proliferation of tumor lines by MTS test. Sterility, endotoxin, and the absence of mycoplasma and adventitious viruses were also assessed.

RESULTS

This process permits to obtain a mean of 522±229x10^6 MSC-PTX (mean±Standard Deviation, SD), after 7 days expansion. Viability was ≥75% (91.39%±2.37% mean±SD); flow cytometry analysis showed that CD90, CD105 and CD73 were >80% and CD31, CD34 and CD45 were <5% for all the produced batches. MSC were able to incorporate 0.55±0.39 pg/cell of PTX (mean±SD) and to inhibit proliferation of the mesothelioma cells line H2052. All batches were sterile and free of mycoplasma and adventitious viruses; the endotoxin content was always <0.5 EU/ml. One year stability of DP was assessed: analysis did not show any difference between the short- and long-term cryopreserved samples. Finally, validation of aseptic production process of MSC-PTX by media fill simulation (three runs) showed conform results.

CONCLUSIONS

The validation of the process for MSC-PTX production, according to GMP rules, was concluded successfully. These data represent the basis for the preparation of the documentation requested by the Regulatory Authorities to obtain the phase I clinical trial approval for the treatment of MPM-patients.

35. ASSESSING MESENCHYMAL STROMAL CELLS DERIVED-EXTRACELLULAR VESICLES IMMUNOMODULATORY ACTIVITY IN VITRO: DEVELOPMENT OF TWO FUNCTIONAL ASSAYS.

Authors: Ricardo Malvicini[1,2,3], Giada De Lazzari[1,2,4], Anna Maria Tolomeo[1,2,4], Michela Pozzobon[4,5], Natalia Pacienza[3], Maurizio Muraca[1,2,4], Gustavo Yannarelli[3]

Affiliation: [1]Laboratory of Extracellular Vesicles as Therapeutic Tools, Fondazione Istituto di Ricerca Pediatrica Città della Speranza, Padua, Italy. [2]L.i.f.e.L.a.b. Program, Consorzio per la Ricerca Sanitaria (CORIS), Veneto Region, Padua, Italy. [3]Laboratorio de Regulación Génica y Células Madre, Instituto de medicina traslacional, trasplante y bioingenieria (IMeTTyB-CONICET), Buenos Aires, Argentina. [4]Department of Women's and Children's Health, University of Padova, Padua, Italy. [5]Laboratory of Stem Cells and Regenerative Medicine, Fondazione Istituto di Ricerca Pediatrica Città della Speranza, Padua, Italy

OBJECTIVE

There is an increasing interest in using extracellular vesicles derived from mesenchymal stromal cells (MSC-EVs) as therapeutic tools, mainly due to their immunomodulatory properties. However, there is a lack of functional assays to test their activity and their relative potency prior to their use. We evaluated the feasibility of using a macrophage and a lymphocyte cell line as reproducible tools to set up functional assays evaluating the immunomodulatory effects of MSC-EVs on the innate and acquired immunity, respectively.

MATERIALS AND METHODS

MSC-EVs were isolated from human umbilical cord-MSC by ion exchange chromatography and quantified by tRPS. For the macrophage assay, the Raw 264.7 cell line was stimulated with LPS (10ng/mL) for 16h in a 96-well plate, measuring NO_2^- production, by Griess assay, as marker of M1 polarization. Furthermore, M1 polarization was confirmed by FACS analysis of CD80 and CD86. To set up the T cell assay, Jurkat cells (clone E6-1) were seeded in a 96-well plate and stimulated with PMA in combination with PHA. As read-outs, IL-2 production was measured in culture supernatant by ELISA, and cells were characterized by FACS to evaluate the activation (CD69+CD25+) and the ratio Th1 /Th2. In both cases, dexamethasone (Dex) was used as internal control to suppress pro-inflammatory activation. Three different doses of MSC-EVs were tested (5E7, 5E8, and 5E9 nanoparticles/mL).

RESULTS

Macrophage stimulation with LPS resulted in strong induction of NO production that was inhibited up to 60% by Dex. A similar effect was observed with MSC-EVs, which inhibited NO production in a dose-dependent manner. Inhibition of NO production correlated with a reduced expression of M1 markers.
Jurkat cell stimulation resulted in increased IL-2 secretion and CD69 expression. Dex inhibited IL-2 secretion by 70% and CD69 expression by 40%, in association with an increase of Th1/Th2 ratio. Moreover, MSC-EVs inhibited the activation of T cells in a dose-dependent manner and increased Th1/Th2 ratio. However, they did not inhibit IL-2 production.

CONCLUSION

We set up a combination of two *in vitro* functional assays, representative of both the innate and acquired immunity, to assess the immunomodulatory effects of MSC-EVs. These assay could allow to test the activity and relative potency of different MSC-EVs batches, prior to their use *in vivo*.

36. (YIA) INFLUENCE OF THE ISOLATION METHOD ON THE PHYSICOCHEMICAL CHARACTERISTICS AND ON THE IMMUNOMODULATORY ACTIVITY OF MESENCHYMAL STEM/STROMAL CELL DERIVED EXTRACELLULAR VESICLES

Authors: **Ricardo Malvicini**[1,2,3], Giada De Lazzari[1,2,4], Anna Maria Tolomeo[1,2,4], Michela Pozzobon[4,5], Natalia Pacienza[3], Maurizio Muraca[1,2,4], Gustavo Yannarelli[3]

Affiliation: [1]Laboratory of Extracellular Vesicles as Therapeutic Tools, Fondazione Istituto di Ricerca Pediatrica Città della Speranza, Padua, Italy. [2]L.i.f.e.L.a.b. Program, Consorzio per la Ricerca Sanitaria (CORIS), Veneto Region, Padua, Italy. [3] Laboratorio de Regulación Génica y Células Madre, Instituto de medicina traslacional, trasplante y bioingenieria (IMeTTyB-CONICET), Buenos Aires, Argentina. [4]Department of Women's and Children's Health, University of Padova, Padua, Italy. [5]Laboratory of Stem Cells and Regenerative Medicine, Fondazione Istituto di Ricerca Pediatrica Città della Speranza, Padua, Italy

OBJECTIVE

In the present work, we compared two different isolation methods that are both scalable and suitable for the production of clinical-grade mesenchymal stromal cells-derived extracellular vesicles (MSC-EV), i.e. ion exchange chromatography (IEX) and ultrafiltration (UF) and we evaluated their impact on the composition and the functional properties of the isolated MSC-EV.

MATERIALS AND METHODS

For IEX, we use an anion exchange resin (Qsepharose). For UF, we use Amicon filters with a molecular cut-off of 100kDa. We assessed both nanoparticle size and distribution by NTA and tRPS and morphology by TEM following isolation from conditioned culture medium by IEX and UF. We also measured protein concentration (BCA assay), lipid concentration (sulfo-phospho-vainillin assay) and RNA concentration (absorbance at 260nm) and immunophenotyped both populations by flow cytometry (Macsplex assay). Moreover, immunomodulatory activity was tested with a macrophage polarization assay and with a T cell stimulation assay.

RESULTS

We found that IEX and UF yielded a comparable amount of total particles (7.43E+10 vs 7.04E+10) with similar size and distribution, (mode 96nm vs 97nm and 94nm vs 101nm by tRPS and NTA, respectively). In addition, a similar amount of proteins (559±126µg vs 479±83µg) and lipids (16±0.21µg vs 18±0.29ug) was obtained with the two procedures. However, IEX yielded a 10-fold higher RNA quantity with respect to UF (13.4 vs 1.54 µg). MSC-EVs isolated from IEX and UF were positive for the exosomal markers CD9, CD63 and CD81, and showed a similar expression pattern of surface markers. Finally, both populations showed a similar immunomodulatory activity *in vitro* as they prevented the acquisition of the M1 phenotype in LPS stimulated macrophages and inhibited the acquisition of the activation markers CD69 and CD25 on T cells.

CONCLUSION

MSC-EVs isolated by the two above-mentioned methods displayed similar physicochemical, phenotypic and functional characteristics. Further studies are ongoing to characterize the higher RNA content in IEX isolates. Funding: Supported by Exo Biologics, Niel, Belgium.

37. STUDY OF CELL-FREE TREATMENTS FOR OSTEOARTHRITIS FROM ADIPOSE-DERIVED STEM/STROMAL CELLS: SECRETOME VERSUS EXTRACELLULAR VESICLES

Authors: Elena Della Morte[1], Chiara Giannasi[1,2], Stefania Niada[1], Sara Casati[2], Anna Teresa Brini[1,2]

Affiliation: [1]IRCCS Istituto Ortopedico Galeazzi, Milan, Italy. [2]Department of Biomedical, Surgical and Dental Sciences, Università degli Studi di Milano, Milan, Italy

OBJECTIVE

Osteoarthritis (OA) is a progressive, age-related disease that affects millions of people all over the world. In the last years, several clinical trials have proved the safety and efficacy of Adipose-derived Stem/Stromal Cells (ASC) in contrasting this disabling disease. It is now widely accepted that ASC act mainly through paracrine mechanisms, i.e. through their secretome (conditioned medium, CM), a complex cocktail of proteins, nucleic acids and lipids released as soluble factors and/or conveyed into extracellular vesicles (EV). Here we compare the therapeutic potential of ASC-CM and ASC-EV in an *in vitro* model of OA based on primary articular chondrocytes (CH) induced towards an OA phenotype by TNFα.

MATERIALS AND METHODS

ASC-CM and -EV were obtained from the growth medium of 90% confluent ASC cultured for 72 hours in starving conditions (no FBS). Collected medium was centrifuged at 2500g to remove debris and large apoptotic bodies. Then, supernatants were either concentrated about 60 times in filter devices (3kDa cut-off) to obtain the CM, or centrifuged at 100000g to isolate the EV. CM and EV were characterized by differential proteomics (nLC-MS/MS). CH were induced towards an OA phenotype by 10 ng/ml TNFα in the presence of either ASC-CM or -EV. 3 and 6 days after the treatments, hypertrophic, catabolic and inflammatory markers were evaluated by enzymatic assays, western blotting and ELISA.

RESULTS

ASC-CM, compared to -EV, contains more chondroprotective factors, among which Bone Morphogenetic Protein 1, Dickkopf-Related Protein 3 and Tissue Inhibitors of Metalloproteinases. The functionality of these last factors was confirmed by the ASC-CM ability to decrease the Matrix Metalloproteinases activity (22% and 29% after 3 and 6 days) induced by TNFα. ASC-CM was also effective in reducing the release of the inflammatory mediator PGE2 (up to 40% at day 6) boosted by the cytokine. Conversely, both treatments could be involved in the reduction of the TNF-mediated hypertrophy as revealed by the down-modulation of collagen X (~30% at the earliest time point).

CONCLUSIONS

Our data reveal a higher therapeutic potential of ASC-CM compared to EV alone, that may be harnessed in the future for OA management. Indeed, the biological and molecular evidences of ASC-CM beneficial action on OA-like CH suggest its promising potential as a cell-free therapeutic in counteracting articular damage.

38. DIFFERENT MOLECULAR WEIGHT HYALURONAN BASED GELS MAY AFFECT BONE REGENERATION THROUGH DIFFERENTIATION OF STROMAL DENTAL PULP STEM CELLS

Authors: Antonietta Stellavato, Virginia Tirino, Valentina Vassallo, Marcella La Noce, Marcella Cammarota, Gianpaolo Papaccio, Chiara Schiraldi

Affiliation: Department of Experimental Medicine, Section of Biotechnology, Molecular Medicine and Medical Histology, University of Campania "L. Vanvitelli", via L. de Crecchio, 7, 80138 Naples (Italy)

OBJECTIVE

Dental pulp stem cell (DPSC) represent an innovative attractive tool of multipotent mesenchymal stem cell for tissue regeneration. In this study we focused our attention on the osteogenic potentiality of DPSC in presence of different hyaluronan based gels.

MATERIALS AND METHODS

Experiments were performed using linear pharmaceutical grade hyaluronans at high (HHA) and low molecular weight (LHA), in comparison to hybrid cooperative complexes (HCC) containing both sizes. Osteogenic differentiation was evaluated using Alizarin Red staining to quantify calcium-rich deposits produced by the cells. Also, the effects on osteogenic differentiation were assessed for 7-14 and 21 days of culture by analyzing specific markers, namely Osteocalcin (OCN), Osteopontin (OPN) and Bone sieloprotein (BSP) at transcriptional and protein level using qRT-PCR and western blotting analyses respectively. In addition, HA based gels were supplemented to DPSC inoculated into Gingistat, a biomaterial frequently used for dentistry applications.

RESULTS

Especially high molecular weight HA and HCC seemed to modulate biomarkers differently from the control and LHA. However HCC presented key biomarkers up-regulation within a shorter incubation time. While at 21 days data were rather similar all indicating a complete differentiation. It can be hypothesized that HA induces osteogenesis differentiation of these specific stem cells through CD44 signaling. HHA and HCC supplementation to growth medium prompted differentiation respect to CTR and LHA.

CONCLUSIONS

Considering all the modulated biomarkers during the time course of the experiment it can be argued that HCC injective gels, beyond the ones based on the sole H- HA, are a promising noninvasive procedures for tissue regeneration with interesting features in bone regeneration.

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39. LOW-INTENSITY PULSED ULTRASOUND STIMULATION ENHANCES CHONDROGENIC DIFFERENTIATION OF ADIPOSE MESENCHYMAL STROMAL CELLS IN A 3D HYDROGEL

Authors: Cristina Manferdini[1*], Elena Gabusi[1], Diego Trucco [1,2,3], Paolo Dolzani[1], Yasmin Saleh[1], Andrea Cafarelli[2,3], Leonardo Ricotti[2,3], Gina Lisignoli[1]

Affiliation: [1]SC Laboratorio di Immunoreumatologia e Rigenerazione Tissutale, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy. [2]The BioRobotics Institute, Scuola Superiore Sant'Anna, 56127 Pisa, Italy; [3]Department of Excellence in Robotics & AI, Scuola Superiore Sant'Anna, 56127 Pisa, Italy

OBJECTIVE

Articular cartilage injuries have a limited potential to heal and, over time, may lead to osteoarthritis, an inflammatory and degenerative joint disease associated with activity-related pain, swelling, and impaired mobility. Regeneration and restoration of the joint tissue functionality remain unmet challenges. Stem cell-based tissue engineering is a promising paradigm to treat cartilage degeneration. In this context, hydrogels have emerged as promising biomaterials, due to their biocompatibility, ability to mimic the tissue extracellular matrix and excellent permeability. Different stimulation strategies have been investigated to guarantee proper conditions for mesenchymal stem cell differentiation into chondrocytes, including growth factors, cell-cell interactions, and biomaterials. An interesting tool to facilitate chondrogenesis is external ultrasound stimulation. In particular, low-intensity pulsed ultrasound (LIPUS) has been demonstrated to have a role in regulating the differentiation of adipose mesenchymal stromal cells (ASCs). However, chondrogenic differentiation of ASCs has been never associated to a precisely measured ultrasound dose. In this study, we aimed to investigate whether dose-controlled LIPUS is able to influence chondrogenic differentiation of ASCs embedded in a 3D hydrogel.

MATERIALS AND METHODS

Human adipose mesenchymal stromal cells at $2*10^6$ cells/mL were embedded in a hydrogel ratio 1:2 (VitroGel RGD®) and exposed to LIPUS stimulation (frequency: 1 MHz, intensity: 250 mW/cm^2, duty cycle: 20%, pulse repetition frequency: 1 kHz, stimulation time: 5 min) in order to assess its influence on cell differentiation. Hydrogel-loaded ASCs were cultured and differentiated for 2, 7, 10 and 28 days. At each time point cell viability (Live&Dead), metabolic activity (Alamar Blue), cytotoxicity (LDH), gene expression (COL2, aggrecan, SOX9, and COL1), histology and immunohistochemistry (COL2, aggrecan, SOX9, and COL1) were evaluated respect to a non-stimulated control.

RESULTS

Histological analysis evidenced a uniform distribution of ASCs both at the periphery and at the center of the hydrogel. Live & Dead test evidenced that the encapsulated ASCs were viable, with no signs of cytotoxicity. We found that LIPUS induced chondrogenesis of ASCs embedded in the hydrogel, as demonstrated by increased expression of COL2, aggrecan and SOX9 genes and proteins, and decreased expression of COL1 respect to the non-stimulated control.

CONCLUSIONS

These results suggest that the LIPUS treatment could be a valuable tool in cartilage tissue engineering, to push the differentiation of ASCs encapsulated in a 3D hydrogel.

This work received funding from the European Union's Horizon 2020 research and innovation program, grant agreement No 814413, project ADMAIORA (AdvanceD nanocomposite MAterIals fOr in situ treatment and ultRAsound-mediated management of osteoarthritis).

40. (YIA) INVESTIGATION OF THE POTENTIAL THERAPEUTIC EFFECT OF AMNIOTIC MESENCHYMAL STROMAL CELL SECRETOME ON SYNOVIAL TISSUE-DERIVED MYELOID AND LYMPHOID CELLS IN RHEUMATOID ARTHRITIS

Authors: Alice Masserdotti[1], Marta Magatti[2], Anna Pasotti[2], Patrizia Bonassi Signoroni[2], Clara Di Mario[3], Luca Petricca[4], Maria Rita Gigante[4], Elisa Gremese[3,4], Barbara Tolusso[4], Stefano Alivernini[4], Antonietta Silini[2], Ornella Parolini[1,5]

Affiliation: [1]Department of Life Science and Public Health, Università Cattolica del Sacro Cuore, Rome, Italy. [2]Centro di Ricerca E. Menni, Fondazione Poliambulanza Istituto Ospedaliero, Brescia, Italy. [3]Division of Rheumatology, Università Cattolica del Sacro Cuore, Rome, Italy. [4]Division of Rheumatology, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy. [5]Fondazione Policlinico Universitario "Agostino Gemelli" IRCCS, Roma, Italy

OBJECTIVE

Amniotic mesenchymal stromal cells (hAMSC) possess interesting immunomodulatory properties. Many in vitro studies showed that hAMSC and hAMSC-derived conditioned medium (hAMSC-CM) modulate the proliferation, maturation, and cytokine secretion profile of cells of the innate and adaptive immune systems. Furthermore, in vivo studies using hAMSC and hAMSC-CM report beneficial effects in different animal models of diseases associated with inflammatory conditions and degenerative processes. Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune joint disease, characterized by persistent synovial tissue (ST) inflammation leading to irreversible disability. The modulation of inflammation with cell therapy is an attractive and innovative strategy. This study aims to assess the effect of hAMSC-CM on ST-derived myeloid and lymphoid cells, and on fibroblast-like synoviocytes (FLS) of RA patients.

MATERIALS AND METHODS

Human ST were obtained from RA patients with active disease by minimally invasive ultrasound guided biopsy at SYNGem biopsy unit. After mechanical and enzymatic ST digestion, immune cells and FLS were cultured in presence or absence of CM-hAMSC. Cytokine production was evaluated after 2 or 3 days by flow cytometry. FLS from biopsies were also expanded up to passage 4 and were used for in vitro assays. After 3, 5 and 7 days of culture with or without CM-hAMSC, the expression of genes involved in RA pathophysiology were evaluated by quantitative real-time PCR.

RESULTS

Preliminary data suggest that CM-hAMSC could affect the production of proinflammatory cytokines, such as TNF**α**, IL-6 and IFN**γ**, by immune cells and FLS derived from ST of RA patients with active disease. Future studies aimed at deeply investing the molecular pathways involved will corroborate these preliminary results.

CONCLUSIONS

These data could be useful to clarify the immunomodulatory properties of CM-hAMSC in RA and to potentially identify CM-hAMSC as a putative therapeutic approach in RA.

41. C-MYC1 SHELVES ITS APOPTOTIC ROLE IN PLATELET LYSATE CELL CULTURE CONDITION

Authors: Marta Nardini[1,3], Anita Muraglia[1,3], Chiara Gentili[2], Patrizio Castagnola[3], Maddalena Mastrogiacomo[1,3]

Affiliation: [1]Biotherapy Laboratory, Department of Internal Medicine (DIMI) University of Genova, Genova, Italy. [2]Cellular Oncology Laboratory, Department of Experimental Medicine (DIMES), University of Genova, Genova, Italy. [3]Biotherapy Laboratory, IRCCS Ospedale Policlinico San Martino, Genova, Italy

OBJECTIVE

Bioactive factors contained in Platelet Lysate (PL) promote the isolation and proliferation of Mesenchymal Stem Cells (MSCs) and the growth of cell lines. It is well known that PL not only enhances cell proliferation compared to Fetal Bovine Serum (FBS) without affecting the cell differentiation capability, but it is also able to activate resting quiescent cells to re-enter cell cycle. The main proliferation-relate pathway, involving Cyclin, Akt and $ERK_{1/2,}$ is activated after a short exposure of the cells to PL but the effect on proliferation is higher in primary cell cultures than in cell lines.

To better understand this effect, we focused our attention on a family of highly conserved proteins involved in different cellular mechanisms: C-MYC's family. This protein has three different isoforms: C-MYC1 particularly expressed in those cells that are in suffering condition; C-MYC2 expressed in growing cells and C-MYCS, a short isoform whose expression is transient during cell growth.

MATERIALS AND METHODS

MSCs from different tissues, Articular Chondrocytes, Osteoblasts, Amniotic Fluid Stem Cells from human samples and HeLa cell line, were cultivated in 10% FBS or 5% PL-supplemented medium. The proliferation capability was evaluated in terms of cell doublings and Ki67 expression by immunofluorescence and the possible apoptotic effect evaluating Annexin V by FACS analysis and C-MYC protein expression by Western Blot.

RESULTS

All primary cell cultures treated with PL showed a high proliferation rate in comparison with FBS treated cultures. C-MYC1 expression was absent in FBS cultured cells but was observed in presence of PL also after a short time of exposure. This pattern of expression was different for the cell line where C-MYC1 was present also in the FBS-cultured cells. C-MYC2 isoform was expressed in all the cells independently from the culture conditions. Immunofluorescence analysis indicated that MSC cultures showing C-MYC1 protein expression were in a proliferative stage.

We also find that the cells treated with PL was not in an apoptotic stage, showing only a 15% of Annexin V positive cells.

CONCLUSIONS: PL induces cell proliferation increasing C-MYC1 isoform expression in primary cell cultures and much more slightly in the cell line, modifying the described apoptotic function of C-MYC1 isoform. These results pave the way to a deeper molecular study in order to understand peculiar differences of PL stimulation on various types of cells.

42. (YIA) INTRATRACHEAL ADMINISTRATION OF ALLOGENEIC MESENCHYMAL STEM CELLS FOR THE TREATMENT OF RECURRENT AIRWAY OBSTRUCTION IN THE HORSE

Authors: Luca Melotti[1], Ludovica Mazzucco[2], Elisa Padoan[2], Serena Ferraresso[1], Vanessa Faillace[3], Marco Patruno[1]

Affiliation: [1]Department of Comparative Biomedicine and Food Science, Agripolis-University of Padua, Legnaro, Padova. [2]Veterinary practitioner. [3]Department of Animal Medicine, Production and Health, Agripolis-University of Padua, Legnaro, Padova

The recurrent airway obstruction (RAO) is a chronic disease of the lower respiratory tract of horses, which resembles human asthma and is characterized by persistent inflammation, mucus accumulation, and bronchospasm. It severely affects the quality of life of horses, dramatically reducing their economic value. Conventional treatments with corticosteroids are effective for containing inflammation but they also might cause different adverse effects. The aim of the study was to assess the effects of allogeneic mesenchymal stem cells (MSCs) administered intratracheally in RAO-affected horses during the acute inflammatory process.

Nine horses presenting clinical signs of RAO were divided into MSC-treated group (n = 5), treated with a single instillation of MSCs at day 0, and untreated group (control, n = 4). At 0 and 30 days, all horses were evaluated for clinical and endoscopic examination. Concomitantly, broncho-alveolar lavage fluid (BALf) was collected for cytology and microbiological examinations, and gene expression analysis of mediators involved in inflammation and tissue remodelling were performed (TNF-α, IL-8, TLR4, NF-kB, CCL17, PI3Kγ, MMP-1, and SMAD7).

Treated horses showed a slight decrease in clinical score ($p < 0.05$) and respiratory effort on day 30, while untreated horses did not show any evidence of clinical improvement. No differences in the percentage of neutrophils were observed between the two groups but, at day 30, a higher percentage of macrophages was observed in treated horses compared to untreated ones. At the same time point, treated horses showed a reduced gene expression of mediators involved in inflammatory cell migration, such as neutrophils (TLR4, IL-8, and NF-kB); on the contrary, untreated horses showed similar levels of mRNA between 0 and 30 days. In addition, control horses presented an increased gene expression of CCL17 and PI3Kγ, which might be related to an enduring airway inflammation. The treatment with MSCs, at day 30, downregulated the mRNA level of MMP-1 while stimulating the gene expression of SMAD7; an opposite trend of gene expression was observed in control untreated horses, which showed bronchial hyperresponsiveness, thus a more severe form of asthma.

Overall, these preliminary results suggest that MSCs may safely improve the clinical status by ameliorating the inflammatory response, therefore reducing airway remodelling in RAO-affected horses and disease severity.

43. (YIA) CANINE ADIPOSE-DERIVED MESENCHYMAL STROMAL CELLS DISPLAY DIFERENT ADHESION TIMES AFTER ISOLATION: A NOVEL APPROACH TO IMPROVE CELL YIELD OR THE EVIDENCE OF DISTINCT SUB-POPULATIONS?

Authors: Gabriele Scattini [1], Martina Pellegrini [2], Giulio Severi [2], Luisa Pascucci [1]

Affiliation: [1]Department of Veterinary Medicine, University of Perugia. [2] Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati"

OBJECTIVE

Isolation of mesenchymal stromal cells (MSC) from adipose tissue (AT) is mostly carried out by collagenase digestion followed by filtration. Non adherent cells are then removed from the culture 48 hours upon seeding. Our hypothesis is that fragments eliminated by filtration or removed after 48h might contain residual MSC that adhere later to the plastic. The aim of this study, therefore, is to evaluate basic features of canine AT-derived MSC that adhere to the plastic at different times after isolation, in order to speculate on the possible existence of distinct subpopulations of MSC that populate different tissue niches.

MATERIALS AND METHODS

Subcutaneous AT collected from 3 dogs was minced and digested with 0.08% collagenase for 75 minutes at 37 °C. The stromal vascular fraction was seeded without filtration in DMEM supplemented with 10% FBS and antibiotics (plate 1). 48 hours after seeding, the unattached residues were recovered and plated again (plate 2). The same was done after 48h, replating unattached cells from plate 2 to a new flask (plate 3). Cells obtained from the 3 adhesions were expanded until passage 3 and were characterized by flow cytometry for positive (CD90, CD44, CD29) and negative (CD14, MHC2) MSC markers as well as for CD31 (endothelial cell marker), CD146 (pericyte marker), alpha-SMA (smooth muscle cell marker). At passage 3, cells from all the three adhesions were evaluated for viability (MTT assay), doubling time and trilineage differentiation ability.

RESULTS

No significant differences between the 3 subpopulations were observed. They all were characterized by the expression of MSC positive markers and by the absence of negative markers. CD31, CD146, and alpha-SMA were expressed by less than 5% of the cells in all the populations. No differences in differentiation ability and viability were detected. Doubling time ranged between 25 and 30 hours for all the 3 populations.

CONCLUSIONS

The three populations were similar in terms of immunophenotype, proliferation and differentiation potential. In this view, this procedure of sequential adhesions seems to be only a useful method to improve MSC yield. Indeed, it allows to optimize cell recovery reducing the amount of sampled tissue and shortening the time necessary to obtain an adequate number of cells for clinical applications. However, functional differences cannot be excluded and potency assays are required in order to explore possible distinct biological attitudes.

44. (YIA) HACKING EXTRACELLULAR VESICLES: EXPLORING A METHOD TO LOAD PROTEINS OF INTEREST INTO EXTRACELLULAR VESICLES FROM MESENCHYMAL STROMAL CELLS

Authors: Gabriele Scattini[1], Laura Musa[1], Samanta Mecocci[1], Maria Rachele Ceccarini[2], Patrizia Casagrande Proietti[1], Stefano Capomaccio[1], Antonello Bufalari[1], Rodolfo Gialletti[1], Olimpia Barbato[1], Luca Avellini[1], Tommaso Beccari[2], Luisa Pascucci[1]

Affiliation: [1]Department of veterinary Medicine, University of Perugia. [2]Department of Pharmaceutical Sciences, University of Perugia

OBJECTIVE

Extracellular Vesicles (EVs) are micro and nanoparticles bounded by a lipid bilayer that contain a large variety of biological molecules. Their parental cells "package the cargo" and release EVs in the extracellular space. Mesenchymal Stromal Cells (MSC) represent an excellent model for the study of EV biology and are considered a possible platform for EV-mediated drug delivery, thanks to their innate biological properties. The short term objective of this study is to define the best tags or signals able to target a protein inside EVs. The long term goal is to develop a method to functionalise EVs from MSC with proteins of interest in order to use them as a tool for protein-based drug delivery.

MATERIALS AND METHODS

In this first phase of the study, we studied the incorporation in EVs of a reporter protein (green fluorescent protein - GFP) associated with different EV specific proteins (tags) with the aim to *hack* the molecular trafficking mechanisms of EVs. cDNAs encoding EV specific proteins or signals (CD63, TSG101 and Syntenin-1, palmitoylation motif) were isolated and cloned into a GFP expression vector in order to obtain plasmids encoding for chimeric proteins. Canine MSCs were transfected with plasmids to allow the expression of GFP labelled EV proteins. Images of fluorescent living cells were acquired 48 hours post-transfection. Immunogold labelling was performed with anti-GFP antibody to detect chimeric proteins and their fine intracellular localization by Transmission Electron Microscopy.

RESULTS

GFP distribution inside cells was characterized by different patterns: diffuse, focal, perinuclear, etc. depending on the tag used. Immunogold labelling revealed the localization of GFP inside cytoplasmic sub-structures, on cell membranes and inside EVs released in the extracellular space revealing the involvement of the endosomal system, as expected.

CONCLUSIONS

The use of genetically engineering molecular tags allowed to describe the localization of GFP in living cells, without the perturbation of antibody labelling, and made possible its detection in immuno-TEM analysis. The study, although in its early phase, confirms the incorporation of GFP inside EVs and opens the way to develop bioengineered MSC-EVs as a new tool in drug delivery. Further analyses are necessary to achieve a more detailed characterization.

45. A NEW HUMAN PLATELET LYSATE FOR MESENCHYMAL STEM CELL PRODUCTION COMPLIANT WITH GOOD MANUFACTURING PRACTICE CONDITIONS

Authors: Elena Marini[1], Katia Mareschi[1-2]*, Alessia Giovanna Santa Banche Niclot[1], Marta Barone[1], Giuseppe Pinnetta[2], Aloe Adamini[2], Manuela Spadea[1,] Luciana Labanca[3], Graziella Lucania[3], Ivana Ferrero[2], Franca Fagioli[1,2]

Affiliation: [1]Department of Public Health and Paediatrics, The University of Turin, Piazza Polonia 94. 10126 Torino, Italy. [2]Stem Cell Transplantation and Cellular Therapy Laboratory, Paediatric Onco-Haematology Division, Regina Margherita Children's Hospital, City of Health and Science of Turin; 10126, Torino. [3]Blood Component Production and Validation Center, City of Health and Science of Turin, S. Anna Hospital, Turin, Italy

OBJECTIVE

Human Platelet Lysate (HPL) is an additive rich in growth factors, cytokines and plasma proteins usually used for the expansion of mesenchymal stem cells (MSCs) in Good manufacturing conditions (GMP). However, various preparation techniques may influence the composition of HPL, potentially conditioning the biological properties of the cells in culture The standard production of HPL consists of repeated freezing and thawing cycles of the platelet pool and the addition of heparin to avoid the formation of gel in the culture medium. We set up a new method for HPL production, which consists of making platelets coagulate through the addition of Ca-Gluconate and the subsequent mechanical wringing of the clot without the addition of heparin- In this work we verified if the new method of HPL production is effective and preserves the characteristics of MSCs when isolated from bone marrow (BM-MSCs) in GMP conditions.

MATERIALS AND METHODS

We compared the new HPL defined HPL-S with the standard HPL (HPL-E). First, we investigated if the treatment of Ca Gluconate could interfere with the chemical characteristic and the growth factor release then if the new HPL was effective in supporting the growth of MSCs.

RESULTS

The HPL-S did not result in PLTs and fibrinogen but the quantity of total protein and growth factors obtained was comparable to that of HPL-E. In the case of HPL-S, avoiding the addition of heparin in the cell culture was more advantageous for MSC production in GMP conditions. We isolated the MSCs from 5 BM samples by testing HPL-S vs HPL-E. The number of colonies forming unit-fibroblasts (CFU-F) showed there were no significant differences between the MSCs isolated in HPL-E and in HPL-S, and showed a cumulative PD higher than that of the MSCs in HPL-S in the earlier passages. At the 4th passage we observed an inverted trend of cell growth in the MSCs cultivated in HPL-S and a maintenance of stem cell markers during expansion. Immunophenotypic analysis showed a significant lower expression of HLA-DR (1.30%) in the MSCs cultivated in HPL-S compared to HPL-E (14.10%) showing an immune-privilege and a higher expression of CD146 probably related to a higher secretory capacity with significantly greater immunomodulatory and anti-inflammatory protein production.

CONCLUSIONS

All data demonstrated that HPL-S is an effective alternative for MSC production in compliance with GMP conditions and their secretory capacity will be investigated.

46. A NEW HUMAN PLATELET LYSATE FOR MESENCHYMAL STEM CELL PRODUCTION COMPLIANT WITH GOOD MANUFACTURING PRACTICE CONDITIONS PRESERVE CHEMICAL CHARACTERISTICS AND BIOLOGICAL ACTIVITY OF LYO-SECRETOME ISOLATED BY ULTRAFILTRATION

Authors: Alessia Giovanna Santa Banche Niclot[1], Katia Mareschi[1-2] *, Elena Marini[1], Elia Bari[3], Luciana Labanca[4], Graziella Lucania[4], Ivana Ferrero[2], Sara Perteghella[3], Franca Fagioli

Affiliation: [1]Department of Public Health and Paediatrics, The University of Turin, Piazza Polonia 94. 10126 Torino, Italy. [2]Stem Cell Transplantation and Cellular Therapy Laboratory, Paediatric Onco-Haematology Division, Regina Margherita Children's Hospital, City of Health and Science of Turin; 10126, Torino. [3]Department of Drug Sciences, University of Pavia, Viale Taramelli 12, Pavia, Italy. [4] Blood Component Production and Validation Center, City of Health and Science of Turin, S. Anna Hospital, Turin, Italy

OBJECTIVE

The effects of Mesenchymal Stromal Cells (MSCs) were associated with soluble proteins and extracellular vesicles (EVs), defined as Secretome. Recently, we defined a scalable Good Manufacturing Practice (GMP)-compliant production process for freeze-dried MSC-secretome (lyo-secretome). Using this methodology, we isolated MSCs cultivate in a new Human Platelet Lysate (HPL). To verify is this new supplement could affect MSC–secretome qualitative and quantitative composition, we analyzed the chemical characteristics and biological activity of lyo-secretome isolated from MSCs cultivate in this new HPL (HPL-S) comparing it with lyo-secretome obtained from MSCs cultivated in the standard HPL (HPL-E).

MATERIALS AND METHODS

From the same pool of platelets, we obtained two batches of HPLs: the standard HPL-E obtained by repeated freezing and thawing cycles and the new HPL-S produced through the addition of Ca-Gluconate and the subsequent mechanical wringing of the clot. MSCs were isolated from bone barrow samples and cultured separately with 2 HPLs At the 3th passage, lyo-secretome was isolated from cell supernatant after serum starvation, collection, ultrafiltration and freeze-drying obtaining a ready and stable powder. Lyo-secretome was analyzed for lipid and protein contents, growth factor releases, immunophenotype, EVs' particle sizes and concentrations. Anti-elastase activity and immunomulant proprieties of Lyo-secretome obtained from MSCs cultivated in 2 HPLs were also analyzed.

RESULTS

We did not observe significantly different chemical characteristics and biological activity between lyo-secretome obtained from MSCs cultivated in HPL-E or HPL-S.

CONCLUSIONS

The use of HPL-S is an effective alternative for MSC production in GMP compliant conditions and the lyo-secretome obtained in this culture condition could replace MSC-cellular therapy. The Lyo-secretome, for their ease of production, conservation and storage and their immunomodulatory capacities, could be a very innovative tools as cell-free surrogates of MSC immunomodulation in multiple disease models.

47. HUMAN TENDON STEM/PROGENITOR CELL FEATURES AND FUNCTIONALITY ARE HIGHLY INFLUENCED BY IN VITRO CULTURE CONDITIONS

Authors: Carlotta Perucca Orfei[1], Annie C Bowles [2,3,4], Dimitrios Kouroupis [2,3], Melissa A Willman[3], Enrico Ragni[1], Lee D Kaplan[2], Thomas M Best[2], Diego Correa[2,3], Laura de Girolamo[1]

Affiliation: [1]Laboratorio di Biotecnologie Applicate all'Ortopedia, IRCCS Istituto Ortopedico Galeazzi, 20161, Milan, Italy. [2]Department of Orthopedics, UHealth Sports Medicine Institute, University of Miami, Miller School of Medicine, 33136, Miami, FL, USA. [3]Diabetes Research Institute & Cell Transplantation Center, University of Miami, Miller School of Medicine, 33136, Miami, FL, USA. [4]Department of Biomedical Engineering College of Engineering, University of Miami, 33136, Miami, FL, USA

OBJECTIVE

In the field of tendinopathies, treatments capable of resolving the pathological condition and restoring tissue functionality are not yet available in the clinic. A deeper understanding of tendon biology is therefore mandatory to develop new effective evidence-based therapies. Much interest is focused on tendon progenitor stem cells (TSPCs). This population plays a crucial role in maintaining tendon homeostasis and actively participates in tissue repair, so assessing their potential to improve tendon repair could fill an important gap in this regard. However, several aspects of TSPCs are still controversial as a consistent heterogeneity of methods observed in *in vitro* studies. Therefore, we investigated whether culture conditions influence the phenotypic and functional characteristics of TSPCs in order to dissect their role in the tissue healing response.

MATERIALS AND METHODS

Human TSPCs were plated at low (50 cells/cm^2, LD) or high density (5000 cells/cm^2, HD), and expanded until passage 2. A shift from low to high density was performed at passage 1 to obtain a hybrid group named as LDHD. The molecular and phenotypic profile, the multipotency, the secretory activity, the anti-inflammatory and the immunomodulatory activities of TSPCs were investigated.

RESULTS

Our results indicated that TSPCs cultured at low density (LD) are the most promising ones for morphological and functional properties respect to the other two groups, composed of TSPCs cultured at high density (HD) and TSPCs who underwent a shift from low to high density in passage 1 (LDHD).

LD showed a cobblestone morphology that was not observed in the other two groups, and possessed a distinct immune-phenotypic profile, with higher levels of several MSC-like markers (CD90, CD44, CD146). Finally, LD showed a stronger secretory and immunomodulatory activity when primed with inflammatory stimuli mediated by TNFα and IFNγ. In contrast, HD showed a more elongated fibroblast-like morphology, a more evident differentiation potential, and a higher expression of tendon-related genes with respect to LD. LDHD demonstrated intermediate features.

CONCLUSIONS

This set of observations allowed us to identify relevant differences in TSPCs based on the conditions they are subjected. This ability to modulate morphology, phenotype, gene expression profile, and functional response advances our current understanding of tendons at the cellular level and suggests high responsiveness to stimuli.

48. (YIA) NANT 001 BIOREACTOR FOR AUTOMATED PRODUCTION OF ADIPOSE-DERIVED MESENCHYMAL STROMAL CELLS FOR CLINICAL USE IN ACCORDANCE WITH GOOD MANUFACTURING PRACTICES

Authors: Elisa Groppelli[1], Elisa [1], Silvia Budelli[1], Cristiana Lavazza[1], Rosaria Giordano[1], Giacomo Cattaruzzi[2], Massimo Moretti[2], Alice Paulitti[2], Nicola Zingaretti[3], Pier Camillo Parodi[3], Paolo Di Benedetto[4], Francesco Curcio[5], Lorenza Lazzari[1], Tiziana Montemurro[1]

Affiliation: [1]Laboratory of Regenerative Medicine - Cell Factory, Department of Transfusion Medicine and Hematology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via Francesco Sforza, 35, 20122, Milan, Italy. [2]VivaBioCell S.p.A., Udine, Italy. [3]SOC Chirurgia Plastica, ASUFC, Udine, Italy. [4]Clinic of Orthopaedics, University of Udine and ASUFC, Udine, Italy. [5]Department of Medicine and Clinical Pathology Institute, University of Udine and ASUFC, Udine, Italy

OBJECTIVE

Adipose-derived mesenchymal stromal cells (ASCs) are considered a promising tool for cell therapy approaches due to their well-known features. NANT 001 System is a closed and automated bioreactor overcoming manual resource-consuming, contamination risks and standard cell culture instruments.
Our aim was to validate a Good Manufacturing Practice (GMP) compliant expansion of ASCs using this system for the treatment of patients with severe knee osteoarthritis.

MATERIALS AND METHODS

ASCs were obtained from sub-abdominal liposuctions after informed consent. All the processes were performed in our GMP facility approved by the national authority (aM 51-2018). The adipose tissue (AT) was mechanically and enzymatically digested with GMP collagenase NB6 to isolate the stromal vascular fraction (SVF). Successively, the SVF resuspended in a GMP-grade medium supplemented with 5% human gamma-irradiated platelet lysate was seeded in the sterile NANT Cartridge. All cell culture steps were automatically performed by NANT 001: 24h after seeding, and at 50% confluence, cells were washed with PBS and medium was fully replaced. Temperature, pH, confluence and real-time imaging were remotely monitored during cell culture directly by the bioreactor. When 90% confluence was reached, the bioreactor automatically harvested the cells and the quality controls (QC) were performed. A stability study was performed mimicking the final investigational Advanced Therapy Medicinal Product (ATMP) packaging and transport condition.

RESULTS

Validation runs (n=3) were performed to verify specific release parameters, based on previous engineering runs and on an appropriate risk assessment. From 10mL of AT, a median of 51x10e6 ASCs were obtained in 9-12 days. Each run gave rise to final cell product with >80% viability, and all the QC were compliant. Validation of aseptic processing by media fill simulation was successfully performed by strictly simulating the manufacturing process. All these data were collected in an Investigational Medicinal Product Dossier submitted to the National Agency for approval.

CONCLUSIONS

We demonstrated the consistent and GMP-compliant applicability of the automated NANT 001 System for a small scale production of expanded ASCs. Patients (50-75 yrs) affected by grade III-IV knee osteoarthritis will benefit of this new ATMP within a phase II clinical trial (EUDRACT 2020-005336-29).
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49. MSC EXOSOMES USED FOR DIABETIC ANIMAL WOUND HEALING MODEL

Authors: Josef Skopalík[1,2], Jaroslav Prucha[1,3], Ivan Justan[4], Tomas Horak[5], Josef Masek[6], Tomas Parak[2]

Affiliation: [1]Technical University in Prague - Faculty of - Biomedical Engineering Czech, Department of Information and Communication Technologies in Medicine, Czech Rep. [2]Masaryk University - Faculty of Pharmacy, Brno, Czech Rep. [3]Czech Technical University in Prague - Faculty of Biomedical Engineering, Department of Health Care Disciplines and Population Protection, Czech Rep. [4]Masaryk Memorial Cancer Institute – Dep. of Surgical Oncology, Brno, Czech Rep. [1]Brno University of Technology - Faculty of Mechanical Engineering, Dep. Of Optics and Precise Mechanics, Czech Rep. [5]Veterinary Research Institute - Dept. of Pharmacology and Toxicology, Brno, Czech Rep.

OBJECTIVE

Chronic wounds are an increasingly common type of the skin injury and they affect about 3% of the population aged over 65 years. The increasing prevalence is found in groups of diabetic patients. Addition of exosomes from fat-derived autologous or allogenic mesenchymal stromal cells (MSC) into the wound could accelerate the curing process, test was prepared on Zucker Diabetic Fatty Rats (ZDF).

METHODS

The full-thickness wounds with diameter 2 cm were produced on the backs of ZDF and covered by gauze to initiate primary inflammation in the next 2 days. After the initial phase, the animal were divided to 3 groups: (1) experimental exosomal application, (2) covering by control collagen cover without exosomes, (3) negative control (gauze cover without any medication). Adipose tissue derived mesenchymal stromal cells (AT-MSCs) were isolated from fatty tissue of three diabetic rats (males, weight 400g). The supernatant from 10 x 10^6 of MSC was filtered by 0,8 micron filters. The filtered solution containing exosomes was sorted by BD FACS Aria III (CD63 surface exosomal marker). Exosomes were resuspended in physiological solution and were injected in to the PCL foam and covered by semi–liquid collagen gel, the complex gel body was incubated at 37°C (contour correlating to the wound cavity), final solidified collagen bodies were applicated into the wounds. The experimental rats groups (application of gel with exosomes, application of gel without exosomes) was monitored until day 21 and compared to negative control group.

RESULTS

The objective comparison was based on three visualization modalities: time-lapse photographing of "wound area" and 3D MRI tomography of "wound depth", post-mortem quantitative histology of dermal and subdermal regeneration (randomly selected animal at day 10, 15, 21), postmortem analysis of amount of capillary in the wound tissue (the same timing). All parameters was recomputed to "healing index". Photographic and thomographic analysis showed the epidermis regeneration and regrow of epidermal layers over the wound at day 21 for the variant of exosomal experimental method. Collagen deposition in the wound healing site was observed using Masson's Trichrome staining, the best regeneration of subdermal layer of collagen was visible at the wound after application of exosomes (variant nondiabetic MSC), the control variants demonstrated very limited regeneration of nature isotropic collagen in central space of wound. The capillary amount was also significantly increased in exosome variant in day 15 and 21.

CONCLUSIONS

The modern curative methods based on exosomes derivation from MSC open the door to modern wound management. After effective test on animal full-thicknes wound model and we are able to prepare the test for another specific chronic wound. Work was supported by Technology Agency of Czech Republic – project FW01010106 and FV30393.

50. ASSESSMENT OF MESENCHYMAL STEM CELL SPHEROIDS AS APPROACH TOWARDS REGENERATIVE APPLICATIONS

Authors: Pasquale Marrazzo[1], Azzurra Sargenti[2], Valeria Pizzuti[1], Francesco Musmeci[2], Francesco Bacchi[2], Simone Pasqua[2], Francesco Alviano[1], Laura Bonsi[1]

Affiliation: [1]Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, 40126 Bologna, Italy. [2]Cell Dynamics i.S.r.l., 40129 Bologna, Italy

OBJECTIVE

Despite the promise of mesenchymal stem cells (MSCs) for tissue regeneration, the loss of their biological functions represents a significant clinical challenge for cell therapies. In this respect, 3D culture conditions are establishing for ameliorating the *in vitro* state and cellular spheroids have been set to better reflect stem cell *in-vivo* microenvironment. Recently, MSCs have been utilized as assembling elements in the formation of 3D spheroid-like culture, however, there is no evidence for whether MSC-based spheroids retain their full differentiation ability. The development of appropriate protocols for generating MSC spheroids could be critical for understanding MSC shared mechanisms of supporting healing tissues *in vivo.*

MATERIALS AND METHODS

Spheroids were generated starting from adult stem cells and different human tissue sources. At different time points, spheroids were measured for their mass density by the W8© instrument. Differentiation potential into osteogenic differentiation has been evaluated by histological staining.

RESULTS

There is a low heterogeneity in spheroid formation within the single cell type, but the morphological analysis showed a different capacity to compact over time among different sources of MSCs. We found that the ability of the different stem cell spheroids to differentiate in osteocytes varied depending on their physical environment, indeed, differences were observed between spheroid differentiation and classical substrate-based culture controls.

CONCLUSIONS

The preliminary results showed how cell culture settings influence stem cell behavior and the importance of optimizing protocols of differentiation for spheroids with different weight and compactness. The possibility to monitor spheroid mass density, a highly sensitive parameter for cell health status, during differentiation will be extremely interesting for quality control of GMP-grade cell banking and future clinical applications.

51. STEMNESS AND ARCHITECTURE OF ADIPOSE TISSUE IN PATIENTS WITH OBESITY AND METABOLIC COMPLICATIONS BEFORE AND AFTER WEIGHT LOSS

Authors: C. Compagnin[1,2], S. Conci[1,2], F. Favaretto[1,2], M. Sanna[1,2], A. Belligoli[1,2], R. Fabris[1,2], C. Dal Prà[1,2], S. Bettini[1,2], M. Foletto2, L. Prevedello[2], C. Da Re[3], C. Mescoli[4], V. Vindigni[5], L. Busetto[1,2], R. Vettor[1,2], G. Milan[1,2]

Affiliation: [1]Internal Medicine 3, Department of Medicine, University of Padova, Padova. [2]Center for the Study and the Integrated Treatment of Obesity, Padova Hospital, Padova. [3]Division of General Surgery, Department of Surgical, Oncological and Gastroenterological Sciences, University of Padova, Padova. [4]Surgical Pathology and Cytopathology Unit, Department of Medicine, University of Padova, Padova. [5]Clinic of Plastic and Reconstructive Surgery, Department of Neurosciences, University of Padova, Padova

OBJECTIVE

Obesity is characterized by the progressive expansion of adipose tissue (AT) by hypertrophy and hyperplasia involving adipose and endothelial precursor cells. We analysed morphological and functional changes of subcutaneous and visceral AT (SAT and VAT) of patients with obesity, different degrees of glucose impairment and after weight loss compared to lean controls.

MATERIALS AND METHODS

SAT and VAT samples were collected during bariatric or abdominal surgery from obese normoglycemic (ob N, n=62), prediabetic (ob preDM, n=58) and diabetic (ob T2DM, n=57) patients compared with lean controls. Moreover, 63 non-diabetic patients with obesity (OB) and 29 weight-loss subjects (WL) underwent bariatric surgery or diet therapy were studied. Clinical data, anthropometric measurements and biochemical parameters of patients were collected. Adipocyte size and capillary density were measured by IHC. Stromal vascular fraction freshly isolated was characterized by cytometry, quantifying AT stem cells (ASCs) (CD45-/34+/31-) and endothelial progenitors (CD45-/31+/34+), and by the evaluation of *in vitro* adipogenic potential.

RESULTS

In both SAT and VAT we measured a hypertrophic and hyperplastic expansion, causing similar vascular rarefaction in obese patients with different degrees of metabolic complications. The largest increase in adipocyte size and decrease in number and adipogenic potential of ASCs occur both in T2DM and in prediabetes. After weight loss both SAT and VAT adipocyte area decreased and the capillary density increased restoring the AT architecture of lean controls. Unexpectedly SAT-ASCs were further increased in WL and changed their phenotype decreasing the expression of both CD105 and CD271 membrane markers. The percentage of endothelial progenitors was higher in SAT than in VAT, increased with obesity and decreased in WL reaching the levels of controls.

CONCLUSIONS

We showed a remodelling of AT architecture in which stemness deficit is associated with early glucose metabolism impairment. After weight loss, we observed the increase of ASCs and capillary number and a size reduction of mature adipocytes. Our results suggested the benefit of controlling hypertrophy and hyperplasia already in prediabetic obese patients. Moreover, these data could provide a biological understanding of how stem cell scaling is maintained during AT growth suggesting a possible explanation for the recurring weight regain.

52. DECELLULARIZED WHARTON'S JELLY MATRIX - BASED SCAFFOLD FOR HUMAN DEGENERATED INTERVERTEBRAL DISC CELLS

Authors: Letizia Penolazzi[1], Elisabetta Lambertini[1], Maria Pina Notarangelo[1], Stefania D'Agostino[2,3], Anna Chiara Moscato[2,3], Michela Pozzobon[2,3], Roberta Piva[1]

Affiliation: [1]Department of Neuroscience and Rehabilitation, University of Ferrara, Ferrara, Italy. [2]Department of Women's and Children's Health, University of Padova, Padua, Italy. [3]Laboratory of Stem Cells and Regenerative Medicine, Fondazione Istituto di Ricerca Pediatrica Città della Speranza, Padua, Italy

Regenerative therapies for intervertebral disc (IVD) injuries are currently a major challenge that is addressed in different ways by scientists working in this field. Tissue engineering approach may rely on extracellular matrix (ECM) from decellularized perinatal tissues that has been established as a biomaterial with remarkable regenerative capacity.

OBJECTIVE

Primary aims were: 1. setting up an in vitro experimental model similar to the one that IVD cells experience in vivo, and 2. investigating the behavior of degenerated/de-differentiated IVD cells when exposed to hypoxic preconditioning and combined with decellularized Wharton's jelly matrix (DWJM) from human umbilical cord.

MATERIALS AND METHODS

An efficient detergent-enzymatic treatment was used to produce DWJM maintaining its native microarchitecture. IVD cells were obtained from surgical hernia biopsies after informed consent. X3 Hypoxia Hood and Culture Combo - Xvivo System device was used for hypoxia condition (2% pO_2). Biochemical assays, electron microscopy and immunofluorescence analysis were used to characterize the properties of DWJM and expression of specific markers.

RESULTS

When combined with IVD cells, DWJM was able to produce sizeable 3D cell aggregates and restore the native chondrocyte-like phenotype of the IVD cells. 3D cell aggregates were able to respond to hypoxia, become HIF-1α positive and, in addition to further increasing the expression of two typical pro-chondrogenic transcription factors, SOX-9 and TRPS1, expressed high levels of FOXO3a, a core regulator of cellular homeostasis, stress response, and longevity.

CONCLUSIONS

For the first time, we demonstrated the efficacy of hypoxia preconditioning of human IVD cells combined with an ECM-based scaffold to improve the IVD degenerated phenotype. Our data are in line with the hypothesis that the strengthening of cell properties in terms of viability and expression of specific proteins at precise times represents an important condition in the perspective of guiding the recovery of cellular functionality and triggering the endogenous regenerative potential. Currently, there are no definitive surgical or pharmacological treatments for IVD degeneration able to restore the disc structure and function. Thus, the efficacy of hypoxia preconditioned cells combined with DWJM in promoting cell differentiation towards a discogenic phenotype paves the way in the near future for innovative ECM-based intradiscal injectable therapies.

53. TETRASPANIN PROFILING IN DECIDUAL STROMAL CELLS AND COGNATE TRAFFICKING OF MICROVESICLES

Authors: George Boyadzhiev[1], Iliya Karagyozov[2], Ivan Bochev[1], Milena Mourdjeva[1], Tsvetelina Oreshkova[1]

Affiliation: [1]Institute of Biology and Immunology of Reproduction "Akad. K. Bratanov" - Bulgarian Academy of Sciences, Department of Molecular Immunology, Sofia, Bulgaria. [2]VITA Hospital Sofia, Department of Gynecology and Pelvic Surgery; Medical University-Pleven, Bulgaria

OBJECTIVE

Tissue integrity is based on physical communications among cells. Cells employ various means for trafficking of information - via receptors engagement, soluble factors release, membrane and microvesicles (MVs) exchange, which influence cells' physiology and fate. Decidual stromal cells (DSCs) are the main component of mature endometrium in pre- and early-pregnancy time with a substantial role for tissue remodeling and placentation. DSCs secrete MVs to communicate with target cells. CD9 and CD63 are membrane-spanning protein receptors (tetraspanins), known to organize integrins and other receptors on cells, which contribute to adhesion, migration, proliferation processes. They are present on cells and on MVs including the smallest MVs – exosomes. The profile of CD9 and CD63 in DSCs and their MVs after decidualization is investigated here.

MATERIALS AND METHODS

DSCs were isolated from decidual tissue from women after elective termination of pregnancy and after receiving of informed consent from patients. Purified and functional DSCs were *in vitro* decidualized by medroxyprogesterone acetate, β-estradiol and cAMP for 10 days. Culture medium was changed at 48 hours and aliquots from day 6 and day 8 were pooled to isolate MVs via ultrafiltration. MVs were loaded on beads for analysis. DSCs and MVs were labeled with antibodies and analyzed by FACS.

RESULTS

Decidualization of DSCs caused decrease of CD9 receptors and significant reduction in the percentage of live cells expressing this molecule. Conversely, CD63 demonstrated more than 2-fold up-regulation of receptor density on membranes and augmentation of $CD63^+$ DSCs when induced to decidualize. CD63 locates on exosomes in trafficking endosomes. Therefore, the total (membrane and intracellular) CD63 quantity in DSCs was evaluated by mean fluorescence intensity. The results showed no change in 2 and increase in 3 DSC lines suggesting an accumulation or induction of CD63 protein synthesis. Respective comparison of MVs released from DSCs showed significant down-regulation for CD9 in decidualized state compared to control and no change for CD63 molecule.

CONCLUSIONS

CD9 and CD63 expression profiles on DSCs show correlation with the functional differentiation of cells, which also reflects on membrane characteristics of the released MVs.

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54. LOW DENSITY CULTURED CHONDROCYTES SHOW PROMISING FEATURES EXPLOITABLE TO IMPROVE AUTOLOGOUS CHONDROCYTE IMPLANTATION

Authors: Alessandra Colombini[1], Francesca Libonati[1], Silvia Lopa[2], Katia Mareschi[3,4], Elena Marini[3], Matteo Moretti[2,5,6], Laura de Girolamo[1]

Affiliation: [1]Orthopaedic Biotechnology Lab, IRCCS Istituto Ortopedico Galeazzi, Via R. Galeazzi 4, 20161, Milan, Italy. [2]Cell and Tissue Engineering Laboratory, IRCCS Istituto Ortopedico Galeazzi, Via R. Galeazzi 4, 20161, Milan, Italy. [3]Department of Public Health and Paediatrics, The University of Turin, Piazza Polonia 94, 10126 Torino, Italy. [4]Stem Cell Transplantation and Cellular Therapy Laboratory, Paediatric Onco-Haematology Division, Regina Margherita Children's Hospital, City of Health and Science of Turin, 10126 Torino, Italy. [5]Regenerative Medicine Technologies Lab, Ente Ospedaliero Cantonale, Via Tesserete 46, 6900, Lugano, Switzerland. [6]Swiss Institute for Regenerative Medicine, Lugano, Switzerland.

OBJECTIVE

To develop an optimized manufacturing protocol to enrich cartilage cells culture in cartilage progenitors (CPs) for the improvement of the autologous chondrocyte implantation (ACI), a clinically effective procedure for the treatment of focal chondral lesions and early osteoarthritis.

MATERIALS AND METHODS

Cartilage cells were obtained from waste surgical material of 5 donors who underwent hip replacement and expanded at low density (50 cells/cm^2) in standard culture medium enriched with human platelet lysate (HPL). Tests for the release of cell-based products for clinical use were performed to exclude replicative senescence and karyotype instability. The expression of CD146 and CD166, surface markers associated with better clinical outcome in ACI patients, was evaluated, as long with MHC-I and –II. The trophic potential of the cells was evaluated by the assessment of matrix deposition after 14 days of pellet culture.

RESULTS

Low-density culture promoted the formation of cell colonies and enhanced cell proliferation yielding a 1.6- and 3.9-fold higher number of doublings/day compared to the standard-density cultured cells cultured in HPL or those cultured in previous experiments in FBS, respectively. In low-density culture, the cells showed an increase in CD146 and CD166 expression (5.66- and 1.52-fold than in standard-density cells, respectively) and low immunogenicity, being almost completely negative for MHC-II. Moreover, these cells did not undergo replicative senescence and showed karyotype stability. After chondrogenic induction in pellet culture, they displayed the typical chondrogenic round morphology and superior matrix deposition, particularly glycosaminoglycans, in comparison with the more elongated and necrotic standard-cultured cells, which produced less matrix.

CONCLUSION

Low-density cultured cartilage cells might represent an advantageous tool to improve ACI or, due to their low immunogenicity, to develop allogenic procedure for the treatment of chondral lesions. In this culture setting it is possible to obtain a high cell number starting from a lower cell number in comparison with standard density culture, thus avoiding long-term expansion. Remarkably, the low-density culture does not induce karyotype alteration, and replicative cell senescence and promotes an enrichment in cells with superior trophic potential. Future plans include the evaluation of these cell potential to modulate the inflammatory microenvironment typical of osteoarthritis.

55. YIA MODULATION OF miR-204 EXPRESSION DURING CHONDROGENESIS

Authors: Jessica Bertacco[1,2], Monica Mottes[2], Arianna Minoia[1], Veronica Li Vigni[1], Luca Dalle Carbonare[1], Maria Teresa Valenti[1]

Affiliation: [1]Department of Medicine, University of Verona, University of Verona, 37100 Verona, Italy. [2]Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, 37100 Verona, Italy

OBJECTIVE

During embryogenesis, the development of cartilage is initiated by a phase of mesenchymal stem cell condensation and chondroprogenitor cell differentiation. Chondrocytes are the cells with cartilage forming activity and maintain cartilage homeostasis by regulating the levels of specific matrix proteins. RUNX2 and SOX9 are two pivotal transcriptional regulators in chondrogenesis. It has been demonstrated that RUNX2 and SOX9 physically interact and SOX9 can inhibit the transactivation of RUNX2. In addition, RUNX2 exerts reciprocal inhibition on SOX9 transactivity. Epigenetic regulation of gene expression plays a pivotal role in multiple differentiation of stem cells and it has been reported that SOX9 can promote the expression of miR-204.

Our aim was therefore to investigate the role of miR-204 during chondrogenesis and to identify the relationship of this miR with the transcription factors and downstream genes involved in the process of chondrogenic commitment and differentiation.

MATERIALS AND METHODS

In order to evaluate the role of miR-204 in chondrogenesis, we performed transfection experiments in vitro, by using mesenchymal stem cells (MSCs). We also evaluated miR-204 expression in adult and larvae zebrafish. In particular, we performed RT Real-Time PCR, Western Blotting analyses and Annexin assay.

RESULTS

By silencing miR-204 during the early phase of differentiation we observed the upregulation of SOX9 and chondrogenic related genes compared to controls. In particular, we observed that SOX9 and COL2A1 expression was 1.5 and 2.2 fold higher compared to controls. In addition, we observed the upregulation of COL1A1 (a RUNX2 downstream gene) whereas the gene expression of RUNX2 was slightly affected compared to controls. However, the RUNX2 protein levels increased in miR-204 silenced cells. The positive effects of miR-204 silencing in osteogenic differentiation was observed also in the middle phase of osteogenic differentiation. On the contrary, the maturation of chondrocytes was considerably affected by miR-204 downregulation. In fact, SOX9 and COL2A1 expression was 0.67 and 0.7 fold in miR-204 silenced cells (30% lower compared to controls). In addition, we observed an increased expression of SESTRIN1 and 2 in miR-204 silenced cells.

CONCLUSIONS

Our preliminary results suggest that miR-204 is a negative regulator of osteochondroprogenitor commitment of mesenchymal stem cells as well as of osteogenic differentiation and a positive regulator of chondrocyte maturation.

56. POSSIBLE ROLE OF LYSOPHOSPHATIDIC ACID TO MEDIATE OVINE BONE MARROW MESENCHYMAL STEM CELLS AUTOCRINE SIGNALING ON PROLIFERATION AND ADHESION TO EXTRACELLULAR MATRIX PROTEINS

Authors: Fabiana Piscitelli[1], Antonella Zannetti[2], Anna Nardelli[2], Arturo Brunetti[3], Alessandra Pelagalli[2,3]

Affiliation: [1]Institute of Biomolecular Chemistry (ICB), National Research Council (CNR), Pozzuoli, Naples, Italy. [2]Institute of Biostructures and Bioimaging (IBB), National Research Council (CNR), Naples, Italy. [3]Department of Advanced Biomedical Sciences, University of Naples "Federico II", Naples, Italy

OBJECTIVE

Ovine bone marrow mesenchymal stem cells (oBM-MSCs) have been characterized in consideration of high similarity with humans with the perspective of the development of novel therapeutic approach for tissue regeneration in veterinary and in translational medicine. Albeit, several biological properties (stemness markers, cell differentiation, etc.) of oBM-MSCs have been studied, some aspects related to the autocrine effect of conditioned medium (CM) have not been completely investigated. Based on our previous results demonstrating potential effect of CM on oBM-MSCs migration and signal transduction, the purpose of this study was to investigate the biological role of lysophosphatidic acid (LPA) released by oBM-MSCs in CM.

MATERIALS AND METHODS

oBM-MSCs were cultured in α-MEM supplemented with 10% fetal bovine serum (FBS). To obtain CM, oBM-MSCs were grown for 24h, in α-MEM with 1% FBS. The effect of CM was tested on proliferation by MTT assay and on cell adhesion on extracellular matrix proteins such as fibronectin and collagen type IV. LPA determination was assessed in CM and oBM-MSCs by lipid extraction using a modified Bligh and Dyer method. The organic phase was collected, dried down by a stream of nitrogen gas and analyzed by LC-MS-IT-TOF. The identification of LPA molecular species was achieved via monitoring for selected transitions from molecular to product (m/z 153) ions specific for each LPA molecular species.

RESULTS

CM produced by oBM-MSCs caused an increase of cell proliferation and adhesion. The CM effect on cell growth was observed up to 24-72 h respect to FBS (1%) whereas its effect on cell adhesion on extracellular matrix proteins reached steady-state values by 2h. At the same time, high levels of LPA were detected in cell lysates and in CM demonstrating that this mediator was produced and released by oBM-MSCs in CM.

CONCLUSIONS

The higher levels of LPA found in CM respect to 1% and 10 % FBS could suggest the involvement of this bioactive phospholipid to mediate autocrine signaling produced by oBM-MSCs on their proliferation and adhesion.

57. BIOMIMETIC AND BIOACTIVE 3D PRINTED BONE SCAFFOLDS TO SUPPORT THE PHYSIOLOGICAL BALANCE BETWEEN OSTEOBLAST AND OSTEOCLAST ACTIVITY

Authors: Giorgia Montalbano[1], Giorgia Borciani[1,2], Priscila Melo[1], Gabriela Ciapetti[2], Sonia Fiorilli[1], Chiara Vitale-Brovarone[1]

Affiliation: [1]Department of Applied Science and Technology, Politecnico di Torino, Italy. [2]Laboratorio di Fisiopatologia Ortopedica e Medicina Rigenerativa, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy

OBJECTIVE

Nowadays, bone tissue engineering has been increasingly indicated as a valid alternative to meet the challenging requirements for a healthy bone regeneration in the case of bone loss or fracture [Jakob et al., 2013]. The reported research study, conducted in the frame of the ERC BOOST project, aims at designing biomimetic 3D printed scaffolds mimicking biochemical and structural features of healthy bone, exploring the potential influence of the 3D composite on the physiological crosstalk between osteoblasts (OB) and osteoclasts (OC).

MATERIALS AND METHODS

Type I collagen has been combined with nano-hydroxyapatite and Sr-containing MBG particles to obtain homogeneous hybrid formulations mimicking the composition of native bone. After confirming the suitability of the developed formulations as biomaterial inks, high resolution scaffolds have been obtained using a commercial 3D Bioprinter. An optimised genipin treatment was further exploited to increase the mechanical strength of the constructs, while modulating the release of strontium ions.
The resulting scaffolds were tested in a co-culture system using human OBs derived from trabecular bone and human peripheral blood mononuclear cells (PBMCs) isolated from buffy coat as OC-precursors. Viability, proliferation, and maturation of cells were explored up to 21 days, without the addition of exogenous osteogenic or osteoclastic inducers.

RESULTS

Bioactive composite scaffolds were fabricated by means of 3D extrusion printing. The subsequent chemical treatment with genipin led to a significant increase of mechanical and thermal stability of printed constructs as well as a sustained release of Sr^{2+} ions up to 7 days.
The indirect co-culture system using human OBs and PBMCs, carried out at Istituto Ortopedico Rizzoli, demonstrated the ability of the designed scaffolds to support OB adhesion and high proliferation rates up to 21 days, as well as the differentiation of OC-precursors between 7 and 14 days after seeding.

CONCLUSIONS

The collagenous hybrid formulations proved their suitability for the design of bioactive 3D printed structures for bone tissue engineering, able to stimulate a positive response on human OBs and OCs. Accordingly, the human OB/OC co-culture system with medium free of exogenous inducers confirmed the ability of the scaffolds to support cell adhesion, proliferation, and differentiation by paracrine activity, i.e., thanks to the OB/OC crosstalk, up to 21 days.

58. CLARIFYING THE ROLE OF MESENCHYMAL PROGENITORS WITHIN THE SKELETAL MUSCLE NICHE: DEVELOPMENT OF AN IN VITRO MODEL OF FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY

Authors: Lorena Di Pietro[1], Flavia Giacalone[1], Elvira Ragozzino[2], Valentina Saccone[1], Andrea Papait[1], Antonietta Silini[3], Pietro Romele[3], Marco De Bardi[4], Mario Picozza[4], Wanda Lattanzi[1,5], Enzo Ricci[2,5], Ornella Parolini[1,5]

Affiliation: [1]Dipartimento Scienze della Vita e Sanità Pubblica, Università Cattolica del Sacro Cuore, Rome, Italy. [2]Dipartimento di Neuroscienze, Università Cattolica del Sacro Cuore, Rome, Italy. [3]Centro di Ricerca E. Menni, Fondazione Poliambulanza-Istituto Ospedaliero, Brescia, Italy. [4]Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Fondazione Santa Lucia, Rome, Italy. [5]Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy

OBJECTIVE

Non-myogenic mesenchymal progenitors have recently been recognized as important players in regeneration/degeneration processes within the skeletal muscle niche. Facioscapulohumeral muscular dystrophy (FSHD) is the third most common muscular dystrophy, for which the pathogenetic mechanisms are not fully clarified. The main objectives of our study are: 1) to assess the role of mesenchymal progenitors and their plasticity/trophic potential in the pathophysiology of FSHD and the interaction between mesenchymal progenitors and satellite cells in FSHD muscles, 2) to evaluate the *in vitro* efficacy of conditioned medium of amniotic cells (CM-hAMSC) in supporting muscle regeneration in FSHD by re-educating mesenchymal progenitors.

MATERIALS AND METHODS

Qualitative and quantitative analysis of patient muscle sections were performed. Mesenchymal progenitors and myoblasts were isolated from muscle specimens of FSHD patients and controls. Myoblasts were induced to differentiate into myotubes, whereas mesenchymal progenitors were alternatively induced with an adipogenic or fibrogenic medium. Co-culture systems were also established. Cells were analysed at different time points and comparisons were performed between patient and control cultures. The ability of CM-hAMSC to enhance proliferation and differentiation of patient-derived mesenchymal progenitors was also evaluated.

RESULTS

Immunofluorescence analysis of muscle sections showed a significant expansion of mesenchymal progenitors in FSHD muscles, compared with healthy muscles, that positively correlated with muscle fibrosis. Our results showed that patient-derived mesenchymal progenitors displayed an altered adipogenic differentiation potential: control cells significantly differentiated after 1 and 2 weeks of induction, while patient-derived cells responded to a lesser extent to inductive stimuli. FSHD mesenchymal progenitors seemed to induce the proliferation of matched myoblasts in co-culture systems, and to reduce their ability to fuse into myotubes. Preliminary results displayed that CM-hAMSC treatment inhibited the adipogenic differentiation of both patient and control mesenchymal progenitors.

CONCLUSIONS

The role of mesenchymal progenitors in tissue damage and regeneration is an emerging topic in current research on muscular dystrophy, nevertheless to date poorly investigated in FSHD. Our data suggest a feasible association among mesenchymal progenitors' impaired plasticity and muscle degeneration.

59. OSTEOINDUCTIVITY OF HYDROXYLAPATITE SCAFFOLDS WITH HUMAN ADIPOSE STEM CELL CULTURES

Authors: Maria Rosa Iaquinta[1], Elena Torreggiani[1], Chiara Mazziotta[1], Andrea Ruffini[2], Simone Sprio[2], Anna Tampieri[2], Mauro Tognon[1], Fernanda Martini[1,3], Elisa Mazzoni[4]

Affiliation: [1]Department of Medical Sciences, Section of Experimental Medicine, University of Ferrara, Ferrara Italy. [2]Institute of Science and Technology for Ceramics, National Research Council, Faenza Italy. [1,3]Laboratory for Technologies of Advanced Therapies (LTTA), University of Ferrara, Ferrara Italy. [4]Department of Chemical, Pharmaceutical and Agricultural Sciences - DOCPAS, University of Ferrara, Ferrara Italy

OBJECTIVE

In vitro biocompatibility and osteoinductive ability of a recently developed biomorphic hydroxylapatite ceramic scaffold (B-HA), derived from transformation of wood structures, were analyzed using human adipose stem cells (hASCs).

MATERIALS AND METHODS

Cell viability and metabolic activity were evaluated in hASCs, parental cells and in recombinant genetically engineered hASC-eGFP cells expressing the green fluorescence protein. B-HA osteoinductivity properties, such as differentially expressed genes (DEG) involved in the skeletal development pathway, osteocalcin (OCN) protein expression and mineral matrix deposition in hASCs, were evaluated.

RESULTS

In vitro induction of osteoblastic genes, such as Alkaline phosphatase (ALPL), Bone gamma-carboxyglutamate (gla) protein (BGLAP), SMAD family member 3 (SMAD3), Sp7 transcription factor (SP7) and Transforming growth factor, beta 3 (TGFB3) and Tumor necrosis factor (ligand) superfamily, member 11 (TNFSF11)/Receptor activator of NF-KB (RANK) ligand (RANKL), involved in osteoclast differentiation, was undertaken in cells grown on B-HA. Chondrogenic transcription factor SRY (sex determining region Y)-box 9 (SOX9), tested up-regulated in hASCs grown on the B-HA scaffold. Gene expression enhancement in the skeletal development pathway was detected in hASCs using B-HA compared to sintered hydroxylapatite (S-HA). OCN protein expression and calcium deposition were increased in hASCs grown on B-HA in comparison with the control.

CONCLUSIONS

This study demonstrates the biocompatibility of the novel biomorphic B-HA scaffold and its potential use in osteogenic differentiation for hASCs. Our data highlight the relevance of B-HA for bone regeneration purposes.

60. (YIA) TARGETING HUMAN MESENCHYMAL STROMAL CELLS WITH FGFR2 GENE KNOCK-DOWN FOR PERSONALIZED NON-INVASIVE THERAPY OF CROUZON CRANIOSYNOSTOSIS SYNDROME

Authors: Federica Tiberio[1], Lorena Di Pietro[1], Martina Salvati[1], Gianpiero Tamburrini[2,3], Ornella Parolini[1,3], Alessandro Arcovito[3,4], Wanda Lattanzi[1,3]

Affiliation: [1]Dipartimento Scienze della Vita e Sanità Pubblica, Università Cattolica del Sacro Cuore, Rome, Italy. [2]Dipartimento di Neuroscienze, Università Cattolica del Sacro Cuore, Rome, Italy. [3]Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy. [4]Dipartimento di Scienze Biotecnologiche di Base, Cliniche Intensivologiche, e Perioperatorie, Università Cattolica del Sacro Cuore, Rome, Italy

OBJECTIVE

Skull sutures represent a unique site of mesenchymal stromal cell (CMSC) niche in the craniofacial complex, regulating skull bone growth and homeostasis. Several interacting osteogenic signalling drive the fate of CMSC and their disruption results in the premature ossification of sutures (i.e. craniosynostosis). Crouzon syndrome (CS) is a rare syndromic craniosynostosis caused by heterozygous missense mutations in the *Fibroblast Growth Factor Receptor 2* (*FGFR2*), resulting in constitutively activation of the receptor as well as its downstream cascade. The current treatment of CS is exclusively based on multiple invasive interventions for cranial vault decompression and modeling.

The aim of this study is to develop a personalized gene therapy targeting MSC within the suture niche through allele-specific siRNA delivered by recombinant human ferritin-based (HFt) nanocarriers. This strategy is intended to downregulate the mutant *FGFR2*, without affecting the wild type allele, thus hampering the overactive FGFR-induced osteogenesis.

MATERIALS AND METHODS

CMSC were isolated from surgical waste of CS patients. HFt was engineered to feature a shield that hampers the CD71 receptor-mediated endocytosis, plus a metalloprotease (MMP) cleavage domain increasing HFt uptake in the presence of an MMP-enriched environment. CMSC were treated with FITC-conjugated HFt and analysed using fluorescence microscopy. CD71 and MMPs expression in CMSC was evaluated through real-time PCR. A set of siRNA specifically targeting the mutant *FGFR2*-allele was designed and transfected into CMSC by cationic lipids. The efficiency of the siRNA silencing was evaluated through real time PCR.

RESULTS

Fluorescence microscopy showed an efficient internalization of HFt in CMSC. Expression analysis revealed that the levels of *CD71*, *MMP-9* and *-13* increase during osteogenic differentiation. Gene expression analysis allowed identifying the specific siRNA with the most remarkable silencing effect on the expression of mutant *FGFR2* after the treatment of CMSC.

CONCLUSIONS

Our data suggested that allele-specific *FGFR2* knockdown by siRNA represents a suitable strategy to reprogram CMSC fate acting on overactive FGFR2 signalling in CS patients. We also demonstrated that CMSC are proper target for HFt-based nanocarriers. On-going studies are addressing to functionalize HFt in order to enhance the encapsulation of selected siRNA and to target CMSC undergoing premature ossification.

61. (YIA) EFFECT OF C9ORF72-NEK1 DOUBLE MUTATION ON DNA DAMAGE RESPONSE IN NEURAL STEM CELLS DERIVED FROM AMYOTROPHIC LATERAL SCLEROSIS PATIENT

Authors: Serena Santangelo[1,2], Claudia Colombrita[2], Patrizia Bossolasco[2], Silvia Peverelli[2], Sabrina Invernizzi[1], Alberto Brusati[2], Valentina Gumina[2], Donatella Bardelli[2], Nicola Ticozzi[2,3], Vincenzo Silani[2,3], Antonia Ratti[1,2]

Affiliation: [1]Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan, Italy. [2]Department of Neurology - Stroke Unit and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy. [3]Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università degli Studi di Milano, Milan, Italy

OBJECTIVE

Emerging evidence suggest that DNA damage and impairment of DNA damage response (DDR) are implicated in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS). *C9ORF72* repeat expansion, the main genetic cause of ALS, associates to DDR defects in motoneurons derived from human induced pluripotent stem cells (iPSC). Moreover, mutations in *NEK1* gene involved in DDR and maintenance of chromosomal stability, have been found in ALS patients. By mutational analysis we have identified an Italian ALS patient carrying a concomitant repeat expansion in *C9ORF72* gene and a loss-of-function mutation in *NEK1* gene (p.Ser1036Ter). We aim to study the effect of the double mutation in *C9ORF72* and *NEK1* genes on DNA damage repair.

MATERIALS AND METHODS

We reprogrammed primary fibroblasts from the double mutant *C9ORF72-NEK1* patient into iPSC, then we characterized and differentiated iPSC into neural stem cells (NSC). We induced DNA damage with the radiomimetic agent Neocarzinostatin in NSC from the double mutant *C9ORF72-NEK1*, three different *C9ORF72* and two healthy control lines. We quantified γH2A.X histone- and BP53-positive nuclear foci as markers of DNA damage and further divided NSC in four arbitrary categories according to the γH2A.X foci number per cell: (I) 2-5 foci, (II) 5-20 foci, (III) 20-30 foci and (IV) >30 foci.

RESULTS

FISH analysis revealed a significant higher number of pathological *C9ORF72* RNA foci in the double mutant *C9ORF72-NEK1* compared to three different mutant *C9ORF72* iPSC lines.

All NSC displayed low and comparable levels of DNA damage in basal condition without significant differences among the experimental groups. After DNA damage induction, we observed a similar increase of γH2A.X- and BP53-positive nuclear foci in all the analyzed cell lines. DNA damage was rescued in a timeframe between 4 and 8 hours after Neocarzinostatin removal, returning to the basal values at 24 hours, with no significant differences among all the analyzed NSC lines.

CONCLUSIONS

Although the *C9ORF72-NEK1* iPSC showed increased pathological RNA foci, the induced DNA damage could be efficiently repaired in the highly-mitotic NSC, independently from the presence of *C9ORF72* or *C9ORF72-NEK1* double mutation. We are now investigating the DDR in iPSC-motoneurons that represents a more differentiated and post-mitotic neuronal model. Our study aims to better understand the possible interplay between *NEK1* and *C9ORF72* genes and assess the relevance of DNA damage and DDR as novel and druggable pathomechanisms in ALS.

62. A NEW PREDICTIVE TECHNOLOGY FOR PERINATAL STEM CELL ISOLATION SUITED FOR CELL THERAPY APPROACHES

Authors: Silvia Zia[1], Giulia Martini[2], Valeria Pizzuti[2,3], Alessia Maggio[2], Giuliana Simonazzi[4], Pierluigi Reschiglian[1,5], Laura Bonsi[2], Francesco Alviano[2], Barbara Roda [1,5], Andrea Zattoni[1,5]

Affiliation: [1]Stem Sel srl, Bologna, Italy. [2]Department of Experimental, Diagnostic and Specialty Medicine, Unit of Histology, Embryology and Applied Biology, University of Bologna, Bologna, Italy. [3]Department of Experimental, Diagnostic and Specialty Medicine, Unit of Nephrology, Dialysis and Renal Transplant, St, Orsola-Malpighi University Hospital, Bologna, Italy. [4]Obstetric Unit, Department of Medical and Surgical Sciences, Policlinico Sant'Orsola-Malpighi, University of Bologna, Bologna, Italy. [5]Department of Chemistry G. Ciamician, University of Bologna, Bologna, Italy

OBJECTIVE

The use of stem cells for regenerative applications and immunomodulatory effect is increasing. Amniotic epithelial cells (AEC) possess embryonic-like proliferation ability and multipotent differentiation potential. Despite the simple isolation procedure, inter-individual variability and different isolation steps can cause differences in isolation yield and proliferation ability, compromising reproducibility observations among centers and further applications. To prevent it, high standards of reproducibility/reliability must be established and maintained in the biomedical research.

MATERIAL AND METHODS

Celector® is our new instrumentation that exploits the NEEGA-DF technology, as a tool for quality control on stem cells isolation. Based on a proprietary fractionation device Celector® separates cells in a label-free mode, based only on their intrinsic physical characteristics. At the fractionator outlet a micro-camera records and counts eluting cells as a function of time to generate a live fractogram. The physical characteristics of all eluting cells are recorded and analysed giving new knowledge on the cell population's morphological state, and ability to discriminate cell subpopulations. Here we show as a case study in which eight amniotic membranes were processed by trypsin, and immediately analysed by Celector®.

RESULTS

Fractograms were reproducible among samples, and two types of profile were typically generated: a monomodal and a bimodal curve. Regardless type of profile, cells were sorted and collected into two fractions: the first part (F1) contained mostly cell aggregates, and the second part (F2) had the single cells.
The monomodal profile represented unsuccessful isolation with all the recovered cells having poorly adherent features and very low proliferate ability. Otherwise, the bimodal profile showed, that the isolation process was successful though we discovered that only F2 cells were alive, and resulted adherent and proliferative. Moreover, morphological analysis showed how F2-cells are statistically smaller and rounder compared to F1 cells, which are important parameters to define stem cells. These results eventually show Celector® as tool to check viability and quality of stem cells isolation procedure before culture processing.

CONCLUSION

We optimized a QC method to define the success of AEC isolation using the fractograms. This predictive outcome is an interesting tool for laboratories and cell banks that isolate and cryopreserve fetal annex stem cells for research and future clinical applications.

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