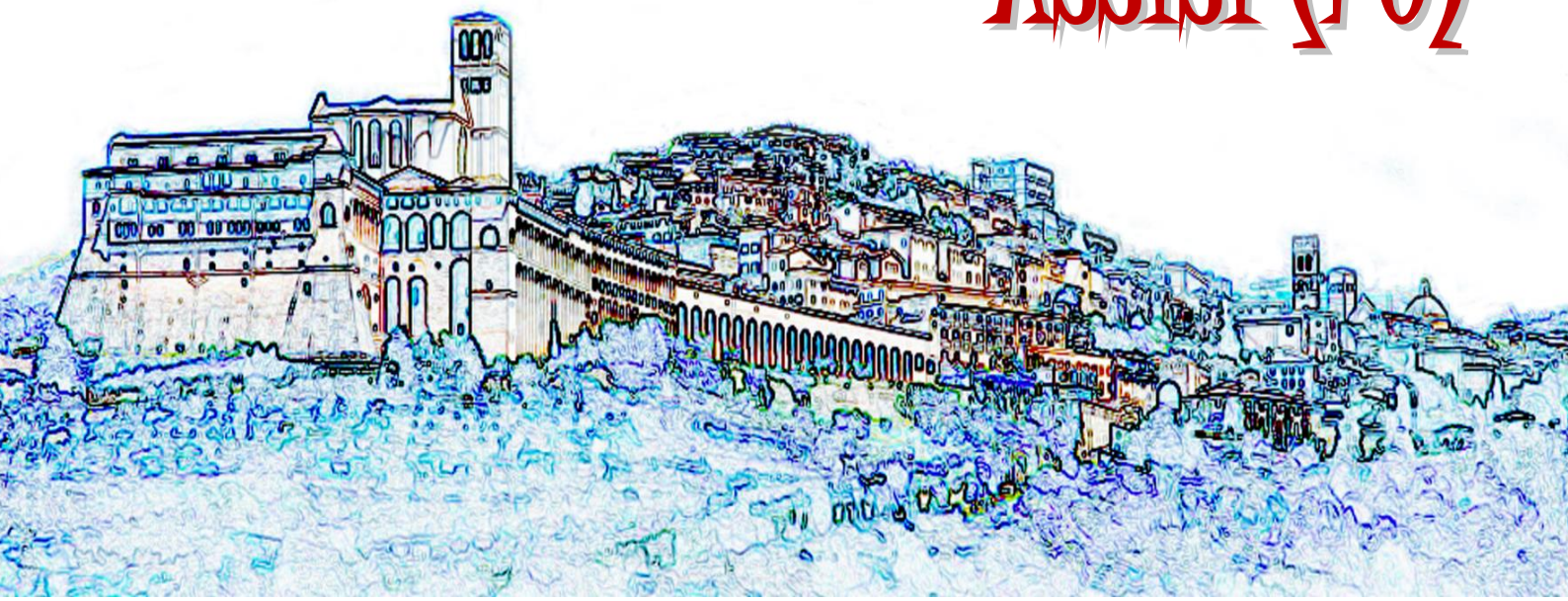
2018 GISM ANNUAL MEETING

APRIL 12-13
2018

Gruppo Italiano Staminali Mesenchimali

Palazzo del Monte Frumentario

Assisi (PG)



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ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELL'UMBRIA E DELLE MARCHE "TOGO ROSATI"

ORDINE dei MEDICI VETERINARI della PROVINCIA di PERUGIA

ACKNOWLEDGEMENTS

GISM acknowledges the following supporting member companies for their contribution to the 2018 GISM Annual Meeting:

ABSTRACT BOOK

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PRESENTATION

The GISM annual meeting will take place in Assisi (Italy) on April 12, and 13, 2018 at Palazzo Frumentario with the support of the University of Perugia, Comune di Assisi, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Department of Veterinary Medicine of Perugia. Assisi was intentionally selected to give to groups in central Italy an opportunity to showcase their research and to give to the meeting the breathtaking atmosphere of the town.

Along the last years the knowledge on the biological properties of Mesenchymal Stromal Cells (MSCs) has significantly increased being recognized that their capacity to regulate tissue regeneration is also related to paracrine mechanisms mediated by soluble molecules and extracellular vesicles. This produced a remarkable attraction to investigate their therapeutic capacity in many field as documented by more than 500 clinical trials managed worldwide.

In this context, the GISM meeting will cover some of the hottest clinical application of the MSCs. In particular, four sessions will be dedicated to the clinical application in neuropathology, gastroenterology, cardiology and respiratory diseases. Two sessions will focus on key issues and solutions of the clinical application of MSCs and to new technological advancements.
Finally, a specific session will be dedicated to the veterinary clinical experience.

All these topics will be presented by world-class speakers with the intent to promote an interactive discussion and eventually to facilitate inter-disciplinary collaborations.

The best three posters will be awarded with the "Young investigator Award" (500 €) to encourage the attendance of the youngest members of our community.
We look forward to meeting you in Assisi.
Yours sincerely,

Augusto Pessina, GISM President
Maura Ferrari, GISM Vice President
Enrico Lucarelli, GISM Secretary
Luisa Pascucci, Local Organizing Committee

SCIENTIFIC PROGRAM

Thursday April 12, 2018

14.00 Registration

15.00 Meeting Opening

Augusto Pessina, *GISM President*
Luisa Pascucci, *Local Organizing Committee*
Invited Authorities: ***Franco Moriconi,*** *Rector of the University of Perugia*
Luca Mechelli, *Director of the Dpt. of Veterinary Medicine, Perugia*
Silvano Severini, *General Director of IZSUM*
Stefania Proietti, *Mayor of Assisi*

15.30-16.40 SESSION 1: MSCs PERSPECTIVES IN NEUROPATHOLOGY

Chairpersons: Anna Brini, Katia Mareschi

15.30-15.50 Mesenchymal stromal cells loaded with Paclitaxel as cell-based therapy for glioblastoma
Maria Laura Falchetti, ***CNR, Roma***

15.50-16.10 Mesenchymal stromal cells for traumatic brain injury: experimental data and clinical perspectives
Elisa Zanier, ***Mario Negri Institute, Milano***

16.10-16.30 Mesenchymal stem cells in neurodegenerative diseases: immunomodulation and neuroprotection
Marina Boido, ***Neuroscience Institute Cavalieri Ottolenghi, University of Turin***

16.30-16.40 Discussion

16.40-17.00 Coffee Break

17.00-18.10 SESSION 2: MSCs IN GASTROENTEROLOGY

Chairpersons: Riccardo Calafiore, Augusto Pessina

17.00-17.20 MSCs for the treatment of Inflammatory Bowel Disease (IBD): the preclinical phase
Maurizio Muraca, ***University of Padova***

17.20-17.40 MSC in immune-mediated intestinal diseases: ready for the clinical use?
Rachele Ciccocioppo, ***University of Verona***

17.40-18.00 Microencapsulated human adult mesenchymal stem cells for the cell therapy of type 1 diabetes mellitus
Riccardo Calafiore, ***University of Perugia***

18.00-18.10 Discussion

18.10-18.40 TECHNOLOGICAL SESSION

18.10-18.20 Cell Factories design: safety as a key factor
Nicola Abbasciano*, ASLAB, Italy*

18.20-18.30 A validated and customized software platform for Cell Factories
Claudia Ottardi, *Gruppo SOL S.p.A., Italy*

18.30-18.40 IncuCyte S3: Live-cell analysis System
Claudio Bencivenga, *Sartorius Corporation, Italy*

18.40-20.00 GISM MEMBERS' ASSEMBLY AND ELECTION OF NEW COUNCIL

20.30 HAPPY HOUR

Friday April 13, 2018

8.50-9.40 LECTIO MAGISTRALIS

MSC: "one" cell, multiple dynamic phenotypes (an adaptive cell or a case of multiple personalities disorder?)
Diego Correa, *University of Miami*, *Miller School of Medicine, USA*

9.40-10.50 SESSION 3: MSCs IN CARDIOLOGY

Chairpersons: Laura De Girolamo, Milena Mastrogiacomo

9.40-10.00 Cell-based therapies for myocardial repair and regeneration in ischemic heart disease and heart failure
Rosalinda Madonna, *University of Chieti*

10.00-10.20 Contribution of oxidized low density lipoproteins to cardiac mesenchymal stromal cells adipogenesis in arrhythmogenic cardiomyopathy
Giulio Pompilio, *Centro Cardiologico Monzino*, *Milano*

10.20-10.40 Mending broken hearts from within: reactivating endogenous regeneration via the stem cell secretome
Sveva Bollini, *University of Genova*

10.40-10.50 Discussion

10.50-11.10 Coffee Break

11.10-12.20 SESSION 4: MSCs IN RESPIRATORY DISEASES

Chairpersons: Giulio Alessandri, Enrico Lucarelli

11.10-11.30 The clinical use of regenerative therapy in obstructive pulmonary disease (COPD)
Luigino Calzetta, *University of Roma Tor Vergata*

11.30-11.50 Experimental and clinical airway restoration by MSC transplantation
Francesco Petrella, *European Institute of Oncology*, *Milan*

11.50-12.10 MSC-based treatment of cystic fibrosis: preclinical studies and challenges ahead
Massimo Conese, *University of Foggia*

12.10-12.20 Discussion

12.20-13.00 SESSION 5: MSCs PITFALLS AND PERSPECTIVES

Chairpersons: Maurizio Muraca, Maria Luisa Torre

12.20-12.40 Updating clinical trials with MSCs
Umberto Galderisi, *Università della Campania*

12.40-13.00 The dark and the light side of advanced therapy medicinal products
Guido Pantè, *AIFA, Roma*

13.00-14.30 LUNCH AND POSTER SESSION

14.30-17.00 SESSION 6: MSCs IN VETERINARY MEDICINE

Chairpersons: Maura Ferrari, Luisa Pascucci

14.30-14.50 MSCs in Veterinary Medicine: why and how do they work?
Eleonora Iacono, *University of Bologna*

14.50-15.10 An update on MSCs-based therapies in Veterinary Medicine, between reality and perspectives
Stefano Grolli, *University of Parma*

15.10-15.30 MSCs in practice: preparation, distribution, quality controls and regulation
Silvia Dotti, *IZSLER*, *Brescia*

Clinical Experiences

15.30-15.45 MSCs in veterinary oncology: a therapy for animals and a model for humans
Offer Zeira, *Ospedale Veterinario San Michele, Lodi*

15.45-16.00 Can I treat my pet with stem cells? Clinical experiences and related issues
Maurizio Del Bue, *Libero professionista, Parma*

16.00-16.15 MSCs in horses: an overview on their application in orthopedic injuries
Raffaello Ciampoli, *Libero professionista*, *Siena*

16.15-16.30 MSCs in the management of muscular injuries in Bovine patients
Piero Boni, *Libero professionista*, *Perugia*

16.30-17.00 Discussion

17.00 GISM HAS GOT TALENTS: YOUNG INVESTIGATOR AWARDS
18.00 CLOSING REMARKS

ABSTRACTS

Invited Speakers

Abstract Index

S-01

MESENCHYMAL STROMAL CELLS LOADED WITH PACLITAXEL AS CELL-BASED THERAPY FOR GLIOBLASTOMA

Simone Pacioni[1,7], Quintino Giorgio D'Alessandris[1], Stefano Giannetti[2], Valentina Coccè[3], Arianna Bonomi[4], Mariachiara Buccarelli[5], Luisa Pascucci[6], Giulio Alessandri[4], Augusto Pessina[3], Lucia Ricci-Vitiani[5], Roberto Pallini[1] and **Maria Laura Falchetti[7]**

Institutes of Neurosurgery[1]and Anatomy[2], Università Cattolica del Sacro Cuore, Rome; Department of Biomedical, Surgical and Dental Sciences, University of Milan[3]; Department of Cerebrovascular Diseases, Fondazione IRCCS Neurological Institute Carlo Besta[4], Milan; Department of Hematology, Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome[5]; Department of Veterinary Medicine, University of Perugia[6]; CNR-Institute of Cell Biology and Neurobiology (IBCN)[7], Rome, Italy.

Abstract

A key goal of cancer chemotherapy is localizing drugs selectively to tumor environment avoiding collateral toxicity. Glioblastoma (GBM), the most aggressive brain tumor, is associated with invariably poor prognosis in spite of extensive surgical resection and adjuvant radio/chemotherapy. The blood brain barrier (BBB) further limits the efficacy of systemic therapies for GBM. Therefore, it is urgent the need for new vehicles that enable local persistent delivery of chemotherapeutic drugs. Mesenchymal stromal cells (MSCs) have recently gained great interest as therapeutic tool. MSCs exert their therapeutic effects by several mechanisms, including the ability to home to pathological tissues. They possess an intrinsic tropism for tumors, cross the BBB, and can reside within the bloodstream as pericytes homing to inflamed blood vessels. Due to these peculiarities, MSCs represent a great opportunity for cell-based cancer therapy. In a first attempt to evaluate the possibility of using MSCs as a vehicle to deliver chemotherapeutic drugs, we demonstrated that bone-marrow-derived MSCs of murine origin uptake and release paclitaxel (PTX) in an amount enough to inhibit GBM cell proliferation.

Objectives. Here we addressed the therapy-relevant issue of using MSCs from human donors (hMSCs) for local or systemic administration in orthotopic GBM models.

Materials and Methods. The GBM cell line U87MG or patient-derived glioma stem cells (GSCs) expressing the green fluorescent protein (GFP) were grafted onto the striatum of immunosuppressed rats. Adipose hMSCs (Ad-hMSCs), fluorescently labeled with the mCherry protein, were inoculated adjacent to or into the tumor. In rats bearing U87MG xenografts, systemic injections of Ad-hMSCs or bone marrow (BM)-hMSCs were done via the femoral vein or carotid artery.

Results. Ad-hMSCs uptake/release PTX with very high efficiency and U87MG exposed to their conditioned medium display cytotoxic damages typical of PTX-treated cells. When grafted adjacently to brain tumors, Ad-hMSCs show remarkable tropism towards the tumor. Intracerebral injection of Ad-hMSCs significantly improved the survival of rats with U87MG xenografts. This effect was associated with a reduction in tumor growth, tumor cell proliferation, and microvascular density. In GSCs xenografts, intratumoral injection of Ad-hMSCs depleted the tumor cell population and induced migration of resident microglial cells. Overall, PTX loading did not significantly enhance the antitumor potential of hMSCs.

Systemically injected Ad- and BM-hMSCs homed to tumor xenografts. The efficiency of hMSC homing ranged between 0.02 and 0.5% of the injected cells, depending both on the route of cell injection and on the source from which the hMSCs were derived. Importantly, systemically injected PTX-loaded hMSCs that homed to the xenograft induced cytotoxic damage to the surrounding tumor cells.

Conclusions. hMSCs have a therapeutic potential in GBM brain xenografts which is also expressed against the GSC population. In this context, PTX loading of hMSCs seems to play a minor role.

S-02

MESENCHYMAL STROMAL CELLS FOR TRAUMATIC BRAIN INJURY: EXPERIMENTAL DATA AND CLINICAL PERSPECTIVES

Elisa Zanier

Mario Negri Institute, Milano

Abstract

Traumatic brain injury resulting from ischemic/hemorrhagic or traumatic damage is one of the leading causes of mortality and disability worldwide, and is a significant burden to the society. Neuroprotective options to counteract brain damage are very limited in stroke and traumatic brain injury. Given the multifaceted nature of acute brain injury and damage progression, several therapeutic targets may need to be addressed simultaneously to interfere with the evolution of the injury and improve the patient's outcome. Mesenchymal stem cells are ideal candidates since they act on various mechanisms of protection and repair, improving structural and functional outcomes after experimental stroke or traumatic brain injury. We will discuss the evidence for the beneficial effects of mesenchymal stem cells in acute brain injury, with the focus on experimental studies of traumatic brain injury and stroke, the engineering strategies pursued to foster cell potential, and characterization of the bioactive molecules secreted by placental cells, known as their secretome, as an alternative cell-free strategy. Results from the clinical application of mesenchymal stem cells for acute brain injury and ongoing clinical trials will also be discussed.

S-03

MESENCHYMAL STEM CELLS IN NEURODEGENERATIVE DISEASES: IMMUNOMODULATION AND NEUROPROTECTION

Marina Boido[1], Ivana Ferrero[2,3], Katia Mareschi[2,3], Letizia Mazzini[4], Franca Fagioli[2], Alessandro Vercelli[1]

[1]Neuroscience Institute Cavalieri Ottolenghi, Department of Neuroscience, University of Torino, Torino
[2]Paediatric Onco-Haematology, Stem Cell Transplantation and Cellular Therapy Division, City of Science and Health of Torino, Regina Margherita Children's Hospital,
[3]Department of Public Health and Paediatrics, University of Torino, Torino, Italy
[4]ALS Centre Department of Neurology, University of Eastern Piedmont, Novara, Italy.

Abstract

Several neurodegenerative diseases (including ALS, PD, HD) still lack of an effective treatment, despite the consistent research efforts in understanding their etiology and pathophysiology and in developing specific therapies.

To counteract neuronal death and inflammation (key events in such pathologies), mesenchymal stem cell (MSC) therapies can represent an intriguing approach, since they can modulate immune response and neuroinflammation, promote neuronal survival, and decrease free radical levels. To this aim, we have evaluated the effects and the mechanism of action of human MSC (hMSC) graft on the progression of disease in the mutant SOD1 G93A mouse, an experimental model of familial ALS. In the past, we demonstrated that the intraparenchymal way of administration of MSCs had beneficial effects on motor neuron (MN) survival, glial activation and motor behavior. The same approach was also used in two open-label pilot studies in which hMSCs were injected into different levels of the thoracic spinal cord of nineteen ALS patients. However this paradigm allows a limited craniocaudal diffusion (requiring multiple injection sites in patients) and can increase the risk for side effects due to surgery and penetration through the dorsal horn.

Therefore, we tested in the ALS murine model a less invasive experimental approach, i.e. the intracisternal delivery of hMSCs labeled with bisbenzimide. At the symptom onset, we injected 300,000 hMSCs into the cisterna lumbaris, which is easily accessible and could be used in outpatient surgery. Two weeks after graft, the animals were sacrificed and the spinal cords analyzed.

Surviving hMSCs were found both in the meninges and into the spinal parenchyma, at considerable distance from the injections site. The decrease in motor performance was significantly delayed in the transplanted mice compared to the sham-operated ones. In addition, stereological counts showed a higher number of lumbar MNs in transplanted animals than in controls, thus indicating a delayed neuronal death. We also investigated the expression of several cytokines in the lumbar spinal cord by microarray strategy, highlighting their modulation after hMSC transplantation: in particular, we observed a significant increase of the anti-inflammatory mouse IL-10, IL-13a and vascular endothelial growth factor a (VEGFa) mRNAs in the lumbar spinal cord of transplanted mice.

Our study confirms the role of stem cell therapy as a promising tool in the treatment of ALS, by providing evidence that a new route of administration, the minimally-invasive injection into the lumbar cistern, can be as effective as intraparenchymal injection.

S-04

MESENCHYMAL STEM/STROMAL CELLS (MSCS) FOR THE TREATMENT OF INFLAMMATORY BOWEL DISEASE (IBD):THE PRECLINICAL PHASE.

Maurizio Muraca

Department of Women's and Children's Health, University of Padova, Italy

Abstract

Animal models of intestinal inflammation are an invaluable tool for the study of mucosal immunology, its role in the maintenance of intestinal homeostasis and the resulting inflammation when such homeostasis is disrupted. Because of the complex pathogenesis of IBD, no single model can reflect the complexity of human disease. Transgenic or gene-targeted mouse models can help understand the role of specific factors. Additional murine models include the adoptive T-cell transfer and spontaneous development of colitis. However, chemically induced models of gut inflammation are the most commonly used because of the rapid onset of inflammation and of their simpler procedures. Therefore, such models have been mostly used to evaluate the therapeutic effects of MSCs and more recently of MSC-derived extracellular vesicles (EVs). Most studies reported a beneficial effect of MSCs on clinical findings, histological score, immune cell infiltration and cytokine expression in colon mucosa, although a few discordant reports have also been published. A single study on MSC-EVs treatment of rodents with dextran sodium sulphate (DSS)-induced colitis reported a striking clinical and histological improvement with a single EV administration. We evaluated the effects of both syngeneic (murine bone marrow) and xenogeneic (human Wharton Jelly) MSCs and MSC-EVs in a murine model of DSS induced colitis. We were unable to observe any significant effect on adaptive immunity, likely because of the short injury time inherent to this model. Moreover, MSCs tended to produce opposite effects compared to MSC-EVs. Analysis of cytokine expression in intestinal mucosa gave conflicting results. Our experience points to several limitations of chemical models of acute colitis and suggests that more chronic models should be preferred.

S-05

MSC IN IMMUNE-MEDIATED INTESTINAL DISEASES: READY FOR THE CLINICAL USE?

Rachele Ciccocioppo, Maria Cristina Conti Bellocchi, Valeria Zuliani, Luca Frulloni

Gastroenterology Unit and Laboratory of Gastroenterology, Department of Medicine, University of Verona

Abstract

Intestinal mucosa is characterized by fast renewal of epithelial cells and rapid trafficking of immune cells that account for its remarkable capacity of repair when tissue injury occurs. However, there are conditions with impaired healing capability, thus resulting in lifelong and invalidating conditions, such as celiac disease, Crohn's disease (CD), and ulcerative colitis. These are caused by a dysregulated immune response towards dietary, bacterial, and self-antigens that develop in genetically susceptible individuals. In spite of the great strides made in understanding the fine mechanisms responsible for tissue injury, a definitive cure is still an unmet need and all the available therapies are encumbered by a series of adverse events or development of refractoriness. The advent of cellular therapies, mainly based on the use of stem cells, represents a great step forward, with mesenchymal stromal cells (MSC) being the most suitable thanks to the lack of immunogenicity and the high safety profile. In this regard, they have been found to play a prominent role in the healing process of gut mucosa, through an array of mechanisms that include protection of both crypt stem cells and enterocytes, and preservation of epithelial barrier. After the benefit of MSC in mouse models of colitis, a number of open-label Phase 1-2 studies were carried out, which tested the use of autologous or allogeneic systemic infusions of bone marrow- and placenta-derived MSC for treatment-resistant CD. Certainly, these studies showed this therapeutic approach to be 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. 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 MSC, a crucial point in establishing the right timing for infusions, which may depend not only on the clinical setting, but also on the phase of the disease. Clearer and more unambiguous results were obtained when using MSC local injections for fistulizing CD. This is a challenging condition for both patients, due to the poor quality of life, and physicians, because of the high relapse rate. This new treatment option gave excellent results in terms of both safety and long lasting efficacy, although only patients refractory to or unsuitable for standard therapies were recruited.

It is conceivable that over the next few years the role of MSC as immunotherapy in inflammatory bowel diseases will become clearer as the biological interactions with injured tissues and the hierarchy by which they deliver their action are unraveled through a continuous moving from bench to bedside and vice versa.

S-06

MICROENCAPSULATED HUMAN ADULT MESENCHYMAL STEM CELLS FOR THE CELL THERAPY OF TYPE 1 DIABETES MELLITUS

Riccardo Calafiore, Giuseppe Basta, Pia Montanucci.

Department of Medicine, Inter-Departmental School of Medicine and Surgery, University of Perugia, Perugia, Italy

Abstract

While subcutaneously administered, exogenous human recombinant insulin, continues to represent the minstay for the management of type 1 diabetes mellitus (T1D), including prevention of acute (ie, diabetic ketoacidosis) and chronic (retinopathy, neuropathy, cardiovascular disease, nephropathy) complications of the disease, it is clear that this is not the cure for T1D. We believe that an early modulatory intervention on the T1D disease process, by means of adult mesenchymal stem cells, retrieved from the post-partum umbilical cord Wharton Jelly (hUCMS), upon envelopment in highly biocompatible, alginate-based microcapsules, would greatly help to rescue still viable Beta cells and possibly prevent use of exogenous insulin. On this purpose, we had shown that hUCMS, enveloped in clinical grade alginate microencapsules (cgaMC) and co-cultured with PBMC's from patients with recent onset T1D exhibited strong immunoregulatory properties, resulting in decline of Th17and rise of Tregs. This outcome was ascribed to both, production of humoral factors (TGFβ, IDO, NO, IL6, PGE2, HGF, VEGF) and expression of HLA-E-F and G molecules (Clin Immun, 2015). On the wave of these in vitro positive results, we aimed to translate the shown hUCMS immunoregulatory properties in vivo. We then emarked upon intraperitoneal graft (TX) of hUCMS/cgaMC, into NOD mice with spontaneous, overt T1D. 15 NOD's were divided into two groups: #1, n=5, with BG of 500 mg/dl; #2: n=10, with BG of 250 to 300 mg/dl and abnormal OGTT. 5 NOD's (with overt DM) served for controls. Group #1 showed poor response to TX (BG levels always erratic from up to down); group #2 showed BG decline to 150 mg/dl throughout over 200 d of TX, and near normalization of OGTT. At 160 d of TX, Tc immunophenotyping, from the NOD's retrieved thymus and spleen, showed CD4+FOXP3+CD25high(Treg) levels that were similar to normal, but not to overtly diabetic control NOD's (where no Tregs were detectable). Pancreatic sections of the long-term remitters were associated with minor insulitis in conjunction with detection of small/intact islet cells, unlike treated-failed or untreated diabetic controls, showing only atrophic endocrine tissue. Some areas of mononuclear cells were detected surrounding a few islets (benign insulitis). CgaMC, retrieved from the remitter group, at 160 after TX, were freely floating in the peritoneal cavity and showed no signs of fibrotic cell overgrowth. From the collected data, we can infer that there is a narrow window, upon the onset of overt DM in NOD's, well trackable by metabolic patterns (OGTT), when induction of acquired central tolerance towards autoreactive Tc clones, by administration of hUCMS in cgaMC, is possible. This would preserve the native pancreas from extensive β-cell destruction, opening a new avenue for initiation of pilot clinical trials in patients with recent onset T1D, in order to prevent exogenous insulin treatment.

S-07

CELL FACTORIES DESIGN: SAFETY AS A KEY FACTOR

Nicola Abbasciano

ASLAB (Italy)

Abstract

In the last years, the attention to the modern developments in the field of biotechnology has risen significantly in the scientific community. From these experimental achievements, we have moved to the “in field” application of these modern techniques of cellular manipulation, tissue and gene therapy, naming these activities as “innovative therapies” and regulating their use in a specific way, both for the procedural side and for the technical requirements of the environments. Since the beginning of the 21st century, new Cells and Gene manipulation Centers (generically named “Cell Factories”) have been built in Italy as well as in Europe; these are by all means pharmaceutical laboratories and therefore, in Italy, subjected to the authorization and control of AIFA. It becomes consequently necessary to design these centers taking into account the peculiarity of the new activities that are performed inside such laboratories. From an engineering point of view, what has then changed in the design of a cell factory, compared to a common pharmaceutical department?

In traditional pharmaceutical production departments, the focus is almost entirely on ensuring the sterility of the product and therefore the safety of the patients needing the drugs. The efforts are therefore aimed at preventing "external" contamination. Inside modern cell factories, in addition to preventing external contamination, it is necessary to carefully evaluate the risk of cross contamination between samples. The risk of contamination is therefore directly inherent in the same biological product to be treated. In the field of Gene Therapy, the common methods for the transduction of the cells can include the use of viral vectors (like CAR-T cells therapy). It is necessary to design laboratories able to guarantee not only the sterility (positive pressure systems) but also the biological containment (negative pressure system), in order to ensure at the same time the product protection and the operator safety. The increased use of biological samples has also introduced the need for their long-term preservation. A correct design of the cryobank is therefore a critical condition to reach this objective, and since the use of liquid nitrogen is required, an adequate risk analysis should be carried on, as well as appropriate technical solutions, to guarantee operator’s safety.

In conclusion, the correct design of these laboratories should require a preliminary adequate risk analysis, starting from the detailed analysis of the production processes highlighting any possible risk connected to this activity.

Once the risk analysis is done, this will ensure the highest level of safety possible for the products and for the operators, by adopting the best available technical and plant engineering solutions.

S-08

A VALIDATED AND CUSTOMIZED SOFTWARE PLATFORM FOR CELL FACTORIES

Claudia Ottardi

Gruppo SOL S.p.A.

Abstract

OBJECTIVE: Good results in advanced therapy can be achieved only having high-quality Cell Factories, authorized by Italian Drug Agency (AIFA). A GMP validated software for the management of samples during the whole production process is fundamental. Therefore the goal of the present project was the development and implementation of a validated and customized software for the management of Cell Factories working procedures.

MATERIALS AND METHODS: A Cell Factory software must follow the entire lifecycle of a product, starting from the registration and approval of raw materials, the production, quality control, storage and release. The system could be fully integrated with the other software and devices, commonly running in a Cell Factory (warehouse management, accounting and biobanks management). SOL Group worked in collaboration with Cell Factories end users in order to develop a flexible and customized tool, able to adapt to each requests.

RESULTS: An easy-to-use software, dedicated to the complete management and traceability of biological samples was developed. The different modules, following the whole lifecycle of the products up to transplants, ensure safety and no risk of errors. The access is allowed only to authorized operators by means of personal and reserved credentials. The collected data are stored with redundancy on several external servers for 30 years. Therefore all the aspects dealing with safety and privacy are ensured. The software manages patient's and donor's data with a connection to the different production and analysis phases. Moreover it is able to manage also the cryopreservation and warehouse data, creating, printing and controlling all the barcodes.

CONCLUSION: SOL Group has developed and successfully implemented in several Cell Factories a new GAMP (5) validated software for the management and production of advanced therapy products, raw materials and reagents. Traceability, privacy and safety are guaranteed during all phases.

S-09

INCUCYTE S3: LIVE-CELL ANALYSIS SYSTEM

Claudio Bencivenga

Sartorius Corporation, Italy

Abstract

The IncuCyte® Live Cell Analysis System, developed by Essen BioScience, Inc., is the first system to quantify cell behavior in real time (from hours to weeks) while cells remain undisturbed inside a standard incubator. The IncuCyte® System automatically collects and analyzes images around the clock, providing continuous insight into active biological processes that is difficult to achieve with endpoint assays. The IncuCyte suite of assays are designed specifically for live cell assays to not perturb cell health.

Whether evaluating general cell health metrics such as proliferation, cytotoxicity, and apoptosis or functionally-specific processes such as immune cell killing, chemotaxis/migration, antibody-dependent cell-mediated cytotoxicity (ADCC), or stem cell differentiation, the IncuCyte System is a purpose-built solution, that will increase lab productivity and deliver new insights into the biology of your cells.

S-10

MSC: "ONE" CELL, MULTIPLE DYNAMIC PHENOTYPES; AN ADAPTIVE CELL OR A CASE OF MULTIPLEPERSONALITIES DISORDER?

Diego Correa

Department of Orthopedics, Division of Sports Medicine, Diabetes Research Institute & Cell Transplant Center, University of Miami, Miller School of Medicine, Miami, FL - USA

Abstract

Mesenchymal Stem Cells (MSCs) have been identified and isolated from multiple vascularized tissues, where they adopt a perivascular localization and a pericytic-like phenotype. From that strategic location, MSCs can exert multiple roles, depending on the environment. Independent of their tissue source, MSCs possess similar features in vitro, namely plastic adherence, multi-lineage differentiation and a particular immunophenotype (positive and negative cell surface markers). However, this equivalent genotype does not necessarily correlate with a universal phenotype, as they can adopt multiple "signatures" depending on their immediate instructive microenvironment and acquired specific functions. It is becoming clearer that multiple subpopulations of MSCs exist in the bone marrow (BM), each with a particular function. In this talk, I will present evidence of the existence of multiple sub-phenotypes of human BM-derived MSCs (hBM-MSCs) that exert totally different functions yet related by the presence of an identifying cell surface marker (CD146). First, CD146Neg hBM-MSCs delineate a bona fide chondroprogenitor capable of sustaining enhanced chondrogenesis, recapitulating the in vivo osteochondroprogenitor present at the endosteal bone surface (CD271Pos; CD146Neg). An inverse relationship between CD146 presence and Sox9 expression explains the molecular readiness and subsequent enhanced chondrogenesis obtained by expanding hMSCs with FGF2, where a significant reduction in the proportion of CD146Pos is associated with a distinct upregulation of Sox9 and their mutually exclusive expression. These cells, later sequentially stimulated with different FGF ligands at particular timepoints of the chondrogenic program induce the maintenance of a pre-hypertrophic chondrocyte and enhanced articular cartilage repair properties in vivo. Second, CD146Pos defines a subpopulation of hBM-MSCs with intrinsic and inducible immunomodulatory properties, recapitulating the perivascular localization of such a subtype of MSC in the BM. This subpopulation is highly sensitive to an inflammatory environment, responding with strong immunosuppressive molecular messengers selectively secreted or packaged into exosome-type of microvessicles for short and long-range signalling, respectively. Third, hBM-MSCs equipped with CD146 expression and a critical perivascular location act as gatekeepers modulating the process of extravasation of circulating melanoma and breast cancer cells (CC) into both BM and liver. CC lacking CD146 fail to colonize those tissues, similar to extraskeletal bones with MSCs lacking of CD146 where CC are unable to invade and establish secondary metastatic tumours. Collectively, CD146 helps to identify hBM-MSCs with distinct phenotypes, reflected in specific functions.

S-11
CELL-BASED THERAPIES FOR MYOCARDIAL REPAIR AND REGENERATION IN ISCHEMIC HEART DISEASE AND HEART FAILURE

Rosalinda Madonna

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Abstract

Despite improvements in modern cardiovascular therapy, the morbidity and mortality of ischemic heart disease (IHD) and heart failure (HF) remain significant in Europe and worldwide. Patients with IHD may benefit from therapies that would accelerate natural processes of postnatal collateral vessel formation and/or muscle regeneration. In this seminar, we discuss the use of cells in the context of heart repair, and the most relevant results and current limitations from clinical trials using cell-based therapies to treat IHD and HF. The lecture will undertake a critical appraisal of where the stem cell field stands and where it appears to be headed, by critically reviewing the current approaches using stem cell or cell-based therapies to treat IHD and HF. We identify and discuss promising potential new therapeutic strategies that include the use of biomaterials and cell-free therapies aimed at increasing the success rates of therapy for IHD and HF. The lecture will also discuss promising new strategies for stem cell therapy enhancement that include ex vivo cell-mediated gene therapy, with the aim of increasing the efficacy and outcome of stem cell therapies in the future

S-12

CONTRIBUTION OF OXIDIZED LOW DENSITY LIPOPROTEINS TO CARDIAC MESENCHYMAL STROMAL CELLS ADIPOGENESIS IN ARRHYTHMOGENIC CARDIOMYOPATHY

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Abstract

Arhythmogenic Cardiomyopathy (ACM) is a genetic condition characterized by progressive fibro-fatty replacement of the ventricular myocardium and malignant arrhythmias. We recently showed that Cardiac Mesenchymal Stromal Cells (C-MSC) in ACM hearts differentiate to adipocytes, through the activation of PPARγ.
The variable penetrance and expressivity of ACM suggest the involvement of co-determinants. Physical exercise is the only accepted co-factor. Strong physical activity increases oxidative stress. 13HODE, a component of oxidized low density lipoproteins (oxLDL), is a marker of exercise-induced oxidative stress, and has been shown, in macrophages, to produce fat accumulation by increasing the expression of both PPARγ and the oxLDL receptor CD36.
To evaluate the effects of oxidative stress and oxLDL on ACM adipogenesis and to dissect underpinning pathways.
We analyzed plasmas (n=34) and ventricular tissues (n=3) from ACM patients and matched healthy controls (HC). In vitro experiments have been carried out on ACM vs. HC human C-MSC (n=5), while in vivo experiments on the heterozygous PKP2 KO mouse model (PKP2+/-; n=5).
Significantly higher plasmatic oxLDL were detected in ACM patients compared to HC (290.90±76.31 vs. 122.40±28.73 ng/ml; p=0.04). Moreover, oxLDL levels can discriminate between ACM patients with overt phenotype vs. their asymptomatic relatives carrying the same causative mutations (456.50±187.80 vs. 93.81±33.39 ng/ml; p=0.03). In human ventricular tissue, we observed higher oxidative stress in ACM hearts vs. HC (malondialdehyde positivity 20.26±6.54 fold higher; p=0.004).
In basal conditions, ACM C-MSC also showed higher oxidative stress (diclorofluorescein emission 5.64±0.80 vs. 3.60±0.36 a.u.; p=0.03) and 2.79±1.32 fold higher expression of PPARγ (p=0.04) compared to HC C-MSC. Administration of 13HODE increased lipid accumulation in ACM C-MSC (Oil Red O (ORO) staining 1.28±0.24 fold vs. untreated; p=0.02). On the contrary, treatment with the antioxidant N-Acetylcysteine (NAC) prevented lipid accumulation in ACM C-MSC (ORO staining 0.63±0.16 fold vs. untreated; p=0.03). Lipid accumulation during adipogenic differentiation in ACM C-MSC paralleled with an increased surface expression of CD36 (R2= 0.93; p=0.03).
Despite PKP2+/- mice do not spontaneously accumulate adipocytes in the heart, C-MSC obtained from PKP2+/- mice hearts are more prone to lipid accumulation in vitro than WT cells (ORO staining 99.49±27.36 fold higher; p=0.007). The increase of plasma cholesterol and oxidative stress by administering a high-fat diet, resulted in fibro-fatty substitution in the heart of PKP2+/- mice only (% ORO positive area 0.47±0.15% in PKP2+/- vs. 0.11±0.01% in WT; p=0.009).
Mutations in ACM genes are necessary but not sufficient for ACM complete penetrance. We showed that elevated oxidative stress and oxLDL are important cofactors of adipogenesis. Further investigations could provide new approaches for pharmacological prevention of ACM adipogenic phenotype.

S-13

MENDING BROKEN HEARTS FROM WITHIN: REACTIVATING ENDOGENOUS REGENERATION VIA THE STEM CELL SECRETOME

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Abstract

Heart failure is related to inefficient cardioprotection, defective repair and lack of myocardial renewal following injury. Yet, the adult mammalian heart retains some regenerative capability, based on cardiac progenitor cell (CPC) activation and resident cardiomyocyte proliferation. Although these mechanisms are widely responsive during cardiogenesis and in the very early stages of post-natal life, they become quiescent and dormant in the adulthood, leaving the heart capable of limited repair potential when facing pathological situations, such as myocardial infarction. Therefore, a working strategy to enhance and restore in full the cardiac endogenous potential for both repair and regeneration will open new frontiers in cardiac medicine.

Recently stem cell biology has been broadly scrutinized in order to define a therapeutic approach. Despite an increasing interest towards the analysis of the cardiovascular differentiation potential of autologous or allogeneic stem cells transplanted into the injured heart, particular attention has been lately addressed to their paracrine modulatory influence instead. Indeed, the so called “paracrine effect” has been proposed as a promising strategy to boost endogenous mechanisms of repair and regeneration from within the cardiac tissue. This has led to a significant paradigm shift: from exploring the stem cell genome and the direct differentiation potential to analysing the stem cell “secretome”, as the whole of growth factors and chemo-attractant molecules produced by the stem cells via paracrine secretion.

Hence, the scientific community is now focusing on identifying the ideal stem cell population endowed with the most effective cardioactive secretome and in such scenario human fetal not-embryonic progenitor cells might represent an appealing source.

S-14

THE CLINICAL USE OF REGENERATIVE THERAPY IN OBSTRUCTIVE PULMONARY DISEASE (COPD)

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Abstract

Regenerative or stem cell therapy is an emerging field of treatment based on stimulation of endogenous resident stem cells or administration of exogenous stem cells to treat diseases or injury and to replace malfunctioning or damaged tissues. Current evidence suggests that in the lung, these cells may participate in tissue homeostasis and regeneration after injury. Animal and human studies have demonstrated that tissue-specific stem cells and bone marrow-derived cells contribute to lung tissue regeneration and protection, and thus administration of exogenous stem/progenitor cells

or humoral factors responsible for the activation of endogenous stem/progenitor cells may be a potent next-generation therapy for chronic obstructive pulmonary disease. The use of bone marrow-derived stem cells could allow repairing and regenerate the damaged tissue present in chronic obstructive pulmonary disease by means of their engraftment into the lung. Another approach could be the stimulation of resident stem cells by means of humoral factors or photobiostimulation.

S-15
EXPERIMENTAL AND CLINICAL AIRWAY RESTORATION BY MSC TRANSPLANTATION

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Abstract

Post resectional broncho-pleural fistula is a pathological connection between the airways and the pleural space that may develop after lung resection. It may be caused by incomplete bronchial closure, impediment of bronchial stump wound healing, or stump destruction by residual neoplastic tissue; its mortality ranges from 12.5 to 71.2%, therefore it is still the most feared complication after curative lung resection; for this reason the healing effects promoted by stem cells – by transformation into mature cells with a specialized function or by enhancing intrinsic repair mechanisms – may represent an effective and only partially explored therapeutic option . Mesenchymal stromal cells have the ability to migrate and engraft at sites of inflammation and injury in response to cytokines, chemokines, and growth factors at a wound site and they can exert local reparative effects through transdifferentiation into tissue-specific cell types or via the paracrine secretion of soluble factors with anti-inflammatory and wound-healing activities.

We proposed, on an animal model, an autologous bone marrow derived mesenchymal stromal cells transplantation: it allowed bronchial stump healing by extraluminal fibroblast proliferation and collagenous matrix development. Encouraged by experimental bronchial wall restoration in large animals and by functional human organ replacement elsewhere, we undertook autologous bone marrow derived mesenchymal stromal cells bronchoscopic transplantation to treat a patient who developed broncho-pleural fistula. The bronchoscopic transplantation of bone marrow-derived mesenchymal stromal cells in our patient appeared to help close this small-caliber post resectional broncho-pleural fistula, further boosting regenerative medicine approach for airway diseases.

Considering the need to specifically track mesenchymal stromal cells following transplantation in order to evaluate different method of implantation, to follow their migration within the body and to quantify their accumulation at the target, we proposed magnetic resonance imaging tracking both by superparamagnetic iron oxide particles and perfluorocarbon nanoemulsion formulations , demonstrating that were both effective, without altering cell viability or differentiation.

Finally we proposed adipose derived mesenchymal stromal cells bronchoscopic transplantation and intra venous injection of Granulocite Colony Stimulating Factors as a faster method and a new frontier in airway restoration.

S-16
MSC-BASED TREATMENT OF CYSTIC FIBROSIS: PRECLINICAL STUDIES AND CHALLENGED AHEAD

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Abstract

Cystic fibrosis (CF) is the most lethal autosomal recessive disorder that arises from alterations in the CF Transmembrane Conductance Regulator (CFTR) gene, with more than 2,000 mutations recognized, divided into six classes. The CFTR protein acts as a regulated chloride and bicarbonate channel expressed mainly in the epithelial lining of absorptive/secretory organs, including the lung, pancreas, liver, sweat glands and reproductive tract. The chief cause of morbidity and mortality of CF patients is represented by lung disease, characterized by accumulation of bronchial mucus due to altered ion and fluid homeostasis, bacterial infections, and a hyperinflammatory state. Other epithelial defects have been described, including an alteration in tight junctions. Recently, an intense molecular pharmacology research has ended in the clinical use of drugs that either correct or potentiate the defective CFTR protein. However, each of them is only tailored for patients bearing specific types of CFTR mutations, being cost-effective treatments. Cell-based therapies may be more advantageous in terms of patient coverage since they would be effective independently of mutation class.

Mesenchymal stem cells (MSCs) have the potency to differentiate into mesodermic cell types, but they have also the capacity to give rise to differentiated cell types belonging to ectodermic and endodermic lineages, including airway epithelial cells. Although MSCs are attracted into the inflamed environment via the intervention of chemotactic factor receptors, their engraftment is limited and not enough to guarantee the generation of a full-thick epithelium. Indeed, they have been shown to provide the repairing tissue with paracrine mediators which act on the different cellular structural components of the lung, with cytoprotective effects, and other factors with inflammatory and immunomodulatory capacities.

In the context of CF pre-clinical mouse models, MSCs obtained from bone marrow (BM-MSCs) have been shown to partially correct the electrophysiological defect of lung epithelium and increase the respirator bacterial clearance. Since BM-MCSCs are obtained by an invasive procedure and their regenerative properties decade with age and pathology, other non-invasive and more ethical sources are being searched, including placenta-derived MSCs. Human amnion-derived MSCs (hAMSCs) in co-culture with CF airway epithelial cells not only acquired CFTR expression over the baseline, but the co-cultures also showed a corrected phenotype as concerning chloride efflux, fluid hyperabsoprtion, tight junction and actin organization. Interestingly, these effects could not be seen when hAMSCs and CF cells were cultured separately, suggesting a role of intercellular communications in the therapeutic effects. Indeed, the rescue of a functional CFTR channel was abrogated when co-cultures were treated with a small interfering RNA (siRNA) directed against a component of gap junction connexin 43. The role of gap junctions should be further investigated in in vivo models, where also the anti-inflammatory, anti-bacterial and anti-scarring properties of hAMSCs should be considered.

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Abstract

In the last year, the promising features of mesenchymal stromal/stem cells (MSCs), including their regenerative properties and ability to differentiate into diverse cell lineages, have generated great interest among researchers whose work has offered intriguing perspectives on cell-based therapies for various diseases. Currently the most commonly used adult stem cells in regenerative medicine, MSCs can be isolated from several tissues, exhibit a strong capacity for replication in vitro, and can differentiate into osteoblasts, chondrocytes, and adipocytes. However, heterogeneous procedures for isolating and cultivating MSCs among laboratories have prompted the International Society for Cellular Therapy (ISCT) to issue criteria for identifying unique populations of these cells. Consequently, the isolation of MSCs according to ISCT criteria has produced heterogeneous, non-clonal cultures of stromal cells containing stem cells with different multipotential properties, committed progenitors, and differentiated cells. Though the nature and functions of MSCs remain unclear, non-clonal stromal cultures obtained from bone marrow and other tissues currently serve as sources of putative MSCs for therapeutic purposes, and several findings underscore their effectiveness in treating different diseases. To date, 783 MSC-based clinical trials, either complete or ongoing, appear in the database of the US National Institute of Health. In the present paper, we provide a comprehensive review of MSC-based clinical trials conducted worldwide that scrutinizes MSCs' biological properties, elucidates recent clinical findings and clinical trial phases of investigation, highlights MSCs' therapeutic effects, and identifies principal criticisms of the use of these cells. In particular, we analyze clinical trials using MSCs for representative diseases, including hematological disease, graft-versus-host disease, diabetes, inflammatory diseases, and diseases in the liver, kidney, and lung, as well as cardiovascular, bone and cartilage, neurological, and autoimmune diseases.

S-18

THE DARK AND THE LIGHT SIDE OF ADVANCED THERAPY MEDICINAL PRODUCTS

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S-19
MSCS IN VETERINARY MEDICINE: WHY AND HOW DO THEY WORK?

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Abstract

The effective management of domestic animals, for their owners, requires sophisticated new treatments and preventive strategies. MSCs are the most promising candidates for tissue engineering and regenerative medicine. BM is the common source of MSCs for clinical applications in veterinary medicine. Alternatively, AT is used; it is ubiquitously available and has several advantages compared to BM. In fact, it is easily accessible in large quantities with minimal invasive harvesting procedures and yields a high amount of MSCs. However, for both sources, an invasive procedure is required and a large variability in cell yield related to the donor was demonstrated. Furthermore, they have limited potential in terms of in vitro proliferation capability and do not appear to noticeably improve long-term functionality compared to MSCs derived from extra-fetal tissues. Foetal adnexa represent a MSCs source readily available and easily procured, without invasive procedures. MSCs from foetal adnexa are defined as an intermediate between embryonic (ESCs) and adult SCs, due to the preservation of some characteristics typical of the primitive native layers. Among foetal adnexal tissues, the major sources of MSCs are: umbilical cord blood, amniotic fluid, amniotic membrane, Wharton's Jelly. Both in human and domestic animals, MSCs from these sources may be useful for immediate use or in later stages of life, after cryopreservation in cell banks.

As previously reported in human, also in domestic animals, MSCs are a population of multipotent cells that meet the following criteria: plastic adherence when maintained in standard culture conditions; differentiation toward different cell types in vitro; expression of CD105, CD73, CD90, lack of CD45, CD14, CD11b, CD79a, CD19, major histocompatibility complex surface molecules. Usually, clinical treatments with MSCs are based on their transplantation but only a small percentage of them engraft successfully. The ability of equine adult MSCs, IFN-gamma and TNG-alpha stimulated, to secrete numerous soluble mediators, implicated in the inhibition of T-cell proliferation, was demonstrated. Moreover, the presence of active genes specific for anti-inflammatory and angiogenic factors was recently observed in non-stimulated cells derived from equine amniotic membrane and Wharton's jelly. In addition, recent findings indicate that EVs are released in culture medium from domestic animals MSCs, derived from both adult tissues and fetal adnexa. In a preliminary study in vitro, the possible use of EVs in equine endometrial and tendon pathologies was evaluated. Furthermore, because of their capacity to encapsulate both hydrophilic and lipophilic molecules and to deliver them, EVs from MSCs have been considered as drug delivery systems, and a preliminary study was conducted on canine MSCs. As reported in the literature, MSCs from different sources have different characteristics that may drive their therapeutic use. These could be noteworthy for domestic animals as well as for other mammalian species, including humans.

S-20

AN UPDATE ON MSCS-BASED THERAPIES IN VETERINARY MEDICINE, BETWEEN REALITY AND PERSPECTIVES

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Abstract

The interest of the veterinary community in regenerative medicine rises with the first successful applications of MSCs-based treatments in the therapy of equine tendonitis. Starting from the beginning of the 2000', thousands of horses have been treated all over the world, so that tendonitis can be considered the real test bench for MSCs based therapy in naturally occurring diseases in veterinary medicine. While initially based on cells whose biology, functions and potentialities were relatively unknown, nowadays MSCs therapies are supported by a much more robust knowledge base. As a consequence, companion animals' spontaneous pathologies are being more and more considered essential models to design safe and efficacious cell therapies not only for veterinary patients but also for the treatment of human diseases. Small pet animals (i.e. dog, cat) and horses reflect – more than laboratory animals- anatomy and physiology of humans, and even share with human environmental risk factors, pathogenesis, symptoms and responses to therapies. Accordingly, literature has shown an increasing number of studies dealing with novel approaches to stem cell therapy, not only related to musculoskeletal diseases, but also to cardiac, gastrointestinal, pulmonary, renal, ophthalmologic and dermatologic systems. Although many of these studies are still aimed to investigate feasibility (how many cells? which route of administration?), safety issues and biological responses to cell administration, useful information can be gathered for the translation in the everyday veterinary practice. Since for many diseases "standard of care" (SOC) protocols have been developed and are successfully used, cell therapy should, at present, be suggested for those animals refractory to SOC procedures, not only to cure the disease, but also with the aim to improve the quality of life of the patients. From this point of view, stem cell therapies can be already considered as an opportunity for the veterinary community. Researchers, practitioners and animal owners will have the chance to contribute to animal health and well-being, but also to improve human health (addressing the so-called One-Health paradigm) implementing rigorous clinical trials.

The presentation will discuss recent advances and major challenges in stem cell therapy in veterinary medicine, outlining cell features, protocol steps and the clinical requirement that, at the best of current knowledge, should be considered to undertake stem cell therapy in the veterinary clinic.

S-21
MSCS IN PRACTICE: PREPARATION, DISTRIBUTION, QUALITY CONTROL AND REGULATION

Silvia Dotti, Riccardo Villa, Maura Ferrari, Guerino Lombardi

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Abstract

In recent years, cell therapy has evolved quickly gaining great interest in both human and veterinary field. In particular, it has evoked considerable excitement in the animal-owning public because of the promise that stem cell technology could deliver tissue regeneration for injuries, for which natural repair mechanisms do not allow functional recovery and for which current therapeutic strategies have minimal effectiveness.

Before tackling the therapeutic approach, and due to the fact that mesenchymal stem cells (MSCs) are identified as a pharmaceutical drug, it is necessary to consider the laboratory process that allows to obtain a suitable product to the medical employment.

The clinical application of cell therapy involves different critical aspects that must be considered during laboratory process. The first step to consider is represented by the manipulation of the sample during in vitro amplification; this aspect is particularly thorny and demanding, because the sample is submitted to different procedures that can result in contamination or cells damages.

After the isolation, all manipulation must be performed in certified laboratories and with standardized procedures approved by the quality management system. Furthermore, quality controls represent a crucial point in order to guarantee a safe and functional final product. Quality testing should be performed in each batch in order to ensure the purity of the final product resulting from the production process.

The quality controls, are made in accordance with European Pharmacopiea and EMA regulations and consist in the evaluation of sterility against bacteria and mycoplasma, the potential viral contaminations by either inoculation of the sample in cell cultures or by molecular biology assays and the maintenance of the amplified cells of the differentiation ability towards the osteogenic, adipogenic and chondrogenic cell line. Furthermore, the evaluation of the absence of tumorigenicity carried out either by in vitro or in vivo tests plays a critical role.

In veterinary field, only autologous MSCs are currently approved although the results of experimental clinical trials already performed and transmitted to Ministry of Health have demonstrated the safety of allogeneic MSCs and no apparent differences in their injury repair capacity. The use of allogeneic MSCs offers the possibility to store quality tested batches of cells available at each time.

Only a strong cooperation among the laboratory staff, the regulatory agencies and, mainly, the veterinarians in the field, will allow to improve the application of the innovative regenerative medicine based on MSCs in veterinary practice.

S-22

INTRACAVITARY AND INTRALESIONAL TREATMENT WITH MICROFRAGMENTED ADIPOSE TISSUE LOADED WITH PACLITAXEL IN CANINE TUMORS – SAFETY, FEASIBILITY AND PRELIMINARY CLINICAL OUTCOME

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Abstract

Safety, feasibility and preliminary clinical outcome of intracavitary and intralesional treatment of canine tumors using micro-fragmented adipose tissue loaded with Paclitaxel (PTX). Five privately owned dogs with spontaneous tumors were selected. Micro-fragmented adipose tissue obtained with Lipogems® (LG) procedure, that allow a minimal manipulation without the use of enzymatic procedures, was taken from dog's lumbar flanks, loaded with PTX (LG-PTX) and cryopreserved without cryoprotectant. LG was loaded with PTX at 1 mg/ml, mixed for 30 minutes and then administrated. Treated tumor types included mastocytoma, squamous cell carcinoma, mesothelioma, meningioma and high grade glioma. In the case of solid tumors, LG-PTX was injected directly into the lesion, while mesothelioma was treated intracavitary by ultrasound (US)-guided injection (thorax, abdomen). The owners of the dogs were informed about the procedure, signed an agreement for the therapy and accepted a post-mortem examination. Results were analyzed by clinical status, blood workup, radiographics, US and magnetic resonance imaging. Eventual adverse effects and clinical outcomes were monitored up to 1 years. In all 5 dogs the procedure was safe, feasible and well tolerated. No major adverse reactions were registered. In the case of local tumors, the treatment managed to slow down the tumor growth, while in the case of mesothelioma it significantly decrease the amount of intracavitary fluid formation. In previous studies we showed that adipose tissue-derived MSCs, once exposed to high doses of the chemotherapeutic drug Paclitaxel (PTX), are able to uptake and release PTX in sufficient amounts to inhibit tumor proliferation, demonstrated both in vitro and in vivo. The drug-releasing mesenchymal cells (Dr-MSCs), were shown to largely reduced lung methastasis in laboratory animals, following intravenous administration, increasing significaly their survival time. However, the use of Dr-MSCs was limited to intravenous injection. In our study, the aim of intracavitary and intralesional chemotherapy was to expose tumors to higher concentrations of chemotherapic agent while reducing drug exposure to the rest of the body. Furthermore, LG elements warranty a slow, long lasting release of PTX. Even though this techniques, particularly intralesional therapy, is not considered standard of care in veterinary oncology, in some cases, where further surgery and/or radiation therapy is not possible, it might be an alternative to preserve patient comfort, function, and survival. As such, they warrant further studies to better define their safety, efficacy, and applications for use. Furthermore, several authors consider dog as a very important spontaneous animal model to evaluate new therapies, because many canine pathologies have high similarity with the human ones. Also in this case the dog should be considered important model, able to provide clinical data with a very strong incidence for human translation, by overcoming the traditional preclinical studies in rat and mouse.

S-23
CAN I TREAT MY PET WITH STEM CELLS? CLINICAL EXPERIENCES AND RELATED ISSUES

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Abstract

After a general review of the literature on the use of Mesenchymal Stem Cell (MSC) therapies in small animal pathologies, the Author reports his personal experiences.
The various methods of application of MSC in small animals are discussed taking into consideration not only clinical situations, but also environmental ones.
Clinical cases are reported for treatment of cutaneous, articular, muscular and tendon lesions.
Furthermore, new fields of applications, particularly autoimmune and spinal cord diseases, are listed.
Finally, particular attention is given to the practical problems faced by the veterinary practitioner who intends to apply these therapies in dogs, cats and horses. Integrative and alternative procedures are proposed.

S-24

MSCS IN HORSES: AN OVERVIEW ON THEIR APPLICATION IN ORTHOPEDIC INJURIES

Raffaello Ciampoli

Practitioner, Siena

Abstract

S-25

MSCS IN THE MANAGEMENT OF MUSCULAR INJURIES IN BOVINE PATIENTS

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Abstract

Three cases of bovines suffering from gastrocnemius injury and treated with MSCs will be presented.
A Chianina calf, suffering from spastic paresis, had undergone tibial nerve resection. In such cases a postoperative rest would be necessary; this is why a mere leap caused the rupture of gastrocnemius muscle which, meanwhile, had become fibrotic because of spastic paresis. Three MSC injections were performed.
An eight-year-old Holstein Frisona cow suffered from gastrocnemius injury, caused by a fall during the parturition at the end of November 2016. When we examined the animal we could diagnose lameness and swelling of the hindlimb. The animal was treated with 2 injections of MSCs.
A three-day-old Chianina calf presented an injury in its hindlimb. The first injection was carried out after twenty days and it was repeated after thirty and sixty days.

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POSTER ABSTRACTS

P-01

PHOTOACTIVATION OF NANOPARTICLES DELIVERED BY MESENCHYMAL STEM CELLS INDUCES OSTEOSARCOMA CELL DEATH IN VITRO

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Abstract

OBJECTIVE: The specific tumour-homing property of Mesenchymal Stem Cells (MSCs) has been successfully exploited to deliver therapeutic agents to the tumour site in several preclinical studies. Furthermore, recently published results from the first 6 patients enrolled in the TREAT-ME clinical trial have demonstrated the safety of genetically engineered MSCs implanted in advanced gastrointestinal adenocarcinoma patients.
The use of MSCs as delivery agents of photoactivatable nanoparticles could overcome the limitations of standard protocols for OS treatment, which currently fail in 30% of cases.
The aim of the present study was to test the in vitro uptake of photoactivatable nanoparticles (NPs) by MSCs and the efficiency of the system in inducing cell death of osteosarcoma (OS) cells upon photoactivation. The results from this in vitro evaluation, performed on MSCs from multiple donors, will set the basis for a preclinical study where the best performing MSC line and the optimal NP-loading and photoactivation parameters will be used in an orthotopic mouse model to evaluate the efficacy of this approach.
MATERIALS AND METHODS: Ptl@PMMA NPs were produced by adding tetrasulfonate aluminium phthalocyanine (Ptl) to an aqueous solution of positively charged poly-methylmethacrylate (PMMA) core-shell nanoparticles. Human MSCs were isolated from the bone marrow of multiple donors. Cell isolation and characterization data were used to set a score system. The five top performing lines were tested for the ability to internalize and retain the NPs, along with their migratory properties. Cell death upon photoactivation (PDT) was evaluated in vitro, both on a monolayer co-culture of MSCs and OS cells and in 3D multicellular spheroids, generated via cell suspension in ultralow attachment plates.
RESULTS: Selected MSCs showed an internalization rate of Plt@PMMA >95%, which did not alter the cell viability and migratory capacity. Ptl@PMMA-MSCs co-cultured in monolayers with human OS cells (SaOS-2) efficiently triggered cell death upon PDT. In particular, AnnexinV/PI and CalceinAM/EthD staining showed 70% cell death in the co-culture system. These results were also validated by the metabolic assay Alamar Blue. Interestingly, in a 3D co-culture of the OS cell line MG63 and Ptl@PMMA-MSCs, a marked reduction of the viability after PDT (<5%) was observed through ATP content measurements. A massive cell necrosis induced by photoactivation of the Ptl in the whole spheroid mass was confirmed by CalceinAM/EthD staining.
CONCLUSION: We demonstrated that photoactivation of MSCs loaded with Ptl@PMMA NPs can successfully induce OS cell death in vitro. These results encourage the continuation of the study to demonstrate the specific targeting of Plt@PMMA loaded MSCs to the tumour stroma and the efficacy of PDT against OS in the in vivo model

P-02

LONG LASTING ANTI-INFLAMMATORY ACTIVITY OF HUMAN MICRO-FRAGMENTED ADIPOSE TISSUE

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Abstract

OBJECTIVE: Since of the high content of MSCs, human micro-fragmented adipose tissue (MFAT) obtained from fat lipoaspirate (LP) through a minimal manipulation by using an innovative system device - named Lipogems® (LG),has been successfully usedin aesthetic medicine as well as in orthopedic and general surgery. Interestingly, in orthopedic diseases, this ready-to-use adipose tissue cell derivative seems to have a prolonged time efficacy even upon a single shot injection intoostio-arthritic tissue. We here investigated the long term survival and content of MSCs as well the anti-inflammatory activity of LP and its derived MFAT secretoma in vitro, with the aim to better understand a possible in vivo mechanism of action.

MATERIALS AND METHODS: The MSCs content of human MFAT and LP specimens obtained from 17 donors was investigated by immunohistochemistry during a long term culture. The analysis of cytokines/chemokines and growth factors secreted by MFAT and LP specimens in the conditioned medium (CM) was performed byusing multiplex bead-based assays based on xMAP technology. The presence of Microvescicles (MVs) in the secretome was analyzed by TEM. The macrophage cell line U937 was used to evaluate anti-inflammatory properties of MFAT and LP derived CM. U937 migration, the release of RANTES and MCP-1 cytokines and the expression of the adhesion molecules (AM) VCAM-1 and ICAM-1 were investigated by chemotaxis assay, ELISA Kit and flow cytometry respectively.

RESULTS: We found that the content of MSCs number in MFAT decreased more slowly if compared to LP specimens during a long term culture. The analysis of cytokines and growth factors secreted in the CM of MFAT and LP specimens was similar during the first week of culture. In contrast, the total amount of cytokines secreted by LP decreased much more rapidly than those produced by MFAT during long term culture. Interestingly, after long periods of culture (up to 30 days) TEM analysis demonstrated that MFAT also maintain a significant capacity of MVs secretion. Similarly, while the MFAT-CM continue to strongly inhibits inflammatory function of U937 during a long term culture, the inhibitory activity of LP-CM on U937 it was drastically reduced.

CONCLUSION: We conclude that MFAT produced by LG device is an effective preparation with a long lasting anti-inflammatory activity probably mediated by a long term survival of their MSCs content that in a continuous way release of a combination of ctytokines that affect several macrophage functions involved in inflammation.

P-03

A COMPARISON BETWEEN TWO OPEN SYSTEMS FOR THE EX-VIVO EXPANSION OF MESENCHYMAL STROMAL CELLS

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Abstract

OBJECTIVE: Clinical scale expansion of cord-blood derived mesenchymal stromal cells (CB-MSCs) can be achieved by using automated closed systems, conventional T-flasks and multilayer flasks. The ultimate goal is the reduction of working times, incubator space, contamination risk, costs and increase of expansion efficiency. We have expanded cord blood derived CB-MSCs in the Corning Cellstack (2-STACK-1,272 cm2) and in the Corning HYPERFlask vessel (1,720 cm2).

MATERIALS AND METHODS: The HYPERFlask uses a gas permeable film to provide gas exchange between the cells, culture medium and the atmosphere and is designed to be filled entirely with medium. The Cellstack has 0.2μ m pore nonwettable membrane sealed directly to the caps to allow gas exchange. CB-MSCs were inoculated in each flask at concentration of 4,000 cells/cm2 and expanded in D-MEM+10% human platelet lysate. After 7 days of culture, cell phenotype, population doubling, cell fold expansion media use and overall handling were evaluated.

RESULTS: Expanded CB-MSCs phenotype was CD73+, CD90+, CD105+, CD45- and CD34-. PD values were comparable (7.3 for HYPERFlask and 7.2 for Cellstack) as well as the cell fold expansion (13.7 and 13.6 for HYPERFlask and Cellstack respectively). After expansion, we have obtained 108±59×106 and 71±18×106 cells in one HYPERFlask and Cellstack respectively. The media volume requested to produce the same amount of cells in the two vessels was comparable.

CONCLUSION: The two devices offers several advantages compared to conventional T-flasks. One HYPERFlask corresponds to ten and a Cellstack to seven conventional T-175 flasks allowing cell seeding and harvesting in a single step with a significant reduction of the working time and the incubator space required. HYPERFlask is easy to handle and emptying can be done by pouring but the cell seeding requires more attention to reach a uniform distribution of cells in the layers.

The use of HYPERFlask and Cellstackfor the ex-vivo expansion of MSCs has several advantages compared to conventional T-flasks reducing contamination risks, working times and increasing overall process optimization.

P-04

EQUINE ADIPOSE-DERIVED MESENCHYMAL STEM CELLS: PRELIMINARY RESULTS OF MICRO-VESICLES AND GROWTH FACTORS SECRETION

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Abstract

OBJECTIVE:
Mesenchymal Stromal Cells (MSCs) have shown therapeutic potential in regenerative medicine. Increasing evidence suggests that their effects depend upon the release of extracellular vesicles (EVs) containing growth factors (GFs) and different species of genetic material. In response to changes in the surrounding environment, MSC adapt EVs content to fine-tune their biological effects.
In order to speculate on possible applications in regenerative medicine, the aim of the study was to evaluate the ability of equine adipose-derived Mesenchymal Stromal Cells (e-AdMSC) to modulate the release of EVs and to adjust their GFs cargo in response to normoxia and hypoxia conditions.
MATERIALS AND METHODS:
AdMSCs were isolated from equine adipose tissue. The cells were cultured at 37°C, 5% CO2 with 20% O2 and 3% O2, respectively, for normoxia and hypoxia conditions. After reaching a confluence of 80%, the supernatants of cells were collected and centrifuged at 2500g for 20 min at 4°C to remove floating cells and debris, and subsequently ultra-centrifuged at 100.000g for 70 min at 4°C. Each pellet obtained was re-suspended in 100 µ L of phosphate buffer and analyzed by Transmission Electron Microscopy (TEM) at CUME (University Centre of Electron Microscopy, University of Perugia). At the same time, one aliquot was stored at -80 °C for ELISA tests. Once assessed the presence of EVs through TEM, ELISA assays were performed to quantify the GFs secretion in the aforementioned two conditions. Tests were preliminary performed on samples diluted 1:10 and 1:20. The equine and human kits were applied according to the manufacturer's instructions to detect TGFß1, PDGF-BB, IGF1, IGF2, EGF, MET, VEGF.
RESULTS:
In both samples, TEM analysis demonstrated the presence of EVs ranging in size from 30 to 1000 nm and displaying a variable electron density. No differences were observed between normoxic and hypoxic samples in terms of number and morphology of EVs. Preliminary results obtained by ELISA tests, demonstrated that MET and IGF1 content were higher under hypoxia condition. On the other hand, the content of TGFß1 and IGF2 resulted higher under normoxia condition. PDGF-BB and EGF showed equal level in both samples. VEGF content was less than the detection range.
CONCLUSION:
In the present study we isolated EVs from AdMSCs and measured the amount of specific GFs.
Morphological analysis evidenced the presence of a mixture of exosomes and microvesicles as revealed by the wide dimensional range. However, no differences in EVs ultrastructure were detected. Preliminary results revealed that the stressful condition of hypoxia does not seem to be relevant for the secretion of specific GFs. In particular, according to Madrigal et al. 2014. MET and IGF1demonstrated higher level under hypoxia. Moreover, differences between equine and human kits were revealed for TGFß1. Further studies will be necessary to investigate our hypothesis.

P-05

RENAL REGENERATIVE POTENTIAL OF EXTRA-CELLULAR VESICLES DERIVED FROM BONE MARROW MESENCHYMAL STROMAL CELLS

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Abstract

OBJECTIVE: Extracellular vesicles (EVs) derived from human bone marrow mesenchymal stromal cells (MSCs) promote the regeneration of kidneys in different animal models of acute kidney injury (AKI) in a manner comparable with the cells of origin. However, due to the heterogeneity observed in the EVs isolated from MSCs, it is unclear which population is responsible for the pro-regenerative effects.

MATERIALS AND METHODS: Two different EV populations were separated by differential ultracentrifugation steps (10K population enriched with microvesicles and 100K population enriched with exosomes). The two vesicles population were characterized by flow cytometry, Nanosight, Electron microscopy and for protein and RNA content. Moreover, pro-regenerative effect of 10K and 100K vesicles populations were tested in vitro and in vivo.

RESULTS: Only the exosomal-enriched population induced an improvement of renal function and morphology comparable with that of the total EV population. The exosomal fraction (100K) exerted a pro-proliferative effect on murine tubular epithelial cells, both in vitro and in vivo. Analysis of the molecular content of the different EV populations revealed a distinct profile. The 100K population, for instance, was enriched in specific mRNAs (CCNB1, CDK8, CDC6) reported to influence cell cycle entry and progression; miRNAs involved in regulating proliferative/antiapoptotic pathways and growth factors (hepatocyte growth factor and insulin-like growth factor-1) that could explain the effect of renal tubular cell proliferation. On the other hand, the EV population enriched in microvesicles (10K) was unable to induce renal regeneration and had a molecular profile with lower expression of proproliferative molecules.

CONCLUSIONS: The different molecular composition of exosome- and microvesicle-enriched populations may explain the regenerative effect of EVs observed in AKI

P-06

MESENCHYMAL STROMAL CELLS IN PROGRESSIVE SUPRANUCLEAR PALSY: AN IN VITRO EVALUATION OF PUTATIVE DISEASE MECHANISMS IN A NON-NEURAL COMPARTMENT.

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Abstract

OBJECTIVE: Progressive Supranuclear palsy (PSP) is a sporadic and progressive neurodegenerative disease linked to the accumulation of Tau protein. No effective therapy is available to date. Since bone marrow (BM) mesenchymal stromal cells (MSC) have recently gained attention as a potential therapeutic tool for their anti-inflammatory, anti-apoptotic and trophic properties, we recently performed a phase I clinical study (NCT01824121) with the aim to evaluate the safety of intra-arterial infusion of autologous BMMSC in patients with PSP. A parallel in vitro study was also conducted to investigate MSC biology and function in this pathologic context.

MATERIALS AND METHODS: BM from twenty-four PSP patients was directly seeded in alphaMEM (Macopharma) supplemented with 10% FBS (Life Technologies). The cells were cultured until reaching the plateau phase and their morphology and growth rate in culture were compared to BMMSC derived from to age-matched healthy donors. To asses the genomic stability and DNA damage

karyotype and comet assay were evaluated during in vitro propagation. For karyotype analysis, colcemid was added to cells in culture to arrests mitotic cycle in metaphase. Chromosomes were stained with quinacrine mustard and at least twenty metaphases were analyzed using Ikaros program (Metasystems). We also performed comet assay, that combines alkaline DNA gel electrophoresis with fluorescence microscopy to visualize migration of DNA strands from individual agarose-embedded cells. Next, we analyzed BMMSC for gene and protein expression of different isoforms of Tau, to investigate whether the typical pathogenetic hallmark of PSP neurons can be found also in MSC. Finally, in order to evaluate if the "culture fragility" of PSP MSC may effect their neuroprotective and neurotrophin secretion capacity, we tested them in an in-vitro model of dopaminergic damage: a human neuroblastoma cell line (SKNB) was exposed to 6-hydroxy-dopamine (6-OHDA) and than co-cultured with BMMSC using a transwell system. Culture media was collected at different time points to quantify the secretion of brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF) by ELISA. SKNB viability was evaluated using the colorimetric MTT assay.

RESULTS: We found that PSP BMMSC were different in terms of morphology, cell growth, and DNA structure (karyotype and comet-assay) in comparison to age-matched healthy controls. Next, PSP BMMSC expressed the total Tau but not its particular 4-repeat and 3-repeat isoforms (4R and 3R). Finally, PSP BMMSC are still able to rescue 6-OHDA damaged neural cell lines and to synthesize and secrete BDNF and GDNF.

CONCLUSION: These original results provide new insights into the pathogenesis of neurodegenerative disorders and on the involvement of non-neural cellular compartments in this context, while still support autologous MSC potential effectiveness in neurological diseases.

P-07

PLATELET DERIVATIVES DRIVE CARTILAGE REGENERATIVE RESPONSE BY ACTIVATING MSC-LIKE PROGENITOR CELLS

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Absract

Injury of articular cartilage often leads to pathogenesis of osteoarthritis, a tissue degenerative disease extending from the articular surface to the subchondral bone. Therapies to regenerate cartilage defects are still challenging due to poor tissue reparative potential, and tissue engineering-based strategies using autologous chondrocytes are hindered due to no self-renewing cell expansion in vitro. In this context, the evidence of the presence of a progenitor cell population within articular cartilage has been considered promising for future clinical application. Furthermore, in last years the use of platelet-derived products in orthopedics has significantly increased with beneficial outcomes.

In our hands, platelet derivatives trigger a strong mitogenic stimulus on articular chondrocytes in vitro, allowing the maintenance of their chondrogenic redifferentiation potential. Platelet products have also beneficial effects during the repair process of a damaged tissue, since they induce a cascade of events leading to cell recruitment, transient activation of inflammation and its subsequent resolution.

Here, we report that differentiated chondrocytes can recover some features of stemness when expanded in vitro in presence of platelet derivatives.These effects could explain their success in the clinical treatment of articular cartilage lesions and pathological states.

OBJECTIVE:In this work we aim to demonstrate that platelet products can recruit and activate MSC- like progenitor cells resident in cartilage that are characterized by the expression of Nestin, an intermediate filament protein considered as marker of proliferating and migrating adult stem cells in several tissue and up-regulated in response to injury or pathology.

MATERIALS AND METHODS: Human cartilage biopsies were regularly cut into several slices, which were further divided into equal parts to be treated in culture with Fetal Bovine Serum (FBS) or Platelet Lysate (PL) for the time necessary to assess cell migration. The remaining bioptic tissue was subjected to repeated enzymatic digestions, and the released articular chondrocytes were cultured in medium supplemented with FBS or PL.

In concurrence with culture passages, Colony Forming Units (CFU) assay and determination of growth kinetics were performed on cells subjected to different treatments. Their phenotype in each condition were characterized on the basis of a specific cell surface markers by flow cytometry and the expression of Nestin and typical chondrogenic markers were evaluated by real-time PCR analysis and immunofluorescence staining. To test multilineage differentiation capacity, cells were exposed to chondrogenic, osteogenic and adipogenic stimulation in vitro. Furthermore, chondrogenesis was investigated also in vivo by implantation of cell-seeded scaffold in nude mice.

RESULTS: The treatment of cartilage slices with PL in vitro induces the spontaneous migration of a cell population showing a fibroblastic-like morphology, whereas no cells emerge from tissue cultured in FBS. This population has a high proliferative rate and shows the capacity to form colonies, as well as mature chondrocytes grown in culture with PL, contrarily to mature chondrocytes cultivated in FBS. Cells obtained in PL show an up-regulated expression of the adult stem cell marker Nestin, that is undetectable in mature chondrocytes. Although there are differences in the expression of Collagen I and II in comparison with mature cells, they are able to differentiate into hyaline-like cartilage both in vitro and in vivo.

CONCLUSION:Our data suggest that PL treatment can activate and bring out stem/progenitor cells resident in adult articular cartilage. These cells might be involved in the maintenance of tissue homeostasis and therefore represent a promising tool for reparative strategies of cartilage defects.

P-08

IN VITRO INHIBITION OF HUMAN MESOTHELIOMA CELLS BY PACLITAXEL-RELEASING MESENCHYMAL STROMAL CELLS

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Abstract

OBJECTIVE: Malignant pleural mesothelioma (MPM) is a rare fatal asbestos-related malignancy originating in the mesothelial cells of the pleura. A platinum-based doublet containing a third-generation antifolate is the front-line standard of care whilst there are no approved second-line treatments for MPM which remains a disease setting to test the efficacy of new therapeutic agents. Recent studies have demonstrated that mesenchymal stromal cells (MSCs) are able to migrate specifically to tumors and their metastatic sites when administered intravenously. Our group previously demonstrated that PTX-primed MSCs provide a new approach for cancer therapy. It also has been reported that a patient with MPM not responsive to standard first-line treatment, had good response after treatment with nanoparticle albumin-bound paclitaxel and carboplatin, suggesting that new delivery strategies might improve the clinical management of this disease. Aim of this study was to evaluate the in vitro antiproliferative effect of PTX-releasing MSCs on human MPM.

MATERIALS AND METHODS: Bone marrow mesenchymal stromal cells (BM-MSCs) were loaded with pemetrexed (PMX) and paclitaxel (PTX) according to a standardized procedure. The primed cells (BM-MSCs/PMX and BM-MSCs/PTX) were lysed and tested in vitro by a 7 days antiproliferation MTT assay against NCI-H28 mesothelioma and a panel of tumor cell lines: T98G (glioblastoma multiforme), U87MG (likely glioblastoma), UPCI-SCC-154 (squamous oral carcinoma) and MOLT-4 (acute lymphoblastic leukemia).

RESULTS: The in vitro anticancer activity of pure PTX was significantly higher than that of PMX against all the cell lines tested; in particular, on NCI-H28, the activity of PTX was 14.7 times higher than that of PMX. No inhibitory activity was exerted by the lysate of BM-MSCs loaded with PMX (BM-MSCs/PMX), whereas a significant antitumor activity was produced by the lysate from PTX-loaded BM-MSCs (BM-MSCs/ PTX). Based on these data, we calculated that a single drug loaded BM-MSCs cell can delivery about 0.15 pg of PTX. By homing 106 BM-MSC/PTX into 1 cm3 of tumor mass, we can estimate a PTX delivery near to the concentration of 150 ng/ml corresponding to a value 26 times higher than IC50.

CONCLUSION: These preliminary results demonstrated the good activity of PTX against a mesothelioma cell line growth in vitro. Furthermore, also PTX-loaded mesenchymal stromal cells can successfully inhibit the in vitro proliferation of human mesothelioma cells. Further studies and in vivo testing are required to confirm these data that could open the way to improve the mesothelioma therapy by apply a cell mediated system for drug delivery.

P-09

IN VITRO INHIBITION OF ORAL SQUAMOUS CELL CARCINOMA BY DRUG LOADED GINGIVAL MESENCHYMAL STROMAL CELLS (GINPA-MSCS)

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Abstract

OBJECTIVE: Mesenchymal stromal cells (MSCs) are widely studied for both their regenerative potential and their anti-inflammatory/immunomodulatory capacity. Furthermore, the capacity of MSCs to uptake and release drugs without any genetic manipulation was recently exploited, and their ability to home pathological tissues suggested a possible use for drug delivery into the tumor microenvironment. Here we explore the effect of drugs-loaded MSCs against squamous cell carcinoma (SCC), a malignant neoplasm which constitute the 95% of the head and neck carcinoma primarily originated from lip-oral cavity, pharynx and larynx.
MATERIALS AND METHODS: MSCs isolated and expanded from gingival papilla (GinPa-MSCs) were loaded with anticancer drugs such as Paclitaxel (PTX), Doxorubicin (DXR) and Gemcitabine (GCB) according to a previous standardized procedure. The capacity of drugs loaded GinPa-MSCs to release the drugs was evaluated in vitro against a tongue squamous cell carcinoma cell line (SCC154) by using a MTT assay.
RESULTS: GinPa-MSCs are less sensitive to the tested drugs than cancer cells and efficiently incorporate and release a sufficient amount of active drugs causing a dramatic inhibition of squamous cell carcinoma cell growth in vitro. The evaluation of the activity of drug released by cells in the conditioned medium, confirmed that after 24 hours, the cells released 62.6% of PTX, 91.8% of GCB and 100% of DXR. The conditioned medium collected from untreated GinPa-MSCs did not affect SCC cell proliferation. Furthermore, the presence in the condition medium of some cytokines such as hIL-6, hIL-8, hVEGF, hGROa, hSCGF-b, at concentration ranging from 13 to 24 ng/ml, does not seem to have any inhibiting or enhancing effect on SCC154 proliferation.
CONCLUSION: The collection of autologous GinPa-MSCs is an easy procedure that can be performed without any discomfort for the patient and it can be easily expanded to obtain large number of mesenchymal stromal cells also for banking. The use gingival MSCs as a carrier for delivery anti-tumor molecules is of great interest also for the anatomic homology of the source with the type of cancer originating into the oral cavity. This means that drug loaded GinPa-MSCs with anticancer agents, GCB or PTX in particular, could be suitable for possible in situ advanced cell therapies (e.g.,in pre-cancerous stage, as adjuvant therapy to reduce the toxicity related to systemic treatment, to prevent cancer recurrences after surgical treatment, to reduce the radiation treatment effects, etc.

P-10

NON-ENZYMATIC AUTOMATED CLOSED-CYCLE PROCESS TO ISOLATE MESENCHYMAL STROMAL CELLS FOR DRUG DELIVERY APPLICATIONS

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Abstract

Mesenchymal stromal cells (MSCs) can be easily isolated from several human organs and tissues and, because of their self-renewing capacity and multipotent differentiation properties; they are important tools for treating immune disorder and for tissue repair. To ensure reproducibility, efficacy and safety for clinical uses, these procedures have to be compliant with Good Manufacturing Practices (GMP). Techniques for harvesting and processing human adipose tissue have rapidly evolved in the last years, and, among them, Lipogems represents an innovative approach to obtain micro-fragmented adipose tissue in a short time, without expansion and/or enzymatic treatment. The aim of this study was to assess the presence of Adipose Tissue Mesenchymal Stromal Cells (AT-MSCs) in the drain bag of the device after the standard processing, and the possibility to use these cells as drug delivery agents.
We used a Lipogems Processor Prototype (PLG-P) to wash and process the lipoaspirate in a standardized condition. The waste fluid was analyses for the presence of mesenchymal stromal cells that were expanded in flask, characterized by CD analysis and differentiation ability. The ability of these cells to uptake Paclitaxel was evaluated by using a previous standardized procedure. The activity of paclitaxel released by drug loaded AT MSCs were tested in vitro against cancer cells.
We found that the drain bag contain red blood cells and oil residues but also significant amount of single isolated cells easy to expand and having the typical characteristics of AT-MSCs. These AT-MSCS were also able to uptake Paclitaxel and then release it in amount active in vitro against the proliferation of a human pancreatic cancer cell line.
Our findings suggest the possibility to develop a new integrated in-line device, implying neither enzymatic treatment, nor centrifugation, which can isolate and expand AT-MSCs in an intrinsically closed system, highly simplifying GMP compliance effort. This system could be used to obtain AT-MSCs not only for regenerative purposes, but also for drug loading procedure, as a new and innovative cell mediated drug-delivery system.

P-11

BIOBANKING, THE EXPERIENCE OF THE IRCCS GALEAZZI ORTHOPAEDIC INSTITUTE

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Abstract

OBJECTIVE: Our goal was to establish a tissue biobank of biological materials to be used in specific studies aimed at defining the pathophysiological mechanisms and/or new diagnostic/prognostic markers and/or therapeutic targets of pathologies of the musculoskeletal system. Cryopreservation is a fundamental step to preserve the integrity of the sample itself, which will be used later in future projects. The main pathologies under forthcoming investigation are primary and secondary tumors of the bone, osteoarthritis of the hip and knee, infections of prosthetic hip and knee implants requiring revision.

MATERIALS AND METHODS: The study includes the collection of biological specimens from patients undergoing biopsies and surgical procedures in the routine diagnostic and/or therapeutic path, following informed consent sign. Only waste biological material, including whole blood, serum, and plasma exceeding from the routine analysis, are being collected. Blood and its derivatives are stored at -80°C while tissue samples at -150°C, in nitrogen vapors. We developed an IT platform for biobank database and giving back a QR-coded label recording all the info of the sample. Each label is glued on the vial of the corresponding sample. At the end of the process all the cryoboxes are stored in a proper repository (BioRep, located in San Raffaele Hospital, Milano) with the best quality standards, after a dedicated transport.

RESULTS: The first patient was enrolled in May 2017 and in 8 months we collected samples from 87 patients from one equipe, for a total of 672 vials (last update, 09/02/18): 532 were stored at -80°C, the other 140 were stored at -150°C. Among tissue samples, 36 specimens were from bone metastasis, 34 from bone primary tumors (including Ewing Sarcoma, Chondrosarcoma, Plasmocitoma, Giant Cells Tumor, Chordoma, and Ostheosarcoma) and 70 from control tissues. Regarding blood and derivatives, samples of whole blood (130), plasma (129) and serum (273) have been collected and stored.

CONCLUSION: Our system is now flowing smoothly and we are planning to include other medical teams soon. Indeed, we will receive the first sample of synovia and synovial fluid in the coming weeks: synovia or synovial fluid can represent a source of Mesenchymal Stromal Cells (MSCs). Furthermore, we are working on IT platform version updating, according to the ongoing needs.

P-12
REGAIN, CLARIFYING REGENERATIVE MEDICINE

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Abstract

OBJECTIVE: Using cutting edge non-invasive methods based on platelet growth factors and cell concentrates derived from adipose tissue, blood and bone marrow, it is possible to adequately stimulate the intrinsic healing capacity of organs and tissues. Many companies have recently proposed different disposable devices for the treatment of musculoskeletal diseases and hundreds of thousands of treatments have been already performed worldwide. However, the objective data about the efficacy of these methods are rather poor, especially when compared to the quality of data available for traditional therapies. The main goal of REGAIN, a new platform located in the IRCCS Galeazzi Orthopaedic Institute, is to clarify the effectiveness of different devices in a number of clinical applications through the collection of longitudinal data. This standardized way of data collection will allow not only for the interpretation of the outcomes, but also for the establishments of proper indications for each given treatment and for the search of a possible correlation between the biochemical/biological properties of the product and the clinical outcomes.

MATERIALS AND METHODS:

Patients of any age who suffer from orthopedic diseases (articular, tendon, muscle, bone) and diseases of the maxillary, periodontal and endodontic bones are regularly seen and treated at REGAIN. The REGAIN team, composed of clinicians and researchers, developed a dedicated app based software to collect pre-, peri- and post-procedural data. The first phase is composed by the "assessment surveys" that allow for the collection of background data of both the pathology and lifestyle of the patients administering them international evaluation scales specific for each given condition. The second phase includes the "procedure surveys" that are aimed to collect data concerning the technical aspects of the treatment such as the device used, the injected volume and the biochemical/biological characterization of the final product. Finally, the third phase consists in collecting all the clinical data at different follow-ups. The patients receive an email containing a link redirecting to the same surveys they filled the day of the procedure.

RESULTS: from October 2017, 59 patients were treated at REGAIN (last update: 27/02/18). Twenty-one affected by OA were treated with a single injection of microfragmented adipose tissue in the knee (8 bilateral), and one in the hip. Thirty-seven patients, still affected by milder knee OA, were treated with three different devices for PRP (7 bilateral). A total of 74 procedures were then performed. The current short term follow up does not allow yet for any interpretation of the results. However, we could evaluate the functionality of the platform and the pretty high compliance of the patients in filling and sending back the surveys in the post-treatment period.

CONCLUSION: Despite the very numerous regenerative medicine treatments performed worldwide everyday, reliable data are still not enough and thus need to be produced. The software used at REGAIN has been specifically developed to generate a set of data crucial in the interpretation of regenerative medicine outcomes. Moreover, the REGAIN experience has increased the interest of other national and international centers, and thus we are creating a network of applied regenerative medicine centers sharing the same protocols and data collections system. This will allow the generation of aggregated data and the possibility to compare the data with a biggest pool, used as a benchmark.

P-13

THE RATIONALE FOR A RESIDENT PROGENITORS- AND MSCS-BASED APPROACH TO TREAT CARTILAGE DEGENERATION

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Abstract

OBJECTIVE: Articular cartilage (AC) is a specialized tissue with limited self-repair ability, which when affected by chronic, degenerative processes (e.g., osteoarthritis - OA) the presence of resident progenitors is not sufficient neither to induce repair nor to restore the tissue homeostasis. For this reason, the present study aims at evaluating the stemness features and the therapeutic paracrine activity of cartilage cells (CCs) obtained from osteoarthritic AC and to compare them with adipose-(ASCs) and bone marrow-derived mesenchymal stem cells (BMSCs), in order to identify the most suitable cell-based therapy to treat AC damage.

MATERIALS AND METHODS: CCs, ASCs and BMSCs were procured from eight (8) de-identified/consented OA donors undergoing hip arthroplasty. From these cells, colony forming unit ability, immunophenotype, multi-differentiation potential and stemness marker expression were systematically evaluated. The release of growth factors (GFs), inflammatory cytokines and expression of metabolic markers such as matrix metalloproteases (MMPs) and tissue inhibitors of metalloproteases (TIMPs) were quantified at basal level and after treatment with 1 ng/ml inteleukin-1 beta (IL-1β) mimicking an inflammatory environment.

RESULTS: CCs exhibit clonogenic potential sustained even after serial expansion; share similar immunophenotype and osteogenic potential (compared to ASCs and BMSCs); did not have adipogenic potential; but, following their physiological commitment, had the best chondrogenic performance. In basal conditions, CCs displayed the highest chondrogenic, anti-angiogenic and pro-mitogenic potential whereas MSCs showed the highest expression of anabolic TIMP1 and TIMP3. After inflammatory stimulation, CCs were the more responsive in terms of production of pro- and anti-inflammatory cytokines, whereas the release of GFs increased only in MSCs. The expression of the catabolic enzymes MMP1, MMP3 and MMP13 increased in all cells types without significant changes in the expression of TIMPs, except from a downregulation of TIMP3 in ASCs. The only anti-inflammatory cytokine significantly upregulated in all the analyzed populations was IL-1Ra.

CONCLUSION: Given their stemness features, chondrogenic commitment and basal paracrine activity, CCs proved to be the most specialized and promising cell type to be exploited as therapeutic targets, as well as promoters for cartilaginous tissue healing and homeostasis restoration. Finally, the cell priming method using inflammatory IL-1βshould be taken with caution. MSCs, and in particular ASCs, generated higher levels of chondrogenic mediators than CCs, but exhibited the most significant angiogenic response, detrimental for cartilage and like CCs, presented a general catabolic and pro-inflammatory behavior.

P-14

STEMNESS FEATURES AND METABOLIC RESPONSE TO INFLAMMATION OF CELLS OBTAINED FROM DIFFERENT REGIONS OF THE HUMAN INTERVERTEBRAL DISC

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Abstract

OBJECTIVE: resident progenitors may represent a potential reservoir of specialized cells for tissue repair and homeostasis re-establishment in degenerated intervertebral disc (IVD). However, the trophic, immunomodulatory and anti-inflammatory activities specifically of nucleus pulposus (NP), annulus fibrosus (AF) and endplate (EP)-derived cells and progenitors, to our knowledge, is lacking. Therefore, this study aims at characterizing disc cells for their stemness features, and to investigate their response to an inflammatory environment in terms of catabolic, anabolic, pro/anti-inflammatory mediators and growth factors production/secretion.

MATERIALS AND METHODS: NP, AF and EP cells from lumbar IVD of 8 de-identified/consented patients affected by discopathy were characterized during expansion for colony forming unit ability, immunophenotype, adipo-osteo-chondro-differentiation potential and the expression of stemness marker genes (NANOG, POU5F1). Moreover, after inflammatory stimulation with 1 ng/ml IL-1β, the release of pro/anti- inflammatory cytokines and the expression of VEGFA, TGFB1, MMP1, MMP3, MMP13, TIMP1 and TIMP3 were evaluated.

RESULTS: all the disc cells were able to form colonies and were negative for CD14, CD34, CD45, CD71, CD146 and positive for CD44, CD73, CD90, CD105, CD151, CD166 surface marker expression. No adipogenic differentiation was observed in any of them. On the contrary, all the cells showed osteo- and chondro-differentiation ability and they expressed NANOG and POU5F1, with a decrease in expression of these two markers in AF and EP cells during expansion. After inflammatory stimulation, all cells exhibited an increase in the expression of catabolic MMPs and angiogenic VEGF, while anti-catabolic TIMP3 and trophic TGFβ1 were found decreased. Moreover, AF cells seem to promote inflammation the most, followed by NP cells. On the contrary, EP cells resulted in the most responsive from a metabolic point of view, while exhibiting the most anti-inflammatory behavior. All disc cells reply with a significant increase of the direct IL-1β antagonist (IL-Ra) release.

CONCLUSION: in conclusion, all IVD cells showed stemness features, confirming the presence of tissue specific progenitors in the disc which can be stimulated for regenerative purposes. Among the three disc cell types, EP cells showed the most promising paracrine activity that can be therapeutically exploited when exposed to inflammatory conditions.

P-15
3D MULTICELLULAR SPHEROIDS: REGULARIZATION TIME FOR OBTAINING A HOMOGENEOUS MODEL

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Abstract

OBJECTIVE: 3D multicellular spheroids with homogeneous morpho-biological features are fundamental to obtain reliable data in biological experiments. This represents a compelling requirement especially for large-scale analysis, like High Content Screening (HCS). In this regard, the spheroidization time has been recently defined as the time required by freshly generated spheroids to lose their variability in sphericity. However, a wider definition of spheroids homogeneity can be attained considering other morphological parameters, such as the volume. Here, we widen the spheroidization time definition introducing the regularization time as the time required by spheroids to reach an asymptotic morpho-biological equilibrium point, ending drifts in sphericity and volume. In this work, we tested three generation systems to analyse initial morphological features and regularization time of the obtained spheroids.

MATERIALS AND METHODS:

Spheroids of Mesenchymal Stromal Cells have been generated using (a) the pellet culture method (PCM); spheroids of A549 cells using (b) a rotatory bioreactor (Synthecon Inc.) and (c) hanging drop plates (GravityPLUS, InSphero). For each generation system we chose an appropriate cell seeding density; additional densities have been tested with the hanging drop plates. The generated spheroids have been maintained in vitro for 30 days, during which we acquired brightfield images and computed the morphological features using AnaSP (http://sourceforge.net/p/anasp/) to determine the associated regularization times.

RESULTS:

We defined for the spheroidization time a threshold of 0.9 for sphericity and a Coefficient of Variation among volumes (CVv) of 0.20 for the regularization time. Since the bioreactor-generated spheroids strongly differed in the initial dimension, without pre-selecting volumes the 70% of the generated spheroids showed a 0.9 sphericity after 7 days, but never reached a 0.20 CVv. However, selecting at the beginning the spheroids with a volume of mean±std, a 0.20 CVv was achieved after approximately one week. 70% of spheroids generated with hanging drop plates showed a 0.9 sphericity after 7 days and a 0.20 CVv already one day after generation (i.e. no regularization time needed). More than 70% of the spheroids generated with PCM showed a 0.9 sphericity and a 0.20 CVv already one day after generation (i.e. no spheroidization and regularization time needed). Regarding the different cell seeding densities tested with the hanging drop plates, it is worth noting that all the generated spheroids evolved toward an equilibrium point of 400-micrometer diameter, which is the theoretical diameter for the origin of the necrotic core.

CONCLUSION:

These preliminary experiments show that each generation system is characterized by a different spheroidization and regularization time. Awareness of these times allows the operators to predict when the generated spheroids can be considered as a homogeneous model, and it is possible using them to obtain reliable and reproducible data in HCS experiments, accordingly.

P-16

BIOBANKING OF CANINE ADIPOSE-DERIVED MESENCHYMAL STEM CELLS (CAD-MSC) FOR CLINICAL APPLICATION: A PRELIMINARY STUDY OF THE EFFECT AFTER A 7 YEARS LONG CRYOPRESERVATION ON STEMNESS FEATURES

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Abstract

OBJECTIVE

MSCs have emerged as prominent candidate for cell-based therapies and tissue repair. They are easily isolated from many tissues, especially from adipose tissue with minimal risk to the donor and abundant yield per extraction volume. In dog, AD-MSCs have been successfully used in regenerative therapies for the treatment of disorders affecting bone and cartilage. However, clinical application requires a steady supply of viable and functional cells. Since MSCs can be cryopreserved and thawed for later clinical autologous use, the challenge becomes the optimization of a long-time freezing protocol that ensure the maintenance of the stemness features. In this preliminary study, the effects of a 7 years long cryopreservation using Dimethyl Sulfoxide (DMSO) and different concentrations of Fetal Bovine Serum (FBS), were evaluated on cAD-MSCs.

MATERIALS AND METHODS

cAD-MSCs were isolated from visceral adipose tissue and cultured. At each passage, cells were collected and resuspended at density of 1x106 cells/cryovials, in cryoconservation media composed of 10% DMSO and increasing percentages of FBS. Cells were frozen by decreasing -1°C/min until -80°C and then were transferred to liquid nitrogen tank for long time storage. Thawing of cells was performed after 7 years. cAD-MSCs were counted by trypan blue and plated in T25 flasks with DMEM low-glucose medium and 10% FBS. Viability was also tested by MTT performed after thawing and after 24h of culture. The colony–forming efficiency on plastic was assayed. Thawed cAD-MSCs were inducted to differentiate towards adipogenic and osteogenic lineages by appropriate culture media.

RESULTS

Trypan blue and MTT assays showed no significant difference from fresh cells in viability of the cAD-MSCs cryopreserved with high FBS percentages (>50%) as opposed to those stored with low serum percentages. Proliferation of cryopreserved cAD-MSCs was comparable to that of fresh cells. The adipogenic and osteogenic differentiation potential was maintained after a long-term storage of 7 years but decreased with in vitro passages.

CONCLUSION

This study demonstrated that cAD-MSCs require high serum concentration for a long-time cryopreservation with DMSO. They maintain the characteristics of stemness after thawing. However, it is essential to cryopreserve them at an early passage in order to maintain a reservoir of healthy and efficacious cells, as the number of cAD-MSCs and their differentiation potential decrease with passages. Biobanking of stem cells would make available a ready off-the-shelf supply of cells suspension for autologous transplant, as well as allow better timing of therapy. Cryopreservation of cAD-MSCs can reduce the constant need for fresh tissues and provide a source of reference cells that can be also used for scientific study.

P-17

ITALIAN CELL FACTORIES PANORAMA FROM THE SUSTAINABILITY POINT OF VIEW: PRIVATE AND PUBLIC, TWO OPPOSITE MODELS

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Abstract

In the past decade, the technological revolution in biomedical research has led to the development of novel clinical procedures and biological medicinal products for human use collectively known as advanced therapy medicinal products (ATMPs). To provide harmonized rules to regulate ATMPs, a specific European Regulation was introduced; this led to the creation the cell factories, Good Manufacturing Practices (GMP) compliant pharmaceutical facilities specialized in development, validation and production of ATMPs. Maintenance of cell factories, as well as development, translation and manufacturing of ATMPs in GMP have impressive costs. To understand how these structures survive the huge amount of money required every year to perform their standard activities, we carried out an analysis on the 15 national cell factories, considering their ATMPs, subdivided in four categories (somatic cell, tissue engineering, not-engineered therapeutic vaccines and gene therapies), as well as their business models. Such analysis unveiled two major sustainability strategies: one is based on direct revenues coming from the sale of products and patents and it's the strategy adopted by private cell factories, while the second one relies on networking activities with hospitals and foundations and it's the strategy adopted by public cell factories. Nature of products also changes. While private structures point their R&D activities toward gene therapy, publics are more oriented toward non-engineered therapeutic vaccines. In any case, research remains the common driving force in both the type of cell factories.

P-18

INVESTIGATING THE IMMUNOREGULATORY POTENTIAL OF HUMAN ADIPOSE-DERIVED STROMAL CELL SECRETOME: PROTEOMIC ANALYSIS OF THEIR CONDITIONED MEDIUM IN COMPARISON TO HUMAN DERMAL FIBROBLASTS' ONE

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Abstract

OBJECTIVE: Human Adipose-derived Stromal Cells (hASCs) possess a well-recognized immunoregulatory potential. Nowadays it is widely accepted that this beneficial action is mainly mediated by released bioactive factors and extracellular vesicles (EVs) rather than depending on cell-cell contact. In this perspective, we recently demonstrated the efficient performance of hASC secretome (Conditioned Medium, CM) in contrasting different inflammatory pathologies, both on an in vitro model of TNFα-induced osteoarthritis and in vivo on diabetic neuropathy. Interestingly, CM deriving from human Dermal Fibroblasts (hDFs) never exerted any effect. Through a proteomic approach we analysed the presence of several soluble molecules in the CM of hASCs and hDFs, focusing on the factors differentially expressed between the two populations that may be involved in hASC immunoregulatory properties.

MATERIALS AND METHODS: Primary cultures were isolated from the subcutaneous fat or the dermal connective tissue of 5 female donors subject to plastic surgery, following the procedure approved by IRCCS Galeazzi Orthopaedic Institute (PQ 7.5.125, version 4). CM was collected from confluent hASCs or hDFs cultured for 72 hours in starving conditions (absence of FBS). Samples were then concentrated through Amicon Ultra-15 Centrifugal Filter Devices with 3 kDa cut-off (Merck Millipore), reducing the initial volume of 43±4 folds for hASCs (n=3) and 46±8 folds for hDFs (n=3). CM protein concentration was assessed through Bradford Protein Assay (Bio Rad) and differences in the protein content between hASC and hDF CM were identified through nanoflow liquid chromatography–tandem mass spectrometry (nLC-MS/MS), following standard procedures. The statistical analysis of the complete dataset of identified and quantified proteins was performed by Student's t-test, followed by hierarchical clustering analysis using MeV software.

RESULTS: 1208 factors were identified in the CM of the two cell types, 976 of which were quantified. 36 secreted proteins resulted significantly different between hASC and hDF CM, with 15 uniquely or preponderantly present in hASC CM. Among these, several molecules with known immune functions, such as CCL2 and Metrnl, were recognized. Interestingly, between the proteins solely released by hDFs, the factor CB4 of the complement system is listed. A full description of the results will be presented in the poster.

CONCLUSION: From a clinical perspective, the choice of CM over cell therapy presents substantial benefits, e.g. in terms of safety and handling. With this study, we lay the basis for the characterization of the factors -released as soluble components or vesicular cargos- involved in the immunoregulatory properties of hASC CM, in the light of giving a solid rationale to its use as novel pharmaceutical in the treatment of inflammatory diseases.

P-19

TREATING SKIN WOUNDS IN VETERINARY MEDICINE: CONVENTIONAL VERSUS INNOVATIVE THERAPIES

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Abstract

The skin is the largest organ of mammals. The loss of skin integrity may induce important dysfunctions. For superficial wounds, the endogenous healing mechanisms in combination with traditional wound care are sufficient to achieve functional repair. Differently, in larger wounds, chronic wounds or deep ulcers it is difficult to obtain the restitutio ad integrum and fibrosis and/or scar tissue develops [1,2].

OBJECTIVE: The aim of this study was to verify the efficacy of conventional and innovative topic treatments on skin regeneration after a provoked lesion, induced experimentally in sheep. To achieve this goal different types of investigations (clinical, molecular, histological, immunohistochemical) were performed.

MATERIALS AND METHODS: Six skin lesions (4x4cm) were surgically created on the back of six healthy adult sheep; every single wound was destined, in a randomized way, to different conventional (Acemannan gel, Manuka honey, Connettivina) or innovative treatments such as allogeneic mesenchymal stem cells (MSCs) and gas ionized plasma [3]. The sixth wound was the placebo.

Biopsies were collected with a surgical punch (0,6x0,6 cm) at time T0, T15 and T42 days. Lesions were clinically evaluated considering the presence and colour of wound fluid, the state of hydration, the wound surface/surroundings and other parameters. Histological examinations considered crust formation, re-epithelization and epidermal thickness, dermis edema and areas of mature and immature granulation tissue, acute and chronic inflammation. Immunohistochemistry for evaluation of the inflammation, vascularization, and cell proliferation was performed using CD3, CD20, MHCII, von Willebrand factor (vWF) and KI67 antibodies. Furthermore, real time-PCR investigated transcripts of genes such as Collagen 1α1 (Col1α1) and hair Keratin (hKER).

RESULTS: Clinically, the lesions treated with MSCs and gas ionized plasma healed more rapidly respect to the conventional treatments and placebo. Reduced bacterial load and macerations were observed in wounds treated with gas ionized plasma compared with placebo and traditional therapies. MSCs significantly up-regulated the expression of Col1α1 and hKER with the formation of new hair follicles while gas ionized plasma induced cell proliferation. Wound closure was observed between day 21 and day 28 in all sheep and after 42 days of treatment all wounds were healed, presenting a 100% of re-epithelialization.

CONCLUSION: To sum up, innovative therapies led to surprising results in terms of quality and rapidity, during the regeneration of mammalian skin. Non-conventional therapies showed better results than the conventional ones. The gas ionized plasma treatment plays a key role in the first phase of the wound healing decreasing dermal and subcutaneous inflammation and stimulating neovascularization and cellular proliferation. On the other hand, the MSCs had a role in the remodelling phase (Col1α1) and in the hair growth (hKER). Further studies should involve a combined usage of these two therapies.

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P-20

EXPRESSION AND IN VITRO MODULATION OF CXCR4 RECEPTOR IN CANINE ADIPOSE-DERIVED MESENCHYMAL STEM CELLS FOR THE ENHANCEMENT OF CELL HOMING

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Abstract

The CXCR4/SDF-1 axis plays an important role in the migration and homing of mesenchymal stem cells (MSCs) towards injured and inflamed tissues. It has been demonstrated that extensive in vitro passaging causes a loss of the expression of CXCR4 protein and gene in human and murine MSCs.

The aim of the present study was to analyze the expression of CXCR4 receptor during in vitro expansion (P0-P3) of canine MSCs and to modulate it, by synergistic pre-conditioning of hypoxia and basic fibroblast growth factor (bFGF). MSCs were isolated from subcutaneous adipose tissue (cAT-MSCs) of adult Beagle dogs (n=3; 3-5 years old, 9-12 kg) and cultured under standard conditions (5%CO2, 37°C). Cells at P0 and P3 were used for FACS and qPCR analysis of CXCR4 expression. Transwell migration assay was performed to assess the role of CXCR4/SDF-1 axis in the migration of cAT-MSCs (P3) towards different chemotactic gradients (10% FBS and 100ng/ml SDF-1) with or without pre-treatment with 5 g/ml AMD3100. CXCR4 expression was modulated by culturing P3 cells in hypoxia (2%O2) or normoxia (21%O2), and stimulating them in both oxic conditions with 1 and 5 ng/ml bFGF for 24h. The modulation of CXCR4 was assessed at protein level by FACS and immunocytochemistry assay, and at gene level by qPCR analysis. Data were analyzed with paired Student's t test and results were considered statistically significant for $P<0.05$.

CXCR4 protein and gene expression decreased during in vitro expansion ($P<0.05$). The statistically significant difference ($P<0.05$) among migrated cells towards different chemotactic gradients demonstrated the role of CXCR4/SDF-1 axis in cAT-MSCs migration. The gene expression of CXCR4 and SDF-1 in cells cultured in hypoxia with bFGF supplementation increased compared to control groups ($P<0.05$). Immunohistochemistry assay revealed increased cytoplasmic expression of CXCR4 protein following bFGF supplementation in hypoxic conditions, however the surface expression of CXCR4 protein analyzed by FACS, remained low in all conditions.

The present study describes for the first time the role of CXCR4/SDF-1 axis in cAT-MSCs migration and presents an in vitro modulation system for the up-regulation of CXCR4 expression as a target for the enhancement of canine MSCs migration. The described pre-conditioning system can have a relevant translational character for the increment of human and murine MSCs migration.

P-21

WHARTON'S JELLY MESENCHYMAL STROMAL CELLS AS FEEDER LAYERS SUPPORT THE EX VIVO EXPANSION OF CORD BLOOD-DERIVED CD34+ CELLS MIMICKING A HEMATOPOIETIC NICHE

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Abstract

OBJECTIVE: Umbilical cord blood (UCB) has been widely used for hematopoietic transplantation as source of hematopoietic stem and progenitor cells (HSPCs). Nevertheless, the low number of UCB-HSPCs represents the main challenge for a successful engraftment. Ex vivo HSPCs expansion may be a valid strategy to overcome this limit. Several attempts to optimize the expansion have been reported, including the use of mesenchymal stromal cells (MSCs) as feeder layer. Wharton's jelly (WJ) of the umbilical cord (UC), known to have hematopoietic supportive function is a rich source of MSCs (WJ-MSCs) that may be an ideal candidate for creating a stromal feeder layer, mimicking an hematopoietic-like niche. Here, we investigated the role of WJ-MSCs in supporting ex vivo UCB-HSPCs expansion either by cell-to-cell direct contact (DC) or by the action of paracrine factors.

MATERIALS AND METHODS: WJ-MSCs and HSPC/CD34+ were isolated from human UC and UCB respectively. Both cell types were phenotyped by flow cytometry analysis. The UCB-CD34+ cells were cultured for 5 days in DC system, or separated from the WJ-MSCs by transwell system (TS), as well as in presence of WJ-conditioned medium (WJ-CM). The number and the frequencies of CD34+ cells were measured by ISHAGE method. Immunocytochemical (ICC) analysis was performed using primary antibodies for the fibronectin, CD34 and vimentin. CFU assays were performed in methylcellulose semi-solid medium. The secretome analysis was performed by reversed-phase HPLC separation and online mass spectrometric detection (HPLC-MS/MS).

RESULTS: We found that the DC system sustained a greater UCB-CD34+ cells expansion degree (15.7 + 4.1-fold increase) with respect to the other conditions. Moreover, in DC system we evidenced two different CD34+ cell populations with different phenotypic and functional characteristics,one floating and one adherent to WJ-MSCs. In particular, DC system supported the expansion of both multipotent (CD34+/CD38-) and lineage-committed (CD34+/CD38+) hematopoietic progenitors. The former were significantly more represented in the adherent cell fraction than in the floating one (18.7 + 11.2% vs. 9.7 + 7.9%). Short-term CFU assays showed that the adherent HSPCs were able to generate much more immature colonies (CFU-GM and BFU-E large) with respect to the floating cells. ICC analysis confirmed the in vitro interactions between WJ-MSCs and HSPCs. Moreover the phase-contrast microscope analysis highlighted pseudopodia-like formations in the HSPCs suggesting their migratory activity toward the WJ-MSCs layer. The secretome analysis identified 16 proteins exclusively expressed in the cell-to-cell contact system analogous to those found in other hematopoietic niches.

CONCLUSION: WJ-MSCs may support the ex vivo UCB-CD34+ cell expansion either by cellular contact and by paracrine signals, even if the former was found to be the elective system, in particular for expansion of primitive HSPCs. Moreover, the WJ-MSCs produced an intricate fibronectin-rich network that favors the interaction between the two cell types.

P-22

HUMAN PLATELET LYSATE IMPROVES WJ-MSCS RECOVERY, PROLIFERATION AND CELL FUNCTIONS USEFUL FOR WOUND HEALING

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Abstract

OBJECTIVE: Mesenchymal stem cells (MSCs), including Wharton's Jelly-derived MSCs (WJ-MSCs) are promising candidates for regenerative medicine applications, as for wound healing. Human platelet lysate (hPL) has been widely used either as cell culture supplement alternative to FBS, and as rich source of factors that could accelerate the wound healing process. Here, we exploited the effect of hPL to optimize the WJ-MSCs isolation protocol and to enhance the ability of these cells to proliferate and migrate.

MATERIALS AND METHODS: All the experiment were conducted in culture medium supplemented with 5% hPL derived from apheresis. Human WJ-MSCs were isolated from umbilical cords by migratory ability toward the plastic surface.WJ-MSCs were characterized by flow cytometry (CD73, CD90, CD105, CD45, CD34, CD31, HLA-DR). The rate of proliferation was measured by the population doubling time. The cell migration rate was determined by the number of WJ-MSCs able to cross a transwell system. Adipogenic and osteogenic differentiation were performed.

Scratch assay was performed on a WJ-MSCs monolayer evaluating the percentage of wound closure after 16 hours.

RESULTS: In four different umbilical cords we found that the number of WJ-MSCs harvested after 14 days from umbilical cord pieces increased of 2.7± 0.8 fold in medium supplemented with 5% hPL compared to 10% FBS. Interestingly no significative differences were observed in the number of WJ-MSCs harvested within the same cord from, distal versus central pieces, either cultured in presence of hPL or FBS. The cell-population doubling time was lower in presence of hPL than in medium with FBS ($p=0.0051$). The hPL did not change the ability of WJ-MSCs to differentiate in adipocytes and osteoblasts.

In addition the ability of hPL to improve WJ-MSC wound healing properties was tested in in vitro assays.We found that hPL significantly enhanced the migration capacity of WJ-MSCs compared to FBS (91,8 % ± 4,9 vs 2,15 % ± 0,7 respectively, $p<0,0001$).

The scratch assay confirmed that WJ-MSCs, in presence of hPL, were able to reduce the wound surface area of 87,3 % ± 6,5 compared to 28,5%± 4,9 scratch closure found in presence of FBS ($p<0,0001$).

CONCLUSION: In this study we showed that an integrated culture platform based on hPL improves both WJ-MSCs recovery and cell proliferation and enhances some of the main functions crucial for the wound healing process.

P-23

OPTIMIZATION OF MESENCHYMAL STEM CELL SEEDING PROTOCOL ON SILK FIBROIN-COATED MICROCARRIERS: DESIGN OF EXPERIMENT APPROACH

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Abstract

OBJECTIVE: Among the most innovative therapeutic approaches in the orthopedic field, an increasing interest is directed towards regenerative medicine, especially on Mesenchymal Stem Cells (MSCs) based therapies in one-step intraoperative procedures. However, for a better targeting of MSCs at the injury site, a cell delivery system is often needed. The aim of this study was to develop and optimize a cell delivery protocol able to guarantee a satisfactory targeting of MSCs, accordingly to the minimal requirements for one-step intraoperative procedure (minimal manipulation of cells and rapid time of the procedure). In this context, the suitability as a cell delivery system of lyophilized fibroin-coated alginate microcarriers (L-FAMs) was evaluated by a Design of Experiment (DoE) approach.

MATERIALS AND METHODS: Starting from a cell-seeding protocol previously developed, the seeding time, the presence of an intermittent dynamic culture, the stirring speed of seeding and the final volume of cell suspension were selected as specific parameters to evaluate in order to identify the final optimized procedure. The DoE approach proposed thirteen cell-seeding protocols, representing the minimal conditions to investigate, each one composed of different combinations of the parameters mentioned above. All the seeding protocols were tested on human Adipose derived stem cells - ASCs (n=3), and the cell adhesion rate on the surface of L-FAMs was evaluated qualitatively by the Live&Dead assay and quantitatively by DNA quantification and cell viability measurements. The outcomes of these experiments were examined by a multivariate analysis that predicted an optimized cell seeding protocol, identified as the best performing one. The predicted protocol was then validated on both ASCs (n=3) and bone marrow derived stem cells - BMSCs (n=3).

RESULTS: Among the 13 protocols tested, those provided of the intermittent dynamic culture of cells resulted to be the more performing ones. In particular, the highest seeding efficiency was obtained by combining the intermittent dynamic cell culture with the stirring speed of 10 rpm and a seeding volume of 400 µl.

Based on these evidences, the DoE prediction identified the protocol composed of the intermittent dynamic cell culture, with a final volume of 400.2 µl, a stirring speed of 12.3 rpm and a seeding time of 85.6 minutes as the most performing protocol. Both the qualitative and the quantitative analysis showed a high adhesion rate of cells, homogenously arranged on the surface of L-FAMs.

CONCLUSION: The DoE approach was useful to discriminate between parameters in term of their influence on the outcome, intended as cell adhesion rate and cell homogeneity on the surface of the delivery system. Even though further optimizations are essential, the present protocol may represents a starting point for the introduction in the clinic of L-FAMs as carriers in autologous one-step applications.

P-24

SPHINGOSINE-1-PHOSPHATE EXERTS PLEIOTROPIC ACTIONS ON AMNIOTIC-FLUID STEM CELLS

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Abstract

OBJECTIVE: In this study, we investigated the effects of Sphingosine-1-phosphate (S1P) on human amniotic stem cells (HASCs). S1P is a bioactive sphingolipid metabolite that influences stem-cell pluripotency, differentiation, mobility, and regulates immune functions. Specifically, S1P can increase proliferation, survival, and differentiation of several stem cells, including human embryonic stem cells; yet, the effects of S1P on HASCs are still unknown.
MATERIALS AND METHODS: This study was performed on a recently-identified stem-cell subtype named fast HASCs (fHASC), present in human amniotic fluid. These cells are characterized by a fast doubling time, have the ability to differentiate into several cell lineages and mediate immunoregulatory functions. Specifcally we studied migration, proliferation, differentiation and immunoregulatory functions of fHASCs in the presence of absence of various concentrations (i.e. 10 nM) of S1P in vitro.
RESULTS: We found that fHASC stimulation with S1P potentiated their migratory and proliferative activity in vitro. Notably, short fHASC exposure to S1P enhanced their differentiation towards multiple cell lineages, including adipocytes, osteocytes and endothelial cells, an effect that was associated with downregulation of the main transcription factors involved in the maintenance of a stem-cell undifferentiated state. A specific crosstalk between S1P and tumor growth factor β1 (TGF-β1) has recently been demonstrated. We found that fHASC exposure to S1P in combination with TGF-β1 promoted the expression of the immune regulatory pathway of indoleamine 2,3-dioxygenase 1 (IDO1). In addition, human peripheral blood mononuclear cells, co-cultured with fHASCs treated with S1P and TGF-β1, expanded regulatory T-cells, via a mechanism requiring IDO1.
CONCLUSION: We demonstrate that S1P activates multiple effects in fHASCs leading to increased migratory activity, promotion of cell differentiation toward multiple lineages and immune-regulatory functions. Most important result was the occurrence of a crosstalk between S1P and TGF-β1 in fHASCs, leading to specific induction of the immune regulatory enzyme IDO1, which was in turn instrumental for in-vitro generation of human Treg cells. The implications of such findings are of interest as S1P may represent an interesting means of potentiating selected functions of stem cells.

P-25

AMNIOTIC FLUID AND STEM CELL DERIVED ESOVESICLES MODULATE PATHOGENIC IMMUNE RESPONSES IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Abstract

OBJECTIVE: Multiple sclerosis (MS) is a complex disease of immune dysfunction and neurodegeneration that affects 2.3 million people worldwide and shows a female preponderance, although late pregnancy is the one condition that most profoundly mitigates MS symptoms. Serum exosomes, released by specific cell types during pregnancy, modulate the immune and central nervous systems and contribute to pregnancy-associated suppression of experimental autoimmune encephalomyelitis (EAE), an induced model of MS.

The aim of our study was to control the inflammatory response and immune dysfunction in EAE, by administration of exovesicles (EVs) derived from Human Amniotic Fluid-derived Stem Cells (HASCs) or directly isolated from Amniotic Fluid. These may represent a novel cell-free regenerative therapeutic approach that can potentially mitigate immune dysfunction and promote remyelination.

MATERIALS AND METHODS: Amniotic Fluid and HASCs were obtained from human amniotic fluids of 16–17-week pregnant women (aged 35–40 years), who underwent amniocentesis during routine prenatal diagnosis. EVs from HASCs or amniotic fluid were collected by centrifugation at 100,000xg for 60 minutes at 4°C. EV pellet were washed with PBS and administrated in female C57/B6 mice immunized with myelin MOG 35-55 peptide on day 0 and treated intravenously with two doses of EVs or vehicle at day 3 and 12 after immunization. Mice were scored daily for clinical signs of EAE. Moreover we evaluated the EV content for proteins, lipids, RNA and micro-RNA expression.

RESULTS: We found that vesicles from HASCs and from amniotic fluid were able to prevent autoimmune responses in EAE, suppressing inflammatory cytokines and promoting immunoregulatory effects. Moreover, we found that treatment with the two different types of exovesicles, significantly reduced disease severity in EAE, relative to controls. Specifically, treatment with EVs reduced neurological deficits and suppressed Rorc, IL-17 and IL-4 in brain lymphnodes (BLN), while increased the percentage of regulatory T cells (Treg-Foxp3+) cells. In addition, treatment with EVs during EAE, promoteed a significant increase of the immune regulatory indoleamine 2,3-dioxygenase 1 (IDO1) mRNA.

CONCLUSION: Our findings unravel the immunosuppressive effects of both exovesicles secreted from HASCs and isolated from amniotic fluid in a model of EAE. Taken together, the immunomodulatory effects observed from exosomes warrant further exploration into the active components mediating the proposed paracrine effects. The initial evidence strongly suggests that HASC-derived exosomes are the active therapeutic entities, and in this regard our study serves as an opportunity to develop biological therapeutic that harnesses the immunomodulatory and protective properties of stem cells.

P-26
CHARACTERIZATION OF NEUROBLASTOMA DERIVED-MSCS IN CHILDREN

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Abstract

OBJECTIVE. Over the last decade, a growing body of literature has highlighted the importance of the tumour microenvironment for the progression and prognosis of different types of cancer. In particular mesenchymal stromal cells (MSCs) have been proposed to promote tumor progression by interacting with tumor cells and other stroma cells in the complex network of microenvironment. The purpose of this study was to characterize MSCs isolated and expanded from tumoral tissue of newly diagnosed neuroblastoma (NB), being the most frequent neoplasm during infancy. MATERIALS AND METHODS. Residual material, for histological analysis obtained from 7 pediatric patients, was used as starting material. NB-MSCs were isolated and in vitro expanded following standard culture procedure, after tumor tissue mechanical disruption and collagenase type II incubation. NB-MSCs were characterized for morphology, phenotype and expression of Sox2, Nanog, Oct3/4, CD117 and O4 by flow-cytometry, proliferative (cPD) and differentiation capacity towards osteogenic and adipogenic lineages. Moreover, the ability of cells to modulate the immune response such as inhibition of phytohemagglutinin (PHA) activated peripheral blood mononuclear cells (PBMCs), NK and cytotoxic T cell function will be evaluated. RESULTS. NB-MSCs were plastic adherent, displayed the typical spindle shape morphology and showed a proliferative capacity superimposable to healthy BM-MSCs. NB-MSCs in vitro expanded showed typical cell surface expression, being positive for CD73, CD90, CD105 and HLA-I and negative for CD34, CD45, CD14, CD31 and HLA-DR. NB-MSCs showed the expression of stemness markers (Sox2, Nanog, Oct3/4) among which a NB stemness marker (CD117) and a differentiation marker (O4). The evaluation of NB-MSCs in vitro differentiation revealed a similar osteogenic potential to BM-MSCs but the lack of adipogenic differentiation capacity. All NB-MSCs reached senescence phases at a median passage of P7 (range, P5-P13). CONCLUSION. Our results show that MSCs isolation from pediatric neuroblastoma tissue is feasible and that NB-MSC can be propagated under standard conditions. NB-MSC represent a population similar, but not identical, to BM-MSC, loosing adipogenic differentiation capacity. Thanks to their multiple properties such as self-renewal, plasticity and ability to modulate immune response as well as strong tropism to tumors, we believe that the characterization of tumor microenvironment derived-MSCs is crucial to understand tumor development and progression

P-27

HUMAN ADIPOSE STEM CELLS INDUCED TO OSTEOGENIC DIFFERENTIATION BY AN INNOVATIVE HYDROXYLAPATITE HYBRID SCAFFOLD

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Abstract

OBJECTIVE: In this study we investigated human adipose stem cells (hASCs) for biocompatibility, osteoconductivity and osteoinductivity effects of an innovative hydroxylapatite hybrid scaffold made from granular hydroxylapatite (Pro Osteon®200) and microfibrillar collagen (Coll/HA).

MATERIALS AND METHODS: In hASCs culture that were grown on this scaffold, cellular cytoskeleton organization, cellular morphology and cell viability were observed. In addition the scanning electron microscopy (SEM) analysis was also performed. Gene expression of ECM, adhesion molecule, and osteogenic genes were evaluated by Q-PCR Array technologies. Osteocalcin, osteopontin, ALP and FAKP Tyr397 proteins expression were detected in hASC. The hASCs biomineralization was evaluated as osteogenic markers.

RESULTS: The cytoskeleton architecture of hASC seeded on biomaterial was well organized. hASC expression of CLEC3B, LAMB3, ITGAM, ITGA3, LAMA2, ITGB5, COL6A2, SELE, COL6A1, and SPP1 genes was up-regulated, whereas expression of CDH1, KAL1,CLEC3B LAMA3, CNTN1, ITGA2, and MMP3genes was downregulated. In hASC culture mRNAs of 22 genes of the ossification process/pathway, i.e. CSF 2/3, SP7, SPP1, TNFSF11, BMPR1B, BMP1/2, BGLAP, IGF1, NOG, RUNX2, TGFB1, EGFR, FGFR1/2, VDR, TWIST1, SOX9, ALPL, IGF1R, COL1A1 were upregulated compared to the control. The hASC culture expressed the osteogenic proteins such as ALP, osteocalcin, and osteopontin). In addition the mineralized matrix was present in hASC culture.

CONCLUSION: The hASC cultures has been tool to evaluate the biocompatibility, osteoconductivity and osteogenic characteristic of biomaterial employed in maxillofacial surgery. Our data demonstrate that the innovative scaffold provides the ideal microenvironment in which hASCs adhesion, morphology, and proliferation, are enhanced, while inducing the up-regulation of osteogenic genes with improvement in matrix mineralization and cell viability.

P-28

MESENCHYMAL STROMAL CELL SENESCENCE IN ABDOMINAL AORTIC ANEURYSM

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Abstract

OBJECTIVE: Abdominal aortic aneurysm (AAA) is a common degenerative vascular disorder associated with sudden death due to aortic rupture. Strong risk factors for AAA development are smoking, advanced age and male sex. The current clinical approaches are (1) to monitor aortic dimensions and (2) to perform an open or endovascular surgical repair when the aortic diameter has attained sufficient expansion, condition that predispose to a high likelihood of aortic rupture. Although several studies have identified many potential mechanisms involved in AAA pathogenesis, a clear depth understanding is still lacking and further studies are needed to facilitate development of effective therapies.

Recent discoveries have demonstrated the presence of mesenchymal stromal cells (MSCs) in human aortic layers. These cells possess high proliferative capacity and potential to generate endothelial, smooth muscle, hematopoietic and mesenchymal cell progeny. Nevertheless, any defect of the proliferation and/or the differentiation process of vascular stem cells may determine the development of human vascular diseases.

The aim of this study was to demonstrate the presence of senescent MSCs residing in human abdominal aortic wall, which could have a role in the AAA pathogenesis.

MATERIALS AND METHODS: MSCs isolated from healthy (HAA - MSCs) or aneurysmal abdominal aortas (AAA – MSCs) were characterized for their proliferation rate, ultrastructural morphology, senescence-associated β-galactosidase activity and differentiation properties.

RESULTS: Results showed low growth potential, high senescence-associated β-galactosidase activity, an increased cell surface area, a reduced amount of autophagic and lysosome vesicles in AAA – MSCs compared to HAA - MSCs, thus indicated a senescent phenotype in AAA MSCs.

CONCLUSION: Vascular wall-resident MSCs are deeply involved in the process of vascular remodeling, that is a dynamic and strictly regulated process of structural changes occurs as a result of a pathological vascular trigger. The presence of a senescent population of AAA MSCs in vascular wall could have implications in the genesis and progression of vascular diseases, such as AAA.

P-29

SERICIN, ALGINATE AND PLATELET LYSATE COMBINED IN A BIOMEMBRANE FOR THE TREATMENT OF SKIN ULCERS.

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Abstract

OBJECTIVE: Chronic skin wounds with different etiophatology heavily compromise the patients' life quality and represent a high and constantly growing cost for National Health Services. Platelet derivatives (Platelet Lysate, PL) has been used in clinical practice for reparative treatments of these pathologies, given their capacity to activate cell recruitment, proliferation, differentiation, as well as to regulate angiogenesis. We here report the development of a sericin/alginate sponge-like membrane as a new delivery system of PL to apply in tissue regeneration.
MATERIALS AND METHODS: Sericin (SS), Alginate (Alg) and PL were solubilized in distilled water and cast into freeze-dried molds. An in vitro test was performed to quantify the growth factor release from sponge/PL membrane by ELISA assay. The in vitro capability of SS and PL contained in the membrane to induce proliferation and protective effects against oxidative stress was performed on BMSCs and human fibroblast (hFB) by MTT assay. In vivo efficacy of sponge/PL was evaluated by skin mouse model. The lesions were treated applying sponge membranes, with or without PL, and covered by Tegaderm™. The animals were sacrificed at different times (3, 7, 14 and 21 days) and a histological investigation was performed.
RESULTS: In vitro results indicated that the release of growth factors and the biodegradation of sponge membrane occurred within 48 hours, a time optimized to burst the healing process. The presence of SS contributes and supports the effect of PL by controlling the release of growing factors.
The proliferation and the protection against the oxidative stress of the membrane, monitored on BMSC cells and hFB, were prevalently due to the presence of PL.
This in vivo analysis showed how Alg:SS:PL membrane led to a faster regeneration of the skin respect to the control one (Alg:SS). The inflammation phase occurred faster in treated lesion to evolve rapidly in the formation of granulation tissue and forming new collagen. This series of events showed a non-complete resolution of the healing process in control lesions, but the initial burst of chronic inflammation probably induced by the presence of PL as revealed in the treatments.
CONCLUSION: This work propose a handle membrane composed of biomaterial (alginate and sericin) that are biocompatible and have particular mechanical properties, in association with a powerful inductor of cell activation and proliferation that lead to complete skin regeneration. The SS contribute and support the effect of PL by controlled release of growth factors into the lesion.

P-30

PLATELET DERIVATIVES EFFECT ON THE PROLIFERATIVE STAGE OF MESENCHYMAL STEM CELLS AND CELL LINES: C-MYC PATHWAY ACTIVATION

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Abstract

OBJECTIVE: Platelet Lysate (PL), which contains platelet growth factors, is able to support isolation, expansion and proliferation of mesenchymal stem cells derived from different tissues (bone marrow, adipose tissue and amniotic fluids) and cell lines as widely reported in the literature. The PL effect on different types of cells and the molecular mechanisms involved in these processes remain poorly understood.

We focused our attention on a family of highly conserved proteins involved in different cellular mechanisms such as cell growth, proliferation and apoptosis, C-MYC's family. The three proteins of C-MYC gene have the same c-terminal part, differing in the N-terminal region due to an alternative translation at the start site: C-MYC1, C-MYC2 and c-MYCS differently expressed during cell growth.

MATERIAL AND METHODS: MSCs obtained from human mesenchymal tissue (BMSC, ADAS, Articular Condrocyte (hAC), human Osteoblast (hOB)), from fetal tissue (Amnotic Fluid Stem Cells (AFS)) and HeLa cell line, were isolated and cultivated in medium supplemented with 10% FBS and/or PL 5% (Lyset, Carlo Erba, Italia). Cells selected in PL or in FBS were compared to cells previously expanded in FBS and after stimulated for different time (5 min, 30 min, 24 hours) with PL. C-MYC1 and C-MYC2 expression was evaluated by immunoblot analysis of cell lysates, while cell proliferation was investigated by immunofluorescence analysis for the proliferative marker ki67 and Vybrant® Apoptosis Assay Kit. The effect of methionine on C-MYC1 expression in addition with PL or FBS was investigated.

RESULTS: BMSC, AD-MSC, hAC, hOB and AFS treated with PL showed a high proliferation rate respect to cultures treated with FBS; this effect didn't occur in Hela cell line. Cells isolated from tissue in FBS and then stimulated at different time with PL or isolated from the tissue directly in PL, expressed C-MYC1 isoform, which was not expressed by the cells cultured in the presence of FBS with statistically significant difference. The same cells expressed the C-MYC2 isoforms independently from the culture conditions. For the cell line, the results showed a scene slightly different with a modest expression of this isoform also in the standard condition (FBS).

Immunofluorescence analysis by Ki67 specific antibody indicated that MSCs showing C-MYC1 protein expression were in a proliferative stage. Considering that C-MYC1 is induced by methionine starvation, the expression of C-MYC1 was resulted in cell treated with PL and supplemented with methionine at increasing concentrations. The results showed that C-MYC1 protein levels in PL treated cells were unaffected by methionine.

CONCLUSIONS: All these experiments showed that PL was responsible for the induction of C-MYC1 isoform in primary cells culture and much more slightly in the cell line, demonstrating an alternative role of this gene in the cell proliferation. Further studies are in progress to understand how different activation of C-MYC1 in primary and cell line correlate to different cellular behaviour to PL stimulation.

P-31

HUMAN ADIPOSE-DERIVED STROMAL CELL SECRETOME BENEFICIAL POTENTIAL IN CONTRASTING OSTEOARTHRITIS

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Abstract

OBJECTIVE: Adipose-derived Stromal Cells (ASCs) possess a strong anti-inflammatory potential, which is mediated by a wide array of released bioactive factors. The use of conditioned medium (CM) instead of cells presents substantial advantages especially in terms of handling and safety. The purpose of this study is to investigate the potential of hASC secretome in contrasting osteoarthritis (OA), an inflammatory disease characterized by hypertrophic differentiation of chondrocytes (CHs) and extracellular-matrix-degrading proteases production.

MATERIALS AND METHODS: CM was collected from hASCs derived from subcutaneous adipose tissue, cultured for 72 hours in starving conditions. The CM was then concentrated trough Amicon Ultra-15 Centrifugal Filter Unit (Merck-Millipore) of about 46±10-folds (n=26). Hypertrophy was induced in vitro in human primary articular chondrocytes with 10ng/ml TNFα. CM (ratio 5:1, hASCs:hCHs) effect on cell proliferation (up to 9 days) was assessed by AlamarBlue. Gene and protein expression of MMPs and other hypertrophic markers were evaluated (24 and 72 hours) by RT-PCR, Western blot and multiplex immunoassay.

RESULTS: hCH proliferation was prompted (+40%) by a 9-day-treatment with 10 ng/ml TNFα, suggesting the induction of a hypertrophic growth status. Despite the lack of CM effect on CH proliferation, the conditioned medium treatment reverted the TNFα–induced significant increase in MMP3 and MMP13 expression (-50% and -30%, respectively), after 24 hours. The reduction in MMP13 protein expression was also evident after both 24 and 72 hours. Moreover, hASC conditioned medium inhibited TNFα-mediated osteocalcin release. The effect of CM on other OA and hypertrophic markers, in TNFα-inflamed CHs, is currently under investigation.

CONCLUSION: Our data reinforce the idea that ASC secretome might be considered a promising source of factors for future therapeutic applications in the OA treatment. The analysis of CM sub-components (EVs and soluble factors) and the comparison of ASC secretome with CM from cells lacking this therapeutic potential, might allow us to reveal the factors responsible for secretome beneficial action.

P-32

PRO-INFLAMMATORY PRIMING OF EQUINE ADIPOSE-DERIVED MESENCHYMAL STROMAL CELLS WITH IL-1β AND TNF-α AFFECTS THEIR INTERACTION WITH VASCULAR ENDOTHELIAL CELLS

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Abstract

OBJECTIVE: Mesenchymal Stromal Cells (MSCs) and endothelial cells closely interact in several biological processes such as inflammation, angiogenesis and tissue regeneration. The idea inspiring this study is that pro-angiogenetic potential of MSCs could be enhanced by an inflammatory microenvironment and possibly exploited in vivo. It is widely known, in fact, that a cell can be prepared by an initial stimulation (priming) to better answer to a second experience.

For that reason, we herein evaluated the effects of pro-inflammatory stimulation of equine adipose-derived MSCs (e-AdMSCs) on their interaction with equine umbilical vein endothelial cells (HoUVEC) in vitro.

MATERIALS AND METHODS:

E-AdMSCs from 4 horses were treated with recombinant equine 10 ng/ml TNF-α and IL-1β for 24 hours. The following tests were performed in order to evaluate e-AdMSCs- HoUVEC interaction.

Migration assay - HoUVEC were exposed to primed and control e-AdMSCs conditioned medium in a Transwell chamber coated with collagen. HoUVEC migrating through the porous membrane during the following 4 hours were fixed and counted.

Adhesion assay - e-AdMSC were seeded on a HoUVEC monolayer in a multiwell plate. After 15 minutes long exposure, the adherent cells were counted.

qRT-PCR - total RNA was extracted from treated and untreated e-AdMSCs and reverse transcribed using commercial kits. Modulation in expression was analysed by means of qRT-PCR with SYBR Green assays on the following genes involved in angiogenesis: ANGPT1 (angiopoietin 1), ANGPT2 (angiopoietin 2), TGF□1 (transforming growth factor □1), and VEGFA (Vascular endothelial growth factor A). Two reference genes were adopted: SDHA and HPRT.

RESULTS: e-AdMSCs adhesion to endothelial cells was significantly increased with respect to controls after pro-inflammatory stimulation. Additionally. primed e-AdMSCs demonstrated to promote migration of HoUVEC if compared to control.

Concerning qRT-PCR, significant differential expression was observed for VEGFA ($p = 0.001$) and ANGPT1 ($p = 0.018$) with log fold change close to 4 for both genes. TGF□ and ANGPT2 were found to be close to the significance threshold that could possibly be reached using a larger sample cohort.

CONCLUSION: The results of this study suggest that inflammatory stimulation significantly increases adhesion of e-AdMSC to HoUVEC and activate their migration. QRT-PCR, in addition, highlighted several aspects of the complex interactions occurring between e-AdMSCs and HoUVEC in an inflammatory environment: in particular, the up-regulation of VEGFA, ANGPT1 and TGF□ and the down-regulation of ANGPT2 depicts the early phases of angiogenesis characterized by proliferation and migration of endothelial cells.

In conclusion, MSCs preconditioning for therapeutic purposes could represents a realistic goal of cell therapy and an effective approach to shape the regeneration environment.

P-33

IN VIVO BIOCOMPATIBILITY AND EFFICIENCY OF SILK FIBROIN-COATED ALGINATE MICROCARRIERS AS DELIVERY SYSTEM OF ADIPOSE-DERIVED STEM CELLS

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Abstract

OBJECTIVE: The use of a carrier in cell-therapy applications may represent a fundamental issue that needs to further investigation to obtain a satisfactory final cell delivery at the target site.
Especially in the context of one-step intraoperative clinical applications, the use of a biocompatible and well performing cell-carrier is crucial. The present study investigated the use of silk fibroin-coated alginate microcarriers (FAMs), a type of microcarrier previously developed and studied in vitro, as a possible cell-carrier for orthopedic intraoperative one-step application of mesenchymal stem cells (MSCs), providing an in vivo characterization of their biocompatibility and suitability as cell carriers.
MATERIALS AND METHODS: To evaluate the biocompatibility of FAMs, 14 immunocompetent rats were used for the study. Two different volumes of un-seeded FAMs were subcutaneously implanted in the back of the animals: 7 animals were treated with a small volume of FAMs whereas the other 7 were treated with a big volume. At 7 days and 28 days post-surgery the animals were monitored by magnetic resonance imaging (MRI). The explants, obtained at 2 days, 8 days and 28 days, were used for the histological analyses. To evaluate the performance of FAMs as cell carriers, fluorescence-labelled human Adipose Stem Cells (ASCs) were seeded on FAMs and subcutaneously implanted in 10 athymic rats. Animals were monitored by MRI at 7 and 14 days and in vivo fluorescence (ivF) imaging (1 day - 3 days - 7 days - 14 days). The histological evaluation was also performed at 8 and 15 days after surgery.
RESULTS: A general consistent decrease of the size of the implants was revealed by MRI, directly proportional with the time after implantation, confirming the erosion and decomposition of the device. No adverse reaction was detected in the animals while evidences of tissue ingrowth within the implants were observed after 2 days from implantation. Hence, the obtained results positively highlight the biocompatibility of the developed carrier.
For the evaluation of FAMs suitability as a cell-carrier, the ivF analyses showed specific fluorescence emission at the implant sites in all the cell-seeded implants, showing a more intense fluorescent signal in FAMs than in the reference material. The histological analysis revealed that hASCs were located on the tissue surrounding the FAMs, rather than on the carriers themselves.
CONCLUSION: the outcomes of this study positively encourage the use of FAMs as a cell-delivery system since they turned out to be a biocompatible and effective device for the delivery of MSCs.
These results represent the premise to verify the efficacy of FAMs in pathological conditions, like osteoarthritis

P-34

F-TRACKER3D: TRACKING FLUORESCENT CELLS IN THREE DIMENSIONS

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Abstract

OBJECTIVE:
Nowadays, the usage of multicellular in vitro models and microscopy techniques optimized for 3D analyses is blooming in the biological laboratories. It has been extensively proved that 3D models represent a more reliable testbed than 2D ones for studying new drugs, cell-cell interaction, and cell-biomaterial compatibility. However, today just few software tools are available for analysing 3D images and none of them is a user-friendly tool for tracking cells. This prevents, for instance, the possibility to monitor the motility of cells seeded on a biomaterial scaffold. In order to provide biologists with a turnkey solution to perform analyses of cell motility in 3D, we designed Fluorescent Cell Tracker in 3D (F-Tracker3D), a user-friendly software tool for tracking individual fluorescent-tagged proteins and cells directly in 3D.
MATERIALS AND METHODS:
F-Tracker3D is an open-source software tool available at: https://sourceforge.net/p/ftracker3d. It provides automatic, semi-automatic and manual methods to track single particles/cells in time-lapse confocal/light-sheet fluorescent microscopy (LSFM) acquisitions. No image processing skills or prior knowledge of the sample is needed. For each tracked cell, F-Tracker3D automatically computes (x, y, z) for each time point t. It provides several measurements computed according to the spatial cell displacement, and an early version of the software was recently used to quantitatively monitor the cell displacement of Mesenchymal Stromal Cells (MSCs) loaded onto silk-fibroin coated alginate beads, so to evaluate the material's biocompatibility.
RESULTS:
By combining LSFM and F-Tracker3D we were able to demonstrate that the motility of MSCs onto fibroin-coated alginate scaffolds one day and three days after seeding significantly differs. One day after seeding, the MSC's shape is rounded, typically referring to cells not yet completely adherent to the surface material, and the cell motion is basically random. After 3 days, MSCs fully attach to the fibroin coating, showing a polarized orientation for motility with the aim of covering the entire surface of the beads. These data suggest that the analysis of cell-biomaterial interactions should be performed several days after cells seeding to permit a perfect cell adhesion and elongation along the surface of the desired biomaterial.
CONCLUSION:
In this work, we described F-Tracker3D, an open-source user-friendly software tool we developed for monitoring cells on 3D scaffolds. By using F-Tracker3D we were able to quantify the MSC's adhesion capacity on a silk-fibroin coated alginate material and detect differences in cell motility along the scaffold surface. Moreover, we were also able to produce a holistic view of the cell distribution during time. We therefore propose the combination of LSFM and F-Tracker3D as a tool to design tissue engineering products, as well as to perform quality control during validation of scaffolds to be used as medical devices.

P-35

ROLE OF CD44 IN THERAPEUTIC MESENCHYMAL STEM CELL-DERIVED EXTRACELLULAR VESICLES FOR JOINT DISEASES

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Abstract

OBJECTIVE: Mesenchymal stem cell (MSC) Extracellular Vesicles (EVs) provide new perspectives in the treatment of joint diseases. CD44, the hyaluronic acid receptor, is a key MSC marker on both cells and EVs and, in cells, its expression in vitro can be tuned up by hyaluronan exposure, a situation mimicking MSC injected in cavity of synovial joints in vivo. Hyaluronan also coats the surface of joint cell types, as chondrocytes and synoviocytes, possibly facilitating EVs docking and incorporation through CD44 binding. We investigated the role of CD44 as a crucial molecule for MSC-EVs homing, together with the hypothesis of increasing EVs uptake through modulation of CD44 abundance.

MATERIALS AND METHODS: MSCs were obtained from adipose tissue (ASCs, n=3). Fibroblast-like synoviocytes were isolated from synovial membrane (hFLS, n=3). ASCs were cultured in presence or absence of hyaluronan coating on cell culture surfaces. EVs were isolated by centrifugation and their number together with physical features scored by Nanosight technology. CD44 presence and modulation in EVs were scored by flow cytometry. EVs ability to fuse with hFLS was tested by FACS, ELISA and confocal microscopy. Role of CD44 in vesicles binding to hFLS was assessed by CD44-masking on EVs with a CD44-blocking antibody.

RESULTS: ASC cultured under hyaluronan exposure for 24h resulted in a 3-fold increase of CD44 expression. Similarly, released EVs showed a 1.6-gain in the abundance of the hyaluronan receptor. On the contrary, no differences in the number of secreted vesicles or in their physical properties were observed. ASC-EVs were able to efficiently fuse with hFLS, with vesicles having higher CD44 levels more prone (1.5-fold) to be incorporated. In a co-colture assay, hFLS resulted to embody up to 1700 EVs per day when 100,000 vesicles per cell were administrated, with no further increase at higher EVs:hFLS ratio. Interfering in CD44-hyaluronan interaction resulted in a decrease of EVs incorporation, suggesting this ligand-receptor synergy as a player involved in EVs docking.

CONCLUSION: This study opens the possibility that presence and modulation of CD44 amount in EVs secreting cells and released vesicles, as it may happen for MSCs injected in hyaluronan rich joint cavity, could direct EV/target recognition and docking. Therefore, future strategies to increase EV-associated CD44 expression, as hyaluronic acid supplemented media, could lead to promising approaches to improve vesicle uptake, especially in cells presenting an extensive hyaluronan coat.

P-36

SENSITIVITY OF MESENCHYMAL STROMAL CELLS TO A NEW IMIDAZOLE-BASED CATIONIC Pt(II) COMPLEX WITH HIGH in vitro ANTICANCER ACTIVITY

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Abstract

OBJECTIVE: Platinum drugs endowed with a novel chemical structure could offer an alternative therapeutic strategy, allowing to enlarge the spectrum of activity and to overcome the many drawbacks of the well-known cisplatin (CisPt) and its derivatives. Our group synthesised a new caPt(II)-complex that showed a very effective cytotoxic effect on triple-negative breast cancer cells and on cell lines partially resistant to cisplatin. In this study, we compared the in vitro stability of CisPt and caPt(II)-complex and their in vitro activity against human tumour cell lines. The drug sensitivity of Mesenchymal Stromal Cells (MSCs) and their ability to uptake and release the drugs was also investigated.

MATERIALS AND METHODS: AT-MSCs were isolated, characterized and expanded from human adipose tissue. Drug stability was studied following incubation at 37°C in complete cell culture medium both in the absence and in the presence of a monolayer of MSCs. The effect of CisPt and caPt(II)-complex was tested against mesothelioma (NCI-H28), glioblastoma (U87MG), pancreatic adenocarcinoma (CFPAC-1) and AT-MSCs by using a MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium) anti-proliferative assay in 96 multiwell plates. The amount of drugs incorporated and released by AT-MSCs drugs was evaluated by inductively coupled plasma mass spectrometry (ICP-MS).

RESULTS: We found that caPt(II)-complex had a high stability until 9 day of treatment while CisPt lost its anticancer activity after only 24 hours of treatment. CisPt was significantly more active (IC50= 9.64 ± 5.10 μM) than caPt(II)-complex (IC50= 21.25 ± 6.68 μM) on CFPAC1 proliferation. On the contrary, caPt(II)-complex showed a significant higher activity than CisPt both against NCI-H28 mesothelioma (19.37 ± 9.57 μM versus 34.66 ± 7.65 μM for CisPt) and U87 MG (19.85 ± 0.97 μM versus 54.14 ± 3.19 for CisPt). AT-MSCs showed to be more resistant to caPt(II) complex than to CisPt (> 35.48 μM versus 11.52 ± 1.99 for CisPt) but they are able to uptake both the drugs at a similar amount (2.49 pM /cell).

DISCUSSION AND CONCLUSION: The high stability of caPt(II)-complex together with its significant anticancer activity against mesothelioma and glioblastoma makes this new platinum derivative a very interesting molecule able to improve cancer chemotherapy. The low sensitivity of MSCs to this drug and their ability to uptake and release the drug will need further investigation to optimize the drug loading procedure in order to set up a possible application for a system of cell mediated drug delivery.

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IN-VITRO ANALYSIS OF QUANTUM MOLECULAR RESONANCE EFFECTS ON HUMAN MESENCHYMAL STROMAL CELLS

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Abstract

OBJECTIVE: Electromagnetic fields play an essential role in cellular functions interfering with cellular pathways and tissue physiology. In this context, Quantum Molecular Resonance (QMR) produces waves with a specific form at high-frequencies (4-64 MHz) and low intensity through electric fields. We evaluated the effects of QMR stimulation on human mesenchymal stromal cells (MSC).

MATERIALS AND METHODS: MSC were treated 10 minutes with QMR for 4 consecutive days for 2 weeks at different nominal powers. Cell morphology, phenotype, multi-lineage differentiation, cell viability and proliferation were assessed. QMR effects were further investigated by cDNA microarray and validated by real-time PCR.

RESULTS: Morphology, phenotype and multilineage differentiation were maintained after 1 and 2 weeks of QMR treatment and no alteration of cellular viability and proliferation were observed between treated MSC samples and controls.

cDNA microarray analysis evidenced more transcriptional changes on cells treated at 40 nominal power than 80 ones. The main enrichment lists belonged to development processes, regulation of phosphorylation, regulation of cellular pathways including metabolism, kinase activity and cellular organization. Real-time PCR confirmed significant increased expression of MMP1, PLAT and ARHGAP22 genes while A2M gene showed decreased expression in treated cells compared to controls.

Interestingly, differentially regulated MMP1, PLAT and A2M genes are involved in the extracellular matrix (ECM) remodeling through the fibrinolytic system that is also implicated in embryogenesis, wound healing and angiogenesis.

CONCLUSION: In our model QMR-treated MSC maintained unaltered cell phenotype, viability, proliferation and the ability to differentiate into bone, cartilage and adipose tissue. Microarray analysis may suggest an involvement of QMR treatment in angiogenesis and in tissue regeneration probably through ECM remodeling.

P-38

APPLICATION OF HPLC-MS AND HPLC-MS/MS SYSTEM TO STUDY THE METABOLISM PATHWAY OF PACLITAXEL: ANALYSES OF TREATED RATS AND BILE SEPARATION PROCEDURE

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Abstract

OBJECTIVE: Recently has been proved that mesenchymal stem cells are able to package and deliver Paclitaxel through their extracellular vesicles, suggesting the possibility of using MSCs to develop drugs with a higher cell-target specificity. The main objective of this study was to exploit the ability of mass spectrometry in order to perform structural studies on paclitaxel molecule because this drug follows a specific fragmentation pathway, which cleaves the molecules in two major fragment ions. These fragments are considered markers in biological fluids. It is possible to take advantage of this mass spectrometric behavior to establish the main features of paclitaxel metabolism, given that the hydroxylation process takes place on these two major fragment ions.

Paclitaxel, tax-11-en-9-one,5β, 20-epoxy-1,2α, 4,7β, 10β, 13α-hexahydroxy-4,10-diacetate-2benzoate-13-(α-phenylhippurate), is a taxane diterpene amide, that was first extracted from the stem bark of the western yew, Taxus brevifolia and identified as a potential antineoplastic agent in 1971. This natural product has proven to be one of the most promising anticancer agents now available. Several clinical studies have been performed in a variety of human neoplastic diseases, including the treatment of ovarian cancer, breast cancer, lung cancer, head and neck cancer.

MATERIALS AND METHODS: the capability of high performance liquid chromatography-ion spray mass spectrometry (HPLC-ISP-MS) and HPLC-ISP-tandem mass spectrometry (HPLC-ISP-MS/MS) were investigated to perform both mass separation as well as structural characterization of paclitaxel metabolites directly in rat bile, without their previous isolation.

HPLC-ISP-MS yield information on molecular weights of several hydroxylated derivatives while HPLC-ISP-MS/MS allowed the on-line structural characterization of all metabolites, present in different ratios in rat bile.

RESULTS: this approach led to the extraction of 9 metabolites and their distinction from the other endogenous contaminants. These metabolites were recognized as three di-hydroxypaclitaxel, four mono-hydroxypaclitaxel, one deacetylpaclitaxel and one containing the taxane ring.

Among these derivatives, we were able to identify four new metabolites of paclitaxel belonging to the di-hydroxy and mono-hydroxy series.

CONCLUSION: these results demonstrate that the high sensitivity of this method, based on the combined use of tandem mass spectrometry with chromatographic separation, can be considered as a valid approach to detect new paclitaxel derivatives directly in biological fluids.

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3D SCAFFOLDS PREPARED FROM PLATELET RICH PLASMA VERSUS CONVENTIONAL PLASTIC SURFACE FOR CULTURING ADIPOSE-DERIVED CANINE MESENCHYMAL STROMAL CELLS AND STROMAL VASCULAR FRACTION CELLS

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Abstract

OBJECTIVE: Mesenchymal Stem Cells (MSCs) are considered a valuable tool for regenerative medicine applications. Stromal vascular fraction (SVF), a heterogeneous population of cells derived from adipose tissue, represents a valid source of MSCs. We evaluated the ability of canine MSCs and adipose-derived SVF cells to be amplified in vitro by exploiting, in addition to the canonical plastic adhesion, a three-dimensional matrix obtained by gelling Platelet Rich Plasma (PRP) or Platelet Poor Plasma (PPP). The aim was to evaluate the possibility of obtaining autologous preparations capable of supporting MSCs and SVF cells growth for the "point of care" application in the veterinary clinic.

MATERIALS AND METHODS: 1. MSCs growth on plastic surface vs 3D fibrin matrix. Total cell number, cell doubling time and cell doubling number of MSCs grown on plastic surface were compared to MSCs cells grown inside a 3D-fibrin matrix prepared by mixing PPP or PPP (50%v/v), DMEM, thrombin (10% v/v) and calcium gluconate (100mg/ml, 10%v/v). 2. SVF growth on plastic surface vs 3D fibrin matrix. SVF cells prepared by collagenase type-I digestion were cultured either on conventional plastic culture dishes or within a 3D fibrin matrix. Total cell number, cell doubling time and cell doubling number were determined for both culture conditions until P3. 3.Phenotypic characterization by RT-PCR of MSCs and SVF cells. Gene expression was compared between MSCs and SVF grown in different culture conditions, i.e. plastic surface versus 3D fibrin matrix. A set of typical MSCs markers and gene involved in their biological properties were evaluated.

RESULTS: Canine MSCs grow within 3D fibrin-based matrices, demonstrating a shorter doubling time and a higher duplication rate when compared to cells grown on the plastic surface: the number of cells obtained is about seven-fold higher in the 3D environment after 144 hours of culture ($p<0.01$). Moreover, the cells included herein can be frozen and sub-cultured. In our experimental setup, the use of PRP instead of PPP in the preparation of the matrix does not modify cell replication rate. RT-PCR characterisation of cells cultured within the 3D matrix confirms the expression framework of MSCs markers. Furthermore, 3D environment improves SVF cells replication rate, producing at the first culture passage, a mean 1.5-fold increase in cell number.

CONCLUSION: 3D matrices prepared with autologous PRP or PPP are suitable for canine MSCs and SVF cells cultures. Cells grow faster than in standard 2D culture on the plastic surface, while they maintain their panel of gene expression. Furthermore, they can be sub-cultured and frozen for future applications. These results could contribute to set-up more effective MSCs-based therapies, with advantages regarding time shortening for the production of adequate amounts of cells to be applied in the clinical practice.

P-40

TREATMENT OF SPONTANEOUS OSTEOARTHRITIS WITH INTRA-ARTICULAR ADMINISTRATION OF AUTOLOGOUS MICRO-FRAGMENTED ADIPOSE TISSUE IN DOGS: SAFETY, FEASIBILITY AND CLINICAL OUTCOMES

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Abstract

OBJECTIVE: Osteoarthritis (OA) is a painful musculoskeletal condition affecting 20% of the adult canine population. Several solutions have been proposed, but the results achieved to date are far from being satisfactory. Recently, new approaches, such as intra-articular delivery of cells, including mesenchymal stromal cells, have been proposed and, among the many sources, the adipose tissue is considered very promising. We evaluated the safety, feasibility and efficacy of a single intra-articular injection of autologous and micro-fragmented adipose tissue (MFAT) in 130 dogs with spontaneous OA.

MATERIALS AND METHODS: MFAT was obtained using a minimal manipulation technique with a disposable product that progressively reduces the size of the adipose tissue clusters while eliminating oily substances and blood residues with pro-inflammatory properties. The entire process, carried out in one surgical step, was performed in complete immersion in physiological solution minimizing any trauma to the cells. The resulting MFAT was collected and injected in the intra- and/or peri-articular space. The amount and site of injected material depended on patient's dimension, type of joint, type of arthropathy and availability of material. Ideally, dogs >30kg body weight had a 2ml injection in or around each treated joint, dogs 15-30kg had 1.5ml and dogs 1-14kg had 1ml. In cases of very small joints or massive osteophytes, MFAT was injected around the joint.

RESULTS: Clinical outcomes were determined using orthopedic examination and owners' scores for up to 6 months. In 78% of the dogs, improvement in the orthopedic score was registered 1 month after treatment and continued gradually up to 6 months when 88% of the dogs improved, 11% did not change and 1% worsened compared to baseline. Considering the owners' scores at 6 months, 92% of the dogs significantly improved, 6% improved only slightly and 2% worsened compared to baseline. No local or systemic major adverse effects were recorded.

CONCLUSION: The results of this study suggest that MFAT injection in dogs with OA is safe, feasible and beneficial. The procedure is time sparing and cost-effective. Post injection cytological investigation, together with the clinical evidence, suggest a long-term pain control role of this treatment. The spontaneous OA dog model has a key role in developing successful treatments for translational medicine.

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CELECTOR® "THE CELL CHROMATOGRAPH" FOR MESENCHIMAL STEM CELL ANALYSIS

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Abstract

OBJECTIVE: A homogeneous, vital and proliferative cell population is a necessity for stem cell research and for clinical application. Stem Sel developed Celector®, the "cell chromatograph", a novel device for the separation and real-time microscopy of living cells. Celector® tag-less and free-flow separates cells from complex and heterogeneous samples on their native physical properties which are fully maintained and not altered. Celector® applications go from cell isolation to quality-control system as it provides a typical profile depending on the cell components. Protocols for the separation of stem cells from different cells, the separation of single cells from aggregates and the enrichment in highly potent stem cells have been developed, and ready for Celector® users.
MATERIALS AND METHODS:Cells are injected into a sterile capillary and are eluted through a biocompatible flow: bigger or more dense cells elute first while smaller and lighter cells elute later. A fractions collector is placed at the capillary outlet to collect viable cells for further analysis or use. A real-time optical system allows live imaging and cells count through a cell count software.
Celector® technology was used to characterize and isolate:
(a) Cells from fresh bone marrow (BM) clinical samples. Fractions have been collected from Celector® outlet, analyzed for colony forming unit (CFU) assay, and characterized for osteogenic and chondrogenic differentiation potential.
(b) A heterogeneous population of mesenchymal stem cells derived by adhesion from lipoaspirate samples. Fractions have been collected and analysed for morphological characteristics and expression of mesenchymal markers such as Vinculin and Phalloidin. The presence of senescent cells has been evaluated by β-Gal staining.
RESULTS: The fractionations process provides typical profiles and cell counts, and:
(a) Fresh BM cells have been selected in three fractions (F1-F2-F3). Only cells isolated from F1 showed the mesenchymal stemness potential. They expressed a significant higher number of CFUs compared to the other fractions and they can differentiate into osteogenic and chondrogenic lineage as shown by Alizarin Red and Alcian Blue staining.
(b) Two fractions have been collected from lipoaspirate derived mesenchymal stem cells. In fraction 2, an enrichment of vital mesenchymal cells with faster proliferative rate and low number of senescent cells (☐20%) has been shown; while cells in fraction 1 have shown bigger cytoplasm, higher expression of Vinculin and stop to proliferate, with more than 70% of senescent cells.
CONCLUSION:Celector® proves an effective depletion of senescent cells from a heterogeneous mesenchymal cell population, able to enrich the vital and proliferative components, and to concentrate mesenchymal stem cells from fresh clinical sample with high purity and viability. Cells are not manipulated and maintain their native characteristics. This allows cell collection for further studies, amplification and potential reuse for stem cell-based applications.

ADDENDUM

ABSTRACTS

Poster Index Addendum

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FREEZE-DRIED MSC-SECRETOME (LYOSECRETOME) FOR REGENERATIVE MEDICINE APPLICATIONS: PHARMACEUTICAL DEVELOPMENT

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Abstract

OBJECTIVE: The aim of this work is to obtain and characterize a freeze-dried and ready to use product, named Lyosecretome, based on MSC-secretome, and able to replace stem cells in regenerative nanomedicine.

MATERIALS AND METHODS: Lyosecretome Production. Secretome release from adipose-derived mesenchymal stem cells (MSCs) was obtained after 24 hours of serum starvation. Supernatants were collected and subjected to a purification process by dialysis. Cryoprotectant was then added and the MSC-secretome was freeze-dried obtaining Lyosecretome. Lyosecretome Characterization. Protein content was determined by BCA-Protein Assay Kit, while Nile Red was exploited as a probe for the quantification of phospholipids. The product was then characterized in terms of vesicles particle size/concentration (nanoparticle tracking analysis, NTA), morphology (scanning electron microscopy, SEM) and ultrastructure (transmission electron microscopy, TEM). For TEM, sample was immune-gold stained with HSP90 monoclonal antibody to detect the Heat shock protein-90β expressed by mesenchymal EVs. Finally, FT-IR spectra of Lyosecretome were obtained in the spectral region of 650–4000 cm−1 with a resolution of 4 cm−1 and a thermal characterization (Thermogravimetric analysis, TGA and Differential Scanning Calorimetry, DSC) was carried out to assess the lyophilization success.

RESULTS: Protein and lipid content, expressed as µg/mg of Lyosecretome, were 27.15 ± 4.000 and 6.23 ± 0.892, respectively (mean values of three batches); NTA detected a vesicle mean diameter of 157.2 ± 13.3 nm, with a concentration of 2.63 x 109 ± 3.59 x 107 EVs per mg of Lyosecretome. SEM images showed that dialyzed and freeze-dried EVs maintained their integrity and spherical structure with smooth surface; TEM images highlighted that EVs maintained their whole phospholipidic bilayer and the presence of HSP90-reactive particles demonstrate their MSC origin. FTIR spectra revealed low intensity bands around 1658 and 1536 cm-1 due to the presence of amide I C=O stretching vibrations and amide II N-H banding vibrations of the peptide groups, respectively. Absorbance bands at around 1457 and 1380 cm-1, related to CH2 and CH3 groups, confirmed the presence of lipid and protein. The bands detected in the spectral region between 1260 and 900 cm-1 are typical of phospholipids, triglycerides and cholesterol esters stretching vibrations. TGA analysis revealed a mass loss of 1.7% w/w due to water content. The DSC analysis revealed the amorphous nature of Lyosecretome, confirming that the process successfully occurred, without any interactions between the formulation components.

CONCLUSION: the dialysis process, coupled with freeze-drying technique, allows to obtain a freeze-dried product in which EVs preserve their integrity and morphology. Moreover, the performed physical/chemical Lyosecretome characterization paved the way for the Lyosecretome pharmaceutical quality definition. This work represents the proof of concept for the obtainment of a reproducible product necessary to carry out the safety and efficacy checks required for regulatory approval.

P-43

INTRATRACHEAL MESENCHYMAL STEM/STROMAL CELLS (MSCS)-DERIVED EXTRACELLULAR VESICLES (EVS) SIGNIFICANTLY IMPROVE MORPHOLOGICAL AND BIOCHEMICAL PARAMETERS IN AN ANIMAL MODEL OF BRONCHOPULMONARY DYSPLASIA

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Abstract

OBJECTIVE: Intravenous administration of Mesenchymal stromal cells (MSCs) – derived extracellular vesicles (EVs) can reverse the development of Bronchopulmonary Dysplasia (BPD) and of the associated pulmonary hypertension in rodent models. However, systemic administration of EVs could cause concern in a fragile patient population such as preterm neonates. Thus, we suggest that intratracheal (IT) administration of MSC-EVs, if proven effective in a reliable animal model, could represent a safer and more convenient tool for future clinical studies on patients with BPD. Moreover, we compared the effects of IT MSCs and MSC-EVs in the same model and we administered human clinical-grade material to increase the translational significance of our study.

MATERIALS AND METHODS: The study was conducted on Sprague-Dawley rat pups (n=10) exposed to normobaric oxygen concentration set at FiO2 0.6 until postnatal day (P) 14. At birth, rats were distributed as follows: rats raised in ambient air for 2 weeks; exposed to 60% oxygen for 2 weeks and treated with intratracheal administration of physiological solution (n=10), mesenchymal stem cells (MSC) (n=10) or MSC-EV (n=10) on P3, P7, P10. Animals were euthanized on P14.

Experimental groups included healthy controls (room air), hyperoxia-exposed pups receiving IT vehicle only and hyperoxia exposed pups receiving IT either human Wharton Jelly-derived MSCs (2x10E6) or MSC-EVs (1.3 x10E10) on days P3, P7, P10. Animals were euthanized on P14. Alveolarization was stereologically assessed and the thickness of the medial layer of small pulmonary arteries was also morphometrically evaluated. Cytokine expression was analyzed in lung lysate.

RESULTS: Sham-treated hyperoxia-exposed animals showed reductions in total surface of alveolar air spaces and total number of alveoli (Nalv) and increased mean alveolar volume (Valv). EVs produced significant increase in Nalv ($P<0.01$) and significant decrease in Valv ($P<0.05$) with respect to sham-treated animals, whereas MSCs only significantly improved Nalv ($P<0.05$). Small pulmonary vessels of sham-treated hyperoxia-exposed rats also showed increased medial thickness, which was significantly prevented only by EVs ($P<0.05$). Reduced IL-10 and TGFb1 concentrations were found in the lungs of hyperoxic animals. Both parameters were significantly increased following both treatments.

CONCLUSION: In conclusion, EVs showed better results than MSCs in improving hyperoxia-induced changes in alveolarization and lung vascularization, suggesting that IT EVs administration could represent a convenient and effective approach to reverse the development of BPD in preterm neonates.

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EFFICACY OF A FREEZE-DRIED PRODUCT BASED ON MSC-SECRETOME (LYOSECRETOME) IN A WOUND HEALING MURINE MODEL

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Abstract

OBJECTIVE: Mesenchymal stem cells (MSCs), including Wharton's Jelly-derived MSCs (WJ-MSCs) are promising candidates for regenerative medicine applications, as for wound healing. Human platelet lysate (hPL) has been widely used either as cell culture supplement alternative to FBS, and as rich source of factors that could accelerate the wound healing process. Here, we exploited the effect of hPL to optimize the WJ-MSCs isolation protocol and to enhance the ability of these cells to proliferate and migrate.

MATERIALS AND METHODS: All the experiment were conducted in culture medium supplemented with 5% hPL derived from apheresis. Human WJ-MSCs were isolated from umbilical cords by migratory ability toward the plastic surface.WJ-MSCs were characterized by flow cytometry (CD73, CD90, CD105, CD45, CD34, CD31, HLA-DR). The rate of proliferation was measured by the population doubling time. The cell migration rate was determined by the number of WJ-MSCs able to cross a transwell system. Adipogenic and osteogenic differentiation were performed.

Scratch assay was performed on a WJ-MSCs monolayer evaluating the percentage of wound closure after 16 hours.

RESULTS: In four different umbilical cords we found that the number of WJ-MSCs harvested after 14 days from umbilical cord pieces increased of 2.7± 0.8 fold in medium supplemented with 5% hPL compared to 10% FBS. Interestingly no significative differences were observed in the number of WJ-MSCs harvested within the same cord from, distal versus central pieces, either cultured in presence of hPL or FBS. The cell-population doubling time was lower in presence of hPL than in medium with FBS ($p=0.0051$). The hPL did not change the ability of WJ-MSCs to differentiate in adipocytes and osteoblasts.

In addition the ability of hPL to improve WJ-MSC wound healing properties was tested in in vitro assays.We found that hPL significantly enhanced the migration capacity of WJ-MSCs compared to FBS (91,8 % ± 4,9 vs 2,15 % ± 0,7 respectively, $p<0,0001$).

The scratch assay confirmed that WJ-MSCs, in presence of hPL, were able to reduce the wound surface area of 87,3 % ± 6,5 compared to 28,5%± 4,9 scratch closure found in presence of FBS ($p<0,0001$).

CONCLUSION: In this study we showed that an integrated culture platform based on hPL improves both WJ-MSCs recovery and cell proliferation and enhances some of the main functions crucial for the wound healing process.

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IN VITRO CYTOTOXICITY AND POTENCY OF FREEZE-DRIED MSC-SECRETOME (LYOSECRETOME)

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Abstract

OBJECTIVE: Aim of this work is to assess in vitro safety and potency of Lyosecretome, a freeze-dried product based on MSC-derived secretome.

MATERIALS AND METHODS: Adipose-derived mesenchymal stem cells were in vitro cultured and secretome release was obtained by serum starvation. Supernatants were collected, dialyzed, added of cryoprotectant, and freeze-dried, obtaining Lyosecretome. Haemolysis of Red Blood Cells (RBCs) was assessed after incubation with Lyosecretome and centrifugation: supernatant absorbance was read at 540 nm to calculate haemolysis percentage of RBC. RBC hemagglutination was identified as formation of 'button like' structure after Lyosecretome-cell co-incubation. Cytotoxicity on human fibroblast, chondrocytes, and nucleus pulposus cells (NP) was performed at three concentrations (5- 25 mg/ml range) and an MTT assay was assessed to determine cell metabolic activity after 24 and 48 hours of incubation. For the potency, two tests were designed: protective effect against oxidative stress, and immunomodulation. The first test was performed on NP cells treated for 24 h with Lyosecretome, at 5 concentrations (5-100 mg/ml range); after this time, hydrogen peroxide solution was added to each well for 24 h, and an MTT assay was performed. Immunomodulation potency was assessed as ability of Lyosecretome to suppress lymphocyte IFN-γ production induced by phytohemaglutinin. Response was quantified after 3-days incubation, as IFN-γ amount (ELISA quantification), and as a function of MSCs (as a control) or the cell-equivalent mg of their Lyosecretome.

RESULTS: Lyosecretome was not hematolytic until a concentration of 150 mg/mL, and induced hemagglutination at the concentration of 150 mg/ml. A dose dependent reduction in cell metabolic activity was observed for all cell lines, but cell viability remained ≥ 70% also at the highest concentration (25 mg/mL). Moreover, the cell metabolic activity of chondrocytes was higher after 48 h of incubation rather than 24 h, suggesting that, in this cell line, exposition to secretome stimulate cell proliferation and/or metabolic activity. Lyosecretome is not cytotoxic for NP cells until a concentration of 50 mg/ml. Oxidative stress results on NP cells showed that the treatment with secretome, at low doses protects cells from oxidative stress damages. On the opposite, at higher doses cell metabolic activity was dramatically reduced. The amount of IFN-γ was reduced in a dose-dependent manner in activated PBMCs incubated with MSCs or their Lyosecretome.

CONCLUSION: Lyosecretome is cytocompatible until 25 mg/mL, non-hematolytic and do not induce hemagglutination until 100 mg/mL. Moreover, it dramatically reduce oxidative stress damage induced on NP cells. Lyosecretome concentration of 3.7 mg/mL (corresponding to 50,000 equivalent cells) showed the same immunomodulatory properties of parental MSCs. Lyosecretome can be proposed as new Active Pharmaceutical Ingredient intended for the treatment of diseases associated with inflammation and oxidative stress, opening new horizons for the ever-closer application of MSC, and overcoming the hurdles related to cell therapy

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ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELLS (ASCS) FOR IN VIVO ANIMAL STUDIES AND CLINICAL APPLICATIONS - COLLECTION, PROCESSING, BANKING AND CHARACTERISATION

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Abstract

OBJECTIVE: Experimental data on adipose stem cells (ASCs) isolated from adipose tissue collected from animals (rat, rabbit) and human patients and applied for tissue regeneration purposes were presented and analysed.

MATERIALS AND METHODS: Adipose tissue was used as a source of mesenchymal stem cells isolated as mixed population with peripheral blood mononuclear and endothelial progenitor cells (SVF). The material was collected by excision of fat tissue (animals) or liposuction (humans), enzyme digested, purified and applied autologically or allogeneically or frozen. Collected and analysed data included the numbers and viabilities of isolated ASCs, in vitro proliferation and differentiation potential, clonal efficiency (CFU-F test), metabolic activity (MTT, NTT) and effects of freezing/thawing cycle on cell quantities and qualities.

RESULTS: Techniques developed for both animal and human ASC isolation result in satisfactory quantitative and qualitative efficiency, allowing to obtain up to 5 - 20 x 107 cells from 100 ml lipoaspirate. Viability of isolated cells was over 95% before freezing and 65% after thawing, they were capable to differentiate into adipo- osteo- and chondrogeneic lineages. Animal cells collected from aged rats (equivalent of human age of 70=80 years) still possessed the basic parameters (proliferation and differentiation potentials, clonal capability, doubling time and metabolic activity) comparable to cells obtained from young animals of age equivalent to 20 year-old humans.

CONCLUSION: Animal mesenchymal stem cells are similar to human cells being used for clinical experiments, hence animal models may be adequate for preclinical studies. Although ASC derived from old age donors may differ in many aspects from cells obtained from young individuals, their basic parameters commonly analysed for evaluation of MSCs intended for regenerative medicine purposes do not differ substantially, which allows to consider the autologous MSC therapies in senescent patients.

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AUTOLOGOUS ADIPOSE STEM CELLS FOR TREATMENT OF MULTIPLE SCLEROSIS (MS) - PHASE I CLINICAL STUDY

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Abstract

OBJECTIVE: According to the approval of local bioethical committee, 20 patients with relapsing-remitting MS or secondary progressive (SP) MS were enrolled into the study. Adipose tissue was collected from patient's femoral or abdominal region using Coleman technique, and the stromal vascular fraction (SVF) consisting of ASCs with the admixtion of another cell subpopulations (peripheral blood cells, endothelial precursor cells) was isolated. Immediately after SVF isolation, cells were injected intrathecally (12 x 106 cells per dose) at the time of enrollment, and the procedure was repeated following 3 and 6 months using cryopreserved ASCs. The follow-up observation time was 12-16 months and the efficacy parameters (EDSS and MS Functional Scale Scores, MRI lesions, relapse incidents and whole brain gray matter atrophy intensity were evaluated and compared to the control patients.

MATERIALS AND METHODS: Adipose tissue was used as a source of mesenchymal stem cells isolated as mixed population with peripheral blood mononuclear and endothelial progenitor cells (SVF). The material was collected by excision of fat tissue (animals) or liposuction (humans), enzyme digestion, gradient purification and applied autologically or frozen. Collected and analysed data included the numbers and viabilities of isolated ASCs, in vitro proliferation and differentiation potential, clonal efficiency (CFU-F test), metabolic activity (MTT, NTT) and effects of freezing/thawing cycle on cell quantities and qualities.

RESULTS AND CONCLUSION: All 20 patients completed the 12- and 18-months follow-ups. The treatment was safe and no adverse events were observed throughout the study period. No difference in 18-months EDSS score changes was found between the groups of RR and SP MS patients. For majority of patients, disease progression-free survival (PFS) was 18 months and did not differ according to disease type, gender, conditioning or EDSS score at transplantation. It may be concluded, that ASC therapy for selected MS patients results in the acceptable equilibrium between safety, efficacy and convenience. The follow-up observations are continued and will allow for collection of more complete data on the clinical efficacy of ASC therapy in MS patients.

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EXTRACELLULAR VESICLES (EVS) SECRETED BY MESENCHYMAL STEM CELLS (MSCS) EXERT OPPOSITE EFFECTS WITH RESPECT TO THEIR CELLS OF ORIGIN IN MICE WITH DSS-INDUCED COLITIS

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Abstract

OBJECTIVE: Several reports have described a beneficial effect of MSC administration in mice with experimental colitis. However, worsening of colitis following MSCtreatment was also reported.

MATERIALS AND METHODS: We compared the effects of murine MSC or MSC-EV administration in a dextran sulfate sodium (DSS)-induced colitis model. Since cytokine conditioning was reported to enhance MSC immune modulatory activity, the cells were kept either under standard culture conditions (naïve, nMSCs) or primed by IL1b, IL6 and TNFalfa (induced, iMSCs) Colitis was induced in C57BL/6 mice with DSS in drinking water for 5 days followed by 2 days on plain water. Healthy controls received plain water. Colitic animals were assigned to one of the following treatments on days 3, 5 and 7: vehicle only (controls), 1x10E7 nMSCs, 1x 10E7 iMSCs, 3x10E10 nMSC-EVs and 3x10E10 iMSC-EVs. Animals were sacrificed on day 8. To assess colitis severity we determined: changes in body wt, Disease Activity Index, colon length, histomorphometric analysis of the whole colon, cytokine expression in intestinal mucosa.

RESULTS: nMSCs and iMSCs administration was associated with clinical and histological worsening with respect to controls. However, mice treated with both nMSCEVs and iMSC-EVs showed clinical improvement, even if no significant difference was found in histological/morphometric score with respect to controls. These opposite effects were particularly evident with iMSCs. Cytokine expression in colon mucosa showed reduced TNFalpha and increased IL-10 in mice treated with iMSC-EVs.

CONCLUSION: In conclusion, both nMSCs and iMSCs worsened DSS-induced colitis, confirming that these cells can behave as pro-inflammatory agents depending on the environment. In contrast, both nMSC-EVs and iMSC-EVs showed a partially beneficial effect, suggesting a more predictable behavior and a safer therapeutic profile with respect to their cells of origin.

ALPHABETICAL INDEX OF AUTHORS

NOTES

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