COMPARING THE IMMUNOREGULATORY EFFECTS OF MESENCHYMAL STEM CELLS ISOALTED FROM BONE MARROW, PLACENTA AND AMNIOTIC FLUID

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Effect of Low-Dose Ionizing Radiations on the Biology of Human Mesenchymal Stromal Cells

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DNA damage induced by ionizing radiations may either trigger a neoplastic transformation of target cells or impair their activity triggering senescence and/or apoptosis. Cellular senescence has been proposed to be an anti-cancer mechanism. Nevertheless, senescence is considered deleterious because it contributed to decrements in tissue renewal and function [1,2].

Exposure of Ionizing radiation (IR) may cause damage to living organisms. The thyroid gland and bone marrow are particularly sensitive to radiations. Several studies have focused their attention on the effect of IR on hematopoietic stem cells and their progeny. However, bone marrow of mammals comprises several different components that support hematopoiesis and bone homeostasis. Among these are mesenchymal stem cells (MSCs) which are non-hematopoietic stem cells that possess multilineage potential. Aside from differentiation in mesenchymal tissues, MSCs support hematopoiesis and contribute to the homeostatic maintenance of many organs and tissues. Impairment of MSC functions can have profound consequences on body physiology. IR may alter functions of the bone marrow microenvironment and hence affect MSCs [3].

We evaluated the effects of IR on the biology of MSCs. In particular, we evaluated if different IR doses may elicit, preferentially, senescence and/or apoptosis.

Human MSCs were irradiated with 40 and 2,000 m Gray gamma rays. The 40 mGray induced a G2/M arrest 6 hours post IR, while at 48 hours we detected a significant reduction of S-phase cells. Same results were obtained with 2,000 mGray IR treatment.

MSCs showed a significant increase in senescence 48 hours post IR treatments. Of note, even the lowest IR treatment (40 mGray) induced a strong increase in senescent cells. At the opposite, the IR treatment reduced the percentage of apoptotic cells. This is in line with the alternative effects of DNA damaging agents on senescence and apoptosis, i.e. some damaging agents may trigger preferentially one or the other phenomenon.

Changes in apoptosis and senescence rates in MSCs prompted us to investigate the degree of DNA damage in our cell system.

DNA damage was evaluated 6 and 48 hours post irradiation. We determined the level of DNA damage by looking at ATM staining in IR exposed MSCs as compared to untreated cells. MSCs were proficient in repairing DNA damage and the percentage of ATM-positive cells, as determined by semi-quantitative immunocytochemistry (ICC), showed a significant reduction 48 hours post irradiation compared with 6 hours

The control of the stem cell properties (self-renewal, multipotentiality) is strictly linked to regulation of the cell cycle; hence it is reasonable to have perturbation in stem cell status in IR treated MSC. We carried out a CFU-F assay on these cells to test their clonogenicity, i.e., their ability to expand at a single-cell level, which is an important feature of self-renewing stem cells. IR treatments reduced the number of clones in all the experimental conditions we tested.

We deciphered also the molecular pathways associated with MSC response to IR radiations. We focused our attention on the retinoblastoma gene family-related pathways, since these genes have a major role in controlling the cell cycle G1/S transition through negative regulation of the E2F family of transcription factors. In addition, this gene family plays an important role in regulating other cellular processes, such as terminal differentiation, senescence and their inactivation is associated with several forms of cancer [4].

References

1. Campisi J, d'Adda di Fagagna F (2007) Cellular senescence: when bad things happen to good cells. Nat Rev Mol Cell Biol 8: 729-740.
2. Kuilman T, Peeper DS (2009) Senescence-messaging secretome: SMS-ing cellular stress. Nat Rev Cancer 9: 81-94.
3. Galderisi U, Giordano A (2014) The Gap Between the Physiological and Therapeutic Roles of Mesenchymal Stem Cells. Med Res Rev 34: 1100-1126.
4. Alessio N, Bohn W, Rauchberger V, Rizzolio F, Cipollaro M, et al. (2013) Silencing of RB1 but not of RB2/P130 induces cellular senescence and impairs the differentiation potential of human mesenchymal stem cells. Cellular and molecular life sciences. Cell Mol Life Sci 70: 1637-1651.

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Anti-Tumor and Anti-Angiogenic Activity of Paclitaxel Primed MSCs

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Mesenchymal stem cells (MSCs) have been shown to home neoplastic environment and to interact with tumor vasculature, thus MSCs may represent an ideal candidate to deliver anti-cancer and anti-angiogenic drugs [1]. Recently our group have demonstrated that *in vitro* MSCs (derived either from bone marrow or adipose tissue), without any genetic manipulation but simply exposing the cells to very high doses of the chemotherapeutic and anti-angiogenic agent Paclitaxel (PTX), can incorporate the drug. MSCs can subsequently release the drug in the culture medium [2,3]. We thus hypothesized that MSCs, once primed *in vitro* with PTX can acquire anti-tumor and anti-angiogenic activity if localized near cancer cells. To verify this hypothesis we performed *in vitro* and in vivo experiments using different tumor models and culture of endothelial cells (ECs) and aorta ring assay to test the anti-angiogenic activity of MSCs primed with PTX (MSCsPTX). In summary, we found that MSCsPTX acquired a potent anti-tumor and anti-angiogenic activity *in vitro* that was dose dependent, and demonstrable by using their conditioned medium or by co-culture assay. MSCsPTX co-injected in immunodeficient mice with DU145 (prostate carcinoma), U87GM (glioblastoma) or B16 (melanoma) cancer cells significantly delayed tumor takes and reduced tumor growth [3]. Additionally, when MSCsPTX were tested on MOLT-4 and L1210, two Leukemia cell (LC) lines of human and mouse origin respectively, we found a strong anti-LC activity. MSCsPTX co-injected with MOLT-4 or intra-tumor injected into an established subcutaneous MOLT-4 nodule strongly inhibited growth and angiogenesis. In syngenic BDF1 mice-bearing L1210, the intraperitoneal administration of mouse MSCs (SR4987PTX) doubled mouse survival time [4]. *In vitro,* both untreated MSCs and MSCsPTX released chemotactic factors, bound and formed rosettes with LCs. By ultrastructural analysis of rosettes, MSCsPTX showed no morphological alterations while the attached LCs were apoptotic and necrotic. Finally, both untreated MSCs and MSCsPTX released molecules that reduced LCs adhesion to microvascular endothelium (hMECs) and down-modulated the adhesion molecules ICAM-1 and VCAM-1 on hMECs [4].

Our data are the first demonstration that, without any genetic manipulation, mature stromal cells can uptake and subsequently slowly release PTX leading the possibility to deliver the drug into tumor environment more efficiently than the standard drug administration.

Tumor neovessels are abnormal, this do not permit a sufficient entry of anti-cancer molecules into tumor microenvironment thus leading many cancer cells to escape from drug cytotoxic activity. We think that to bypass this problem the most effective way is to use cells for drug delivery. To our opinion, among the different approaches and kind of stem cells that can be applied in cancer therapy, MSCs could be the most effective since of their unique properties to uptake/release significant amount of anti-cancer drugs.

References

1. Hall B, Dembinski J, Sasser AK, Studeny M, Andreeff M, et al (2007) Mesenchymal stem cells in cancer: tumor-associated fibroblasts and cell-based delivery vehicles. Int J Hematol 86: 8-16.
2. Bonomi A, Coccè V, Cavicchini L, Sisto F, Dossena M, et al (2013) Adipose tissue-derived stromal cells primed *in vitro* with paclitaxel acquire anti-tumor activity. Int J Immunopathol Pharmacol. 26: 33-41.
3. Pessina a, Bonomi a, Coccè V, Invernici G, Navone S, et al (2011) Mesenchymal stromal cells primed with paclitaxel provide a new approach for cancer therapy. PLoS One 6: e28321.
4. Pessina A, Coccè V, Pascucci L, Bonomi A, Cavicchini L, et al (2013) Mesenchymal stromal cells primed with Paclitaxel attract and kill leukaemia cells, inhibit angiogenesis and improve survival of leukaemia-bearing mice. Br J Haematol 160: 766-78.

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Encapsulation of Dedifferentiated Nasal Septal Chondrocytes in Alginate Based Scaffolds for Cartilage Tissue Engineering

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The use of mesenchymal stromal stem cells (MSCs) in the field of tissue engineering for cartilage repair is a very promising tool since these cells are readily expandable and capable of differentiating into chondrocytes. However, *in vitro* manipulation of these cells for the production of an implantable construct for cartilage defect healing presents still many challenges [1]. In this regard, several studies are aimed at developing alternative protocols to obtain chondrogenic differentiation from cells able to express those proteins required for optimal in vivo chondrogenesis and subsequent tissue repair. The use of MSCs traditionally requires the use of TGFβ since *in vitro* treatment with TGFβ is a well accepted system by which MSCs become chondrocyte-like cells. However, it is taken into account that this is a forced system and, according to recent evidences, it may give rise to unexpected side effects which are worth some considerations. On the other hand, clinical outcome of the use of mature chondrocytes in conventional autologous chondrocyte implantation (ACI) is not entirely satisfactory since stable hyaline-like cartilage in vivo is not convincing produced. Recent evidences suggest that the employment of heterogeneous populations of uncharacterized cells may also account for the disparate results in cell therapy studies. In search of new strategies for the assessment of chondrogenic differentiation we focused on the potential of human de-differentiated chondrocytes (p3Ch) obtained after subculturing chondrocytes (Ch) from human nasal septum. We demonstrated, by immunocytochemical analysis, that p3Ch lost cartilaginous phenotype and acquired an expression profile really close to that of hMSCs [2]. In addition, FACS analysis of specific markers showed that p3Ch population had a degree of homogeneity higher than Ch from which they derived. These evidences prompted us to use p3Ch cells in combination with alginate based scaffolds to generate a construct to be proposed for cartilage tissue engineering. We produced alginate microfibers (2% w/v) in combination or not with gelatin (2.25% w/v) or urinary bladder matrix (UBM) (0.5% w/v), in the purpose of taking advantage from association of different biomaterials and improving the differentiation ability of encapsulated cells without adding chondrogenic inducers. Microfibers were produced by an extrusion method [3] where biomaterial solution containing p3Ch has let been flowed by a syringe-pump in $BaCl_2$ gelling solution. Interestingly, microfibrous shape makes our scaffold manageable and adaptable to the structure and size of damaged tissue site for a possible in vivo application. Cells were successfully embedded in all tested conditions, showing rounded morphology and maintaining specific alignment and high viability. Scaffolds containing cells were maintained in culture without adding chemical inducers and monitored for 14 days. After an adequate set up of cell recovery, we were able to analyse gene expression by immunocytochemistry, RT-PCR and FACS. An effective redifferentiation ability of encapsulated cells was obtained as demonstrated by increase of typical chondrogenic markers including collagen type II and aggrecan, Alcian Blue staining of cartilage specific proteoglycans, associated with a desirable low level of collagen type I and X (a typical marker of hypertrophic cartilage), since day 7 of culture. In addition, transmission electron microscope (TEM) analysis showed that encapsulated p3Ch during redifferentiation process presented an appreciable production of secretory vesicles, containing extracellular matrix (ECM) dense materials, and collagen fibers with their typical banding pattern, in the surrounding lacuna, remembering the natural microenvironment of chondrocytes. As a whole, our data indicate that the cell-microfiber combinations here produced may be efficient in the production of the ECM in its correct form in a short time to support cartilage repair and regeneration.

References

1. Johnstone B, Alini M, Cucchiarini M, Dodge GR, Eglin D, et al. (2013) Tissue engineering for articular cartilage repair - the state of the art. Eur Cell Mater 25: 248-267.
2. Lolli A, Lambertini E, Penolazzi L, Angelozzi M, Morganti C, et al. Pro-chondrogenic effect of miR-221 and Slug depletion in human MSCs. Stem Cell Rev 10: 841-855.
3. Mazzitelli S, Capretto L, Quinci F, Piva R, Nastruzzi C (2013) Preparation of cell-encapsulation devices in confined microenvironment. Adv Drug Deliv Rev 65: 1533-1555.

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Amniotic Epithelial Cells: Path from Lab to Muscle-Skeleton Therapy

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The lack of a consistent therapeutic approach to tendon injury represents a great worldwide medical, social and economic challenge. In fact, after tendon injury the spontaneous healing results in a fibrotic scar formation which is biomechanically inferior to normal tendons by making chronic the pain symptoms and leaving high the risk of recurrence. None of the surgical techniques provide long term repair, thus, cell based therapy may represent a promising alternative. Several progenitors cell sources have been tempted to date even if none of them fulfill a complete clinical outcome. In our lab [1], it has been recently verified that ovine amniotic epithelial cells (oAEC) display the ability to step differentiating through the tenogenic lineage *in vitro* after a preliminary epithelial mesenchymal transition induced by the paracrine factors released in culture by tendon explants. The process of *in vitro* differentiation became quite efficient when oAEC were stimulated by fetal tendon explants that were able to induce in 2-3 weeks a spontaneous organization into 3D tendon-like structures. The ability of AEC to support tendon tissue regeneration was then documented *in vivo* on ovine tendon experimental defects [2-3] and on equine spontaneous tendinopaties [4]. Either in allo (oAEC) and xenotransplantation (equine AEC and 85-90% human AEC+ 10-15% AMSC in ovine host tendon) experiments the cells are able to accelerate tendon regeneration by improving the microarchitecture and the biomechanical properties of ovine injured tissues. Taking advantage of chimerism the cross-talk between transplanted hAEC and the ovine host tissue started to be clarified. The hAEC actively modulated tendon regeneration by displaying a direct contribution that involves either the synthesis *ex novo* of tissue specific extracellular matrix proteins (COL 1, SCX, TNMD) or the up regulation of several mesenchymal specific genes. In parallel, paracrine mechanisms are involved. Amongst them, the synthesis of growth factors modulating the process of tendon repairing (TGF1β, NGF and VEGF) and the active modulation of the inflammatory tissue response with an early recruitment of the reparative M2 macrophages has been demonstrated. Altogether these findings indicated that AEC conserved the ability to support early tendon healing thus indicating that this cell source may represent a valid alternative to develop therapeutic protocols leading tissue regeneration and not a simply transitory repairing event.

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References

1. Barboni B, Curini V, Russo V, Mauro A, Di Giacinto O, et al. (2012) Indirect co-culture with tendons or tenocytes can program amniotic epithelial cells towards stepwise tenogenic differentiation. PlosOne 7: e30974.
2. Barboni B, Russo V, Curini V, Mauro A, Martelli A, et al. (2012) Achilles tendon regeneration can be improved by amniotic epithelial cell allotransplantation. Cell Transplant 21: 2377-95.
3. Muttini A, Valbonetti L, Abate M, Colosimo A, Curini Vet al. (2013) Ovine amniotic epithelial cells: in vitro characterization and transplantation into equine superficial digital flexor tendon spontaneous defects. Res Vet Sci 94: 158-169.
4. Colosimo A, Curini V, Russo V, Mauro A, Bernabò N, et al. (2013) Characterization, GFP gene Nucleofection, and allotransplantation in injured tendons of ovine amniotic fluid-derived stem cells. Cell Transplant 22: 99-117.

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Amniotic Membrane from Human Term Placenta: A Morphological Study

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Human term placental tissues arouse great interest as a source of stem/progenitor cells useful in regenerative medicine [1]. Human term placenta, a discarded organ after parturition, appears ethically accepted as a new source of stem/progenitor cells and easy to obtain with a non-invasive method. Moreover, placental stem cells show interesting immunomodulatory properties as well as phenotypic plasticity. Due to the complexity of human term placenta, it is necessary to clearly define its histological structure, to better understand morphology, function and appropriate use of its cells for cell therapy. Our study was focused on the amniotic membrane, the innermost layer of human term placenta consisting of a thin epithelium placed on an avascular stromal matrix on foetal and amniotic fluid side. Human term placentas were obtained from donors after informed consent according to the guidelines of the Ethical Committee of the Catholic Hospitals (CEIOC). The amnion was manually separated from the chorion as previously described [2]. Three tissue samples were taken from the area in close proximity to the umbilical cord toward the periphery and processed for light and transmission electron microscopy. Light microscopy observations showed changes in the epithelium organization but not in the structure of the underlying loose avascular connective tissue, containing several star-shaped mesenchymal stem cells. In particular, from the centre toward the periphery, the surface epithelium changed from squamous stratified, with a number of exfoliating cells, to a monolayer of cubic and cylindrical cells with a brush border and, at the periphery, to a monolayer of squamous epithelial cells covering a thinner connective tissue. Immunocytochemical analysis demonstrated the expression of cKit and OCT3/4, known markers of pluripotency. Transmission electron microscopy observations showed a mixed cell population mainly of epithelial nature. Both cell types displayed euchromatic nuclei and prominent nucleoli and aspects of secretory activity. At cytoplasmic level numerous mitochondria and different types of vesicles and granules, both in size and in chemical nature (protein and/or lipid), were detected. The surface of the epithelial cells had numerous microvilli of different length, with functions of resorption/secretion of the amniotic fluid. All the observed morphological features are closely related to the characteristics of plasticity and immunomodulation of these cells.

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References

1. Parolini O, Alviano F, Bagnara GF, Bilic G, Bühring HJ, et al. (2008) Concise review: isolation and characterization of cells from human term placenta: outcome of the first international Workshop on Placenta Derived Stem Cells. Stem Cells 26: 300-311.
2. Soncini M, Vertua E, Gibelli L, Zorzi F, Denegri M, et al. (2007) Isolation and characterization of mesenchymal cells from human fetal membranes. J Tissue Eng Regen Med 1: 296-305.

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Increased Frequency Rate and Alteration of "If" Current in iPSC-Derived Human Cardiomyocytes from Atrial Fibrillation Patients

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Atrial fibrillation (AF) is the most common sustained arrhythmia worldwide. The mechanisms underlying AF are not fully understood, but multiple pathophysiological pathways have been suggested. On the contrary, the well-established AF risk factors include male sex, advancing age, diabetes, hypertension, heart failure, myocardial infarction, and valvular heart disease. Nevertheless, much of the AF variability remains unexplained, leading investigators to look for novel and genetic risk factors. Since 1940, a number of reports have described rare inherited AF disorders mainly associated with cardiac channel mutations. Recently several GWAS studies have shown the association of AF with peculiar chromosomal loci, and, nowadays, parental AF is considered as one of the main risk factor in offspring.

We started characterizing a family where three siblings suffered a common persistent AF. The consanguinity, the variability in risk factors, and the young age of onset of AF in these patients (from 44 to 52 years old) strongly suggested a genetic basis for this form of arrhythmia. Since an extensive single candidate gene analysis, comprising several known AF-associated genes, did not reveal any mutation, we approached the entire exome analysis to identify other sequence abnormalities. Preliminary results show that the three siblings are carrying single nucleotide mutations in cellular filaments that we will soon characterize. In parallel, we built the cellular-based human *in vitro* AF model, starting from patients-derived primary cultures of dermal fibroblasts [1]. These cells were transduced with Yamanaka's factors OCT4, KLF4, SOX2 and c-MYC, using two different systems: either a mix of retroviruses each of them carrying a single gene, or a single lentiviral particle carrying an omni-comprehensive polycistronic RNA [2,3]. Colonies of pluripotent stem cells (iPSC) were generated using both systems, but the lentiviral infection showed a higher reprogramming efficiency. Nevertheless, karyotype analysis of five independent lentiviral-derived clones showed a common chromosomal translocation (t17;19) that was absent in fibroblast primary culture, as well as in retroviral infected iPS clones.

Following the assessment of pluripotency, we differentiated AF-derived iPSC into cardiomyocytes (CMs) by a standard differentiation protocol involving embryoid bodies formation [4]. The presence of beating cells allowed us to identify and to isolate clusters of CMs whose electrophysiological properties have been analyzed. Cellular spontaneous action potentials were recorded revealing different traces and frequencies in AF-derived CMs versus the normal counterpart. No significant differences in action potential duration (APD) and maximum diastolic potential (MDP) were found between AF- and control-derived CMs, while 4-aminopyridine (4-AP), a modulator of potassium ultra rapid current, increased the APD of control-derived CMs, but was mostly ineffective in the AF-counterpart. Beating frequency was slightly but significantly higher in AF-derived CMs, that, despite their increased frequency, still responded to isoproterenol stimulation as much as control. We dissociated single CMs from the clusters and we directly measured on isolated cells the funny current (If) that is related to pacemaker activity, and is conducted through HCN channels. We found that AF-derived CMs open the If-related channels before the normal counterpart, thus suggesting a higher cellular excitability. qPCR data confirmed higher HCN4 expression levels in AF-derived CMs.

In conclusion we obtained iPSC-derived human CMs that, as shown, represent a valuable and reliable model of AF. We are presently using our model to understand the link between the identified mutations and the recorded functional alterations. Using this tool we will ultimately be able to identify novel therapeutic targets for this form of cardiac arrhythmia.

References

1. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, et al. (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131: 861-872.
2. Trond Aasen, Angel Raya, Maria J Barrero, Elena Garreta, Antonella Consiglio, et al. (2008) Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. Nature biotechnology 26: 1276-1284.
3. Sommer CA, Stadtfeld M, Murphy GJ, Hochedlinger K, Kotton DN, et al. (2009) Induced pluripotent stem cell generation using a single lentiviral stem cell cassette. Stem cells 27: 543-549.
4. Di Pasquale E, Song B, Condorelli G (2013) Generation of Human Cardiomyocytes: A Differentiation Protocol from Feeder-free Human Induced Pluripotent Stem Cells. Journal of Visualized Experiments (JoVE) 28: (76).

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Cancer-Stroma Interaction Increases Thyroid Tumor Growth

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Tumors are heterogenic cellular entities whose growth depends on mutual interaction between genetically altered neoplastic cells and non-neoplastic cells in the stromal microenvironment. Mesenchymal stromal cells (MSC) are able to influence the growth abilities of transformed cells while cancer cells modify the phenotypic characteristics of the stromal cells. Here, we show that papillary thyroid cancel TPC1 and HEK 293T cells interact physically with human primary bone marrow- derived MSCs followed by evanescence of MSC cytoplasm. Interestingly, transformed cells were able to connect only to apoptotic MSCs that had lost their migration ability, whereas naïve MSCs, avoided the direct contact. The interaction stimulated the proliferation of the co-cultured transformed cells, activated mitogen and stress signaling and increased resistance to cytotoxins. Consistent with *in vitro* data, the MSC interaction stimulated transformed cells had enhanced ability to grow and metastasize in vivo. The parental control cells showed mild tumorigenicity as compared to MSC interaction stimulated cells yielding measurable tumors in 31 days and seven days respectively. Additionally, we have shown the MSCs isolated from papillary thyroid cancer stroma (PTC-MSC) have differential phenotypic characteristics as compared to MSCs isolated from normal thyroid (Norm-MSC/ peritumoral MSC). While PTC-MSCs increased papillary thyroid cancer cell proliferation as compared to Norm-MSC controls, PTC-MSCs had lower affinity to cancer cells in migration assay. Our data therefore describe how adjacent transformed cells absorb stromal cells thus leading to the stroma-driven evolution of moderately carcinogenic cells to highly aggressive metastatic cells. Further, the results suggest the thyroid cancer cell derived modification of stromal MSCs.

References

1. Lisanti MP, Martinez-Outschoorn UE, Chiavarina B, Pavlides S, Whitaker-Menezes D, et al. (2010) Understanding the “lethal” drivers of tumor-stroma co-evolution: emerging role(s) for hypoxia, oxidative stress and autophagy/mitophagy in the tumor micro-environment. Cancer Biol Ther 10: 537-542.

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Immunomodulatory and Pro-Healing Properties of Periodontal Ligament Stem Cells: A Role of Pro-Resolving Lipid Mediators

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The well-known stemness properties of periodontal ligament stem cells (PDLSC) make these cells the possible key players in tissue healing and resolution of inflammation in periodontal tissue [1,2]. Recently, the potential therapeutic applications of stem cells were dramatically increased by experimental evidences that demonstrated the capability of human stem cells to exert Immunomodulatory properties [3,4]. Here we demonstrated the Immunomodulatory functions of PDLSC on neutrophil (PMN), the key cells in acute inflammatory reactions and investigate the involvement of specialized pro-resolving lipid mediators (SPM) on PDLSC functions. Co-incubation of PDLSCs with PMN significantly reduced (from 90% for PMN alone to 36% for PMN with PDLSC) spontaneous apoptosis of resting and activated PMNs, after 18 hour of culture. Interestingly, this anti-apoptotic effect occurred also when PMNs were incubated with the PDLSC-derived conditioned medium, suggesting the involvement of soluble factors released by PDLSCs. In this regard, performing a metabololipidomics profiling of PDLSCs we observed the production of pro-resolving metabolites deriving from docosahexanoic acid (DHA) (RvD6, PD1); the arachidonic acid (AA) (PGE_2,12-HETE) and the eicosapentenoic acid (EPA) metabolome (12-HEPE). Notably, we found that PDLSCs express the pro-resolving LXA_4 specific receptor, FPR2/ALX, and we observed that treatment with LXA_4 increases PDLSCs proliferation and migration, considered the key step of healing process. These effects occurred both with a FPR2/ALX-mediated mechanism. Together these results establish the first evidence of immunomodulatory properties of PDLSCs, elucidating a possible involvement of SMPs directly produced by PDLSCs. Moreover, they provide evidence for the regulation of key periodontal healing functions of PDLSCs by the LXA_4-FPR2/ALX system.

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References

1. Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, et al. (2004) Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 364: 149-155.
2. Park JY, Jeon SH, Choung PH (2011) Efficacy of periodontal stem cell transplantation in the treatment of advanced periodontitis. Cell Transplant 20: 271-285.
3. Krampera M, Glennie S, Dyson J, Scott D, Laylor R, et al. (2003) Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. Blood 101: 3722-3729
4. Baraniak PR, McDevitt TC (2010) Stem cell paracrine actions and tissue regeneration. Regen Med 5: 121–143.

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Reprogramming of Bovine Mammary Epithelial Cells and Bovine Skin Fibroblasts with Pluripotency Factors: A New Perspective in Veterinary Science and Milk Production

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Induced Pluripotent Stem Cells (iPSCs) represent an interesting study model in the research on genetically based diseases and a perspective for the regenerative medicine. Our aim is to generate and characterize iPSCs clones by reprogramming bovine mammary epithelial cells and bovine skin fibroblasts and to set up a procedure to commit reprogrammed clones towards a mammary phenotype in order to have a permanent source of mammary epithelial cell to differentiate in vivo study. We used a retroviral system (with four factors: Oct-4, Sox-2, Klf-4, c-Myc) to reprogram bovine mammary epithelial cells and skin fibroblast obtained from a 5-years old dairy cow. After reprogramming, 30 clones were harvested (20 derived from mammary epithelial cells population and 10 from skin fibroblasts). Five clones were selected by cell morphology for further analysis. Three clones (R5, 5AB, 2BA) derived from mammary epithelial cells and 1BB clone derived from skin fibroblast were positive for Alkaline Phosphatase activity. Pluripotent markers like Oct-4 (5AB, R5, 2BA, 1BB), Rex-2 and Lin-28 (1BB, 2BA) were detected by IFA. PCR analysis showed Nanog (5AB, 2BA and R5), Oct-4 (5AB, R5, 1BB, 2BA), Lin-28 (in 2BA and 1BB) and c-Myc (2BA, 1BB) expression. Subcutaneous injections of 2BA, 1BB and R5 clones (2×10^6 cells for each sample) have been performed using NOD/SCIID mice to test teratome formation. Tumor samples were collected after 6-8 weeks and then analyzed by immunochemistry and IFA. In tumors derived from the R5 and 1BB, it has been possible to observe cartilage, membranous ossification, and epidermis with presence of stratified squamous epithelial tissue, hair follicle, neural pinwheels, and different type of glandular tissues. Specifically, immunofluorescence assay revealed the presence of Cytokeratin 14, 18, P63, Muc-1 and Milk Proteins in ducts and alveoli of the R5 derived tissues according with the mammary phenotype. Taken together these results demonstrate the possibility to obtain iPSCs clones from bovine mammary epithelial cells and bovine skin fibroblasts, with the in vivo potential to differentiate into functional mammary gland tissue. Different hormonal association have been tested to differentiate a specific mammary phenotype *in vitro* cell cultures experiments to isolate cell clones able to regenerate outgrowths in vivo xenotransplantation.

References

1. Stingl J, Eirew P, Ricketson I, Shackleton M, Vaillant F, et al. (2006) Purification and unique properties of mammary epithelial stem cells. Nature 439: 993–997.
2. Peter Eirew, John Stingl, Afshin Raouf, Gulisa Turashvili, Samuel Aparicio, et al. (2008) A method for quantifying normal human mammary epithelial stem cells with in vivo regenerative ability. Nat Med 14: 1384– 1389.
3. Eugenio Martignani, Peter Eirew, Paolo Accornero, Connie J Eaves , Mario Baratta (2010) Human Milk Protein Production in Xenografts of Genetically Engineered Bovine Mammary Epithelial Stem Cells. PLoS ONE 5: e13372.
4. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126: 663-676.
5. Sumer H, Liu J, Malaver-Ortega LF, Lim ML, Khodadadi K, et al. (2011) NANOG is a key factor for induction of pluripotency in bovine adult fibroblasts. J Anim Sci 89: 2708-2716

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Placental Pericytes: The Initial Characterization Shows Similarities, as Well as Peculiar Differences, With Mesenchymal Stem/Stromal Cells

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Pericytes (PCs) represent a unique type of contractile cells surrounding the endothelium of small blood vessels, identified and described firstly in late 18th century. Since then, several studies revealed that these cells are not only involved in blood vessel architecture, but exert various angiogenic functions [1]. From recent literature, it has been suggested that blood vessels harbor also perivascular native ancestors of the mesenchymal stem/stromal cells (MSCs) that, depending on the anatomy of the vessel, can be classified as Pericytes- or adventitial-derived MSCs [2]. MSCs are a heterogeneous population of cells with extensive proliferative capacity and differentiation potential. Human MSCs have been initially isolated and characterized from bone marrow as plastic adherent fibroblast-like cells carrying a peculiar phenotype identified by co-expression of specific CD markers. Nevertheless, following studies identified cells with similar properties in various tissues including muscle, skin and adipose tissue. We have previously analyzed several biological properties of plastic adherent CD105$^+$/CD90$^+$/CD73$^+$/CD45$^-$ cells isolated from subcutaneous and visceral adipose tissues, identifying the lack of osteogenic differentiation in cells isolated from omental tissue [3]. Since omentum is a highly vascularized mass, our finding raised the possibility that the isolated cell line was mainly composed by PCs rather than MSCs. To address this clue we isolated PCs from highly vascularized human placenta and we are in process of comparing the biological properties of the two cell lines.

We initially performed immunohistochemistry assays on paraffin- or OCT-embedded placental sections to confirm the presence and abundance of microvascular cell types as indicated by specific staining for CD31 and CD146. Then, fresh human placenta samples were collected, the chorionic villi portions were minced into small pieces, digested with collagenase and the cell suspension was seeded in M199 medium containing 20% FBS and 1% penicillin/streptomycin solution. After 2-3 days non-adherent cells were removed and the complete medium was replaced twice a week until cellular confluence [4]. Adherent cells were morphologically analyzed daily and, at confluence, trypsinized, expanded, and subjected to qPCR, immunofluorescence, and flow cytometry analyses for Pericytes markers expression (CD146, PDGFRβ and Calponin). Preliminary results indicate similarities as well as peculiar differences, between MSCs and placental-derived PCs, thus expanding the range of cells of mesenchymal origin that share some of the properties of MSCs.

References

1. Armulik A, Genové G, Betsholtz C (2011) Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. Dev Cell 21: 193-215.
2. Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, et al. (2008) A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 3: 301-313.
3. Angela De Luca, Rosanna Verardi, Arabella Neva, Patrizia Benzoni, Elisabetta Crescini, et al. (2013) Comparative Analysis of Mesenchymal Stromal Cells Biological Properties". ISRN Stem Cells 2013: 9.
4. Bryan BA, D'Amore PA (2008) Pericyte isolation and use in endothelial/pericyte coculture models. Methods Enzymol 443: 315-331.

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Mitochondria Toxicity of PEI-Based Nanoparticles is Reduced in MSC By Acetylation of Amines

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Polyethylenimine (PEI) is an efficient non-viral gene delivery vector, known to have varying degree of toxic effect to cells based on its chemical structure (i.e, amount of primary and secondary amine) [1]. Previous research has shown that PEI is able to depolarize mitochondria via direct mitochondrial membrane permeabilization, activation of the mitochondrial permeability transition pore, and interference with mitochondrial membrane proton pumps allowing for release of pro-apoptotic factors and eventual cell death [2].

In this study, gene delivery carriers such as PEI-PLGA Nanoparticles and acetylated PEI-PLGA Nanoparticles (PEI-NPs and AcPEI-NPs respectively) were utilized to examine the effect of Acetylation on PEI toxicity onto mesenchymal stem cells (MSC). Our recent studies evidenced that the intracellular level of ROS generation and DNA damage were significantly reduced using AcPEI-NPs in comparison with PEI-NPs. The results indicate that the surface charge of the NPs influences their capability to induce intracellular ROS formation and oxidative stress and point at a role of mitochondria in this NP-induced ROS production at the sub cellular level [3].

In order to find out the effect of Acetylation on mitochondria, we performed an assay to determine the Mitochondrial Membrane Potential (MMP) change using the lipophilic fluorochrome JC-1. Uptake of JC-1 into mitochondria is driven by the MMP. The accumulation of JC-1 in mitochondria leads to the formation of JC-1 aggregates detected in the red channel by flow cytometry. JC-1 does not accumulate in mitochondria with depolarized MMP, and remains in the cytoplasm as monomers which are detectable in the green channel by flow cytometry. Carbonyl cyanide 3-chlorophenylhydrazone (CCCP), a mitochondrial depolarizing agent, was used as a positive control for MMP reduction. Our results indicate that more cells with green fluorescence were seen in the presence of PEI-NPs. To study mitochondrial morphology in the presence of NPs, mitochondria were stained with a red fluorescent emitting mitochondria-specific dye after 60 min of incubation with 50 and 300 μg/ml of Nanoparticles. Imaging of mitochondria in cells with AcPEI-NPs showed distributed filamentous mitochondria in the cytoplasm even at high concentration. Treatment with PEI-NPs induces mitochondria disorganization also at 50 μg/ml.

The amount of cytochrome C in the cytosolic fraction of the MSC cells treated with PEI-NPs was 4.3-fold higher than control (cells treated only with NPs). Furthermore, AcPEI-NPs treatment markedly reduced this increase to 1.2 ± 0.2-fold of control, indicating that AcPEI could inhibit cytochrome c translocation from mitochondria to cytosol, preventing the triggering of the apoptotic process.

Our results evidenced that mitochondrial toxicity were significantly reduced by PEI Acetylation.

References

1. Godbey WT, Wu KK, Mikos AG (2001) Poly(ethylenimine)-mediated gene delivery affects endothelial cell function and viability. Biomaterials 22: 471–480.
2. Beyerle A, Irmler M, Beckers J, Kissel T, Stoeger T (2010) Toxicity pathway focused gene expression profiling of PEI-based polymers for pulmonary applications. Mol Pharm 7: 727–737.
3. Calarco A, Bosetti M, Margarucci S, Fusaro L, Nicolì E, et al. (2013) The genotoxicity of PEI-based nanoparticles is reduced by acetylation of polyethylenimine amines in human primary cells. Toxicol Lett 218: 10-7.

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Energy Restriction as a Means to Select Chronic Myeloid Leukaemia Stem Cells Resistant to Tyrosine Kinase Inhibitors

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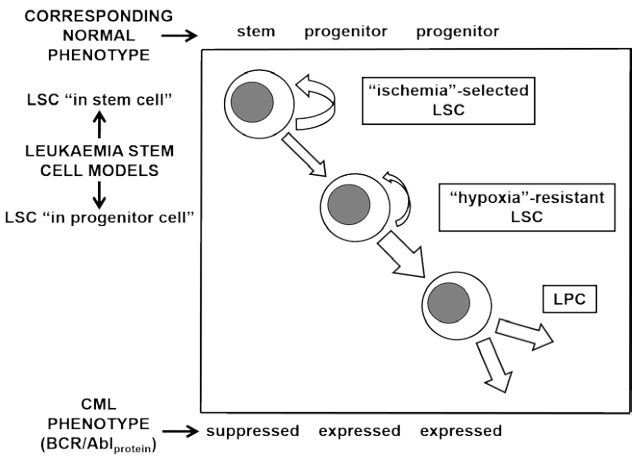
The concept of *stem cell niche* was introduced in 1978 to model bone marrow sites suited to host haematopoietic stem cells (HSC) and favour their self-renewal, while restraining clonal expansion and commitment to differentiation. In 1993, studies of the effects of low oxygen tension on HSC maintenance *in vitro* led us to hypothesize that HSC niches are located within bone marrow (BM) areas where oxygen tension is lower than elsewhere. We named these areas "hypoxic" stem cell niches, although oxygen tension is physiologically low in the sites where HSC are maintained [1]. We later showed that HSC are capable to cycle in low oxygen and that low oxygen steers cycling towards HSC self-renewal.

Leukaemia cell populations, including chronic myeloid leukaemia (CML), also comprise cell subsets capable to stand low oxygen. In these subsets, the oncogenic BCR/Abl$_{protein}$ is completely suppressed, indicating that CML cells resistant to low oxygen are independent of BCR/Abl signalling for persistence in culture, although they remain genetically leukaemic. Indeed, when shifted to standard incubation conditions (air), these cells re-express, or generate a progeny expressing, BCR/Abl$_{protein}$, which boosts BCR/Abl-dependent self-renewal and CML growth. On this basis, we found that CML stem cells (LSC) selected in low oxygen are refractory to the tyrosine kinase inhibitor (TKi) imatinib-mesylate (IM; Gleevec®), the prototype drug used for CML therapy, because BCR/Abl$_{protein}$, the molecular target of IM, is suppressed [2]. This novel pathway to insensitivity to IM, environment-enforced *primary* resistance (better referred to as *refractoriness*), relies on a phenotypical adaptation of LSC and is therefore fully reversible. This is well in keeping with the clinical finding that most relapses of disease in CML are sustained by cells with wild-type *BCR/abl*. Interestingly, refractoriness is independent of whether LSC are cycling or quiescent.

Further studies indicated that BCR/Abl$_{protein}$ suppression is actually determined when glucose shortage complicates the effects of low oxygen, indicating that ischemia-like conditions are the driving force of LSC refractoriness to IM [3]. Thus, we hypothesized the existence of "ischemic" stem cell niches as the very sites where LSC are maintained via BCR/Abl-independent self-renewal and where treatment-insensitive minimal residual disease (MRD) of CML persists. Data obtained by others indicate that blood perfusion rather than oxygen diffusion controls the hierarchical distribution of HSC in normal BM and that the most primitive HSC reside in BM areas at lowest blood perfusion.



On the basis of all above, we focused on the relationship of the "hypoxic" to the "ischemic" LSC niche and undertook the characterization of the effects of oxygen *vs* glucose shortage on the expression of BCR/Abl$_{protein}$ and the maintenance of LSC potential. We found that oxygen shortage reduces BCR/Abl$_{mRNA}$ levels (and possibly half-life), while glucose shortage does not, in spite of the fact that either condition suppresses BCR/Abl$_{protein}$. On the other hand, BCR/Abl$_{protein}$ half-life is reduced under glucose, but not oxygen, shortage. Thus, BCR/Abl expression is regulated differently within the two different niche environments. Preliminary data suggest that glucose shortage alone is less efficient than oxygen shortage

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in selecting LSC. These differences might reflect how "deep" is BCR/Abl suppression in the two cases. MRD may reside within areas where suppression is deepest. The model is summarized in (Figure 1) (LPC: leukaemia progenitor cell).

A translational aspect of our research is the use of CML cell selection under energy restriction to identify and test treatments potentially active on TKi-resistant LSC sustaining MRD. A number of different drugs have been, and are being, tested. We found drugs which are active on CML cell bulk but inactive on LSC and vice versa [4]. The latter drugs are promising in view of their use in combination with IM to induce remission of disease while suppressing cells potentially responsible for MRD.

References

1. Cipolleschi MG, Dello Sbarba P, Olivotto M (1993) The role of hypoxia in the maintenance of hematopoietic stem cells. Blood 82: 2031-2037.
2. Giuntoli S, Rovida E, Barbetti V, Cipolleschi MG, Olivotto M, et al. (2006) Hypoxia suppresses BCR/Abl and selects imatinib-insensitive progenitors within clonal CML populations.. Leukemia 20: 1291-1293.
3. Giuntoli S, Tanturli M, Di Gesualdo F, Barbetti V, Rovida E, et al. (2011) Glucose availability in hypoxia regulates the selection of chronic myeloid leukemia progenitor subsets with different resistance to imatinib-mesylate. Haematologica 96: 204-212.
4. Michele Tanturli, Serena Giuntoli, Valentina Barbetti, Elisabetta Rovida, and Persio Dello Sbarba (2011) Hypoxia Selects Bortezomib-Resistant Stem Cells of Chronic Myeloid Leukemia. PLoS One 6: e17008.

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Satellite Cell Ageing and Apoptosis

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Adult skeletal muscle retains a remarkable capacity of regeneration and repair after injury, mainly through muscle-specific stem cells, known as satellite cells (SCs). However, the sarcopenic process developing with ageing has been linked to an impaired regeneration: SCs derived from elderly subjects are unable to carry out an adequate program of differentiation to maintain the muscle mass in a stable and functional status. Several studies have suggested that both cell intrinsic factors and the niche where SCs lie are able to influence each other [1]. This reduced capability seems to be due to an impairment of myoblast differentiation and an alteration of gene profile. In particular, specific gene pathways related to modulation of miRNAs and involved in muscle remodeling are entailed. Moreover, we also highlighted the involvement of altered ROS homeostasis along with the reduction of the antioxidant activity. This evidence was confirmed by our results using microarrays platform and analysis showing an age-related deregulation of some genes involved in oxidation management and in protein balance (Polimerase K, SHC1 and FOXO1A). The last two genes could also be implicated in apoptotic process activation. Further RT-PCR analysis demonstrated an alteration of differentiation and apoptotic processes and miRNAs expression. Parallel analyses in flow cytometry and immunofluorescence demonstrated an increased rate of apoptotic cells in aged subjects and a possible relationship with caspase-9 and caspase-3 activation. Our data indicate apoptosis as one of the possible mechanisms responsible for the impaired SC differentiation and muscle regeneration in the elderly.

Reference

1. Fulle S, Centurione L, Mancinelli R, Sancilio S, Manzoli FA, et al. (2012) Stem cell ageing and apoptosis. Curr Pharm Des 18: 1694-1717.

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A Sphingosine-1-Phosphate Autocrine Loop Promotes Proliferation and Stemness of Glioblastoma Stem Cells

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Sphingosine-1-phosphate (S1P) is an onco-promoter lipid that, after interaction with specific membrane receptors, can influence different cell properties strictly related to cancer [1]. Increasing evidence indicates that S1P acts as a key regulator of growth, invasion, and therapy-resistance in human glioblastoma (GBM), the most common and fatal intracranial cancer in adults [2]. Recent studies support that GBM contain a subpopulation of cells, named glioblastoma stem cells (GSCs), that plays a crucial role in tumor initiation, maintenance, and malignant progression [3]. In this context S1P is emerging as a key molecule in determining GSC properties [4]. However, little is known on the origin and role of S1P in GBM.

In this study, we investigated the possible role of S1P in GSC proliferation and stemness.

To this purpose, among different GSC lines prepared in our laboratory from GBM specimens, we selected two lines, representative of slow- and fast-proliferating cells, and named S-GSCs and F-GSCs, respectively. These GSCs demonstrated heterogeneity not only in their proliferative potential, but also in the expression of stemness markers. Indeed, the fast proliferative status of the F-GSC population was paralleled by a significant higher expression of stemness parameters than that of S-GSCs. Metabolic studies revealed that both GSC lines constitutively exhibit the property to rapidly export S1P into the extracellular microenvironment. Intriguingly, the proliferative properties of GSCs were related to an efficient secretion of newly produced S1P. Indeed, in the fast-proliferating cells, the extracellular S1P level was found up to 10-fold higher than that of slow-proliferating ones, suggesting that the high extent of S1P released by F-GSCs reflects, and most probably participates to their proliferative and stemness features. In addition, the presence of EGF and bFGF potentiated the constitutive capacity of GSCs to secrete newly synthesized S1P, indicating that cooperation between S1P and these growth factors is of relevance in the maintenance and proliferation of GSCs. For the first time, we then report that S1P is able to act as a survival, proliferative, and pro-stemness factor for GSCs, promoting both cell cycle progression and stemness phenotypic profile. These effects were counteracted by FTY720 (a precursor of S1P receptor inhibitor), implying S1P specific receptors in its GSC-stimulating effects.

In conclusion, this work implicates S1P to be an autocrine/paracrine mediator acting as a mitogenic and stemness-favoring factor, through direct effects in GSCs, and possibly through the induction of their niche. This suggests that the inhibition of S1P release from GSCs and/or of S1P receptors could be a valuable strategy to curtail GBM progression.

References

1. Yester JW, Tizazu E, Harikumar KB, Kordula T (2011) Extracellular and intracellular sphingosine-1-phosphate in cancer. Cancer Metastasis Rev 30: 577-597.
2. Wen PY, Kesari S (2008) Malignant gliomas in adults. N Engl J Med 359: 492-507.
3. Chen J, Li Y, Yu TS, McKay RM, Burns DK, et al. (2012) A restricted cell population propagates glioblastoma growth after chemotherapy. Nature 488: 522-526.
4. Riccitelli E, Giussani P, Di Vito C, Condomitti G, Tringali C, et al. (2013) Extracellular sphingosine-1-phosphate: a novel actor in human glioblastoma stem cell survival. PLoS One 8: e68229.

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Xeno-Free Culture of Human Periodontal Ligament Stem Cells: A New Challenge for Tissue Engineering

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The possibility of transplanting adult stem cells into damaged organs has opened new prospective for the treatment of several human pathologies. Currently, *in vitro* expansion and culture of mesenchymal stem cells is founded on supplementing cell culture and differentiation media with fetal calf serum (FCS), that contains numerous growth factors inducing cell attachment to plastic surfaces, cell proliferation and differentiation. Although these traditional formulations provide an high expansion of stem cells, the presence in the culture medium of FCS may trigger a xenogenic immune response, immunological reactions and the potential of transmission of prion diseases and zoonoses, once used in regenerative medicine [1].

The purpose of this study was to develop a culture system for the expansion and production of human Periodontal Ligament Stem Cells (hPDLSCs) using a new xeno-free media formulation ensuring the maintenance of the stem cells features comprising: the multiple passage expansion, mesengenic lineage differentiation, cellular phenotype and genomic stability, essential elements for conforming to translation to cell therapy [2].

Somatic stem cells were isolated from the human periodontium using a minimally invasive periodontal access flap surgery in healthy donors. Expanded hPDLSCs in a xeno-free culture showed the morphological features of stem cells, expressed the markers associated with Pluripotency, and a normal karyotype.

Under appropriate culture conditions, hPDLSCs presented adipogenic and osteogenic potential; indeed, a very high accumulation of lipid droplets was evident in the cytoplasm of adipogenic induced cells, and indisputable evidence of osteogenic differentiation, investigated by transmission electron microscopy, and analyzed for gene expression analysis has been shown.

Based on these data, the novel xeno-free culture method might provide the basis for GMP culture of autologous stem cells, readily accessible from human periodontium, and can be a resource to facilitate their use in human clinical studies for potential therapeutic regeneration.

References

1. Eleuterio E, Trubiani O, Sulpizio M, Di Giuseppe F, Pierdomenico L, et al. (2013) Proteome of human stem cells from periodontal ligament and dental pulp. PLoS one 8: e71101.
2. Trubiani O, Orsini G, Zini N, Di Iorio D, Piccirilli M, et al. (2008) Regenerative potential of human periodontal ligament derived stem cells on three-dimensional biomaterials: a morphological report. J Biomed Mater Res A 87: 986-993.

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Is the G-CSF-Based Treatment Effective in Limiting Restenosis? Observations in a Rat Model of Carotid Arteriotomy

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Restenosis is a complex pathophysiological disease, which causative mechanisms have not been yet fully identified. It has been widely accepted that the vascular damage occurring in revascularization procedures triggers a cascade of events (i.e. inflammation, proliferation and matrix remodeling) leading to pathological repair and remodeling of the injured vessel. Therapeutic strategies promoting early repair on the injured vessel would be effective in inhibiting intimal lesion, reducing vascular remodeling and improving long-term vessel patency [1,2].

Bone marrow mesenchymal stromal cells (MSCs) are a population of stromal cells, which contains a subpopulation of self-renewing multi-potent cells. MSCs are currently used in several clinical trials. Previous studies allowed us to demonstrate the efficacy of MSC transplantation in limiting the pathophysiologically phenomena leading to stenotic progression in a rat model of carotid arteriotomy. Interestingly, our results pointed out their immunomodulatory and secretory properties suggesting a paracrine effect [3].

Experimental evidences showed the effectiveness of drugs and natural compounds in mobilizing bone marrow-derived stem cells; granulocyte-colony stimulating factor (G-CSF) is one of these. It is hematopoietic cytokine involved in release of stem cells and endothelial progenitor cells (EPCs) from bone marrow into peripheral blood circulation. Interestingly, the G-SCF anti-inflammatory properties were documented [4].

In this context, we evaluate the efficacy of a G-CSF-based pre- and post-treatment as a strategy for promoting an early repair of the vascular wall of carotid artery submitted to Arteriotomy. Mobilization of endogenous bone marrow stem cells by G-CSF may provide a potentially effective non-invasive approach to enhance recovery of arterial wall of injured carotid.

Eight-week male rats were injected subcutaneously with recombinant human G-CSF or normal saline for about 1 week and then were submitted to Arteriotomy of the left common carotid artery. Uninjured carotids were used as control for the analyses. Carotids were harvested 7 and 30 days following injury.

The mRNA expression profiles of genes involved in processes leading to vascular remodeling such as cell proliferation (FOXO, MURF, VEGF, FLK1), SMC markers (MYO, SMTN, SM22α) and immune response (HIFα) were assessed by RT-PCR.

Histological staining and immunhistochemical analyses were used to detect signs of vascular remodeling and the expression of SMC marker (SMTN), respectively.

Our data suggest that G-CSF treatment seems to contribute to modulating the expression profiles of genes involved in cell proliferation and inflammation along with a concomitant increase of the levels of mRNA and protein known as markers of contractile SMC phenotype. This effect seems to be mainly related to the early phase of the (re) stenotic process and could, in turn, lead to a recovery of the vascular wall architecture 30 days following injury.

References

1. Forte A, Finicelli M, Mattia M, Berrino L, Rossi F, et al. (2008) Mesenchymal stem cells effectively reduce surgically induced stenosis in rat carotids. J Cell Physiol 217: 789-799.
2. Forte A, Rinaldi B, Sodano L, Berrino L, Rossi F, et al. (2012) Stem cell therapy for arterial restenosis: potential parameters contributing to the success of bone marrow-derived mesenchymal stromal cells. Cardiovasc Drugs Ther 26: 9-21.
3. Galderisi U, Giordano A (2014) The Gap Between the Physiological and Therapeutic Roles of Mesenchymal Stem Cells. Med Res Rev 34: 1100-1126.
4. Hasegawa H, Takano H, Ohtsuka M, Ueda K, Niitsuma Y, et al. (2006) G-CSF prevents the progression of atherosclerosis and neointimal formation in rabbits. Biochem Biophys Res Commun 344: 370-376.

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Generation of iPSCs from CD133+ Cord Blood Stem Cells Using a Non Integrative Strategy

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The ability to generate induced pluripotent stem cells (iPSCs) from somatic cells has opened up a new avenue for regenerative medicine. iPSCs can provide a cell source for disease-modelling, drug-screening platforms, and transplantation strategies to treat incurable degenerative diseases.

Human iPSCs were first established from dermal fibroblasts derived from skin biopsy by over expression of Yamanaka factors (*OCT4, SOX2, MYC* and *KLF4*, or OSMK) or Thomson/Yu factors (*OCT4, SOX2, NANOG,* and *LIN28*). Later, hematopoietic stem/progenitor cells from cord blood (CB) captured much attention because blood cells can be used immediately for reprogramming.

Umbilical cord blood is a tissue rich in stem cells and readily available. In comparison with stem cells isolated from aged individuals, cord blood stem cells are expected to be superior because nuclear and mitochondrial mutations tend to accumulate in adult stem cells and differentiated somatic lineages over an organism's lifetime. In addition, >400,000 fully characterized and HLA-typed CB units are stored in public banks and are readily available for clinical therapy.

For clinical applications, transgene-free or footprint-free iPSCs need to be used to prevent potential adverse effects due to retroviral or lentiviral integration or due to the interference of residual expression of reprogramming factors on the differentiation of iPSCs into progenies of clinical interest. Toward this goal, several approaches have been used for obtaining integration or transgene-free iPSCs, including the use of plasmids, theCre/loxP system, adenoviruses, piggyback transposon, minicircle DNA, protein transduction, miRNA and Sendai virus.

The reprogramming of iPSCs using the SeV system has a significant advantage over presently available methods for its safety, efficiency and convenience. In fact, the SeV allows transgenes expression without modification of the host genome, so the resulting iPSCs are genetically intact and carry the same genome DNA as the original cells. In contrast with many available protocols, the SeV vectors are non-integrating and remain in the cytoplasm (zero footprints). In addition, the host cell can be cleared of the vectors and reprogramming genes by temperature sensitivity.

Here we show that cord blood CD133+ cells can be reprogrammed into iPSCs using Sendai virus with a very high efficiency. CD133+ stem cells, isolated from different CB units by immunomagnetic selection, were reprogrammed into hiPSCs using the four Yamanaka factors (Oct4, Sox2, Klf4, c-Myc). All the CB-iPSCs lines tested showed strong alkaline phosphatase and expression of pluripotency markers such as OCT4, SOX2, TRA-1-81, TRA-1-60, SSEA3, SSEA4, and NANOG, revealed by citofluorimetry and immunofluorescence. The expression of the Sendai virus genome and transgenes in the vector-free CB-iPSCs was determined by RT-PCR in order to confirm the complete adsence of viral vectors. The ability to generate integration-free iPSCs from the most readily available source, the Cord blood, has the potential to expedite the advances of iPSC-based therapies. Due to its unique advantages as donor cells for the production of clinical-grade human iPSCs, CB is believed to be one of the best sources for reprogramming. An additional advantage is the potential of converting CB banks into iPSC banks for allogeneic cell-based therapy.

Although the details and mechanisms of the reprogramming process during iPSC generation are still being elucidated, the products are promising for many purposes, such as drug discovery, pathological studies, toxicology studies, the evaluation of secondary drug effects and regenerative medicine.

The high scientific relevance of this new methodology can overcome the major issues of iPSCs-based therapy and may definitely shorten the time for their application in the clinic.

References

1. Bana H, Nishishitab N, Fusakia N, Tabataa T, Saeki K et al. (2011) Efficient generation of transgene-free human induced pluripotent stem cells (iPSCs) by temperature-sensitive Sendai virus vectors. Proc Natl Acad Sci 108: 14234-14239.
2. Churko JM, Burridge PW, Wu JC (2013) Generation of human iPSCs from human peripheral blood mononuclear cells using non-integrative Sendai virus in chemically defined conditions. Methods Mol Biol 1036: 81-88.
3. Giorgetti A, Fazzina R, Li M, Belmonte JCI (2011) Human cord blood reprogrammed into embryonic-like stem cells. ISBT Science Series 6: 107-111.
4. Giorgetti A, Montserrat N, Aasen T, Gonzalez F, Rodríguez-Pizà I, et al. (2009) Generation of induced pluripotent stem cells from human cord blood using OCT4 and SOX2. Cell Stem Cell 5: 353-357.

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Full-Length Dysferlin Expression Driven By Engineered Human Dystrophic Blood-Derived CD133+ Stem Cells

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The protein dysferlin is abundantly expressed in skeletal and cardiac muscles, where its main function is membrane repair. Mutations in the dysferlin gene are involved in two autosomal recessive muscular dystrophies: Miyoshi myopathy and limb-girdle muscular dystrophy type 2B [1,2]. Development of effective therapies remains a great challenge. Strategies to repair the dysferlin gene by skipping mutated exons may be suitable only for a subset of mutations, while cell and gene therapy can be extended to all mutations [3]. Herein, we show for the first time the *in vitro* production of full-length dysferlin mediated by a lentiviral vector in blood-derived CD133+ stem cells isolated from patients with Miyoshi myopathy. Transplantation of engineered blood-derived CD133+ stem cells into scid/blAJ mice resulted in sufficient dysferlin expression to correct functional deficits in skeletal muscle membrane repair. Multi-exon skipping of blood-derived CD133+ stem cells isolated from the same patients led to partial dysferlin reconstitution in vitro, but failed to ameliorate the dystrophic phenotype in vivo. Our data suggest that lentivirus-mediated delivery of full-length dysferlin in stem cells isolated from Miyoshi myopathy patients is a feasible strategy to develop novel therapeutic approaches for treatment of dysferlinopathies.

References

1. Bashir A, Liu YT, Raphael BJ, Carson D, Bafna V (2007) Optimization of primer design for the detection of variable genomic lesions in cancer. Bioinformatics 23: 2807–2815
2. Liu X, Jiang Q, Mansfield SG, Puttaraju M, Zhang Y, et al. (2002) Partial correction of endogenous ΔF508 CFTR in human cystic fibrosis airway epithelia by spliceosome-mediated RNA trans-splicing. Nat Biotechnol 20: 47–52.
3. Lu QL, Morris GE, Wilton SD, Ly T, Artem'yeva OV, et al. (2000) Massive Idiosyncratic Exon Skipping Corrects the Nonsense Mutation in Dystrophic Mouse Muscle and Produces Functional Revertant Fibers by Clonal Expansion. J Cell Biol 148: 985–996.

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Wharton's Jelly Mesenchymal Stem Cells Immunomodulatory Molecules: Their Journey from Umbilical Cord to Differentiated Cells Wharton's Jelly Mesenchymal Stem Cells Immunomodulatory Molecules: Their Journey from Umbilical Cord to Differentiated Cells

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Wharton's jelly mesenchymal stem cells (WJ-MSCs) can differentiate into diverse cell types, showing a unique ability to cross lineage borders. This, together with their immunomodulatory and anti-inflammatory features, renders these cells promising for regenerative medicine applications in different pathological settings [1]. Few data are present in literature on the expression of structural and immunomodulatory molecules in umbilical cord tissue and the maintenance of their expression in paired cultured WJ-MSCs, an aspect of key importance in cellular therapy applications [2,3]. In addition, very few data do exist on the maintenance of expression of immunomodulatory molecules into the mature cell types differentiated from MSCs [4]. Therefore we intended to deeply investigate, in vivo (in umbilical cords at full term) and *in vitro* in the undifferentiated or differentiated WJ-MSCs populations, the levels of expression of different markers and their maintenance alongside cell culture, ex vivo expansion and differentiation.

Immunohistochemistry (IHC), immunocytochemistry (ICC), RT-PCR and Flow cytometry have been used to detect expression of several markers in both paired UC sections and WJ-MSCs. Differentiation has been performed towards the standard three lineages.

Paired ICC and IHC analyses on cord sections showed that for most of the analyzed molecules the expression at the protein level is maintained in both umbilical cord tissue and WJ-MSC. Structural molecules were expressed in both WJ and umbilical epithelium (UE), as well in WJ-MSCs. This was demonstrated for different classes of intermediate filaments (as cytokeratins, vimentin, desmin). Regarding HLA molecules expression, we showed for the first time that UE and WJ were positive for both HLA-ABC and HLA-E, while HLA-DR was not detectable. The same data were confirmed on WJ-MSCs. For the B7 co-stimulators, we showed for the first time that both B7-1 and B7-2 were absent in the whole UC, and in WJ-MSCs, while B7-H3 was highly expressed in both WJ and WJ-MSCs. Differentiation experiments showed that most immunomodulatory molecules are maintained without significant changes in their expression levels also after application of complex differentiation protocols, in parallel to the acquisition of mature markers or functions of the desired cell types.

Our study increases the amount of information regarding the expression of structural and immunomodulatory molecules in paired UC tissues and WJ-MSCs. WJ-MSCs mostly maintain the expression of molecules just present in their "niche", under standard culture conditions. The parallel expression of immunomodulatory molecules sheds new light on the ability of WJ-MSCs to modulate host immune responses. This is particularly of interest for the expression of HLA-E and B7-H3, which we demonstrated for the first time in both umbilical cord tissue and WJ-MSCs.

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References

1. Anzalone R, Farina F, Zummo G, La Rocca G (2011) Recent Patents and Advances on Isolation and Cellular Therapy Applications of Mesenchymal Stem Cells from Human Umbilical Cord Whartons Jelly. Rec Patents Regen Med 3: 216-227.
2. La Rocca G, Corrao S, Lo Iacono M, Corsello T, Farina F, et al. (2012) Novel Immunomodulatory Markers Expressed by Human WJ-MSC: an Updated Review in Regenerative and Reparative Medicine. Open Tissue Eng Regen Med J 5: 50-58.
3. La Rocca G, Anzalone R (2013) Perinatal stem cells revisited: directions and indications at the crossroads between tissue regeneration and repair. Curr Stem Cell Res Ther 8: 2-5.
4. La Rocca G, Lo Iacono M, Corsello T, Corrao S, Farina F, et al. (2013) Human Wharton's jelly mesenchymal stem cells maintain the expression of key immunomodulatory molecules when subjected to osteogenic, adipogenic and chondrogenic differentiation in vitro: new perspectives for cellular therapy. Curr Stem Cell Res Ther 8: 100-113.

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Characterization of the *InVitro* Immunomodulatory Properties of Mesenchymal Stem Cells Isolated from Wharton's Jelly: New Actors at Play

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Extraembryonic tissues such as umbilical cord are considered a promising source of stem cells, potentially useful in therapy [1,2]. The characterization of cells from the umbilical cord matrix (Wharton's Jelly) and amniotic membrane revealed the presence of a population of mesenchymal-like cells, sharing a set of core markers expressed by mesenchymal stem cells (MSCs) [3,4].

We aimed to evaluate the global phenotype and immunoregulatory molecules expression in undifferentiated WJ-MSCs cultured at different passages. The evaluation of in vitro immunomodulatory potential of WJ-MSCs was performed through MLR (Mixed Lymphocyte Reaction) by incubating cells with enriched lymphocytes (obtained from PBMCs). Quantitative assessment of lymphocyte proliferation was performed by flow cytometry analyses of responding cells which were subjected to incorporation of CFSE previous to start MLR.

Freshly isolated WJ-MSCs were cultured in standard media. Flow cytometry was used to characterize cells at 2nd, 5th, 10th passage. Further characterization was made by immunocytochemistry analysis. To perform MLR, cells were incubated with enriched lymphocytes previously loaded with CFSE, in order to track their proliferation using flow cytometry. After 5 days of incubation, marked lymphocytes were counted and data plotted and analyzed. Positive and negative controls were run simultaneously.

WJ-MSCs did express the classical MSC markers at all the tested passages. Moreover they resulted highly positive for class I MHC and negative for class II ones. MLR was performed at different MSC/lymphocyte ratios, showing a global trend which demonstrated that MSCs are able to reduce proliferation of responder cells in MLR. In particular, ratios of 1:1 and 1:2 cells:lymphocytes showed results which reached the statistical significance. Experiments of selective blockade of immunomodulatory molecules have been also performed to better characterize the immunomodulatory effect of WJ-MSCs.

Cells extracted from Wharton's jelly are adherent to the MSCs phenotype. In addition, we characterized the expression of a number of novel molecules by these cells, some of which are known for their immunomodulatory activity. As a result, WJ-MSCs were able to inhibit lymphocyte proliferation, both mitogen-induced or in response to allogeneic cells. These data confirm the great interest on the phenotype of these cells, for which growing roles are proposed in regenerative medicine. The better definition of the precise molecules actually involved in blocking lymphocyte proliferation in vitro will be mandatory for safe applications of these cells *in vivo*.

Acknowledgments

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References

1. Anzalone R, Lo Iacono M, Loria T, Di Stefano A, Giannuzzi P, et al. (2011) Wharton's jelly mesenchymal stem cells as candidates for beta cells regeneration: extending the differentiative and immunomodulatory benefits of adult mesenchymal stem cells for the treatment of type 1 diabetes. Stem Cell Rev 7: 342-363.
2. Lo iacono M, Anzalone R, Corrao S, Giuffrè M, Di Stefano A, et al. (2011) Perinatal and wharton's jelly-derived mesenchymal stem cells in cartilage regenerative medicine and tissue engineering strategies. Open Tissue Eng Regen Med J 4: 72-81.
3. La Rocca G (2011) Connecting the dots: The promises of wharton's jelly mesenchymal stem cells for tissue repair and regeneration. Open Tissue Eng Regen Med J 4: 3-5.
4. La Rocca G, Anzalone R, Farina F (2009) The expression of CD68 in human umbilical cord mesenchymal stem cells: new evidences of presence in non-myeloid cell types. Scand J Immunol 70: 161-162.

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Isolation and Phenotypical Characterization of Mesenchymal Stem Cells from the Wharton's Jelly of Pre-Term Human Umbilical Cord

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Extraembryonic tissues such as umbilical cord are considered a promising source of stem cells, potentially useful in therapy. The characterization of cells from the umbilical cord matrix (Wharton's Jelly) and amniotic membrane revealed the presence of a population of mesenchymal-like cells, sharing a set of core markers expressed by mesenchymal stem cells [1-3]. Pre-term umbilical cords may be also a useful source of mesenchymal populations, also considering that pre-term birth infants may develop pathologic conditions during childhood which may be reverted by a therapeutic approach based on cell therapy. In addition, pre-term cords can be also made available from therapeutic abortions and may constitute a further cell source to obtain high numbers of WJ-MSCs. Little is known about the phenotype and differentiative potential of these cells.

Preterm UC were obtained following therapeutic abortions after mothers' informed consent and processed within 12 hours from tissue collection. The isolation protocol is based on that previously described by our group for term UC. Isolated cells were routinely cultured and expanded using standardized media. Characterization of cells was performed at early passages (typically P2 and P5), by both flow cytometry (FC) and immunocytochemistry (ICC), for the detection of classical MSCs markers, immunomodulatory molecules, tissue-specific markers.

The isolation protocol allowed to successfully derive WJ-MSCs from cord specimens. The derived cells showed the typical morphology of MSCs, and were routinely passaged up to passage 10. Multi-color flow cytometric analysis showed that isolated cells were positive for classical MSCs markers (CD29, CD44, CD73, CD90, CD105) and. negative (or weakly positive) for typical hematopoietic and endothelial markers (CD45, CD34, CD14, CD68, CD39 and CD31). Further data were obtained regarding other markers which are known to be expressed in MSCs populations (CD10, CD13, CD54, CD71, CD106, CD117, CD166, STRO-1 and vimentin). In addition, we demonstrated the expression of tissue specific markers, both endodermal (CK18, CK19, alpha-fetoprotein and albumin) and neuro-ectodermal (nestin). This may indicate the potential of preterm WJ-MSCs to undergo multiple differentiation pathways, as demonstrated for cells isolated from term UC. Preterm WJ-MSCs showed MHC class I expression (but not class II), suggesting hypoimmunogenic properties for these cells. Moreover, B7H3 expression (an immunomodulatory protein that has been reported in placental tissue), should favor immune tolerance by the host following cellular transplantation [4]. ICC allowed confirming part of the FC data and was used to assess the expression of further antigens. Present data demonstrate that preterm WJ-MSCs can be isolated and expanded, with high reproducibility. A homogeneous and phenotypically stable fibroblast-like cell population can be easily isolated and expanded. Our data showed that these cells do express classical MSC markers and immunomodulatory molecules, independently from the underlying pathology which led to abortion. Further studies are underway to determine the degree of differentiability of these cells towards mature cell types derived from all of the three germ layers.

Acknowledgments

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References

1. Corrao S, La Rocca G, Lo Iacono M, Corsello T, Farina F, et al. (2013) Umbilical cord revisited: from Wharton's jelly myofibroblasts to mesenchymal stem cells. Histol Histopathol 28: 1235-1244.
2. Corrao S, La Rocca G, Lo Iacono M, Zummo G, Gerbino A, et al. (2013) New frontiers in regenerative medicine in cardiology: the potential of Wharton's jelly mesenchymal stem cells. Curr Stem Cell Res Ther 8: 39-45.
3. Huang YC, Parolini O, La Rocca G, Deng L (2012) Umbilical cord versus bone marrow-derived mesenchymal stromal cells. Stem Cells Dev 21: 2900-2903.
4. Anzalone R, Corrao S, Lo Iacono M, Loria T, Corsello T, et al. (2013) Isolation and characterization of CD276+/HLA-E+ human subendocardial mesenchymal stem cells from chronic heart failure patients: analysis of differentiative potential and immunomodulatory markers expression. Stem Cells Dev 2013 22: 1-17.

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Lamin B Participates in Cartilage Senescence and Degeneration

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It has been demonstrated that nuclear lamins play a key role in a wide range of nuclear functions, including transcription, DNA replication and repair, cell proliferation and differentiation of specific lineages during development and adult life [1]. Given the role of lamins in aging as well as disease (the premature aging disease, Hutchinson-Gilford Progeria Syndrome, HGPS, is the most intensively studied laminopathy [2], direct and indirect approaches to understanding the significance of the differential expression of lamins could be important to human health. More recently, lamins have been linked tostem cell niche function suggesting that the nucleoplasmiclamin pool and its associated proteins may have important function in chromatin organization, cell signaling and cell cycle control in adult tissue stem cells [3]. Consequently, tissue homeostasis based on stem cell activity could be disrupted by different mutations and expression levels of lamins that can lead to premature senescence and tissue degeneration. This notion is supported byrecent demonstration of the relationship between lamins, tissue stiffness and environmental signals that drive differentiation from progenitor cells. Therefore, a defect of adult stem cell functions, coupled with a potentially increased mechanical sensitivity, could result in an inefficient repair of damaged tissues in laminopathies. The information gathered to date on the lamins can be relevant not only for the understanding of molecular mechanisms underlying the aging process, but also for the developing of more effective regenerative medicine techniques and better drug targets.

It has been recently shown that overexpression of lamin A occurs in chondrocytes from osteoarthritis (OA) leading to cellular senescence [4]. OA is a common complex degenerative joint diseasewhose prominent risk factor for its development is aging. In this study, we wondered whether the process of de-differentiation and senescence in OA chondrocytes may resemble what happens in the joint of individuals with premature aging. At the same time we are interested in understanding whether an OA in vitro model may help to understand the mechanisms that may occur in premature ageing syndromes. As experimental models we used human primary chondrocytes from healthy subjects and from OA patients. A first set of experiments demonstrated that OA chondrocytes strongly resemble the behavior of de-differentiated chondrocytes obtained through prolonged passages in culture and mesenchymal stem cells (MSCs) as they express very low levels of typical chondrogenic markers including collagen type II, aggrecan, Sox9 and TRPS1. Conversely, they express high level of Slug transcription factor, a negative regulator of chondrogenesis [5]. For the first time, a marked Slug expression was observed in a sample of HGPS patient derived fibroblasts associated with a strong nuclear localization, suggesting that Slug may be a new potential marker of senescence. Consistent with these observations, cartilage histological sections and chondrocytes from OA showed an increase of both Lamin A and Lamin B1 expression, and a co-localization of lamin B1 and Slug expression. This prompted us to further this correlation. After bioinformatics analysis showing the existence of putative Slug consensus sites (E boxes) in the LMNB1 promoter, we demonstrated an in vivo recruitment of Slug at Lamin B1 promoter by chromatin immunoprecipitation (ChIP analysis). This suggests for the first time that Lamin B1 expression is dependent on Slug regulation and that this may play a critical role in the process of cartilage degeneration.

Considering that cellular senescenceis emerging as an important issue in stem cell–based therapies, our data may be relevant for two main issues: i. improvement of information about the regulation of lamin B gene expression, and ii. Characterization of a new cellular approach to study senescence and connective tissue degeneration processes.

References

1. Zuela N, Bar DZ, Gruenbaum Y (2012) Lamins in development, tissue maintenance and stress. EMBO Rep 13: 1070-1078.
2. Maraldi NM, Capanni C, Cenni V, Fini M, Lattanzi G (2011) Laminopathies and lamin-associated signaling pathways. J Cell Biochem 112: 979-992.
3. Pekovic V, Hutchison CJ (2008) Adult stem cell maintenance and tissue regeneration in the ageing context: the role for A-type lamins as intrinsic modulators of ageing in adult stem cells and their niches. J Anat 213: 5-25.
4. Attur M, Ben-Artzi A, Yang Q, Al-Mussawir HE, Worman HJ, et al. (2012) Perturbation of nuclear lamin A causes cell death in chondrocytes. Arthritis Rheum 64: 1940-1949.
5. Torreggiani E, Lisignoli G, Manferdini C, Lambertini E, Penolazzi L, et al. (2012) Role of Slug transcription factor in human mesenchymal stem cells. J Cell Mol Med 16: 740-751.

http://dx.doi.org/10.4172/2325-9620.S1-001

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Amniotic Derived Progenitor Cells in Different Animal Species: From Bench to Therapy Outcomes

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The use of stem cells in the veterinary practice is mainly focused on the treatment of orthopedic injuries, especially in the horse. The first treatment regime was published in 2001, and was followed by several experimental and clinical studies which gave encouraging results, providing evidence of the benefit and safety of applying mesenchymal stem cells (MSCs) for tendon repair. Although bone marrow-derived MSCs (BM-MSCs) represent the most widely investigated cells for application in veterinary regenerative medicine, it is important to underline the fact that in the horse, these cells have a limited potential in terms of in vitro proliferation capacity (about 32 days for expansion from isolation to implantation) [1]. To overcome this problem, other sources of MSCs could be indicated as potentially useful for the same purpose, for example extra-fetal stem cells.

The human amnion membrane is a known source of mesenchymal stem cells (AMCs). For the first time, AMCs in horse, cow, dog and cat were characterized by immunocytochemical studies which showed the expression of specific embryonic markers (TRA-1-60, SSEA-3, SSEA-4 and Oct-4) and by molecular studies which proved the positivity to CD105, CD73, CD90, CD29, CD166, and CD44, confirming the stemness of these cells. AMCs showed a high proliferative capacity due to a prominent telomerase activity and a high differentiative potential toward osteogenic, adipogenic, chondrogenic and neurogenic lineages (mesodermic and ectodermic) [2].

Moreover, equine AMCs were compared to bone marrow-derived cells (BM-MSCs) demonstrating, *in vitro*, higher proliferative and differentiative potential [1] and, *in vivo*, lower rate (4%) of re-injury when allogeneic cryopreserved AMCs were, for the first time, transplanted in spontaneous tendon injuries in horses compared to fresh autologous BM-MSCs group (23.08%) [2]. These data suggest that the appropriate time of implantation, together with the higher plasticity and proliferative capacity of AMCs represented the main features of interest for this novel heterologous approach for the treatment of equine tendon diseases.

Moreover, horse AMCs has immunomodulatory capacity demonstrated by their ability to inhibit PBMC proliferation not only when cultured in cell–cell contact with responder cells but also when separated from them by a transwell membrane. This ability could be attributed to soluble factors released by AMCs. This hypothesis was further supported by the inhibition of the PBMC proliferation exerted by the conditioned medium secreted by AMCs (AMC-CM). When AMC-CM was used to treat spontaneous horse tendon and ligament injuries, regenerative/reparative responses were achieved. Our outcomes demonstrated that the AMCs transplantation results in improved tendon healing and that the AMC-CM could be a novel therapeutic biological cell-free product in spontaneous tendon and ligament diseases [3].

In the horse, in addition to tendon disorders, endometrial diseases are also widespread. In view of the potential application of AMCs in endometrial regenerative medicine we further characterised equine amniotic derived cells. Since endometrial stromal cells proliferate and specialize during pregnancy under the control of progesterone, the expression of endometrial genes involved in early pregnancy (AbdB-like Hoxa genes), in conceptus pre-implantantion development (*ERα*, *ERβ*, *PR*, *PGRMC1* and *mPR*), and their regulators (*Wnt7a*, *Wnt4a*) were evaluated on AMCs cultured with and without progesterone in comparison to cells isolated from endometrium (EDCs) at diestrus stage. All the genes studied were expressed abundantly in endometrial tissue and amniotic membrane. Similarly, AMCs at P0 expressed the same genes identified in tissues, but *Hoxa-9* was the only gene observed in AMCs at passage 1 (P1). After culture of AMCs with progesterone, *Hoxa-9*, *PGRMC1* and *mPR* expressions were conserved in this cell line until P5. Moreover, EDCs, when in co-culture with AMCs by transwell system or by CM, showed a 1.23 and 1.34 fold increase in proliferation, respectively. These data suggest that AMCS or their CM can promote EDCs proliferation and may be used in regenerative medicine when poor endometrial proliferation is associated with infertility or poor pregnancy outcomes [4].

All of our data provide an intriguing indication of the possible implication of AMCs in regenerative medicine.

References

1. Lange-Consiglio A, Corradetti B, Meucci A, Bizzaro D, Cremonesi F (2013) Characteristics of equine mesenchymal stem cells derived from amnion and bone marrow: in vitro proliferative and multilineage potential assessment. Equine Vet J 45: 737-744.
2. Lange-Consiglio A, Corradetti B, Bizzaro D, Magatti M, Ressel L et al. (2012) Characterization and potential applications of progenitor-like cells isolated from horse amniotic membrane. Journal of Tissue Engineering and Regenerative Medicine 6: 622-635.
3. Lange-Consiglio A, Rossi D, Tassan S, Cremonesi F, Parolini O (2013) Conditioned medium from horse amniotic membrane-derived multipotent progenitor cells: immunomodulatory activity *in vitro* and first clinical application in tendon and ligament injuries *in vivo*. Stem Cell Development 22: 3015-3024.
4. Corradetti B, Perego R, Meucci A, Cremonesi F, Lange-Consiglio A (2013) Equine amnion-derived mesenchymal stem cells: possible implication in endometrial re generation Reproduction, Fertility and Development 25: 295.

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Adipose Stromal Cells-Conditioned Medium is Less Efficient in Modulating Osteoarthritic Chondrocytes and Synoviocytes Behavior

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Introduction

Adipose stromal cells (ASC) have been shown to exert anti-fibrotic, anti-inflammatory and anti-apoptotic properties, through secreted growth factors [1,2]. Basing on these characteristics has been proposed their use for stem cell-based therapy of degenerative disease for the treatment of osteoarthritis (OA). Therefore the aim of the study was the analysis of trophic potential effects of ASC-conditioned medium (CM) on chondrocyte and synoviocyte from OA patients.

Materials and Methods

Good manufacturing practice (GMP)-clinical grade ASC was isolated from subcutaneous adipose tissue. Chondrocytes and synoviocytes were isolated from cartilage and synovial of OA patients undergoing total joint replacement. Chondrocytes or synoviocytes were treated with different ratio of ASC-CM or co-cultured with ASC in transwell. Specific markers of fibrosis (collagen type 1 and 3), matrix degrading factors and inhibitors (ADAMTS4, ADAMTS5, TIMP1, TIMP3), inflammatory factors (IL6, CXCL1/GROα, CXCL8/ IL8, CCL2/MCP-1, CCL3/MIP-1α, CCL5/RANTES) and anabolic (HGF, PGE) factors were evaluated by qRT-PCR or immunoassays.

Results

ASC-CM was capable of reducing collagen type 1 and 3 both on chondrocytes and synoviocytes as we found using the co-culture condition. However, ASC-CM was less efficient both on basal inflamed OA chondrocytes and synoviocytes, in reducing inflammatory factors (IL6, CXCL1/GROα, CXCL8/IL8, CCL2/MCP-1, CCL3/MIP1-α, CCL5/RANTES), as well as proteinases, such as metalloproteinase (MMP13), a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS4, ADAMTS5) and their tissue metalloproteinase inhibitors (TIMP1, TIMP3) compared to ASC in co-culture. HGF and PGE2 immunomodulators confirmed their role in exerting anti-fibrotic and anti-inflammatory effects.

Discussion and Conclusions

These data demonstrate that all the markers analyzed on chondrocytes or synoviocytes were mainly down-modulated in co-culture condition suggesting the importance of the cross-talk between cells and supporting the importance of using ASC in the treatment of OA. Moreover, even if HGF and PGE2 were involved in down-modulation of fibrosis and inflammation, however, ASC effects were strictly dependent from their presence indicating that ASC-CM could be less efficacious in preventing OA evolution.

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References

1. Manferdini C, Maumus M, Gabusi E, Piacentini A, Filardo G, et al.(2013) Adipose-derived mesenchymal stem cells exert antiinflammatory effects on chondrocytes and synoviocytes from osteoarthritis patients through prostaglandin E2. Arthritis & Rheumatism 65: 1271-1281.

2. Maumus M, Manferdini C, Toupet K, Peyrafitte JA, Ferreira R, et al. (2013) Adipose mesenchymal stem cells protect chondrocytes from degeneration associated with osteoarthritis. Stem Cell Res 11: 834-44.

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Human Mesenchymal Stem Cells Reendothelize Porcine Heart Valve Scaffolds: New Perspectives in the Heart Valve Tissue Engineering

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Keywords

Heart valve diseases; Endothelium; Heart valve tissue engineering; WJ-MSC; HUVEC

Heart valve substitution, based on biosynthetic or mechanical prosthesis replacement, is one of the most frequent surgical approaches to treat heart valve diseases. Even if the prosthesis implantation gives a good life quality for patients, there are many long-term disadvantages related to the substitution, such as structural deterioration, non-structural dysfunction and re-intervention. The heart valve tissue engineering (HVTE), a novel branch of regenerative medicine, is developing innovative models and testing new methods to overcome the above reported limitations. In the present study, we investigated the possibility to reendothelize a porcine heart valve scaffold, previously decellularized, by using two cell types: Wharton's Jelly mesenchymal stem cells (WJ-MSC) and human umbilical vein endothelial cells (HUVEC), the last used as control cells for the reendothelialization process. Both cell types showed, by fluorescence microscopy that they were able to reconstitute a valid and functional monolayer of neo-endothelium, characterized by the surface expression of typical endothelial markers (i.e. CD144 and CD146). All together, these data suggest that both HUVEC and WJ-MSC are suitable for *in vitro* autologous endothelium regeneration, opening new perspectives in the field of HVTE.

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References

1. Weymann Patil NP, Sabashnikov A, Jungebluth P, Korkmaz S, Li S, et al. (2013) Bioartificial Heart: A Human-Sized Porcine Model – The Way Ahead. PLoS One 9: e111591.
2. Angelucci (2010) Proteome Science 8: 18.
3. Di Tomo P, Canali R, Ciavardelli D, Di Silvestre S, De Marco R, et al. (2012) β-Carotene and lycopene affect endothelial response to TNF-α reducing nitro-oxidative stress and interaction with monocytes. Mol Nutr Food Res 56: 217-227.
4. Lachmann R, Lanuti P, Miscia S (2012) OMIP-011: Characterization of circulating endothelial cells (CECs) in peripheral blood. Cytometry Part A 81: 549-551.

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Comparing the Immunoregulatory Effects of Mesenchymal Stem Cells Isolated From Bone Marrow, Placenta and Amniotic Fluid

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Although bone marrow (BM) represents the main source of MSCs, the need remains to identify a stem cell source that is safe, easily accessible, providing high cell yield and for which cell procurement does not provoke ethical debate.

In this study, MSCs isolated from amniotic fluid (AF) and placenta (PL) were compared with BM-MSCs. First, immunophenotype, differentiative potential and embryonic markers were analysed, then immunomodulant proprieties. In particular, *in vitro* co-culture system of MSCs with total activated T-cells with Phytoemoagglutin (PHA-PBMC) were performed to study: 1) the effect on T Lymphocytes (Ly) proliferation, 2) the presence of T regulatory Ly (Treg), 3) the immunophenotype of different T subsets, 4) the cytokine release and master gene expression to verify the Th1, Th2 and Th17 polarization, 5) the production of IDO as a potent immunomodulant soluble factor.

The results show that MSCs derived from foetal tissues have a greater proliferative and differentiative potential associated with the presence of embryonic markers than BM-MSCs.

In all co-culture conditions with PHA-PBMC and MSCs (independently from the tissue origin) data showed: 1) T proliferation inhibition; 2) Naïve T Increase and Memory T decrease; 3) Treg increase; 4) strong Th2 polarization associated to increase IL-10 and IL-4; Th1 inhibition (IL-2, TNF-α, IFN-γ and IL-12 significantly decrease) and Th17 induction confirmed from the production of high concentration of IL-6 and IL-17; 5) IDO mRNA induction in MSCs co-cultured with PHA-PBMC. Real time PCR performed on transcriptional factors involved in these polarization confirmed the data obtained at protein level. **A**F-MSCs showed a more potent immunomodulant effect on T-cells than BM-MSCs and only slightly higher than PL-MSCs. This study shows that MSCs isolated from foetal tissues may be considered a good alternative to BM-MSCs for clinical applications.

References

1. Mareschi K, Rustichelli D, Comunanza V, De Fazio R, Cravero C, et al. (2009) Multipotent mesenchymal stem cells from amniotic fluid originate neural precursors with functional voltage-gated sodium channels. Cytotherapy 11: 534-547.
2. Mareschi K, Novara M, Rustichelli D, Ferrero I, Guido D, et al. (2006) Neural differentiation of human mesenchymal stem cells: Evidence for expression of neural markers and eag K+ channel types. Exp Hematol 34: 1563-1572.

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In Vitro Generation of Vascular Grafts from Lipoaspirated Human Adipose Tissue Derived Stem Cells

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Adipose tissue is a readily available source of multipotent adult stem cells for use in tissue engineering/regenerative medicine. Various materials have been used to develop Vascular Grafts. Synthetic vascular grafts have disadvantages in terms of availability and functionality that have limited their adoption in clinical routine. Herein we study the adhesion, growth and endothelial differentiation potential of ADSC seeded onto a tubular porous polycaprolactone (PCL) scaffold. The objective of this study is to demonstrate that tubular scaffold PCL can to be used as support for construction of Vascular Graft using adipose stem cells. We have previously demonstrated that undifferentiated ADSC adhere and grow on round PCL dishes. In this study for the first time demonstrate that ADSC can adhere, grow and differentiate into endothelial cells on a matrix of three-dimensional tubular *PCL*. After culture in endothelial differentiation medium, ADSC were positive to LDL uptake and expressed molecular markers characteristic of endothelial cells (CD31; eNOS and vWF). PLC has excellent mechanical properties and a slow degradation rate. In addition, our study defines the time required for the differentiation of ADSC directly onto PCL. This study suggests that tubular PCL can be used as a scaffold to generate vascular grafts in vitro.

References

1. Marino G, Rosso F, Ferdinando P, Grimaldi A, De Biasio G, et al. (2012) Growth and endothelial differentiation of adipose stem cells on polycaprolactone. J Biomed Mater Res A 100: 543-548.
2. Cafiero G, Papale F, Grimaldi A, Rosso F, Barbarisi M, et al. (2011) Immunogold labelling in environmental scanning electron microscopy: applicative features for complementary cytological interpretation. J Microsc 241: 83-93.
3. Papale F, Cafiero G, Grimaldi A, Marino G, Rosso F, et al. (2013) Galectin-3 expression in thyroid fine needle cytology (t-FNAC) uncertain cases: Validation of molecular markers and technology innovation. J Cell Physiol 228: 968 – 974.

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Functional Characterization of GAP-43 Knockout Satellite Cells during the Myogenic Processes

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The Growth Associated Protein 43 (GAP-43) is involved in neuronal plasticity during development and regeneration. Our recent data revealed that this protein is expressed in both myoblasts and myotubes, and its cellular localization changes dramatically during differentiation. In adult fibers, GAP-43 localization is found nearby the calcium release units suggesting a functional role for this protein. The aim of this study is to define the role of GAP-43 during myogenesis and in myotubes. This was obtained analyzing the main cellular properties and intracellular calcium signaling of myogenic satellite cells isolated from wild type and GAP-43 knockout hetero- and homozygous mice [1-3].

The results showed similar proliferative and differentiative properties (assayed by morphological analyses) in all tested models. All myotube populations were responsive to KCl or caffeine with intracellular calcium increases. Interestingly, the myotubes from GAP-43 knockout hetero- and homozygous satellite cells, showed different intracellular calcium dynamics in respect to wild type ones. In particular GAP-43 knockout homozygous myotubes showed high amplitude spontaneous waves differently from the significantly lower ones expressed in wild type myotubes.

These data suggested that the absence of GAP-43 could not affect the myoblast differentiation and myotube formation, but it had a significant role in modulating intracellular calcium handling.

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References

1. Dent EW, Meiri KF (1998) Distribution of phosphorylated GAP-43 (neuromodulin) in growth cones directly reflects growth cone behavior. J Neurobiol 35: 287–299.
2. Korshunova I, Novitskaya V, Kiryushko D, Pedersen N, Kolkova K, et al. (2007) GAP-43 regulates NCAM-180-mediated neurite outgrowth. J Neurochem 100: 1599–1612.
3. Guarnieri S, Morabito C, Paolini C, Boncompagni S, Pilla R et al. (2013) Growth associated protein 43 is expressed in skeletal muscle fibers and is localized in proximity of mitochondria and calcium release units. PlosOne 8: e73816.

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Extracellular Vesicles: Can Paracrine Signaling Be Exploited as a Therapeutic Tool?

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Mesenchymal stromal cells (MSCs) have immunomodulatory properties demonstrated in vitro, in animal studies and in clinical applications such as the treatment of severe GVHD. However, the underlying mechanisms have not been fully clarified. The role of soluble factors is generally recognized, both in mediating the effect on B and on T lymphocytes [1]. Recently, it was demonstrated that several interactions between immune cells are mediated by secreted membrane vesicles (EVs) [2]. Various types of secreted EVs have been described (the two major species termed "shedding vesicles" and exosomes) ranging from 50 to 1000 nm diameter size and exhibiting distinct structural and biochemical properties according to their intracellular site of origin, features probably also affecting their function. An increasing body of evidence indicates that they play a pivotal role in cell-to-cell communication. In particular, EVs can play a role in intercellular signaling by exchanging mRNA, microRNA, and proteins among cells within a defined microenvironment.

We observed that the inhibitory effects of MSCs on B-cell proliferation and differentiation in a CpG-stimulated peripheral blood mononuclear cell coculture system could be fully reproduced by EVs isolated from MSC culture supernatants in a dose-dependent fashion. A dose-dependent inhibitory activity of MSC-EVs was also observed for IgM, IgG and IgA production. Moreover, in the same coculture system 7-AA-negative and Annexin-positive MSC-EVs isolated from mesenchymal stromal cells were internalized in a subset of CD86/CD19 positive cells corresponding to activated B lymphocytes [3].

We also investigated the effect of MSC-EVs on T cells in PBMC cultures stimulated with aCD3/CD28 beads. Stimulation significantly increased the number of proliferating CD3+ cells as well as of CD4+/CD25+/CD127low T regulatory cells (Treg). Stimulation did not affect the number of apoptotic cells. Co-culture with MSCs inhibited the proliferation of CD3+ cells, with no significant changes in apoptosis. Addition of MSC-EVs to PBMCs did not affect proliferation of CD3+ cells, but induced the apoptosis in CD3+ cells and the CD4+ sub-population and increased the proliferation and (to a lesser extent) the apoptosis of Treg. Moreover, MSC-EV treatment increased of the Treg/Teff ratio and the immunosuppressive cytokine IL-10 concentration in culture medium. The activity of indoleamine 2,3-dioxygenase (IDO), an established mediator of MSC immunosuppressive effects, was increased in supernatants of PBMC co-cultured with MSCs, but was not affected by the presence of MSC-EVs. In conclusion, MSC-EVs demonstrate immunomodulatory effects both on B and on T cells in vitro. However, both these effects and the underlying mechanisms may be different from those exhibited by the parent MSC cells.

The effect of EVs on T cells was also investigated by Mokarizadeh et al. [4]. These authors showed that EVs isolated from murine BMSCs inhibited the proliferation of both syngenic and allogenic T lymphocytes. Additionally, they demonstrated that these microparticles were able to induce apoptosis in activated T cells. Interestingly, this inhibition was associated with an increased proportion of regulatory T CD4+-CD25+-FoxP3+ cells. Moreover, an increased secretion of IL-10 and TGFβ1 by cultured splenic cells added with MSC-EVs was observed. These results suggest that MSC-EVs can induce tolerogenic signaling.

In vitro results are also supported by our in vivo observations in an animal model of inflammatory bowel disease induced by Dextran Sulfate Sodium (DSS). Mice injected daily with MSC-EVs showed less weight loss, improved disease activity index and a less severe reduction in colon length when compared to DSS/vehicle-treated controls. Real time RT-PCR analysis performed on RNA extracted from colon tissue revealed a strong inhibition of the induction of inflammatory cytokines with respect to untreated animals. Collectively, these data suggest that EVs isolated from MSCs could reproduce the immunomodulatory effect of MSCs. MSC-derived EVs appear to be potent organelles for induction of peripheral tolerance and modulation of immune responses.

References

1. Doorn J, Moll G, Le Blanc K., van Blitterswijk C, de Boer J (2012) Therapeutic applications of mesenchymal stromal cells: paracrine effects and potential improvements. Tissue Eng Part B Rev 18: 101-115.
2. Fierabracci A, Del Fattore A, Luciano R, Muraca M, Teti A, et al. (2013) Recent advances in mesenchymal stem cell immunomodulation: the role of microvesicles. Cell Transplant 24: 133-149.
3. Budoni M, Fierabracci A, Luciano R, Petrini S, Di Ciommo V, et al. (2013) The immunosuppressive effect of mesenchymal stromal cells on B lymphocytes is mediated by membrane vesicles. Cell Transplant 22: 369-379.
4. Mokarizadeh A, Delirezh N, Morshedi A, Mosayebi G, Farshid AA, et al. (2012)Microvesicles derived from mesenchymal stem cells: potent organelles for induction of tolerogenic signaling. Immunol Lett 147: 47-54.

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Amniotic Membrane Derivatives: From Preclinical Data towards Future Clinical Applications

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The human placenta is an unexpected source of stems cells, which has gained increased interest over the past several years. The interest raises for their differentiation capabilities but mostly for their immunomodulatory properties, along with the fact that they are easily obtained and impervious to ethical concerns [1]. Our studies have significantly contributed to elucidating the immunomodulatory action of these cells, and more importantly, to that of the conditioned medium derived from their culture [2].

We have shown that mesenchymal cells from the human amniotic membrane (hAMSC) can reduce T cell proliferation, induce modulation of T helper cell *subsets,* and induce polarization towards T regulatory cells. Additionally, we have demonstrated that hAMSC can inhibit monocyte maturation towards dendritic cells, and promote macrophage polarization towards an M2 phenotype. We confirmed these observations by the comparative studies using amniotic membrane-derived cells and conditioned medium derived from the culture of these cells in several in vivo models, such as lung and liver fibrosis, and rheumatoid arthritis [3,4]. Interestingly, we have shown that the therapeutic effects, as demonstrated by fibrosis attenuation after injection of conditioned medium, are mostly due to the release of paracrine-acting bioactive molecules derived from the culture of hAMSC [3].

In conclusion, human placenta cells and their derivatives such as conditioned medium could represent a valuable therapeutic tool in degenerative diseases through the alteration of immune reactions and inflammatory processes. Much work is still underway to unveil the mechanisms underlying their therapeutic capacities.

References

1. Parolini O, Alviano F, Bagnara GP, Bilic G, Bühring HJ, et al. (2008) Concise review: isolation and characterization of cells from human term placenta: outcome of the first international Workshop on Placenta Derived Stem Cells. Stem Cells 26: 300-311.
2. Silini A, Parolini O, Huppertz B, Lang I (2013) Soluble factors of amnion-derived cells in treatment of inflammatory and fibrotic pathologies. Curr Stem Cell Res Ther 8: 6-14.
3. Cargnoni A, Piccinelli EC, Ressel L, Rossi D, Magatti M, et al. (2014) Conditioned medium from amniotic membrane-derived cells prevents lung fibrosis and preserves blood gas exchanges in bleomycin-injured mice-specificity of the effects and insights into possible mechanisms. Cytotherapy 16: 17-32.
4. Parolini O, Souza-Moreira L, O'Valle F, Magatti M, Hernandez-Cortes P, et al. (2014) Therapeutic effect of human amniotic membrane-derived cells on experimental arthritis and other inflammatory disorders. Arthritis Rheumatol 66: 327-339.

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Expression of Aquaporin-1 and CXCR4 in Ovine Mesenchymal Stem Cell

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Mesenchymal stem cells (MSCs) are a well characterized and highly adaptable cell source for regenerative medicine and tissue engineering. Ovine species is considered as a valuable model for human bone turnover and remodeling activity, due to the fact that adult animals show similar bone structure and composition [1]. Therefore, in orthopedic research, sheep are frequently employed for critical-size bone defects, which are then treated with different biomaterials combined with (predifferentiated) MSCs. Despite a considerable number of reports employing ovine MSCs (oMSCs) in tissue engineering, their molecular characterization is limited to few studies. Unlike human MSCs, ovine MSC are not well studied regarding isolation, expansion and surface antigen expression of oMSC.

Recently, a family of 13 water-transporting membrane channel proteins, called aquaporins (AQPs) known for their key role in fluid transport in various epithelial and endothelial tissues, have been extensively studied [2]. Such proteins seem to have important functions in diverse biological processes related to cell migration, proliferation, apoptosis and cell volume-regulation. Furthermore, many studies have demonstrated that stem cell migration and organ-specific homing are regulated by chemokine and their receptors [3].

Therefore, the aim of this work was to determine whether water channel molecule, aquaporin 1 (AQP1) and chemokine receptor type 4 (CXCR4) could be expressed in o-MSCs to regulate some cellular events. MSC isolated from fresh bone marrow obtained from the posterior iliac crest of sheep were cultured on flasks by incubation in a humidified atmosphere at 37 °C with 5% CO2 in culture medium (a-MEM) supplemented with 10% fetal bovine serum (FBS). Further phenotypical characterization of MSC was performed via cytofluorimetric analysis of cell surface markers at passage 2. MSC resulted positive for CD44, CD73, CD90, CD105, CD146 and negative for CD34 and CD45. The analysis was performed using a FC500 flow cytometer (Beckman Coulter, Brea, CA, USA). To obtain conditionated Medium (CM), oMSCs were grown for different times 24 h, 48 h and 72 h in cultured medium with 1% FBS. Then, CM was collected, filtered and stored in aliquots at -20 °C until required, to investigate the impact of CM composition on AQP1 and CXCR4 expression. Morphological changes of cells exposed to CM were observed by light microscope. Whole protein extracts were prepared and analyzed by western blotting. Furthermore, oMSCs proliferation and migration analysis were performed also in presence of CM.

Our results showed that oMSCs cultured in 10% FCS medium express high level of AQP1 and CXCR4. Furthermore, CM after an exposure to oMSCs for 48 h, causes an increase of AQP1 and CXCR4 expression as well as an enhancement of cell proliferation. In conclusion, the results of this study suggest that oMSCs produce some regulatory factors (cytokines, chemokines or growth factors) that promote their migration by autoctine pathways [4]. It is well known that these soluble mediators may act directly, triggering intracellular mechanisms of injured cells, or indirectly, inducing secretion of functionally active mediators by neighboring cells. For these reasons, the results of this study could improve the knowledge on oMSC characterization in consideration of their application in regenerative medicine because of handling and housing, cost, and ethical acceptance.

References

1. Guo X, Wang C, Duan C, Descamps M, Zhao Q, et al. (2004) Repair of osteochondral defects with autologous chondrocytes seeded onto bioceramic scaffold in sheep. Tissue Eng 10: 1830–1840.
2. Verkman AS (2008) Mammalian aquaporins: diverse physiological roles and potential clinical significance. Expert Rev Mol Med 10: e13.
3. Miller RJ, Banisadr G, Bhattacharyya BJ (2008) CXCR4 signaling in the regulation of stem cell migration and development. J Neuroimmunol 198: 31–38.
4. Li L, Jiang J (2011) Regulatory factors of mesenchymal stem cell migration into injured tissues and their signal transduction mechanisms. Front Med 5: 33–39.

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Paclitaxel Uptake Release by Mesenchymal Stromal Cells

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Mesenchymal stromal cells (MSCs) are easy to isolate and expand, have good migration capacity and after injection "*in vivo*" show the ability to home pathological tissues expressing a significant tumour tropism. These characteristics have contributed to candidate MSCs as an interesting vehicle to deliver drugs. For this purpose several strategies have been setup for using engineered MSCs to deliver anti-neoplastic agents in the tumor microenvironment to limit the collateral toxicity making more specific the therapy. Our previous studies showed that exposure of bone marrow derived MSCs to high doses of Doxorubicin led them to acquire anti-proliferative potential towards co-cultured haematopoietic stem cells (HSCs). We thus hypothesized whether MSCs isolated from different tissues (eg: bone marrow, adipose tissue and dermis) after *in vitro* loading with the anti-cancer drug and then localized near tumor cells, could inhibit their proliferation.

In our recent studies we used Paclitaxel (PTX) that is an important drug able to block cell proliferation binding tubulin and also expressing strong anti-angiogenic properties. The incorporation of PTX into MSCs was studied by using FITC-labelled-PTX and analyzed by FACS and confocal microscopy. The ultra structural analysis performed by electron microscopy revealed that the loading with PTX did not induce morphological alterations in PTX primed MSCs (MSCs-PTX). The results showed that MSCs rapidly incorporate PTX and only a little fraction of cells were lost, due to chemo-induced apoptosis. More than 80% of viable MSCs were able to release PTX in the conditioned medium (CM) in a time dependent manner. The release of PTX in culture medium by MSCs-PTX was demonstrated by HPLC. The activity of the culture medium and the direct anti-tumor activity of MSCs-PTX (by co-culture systems) were tested *in vitro* by evaluating the inhibition of the proliferation of different tumor cell lines as DU145, MOLT-4, T-98, B16 and CF-PAC. Our results demonstrated that MSCs-PTX (both from bone marrow, adipose tissue and dermis) exerted a potent anti-tumor effect that has been also confirmed by the *in vivo* studies performed in nude mice. Although genetically modified cells provided encouraging results in animal models, they are not devoid of risks if considered for clinical applications. Our data demonstrate, for the first time, that without any genetic manipulation, MSCs can uptake and subsequently release PTX, thus becoming a potential safer tool for drug delivery in new therapeutic approaches.

References

1. Pessina A, Bonomi A, Coccè V, Invernici G, Navone S, et al. (2011) Mesenchymal stromal cells primed with paclitaxel provide a new approach for cancer therapy. PLoS One 6: e28321.
2. Pessina A, Coccè V, Pascucci L, Bonomi A, Cavicchini L, et al. (2013) Mesenchymal stromal cells primed with Paclitaxel attract and kill leukaemia cells, inhibit angiogenesis and improve survival of leukaemia-bearing mice. Br J Haematol 160: 766-778.
3. Pessina A, Coccè V, Bonomi A, Cavicchini L, Sisto F, et al. (2013) Human skin-derived fibroblasts acquire in vitro anti-tumor potential after priming with Paclitaxel. Anticancer Agents Med Chem 13: 523-530.
4. Bonomi A, Coccè V, Cavicchini L, Sisto F, Dossena M, et al. (2013) Adipose tissue-derived stromal cells primed in vitro with paclitaxel acquire anti-tumor activity. Int J Immunopathol Pharmacol 26: 33-41.

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Calcimimetic R-568 Improves Osteogenic Differentiation of Human Amniotic Fluid Mesenchymal Stem Cells (hAFMSCs) through Calcium Sensing Receptor (CaSR) Activation

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Human mesenchymal stem cells derived from amniotic fluid (hAFMSCs) have been identified as a promising model for therapeutic applications in bone traumatic and degenerative damage. Calcium Sensing Receptor (CaSR), a G protein-coupled receptor, plays a physiological role in the regulation of bone metabolism. Thus, the bone CaSR could be targeted by allosteric modulators, in particular by agonists such as the calcimimetic R-568, which may be potentially helpful in treating bone diseases [1].

The aim of our study was to characterize CaSR expression in hAFMSCs and to evaluate the activity of calcimimetic R-568 during *in vitro* osteogenesis. Using western blotting, immunofluorescence and flow cytometry, we demonstrated basal CaSR diffuse expression in hAFMSCs which increased along osteogenic differentiation. Notoriously, both R-568 and calcium significantly enhanced hAFMSC osteogenic differentiation after exposure to osteogenic medium.

To provide additional evidence of the involvement of CaSR in osteogenesis, we correlated its expression with that of established osteogenic markers, i.e. alkaline phosphatase (ALP), runt-related transcription factor 2 (Runx2) and osteopontin (OPN), as well as novel, not yet completely defined regulators of osteogenesis. Among these, β-catenin and Slug, which are mediators of Wnt signalling [2], and Nuclear factor of activated T cells c1 (NFATc1), which plays a critical role in calcium/calcineurin signalling [3].

Taken together, our results demonstrate, for the first time, that CaSR is expressed in hAFMSCs, positively correlates with osteogenic markers and is activated by R-568. Importantly, down regulation of CaSR by RNA interference supports the conclusion that CaSR activation plays a fundamental role in hAFMSC osteogenesis.

Therefore, this study provides relevant information on the mechanisms of hAFMSC osteogenesis, which could provide additional molecular basis for the application of calcimimetics in bone regenerative medicine.

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References

1. Di Tomo P, Pipino C, Lanuti P, Morabito C, Pierdomenico L, et al. (2013) Calcium sensing receptor expression in ovine amniotic fluid mesenchymal stem cells and the potential role of R-568 during osteogenic differentiation. PLoS One 8: e73816.
2. D'Alimonte I, Lannutti A, Pipino C, Di Tomo P, Pierdomenico L, et al. (2013) Wnt signaling behaves as a "master regulator" in the osteogenic and adipogenic commitment of human amniotic fluid mesenchymal stem cells. Stem Cell Rev 9: 642–654.
3. Penolazzi L, Lisignoli G, Lambertini E, Torreggiani E, Manferdini C, et al. (2011) Transcription factor decoy against NFATc1 in human primary osteoblasts. Int J Mol Med 28: 199–206.

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Characterization of Human Amniotic Fluid-Derived Cells through a Morphological and Proteomic Study

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Mesenchymal Stem Cells derived from Amniotic Fluid (AFMSCs) are multipotent cells able to give rise to multiple mesenchymal lineages and therefore of great interest for regenerative medicine [1]. Although the presence of different cell populations in the amniotic fluid has been documented, the two predominant cell types, i.e. Epithelial-like (E-like) and Fibroblast-like (F-like), need to be fully characterized.

In the present study, we isolated the cell populations from second trimester amniotic fluid of pregnant women and classified as E-like or F-like phenotype (by phase contrast and toluidine blue-stained semithin section microscopy) on the basis of the prevalence (60-70%) of epithelial or fibroblast morphology of the cultured cells, respectively. Importantly, we tested the immunophenotype of cultured AFMSCs by flow cytometry analysis using large types of markers. These phenotypes showed slight differences in surface and stemness markers, with higher CD90 and lower Sox2 and SSEA-4 expression in F-like compared to E-like cells, whereas CD326 (Ep-CAM) was expressed only in the E-like phenotype. Nevertheless, they displayed similar capability to differentiate into osteogenic and adipogenic lineages. In fact both E-like and F-like AFMSCs maintained in osteogenic medium, exhibited a greater time-dependent (7-14-21 days) increase in calcium deposition compared to cells kept in normal medium and a comparable increment in Oil Red O staining as well as in the presence of single adipocytes with multiple vacuoles [2]. Furthermore, proteomic analysis [3] revealed proteins specifically expressed in each cluster (HC1, epithelial; HC2, fibroblast). Of note, twenty-five and eighteen protein spots were differentially expressed in HC1 and HC2 classes, respectively. The protein-interaction networks (PIN) for both phenotypes, showed strong interactions between specific AFMSC proteins and molecular chaperones, such as pre-proteasomes and mature proteasomes, both important for cell cycle regulation and apoptosis.

Thus, our results provide the first evidence that, although cultured E-like and F-like AFMSCs displayed a differential proteomic phenotype, they showed similar *in vitro* properties. Then, proteomic profile of E-like and F-like AFMSCs could be uncoupled from the differentiation potential and therefore both cell phenotype might be considered an excellent source for basic research and for potential therapeutic use.

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References

1. Cananzi M, Atala A, De Coppi P (2009) Stem cells derived from amniotic fluid: new potentials in regenerative medicine. Reprod Biomed Online 18 Suppl 1: 17–27.
2. D'Alimonte I, Lannutti A, Pipino C, Di Tomo P, Pierdomenico L, et al. (2013) Wnt Signaling Behaves as a "Master Regulator" in the Osteogenic and Adipogenic Commitment of Human Amniotic Fluid Mesenchymal Stem Cells. Stem Cell Rev 9: 642–654.
3. Angelucci S, Marchisio M, Di Giuseppe F, Pierdomenico L, Sulpizio M, et al. (2010) Proteome analysis of human Wharton's jelly cells during *in vitro* expansion. Proteome science 8: 18.

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Increase in Post-Thaw Viability of Cord Blood HSC by adding rMnSOD: Preliminary Results

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Abstract

Umbilical cord blood (UCB) represents a source for hematopoietic stem cells (CB-HSC), and transplantation of cord blood has been part of clinical practice since more than 10 years [1]. The UCB is usually collected by public or private cord blood banks, which store it for an unknown recipient for an undetermined time. The cryopreservation process, used for stem cell storage, is particularly critical for umbilical cord blood [2]. The most used cryoprotectant agent (CPA) to avoid or reduce cell damage caused by ice crystals, is 10% dimethyl sulfoxide (Me_2SO). Me_2SO, while protecting the cells from membrane rupture, however has a toxic effect on them, depending on the temperature and the exposure time for both pre-freeze and post-thaw periods [2]. Generation of oxygen-free radicals (ROS) is a major cause of cell damage during low-temperature storage. Cells counteract ROS accumulation through antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPX). The addition of antioxidants to the conventional freezing medium improves the post-thaw cell recovery [3]. The purpose of this study is the improvement of cord blood HSC's (CB-HSC) cryopreservation method by addition of a new isoform of recombinant manganese superoxide dismutase (rMnSOD), obtained from the native liposarcoma derived form (LSA-MnSOD) [4]. rMnSOD, while having the same enzymatic activity common to all SODs, differs for its higher molecular weight (30 kDa versus 24 kDa) due to the presence of an additional sequence, called leader peptide, not cleaved during its maturation. As demonstrated by Mancini *et al.* [4], recombinant LSA-type MnSOD (rMnSOD) has a distinctive capacity to penetrate cells and converts ROS to H_2O_2. The latter is rapidly converted to O_2 and H_2O by catalase, thus resulting in a beneficial oxygenation of healthy cells.

Materials and Methods

In this study, we isolated mononuclear cells [5] by lymphoprep gradient tube (Pan Biotech, Germany) from cord blood after informed consent of healthy donors, received by the Cord blood Bank Ba.S.C.O of "*Santobono Pausilipon*" hospital of Naples. HSCs CD43+ were detected by flow cytometry and immunocytochemical methods at L.M., by using specific stem cell antibodies before and after the following experimental treatment. Cultured CB-HSCs were incubated with scalar concentrations from 1.5 μM to 0.015 μM of rMnSOD for 5 hours (with StemPro®-34 SFM, Life Technologies) and frozen using standard freezing protocol with 10% Me_2SO. After thawing, CD34+ viable cells were counted by flow cytometry FACSCalibur II (Becton & Dickinson) by using BD Stem Cell Enumeration Kit (BD Biosciences).

Results

The viability of CB-HSC CD34+ increased from 10% of the control to 45% by adding 0.15 μM rMnSOD. Further experiments are in progress to define the rMnSOD concentration able to obtain the greatest increase of post-thaw CB-HSC viability. The addition of rMnSOD results in a beneficial effect when the cell resumes its post-thaw activity, due to its oxygenating toxicity free action. This is why the rMnSOD does not need to be removed from the sample, unlike other cryoprotectant agents.

In conclusion, the rMnSOD seems to be a new promise as a cryoprotectant agent to improve the efficiency of stem cell transplants.

References

1. Lee OK, Kuo TK, Chen WM, Lee KD, Hsieh SL, et al. (2004) Isolation of multipotent mesenchymal stem cells from umbilical cord blood. Blood 103: 1669-1675.
2. Berz D, McCormack EM, Winer ES, Colvin GA, Quesenberry PJ (2007) Cryopreservation of Hematopoietic Stem Cells. Am J Hematol 82: 463–472.
3. Limaye LS, Kale VP (2001) Cryopreservation of human hematopoietic cells with membrane stabilizers and bioantioxidants as additives in the conventional freezing medium. J Hematother Stem Cell Res 10:709–718.
4. Mancini A, Borrelli A, Schiattarella A, Fasano S, Sehr P, et al. (2006) Tumor suppressive activity of a variant isoform of manganese superoxide dismutase released by a human liposarcoma cell line. Int J Cancer 119: 932-943.
5. https://www.youtube.com/watch?v=Cl4sTwKFe9k

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Immunoregulatory Effects of *Fast* Human Amniotic Stem Cells on Experimental Type 1 Diabetes

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Type I diabetes mellitus is caused by autoimmune destruction of pancreatic β cells. Effective treatment might require rescuing β-cell function in a context of reinstalled immune tolerance. The Nonobese diabetic (NOD) mice have become a prototypic model of autoimmune disease. Recently, Villani *et al.* have shown that amniotic fluid stem cells protect NOD mice from β-cell damage while increasing β–cell regeneration [1]. We isolated human amniotic stem cells characterized by a doubling time of 14 hours (*fast* human amniotic stem cells or fHASCs). Those cells are pluripotent stem cells with immunoregulatory properties. When treated with interferon (IFN)-γ, fHASCs induce the immunomodulatory enzyme indoleamine 2,3-dioxygenase 1 (IDO1). On coculture with human peripheral blood mononuclear cells, IFN-γ–treated fHASCs will thus cause a significant decrease in T-cell proliferation and an increased frequency of regulatory T cells [2]. Both effects require functional IDO1 and are cell contact-independent. *In vivo*, fHASCs can prevent and/or cure type I diabetes in most NOD recipients of the transferred cells. In this setting, fHASCs were found to migrate to the pancreas, contributing to the suppression of the autoimmune process. These findings indicate that fHASCs may represent a novel type of stem cells from amniotic fluid, potentially useful in both regenerative medicine and modulation of the immune system.

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References

1. Villani V, et al. (2013) Cytotherapy 1-15.
2. Romani R, et al. (2012) J Tissue Eng Regen Med 1: 464.

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Cells with Characteristics of Cancer Stem/Progenitor Cells Isolated from Clear Cell Renal Cell Carcinoma Expressed Genes Conferring Resistance to the Cisplatin Treatment

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Recent studies demonstrate the presence of a stem-like cell population in several human cancers that is crucial for the tumor development. It is becoming clear that these "cancer stem cells" maintain the ability to self-renew and sustain the tumor via the expression of tumor-progenitor genes. They are often biologically distinct from the differentiated cancer cells that comprise most of the tumor bulk [1]. Because cancer stem cells are believed to be primarily responsible for tumor initiation as well as resistance to chemo- and radiotherapy, their persistence may account for relapsing disease [2].

With the aim to study and characterize Renal Cancer Stem Cells (RCSCs) derived from clear cell renal cell carcinoma (cc-RCC), we isolated CD133+/24$^+$ from healthy and tumoral renal tissue of 40 patients. Cells were characterized for their mesenchymal phenotype and stemness proteomic profile. The colony-forming efficiency and self-renewal ability were tested with low-density plating and serial passaging. The tumorigenic properties of RCSCs were evaluated with soft agar assay *in vitro*. Microarray analysis was performed on 24 healthy Adult Renal Stem/Progenitor Cells (ARPCs) and 24 RCSCs. An additional control group consisting of CD133$^-$ tumoral cells was used. Results were validated using qRT-PCR analysis.

Using fluorescence activated cell sorting (FACS) analysis it was shown that the CD133$^+$/CD24$^+$ tumoral cells did not express the mesenchymal stem cell markers. We showed, by protein array, that CD133$^+$/CD24$^+$ tumor cells were more undifferentiated than ARPCs. RCSCs were clonogenic and able to differentiate in adipocytes, epithelial and osteogenic cells. Microarray analysis identified 52 genes differently modulated between RCSCs and ARPCs. 23 genes were expressed exclusively in RCSCs but not in ARPCs and CD133- tumoral cells. Among the most significant pathways and biological processes differently modulated we identified the cancer biological processes and in particular the proliferation of vascular endothelial cells.

The gene expression profile identified NRCAM and CTR2 as putative markers for renal cancer stem cells. Moreover, we identified 2 genes (ERCC1 and CTR2) that are involved in the mechanism conferring resistance to the cisplatin treatment in patients. The identification of these cancer cells in ccRCC and related markers could have a role in supporting the diagnosis and prognosis of patients with RCC and the improving of the therapeutic strategies.

References

1. Rosen JM, Jordan CT (2009) The Increasing Complexity of the Cancer Stem Cell Paradigm. Science 324: 1670-1673.
2. Bertolini G, Roz L, Perego P, Tortoreto M, Fontanella E, et al. (2009) Highly tumorigenic lung cancer CD133+ cells display stem-like features and are spared by cisplatin treatment. Proc Natl Acad Sci U S A. 106: 16281- 16286.

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Disease-Specific Induced Pluripotent Stem Cells from Fetal and Somatic Cells: A Platform for Human Genetic Disease Modeling

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Many diseases or disease predispositions have genetic components that vary from person to person. Now cells from individuals can be readily reprogrammed to form pluripotent cells, and then directed to differentiate into the lineage and the cell type in which the disease manifests. Those cells will contain the genetic contribution of the donor, providing an excellent model to delve into human disease at the level of individuals and their genomic variants [1]. iPSCs is a innovative personalized-regenerative technology, which can transform own-self cells into embryonic stem –like cells.

The objective of the present study is to reprogram patient-specific fetal cells deriving from prenatal diagnosis for several genetic disorder as Cystic Fibrosis (CF, OMIM #219700), Myotonic Dystrophy (DM1; Steinert's disease; OMIM #160900), β-Thalassemia (β-Thal; OMIM #613985), Spinal Muscolar Atrophy (SMA1; OMIM #253300), Lymphema-Distichiasis Syndrome (LDS; OMIM #153400) or from skin biopsy of a patient affected by Marfan Syndrome (MFS1; OMIM #154700) and of an healthy individual, as control.

The cell type used for create iPSCs can significantly influence the reprogramming efficiency and kinetics. Here, we show that amniotic fluid (AF) and chorionic villus sampling (CVS) represent an ideal cell resource for more rapid and efficient generation of human iPSCs respect to adult somatic dermal fibroblasts (Fib) [2,3]. The reprogramming were done using a polycistronic lentiviral vector (hSTEMCCA-loxP) encoding Oct4, Sox2, Klf4 and c-Myc genes necessary to cell reprogramming. Moreover loxP sites can be excised with Cre recombinase [4]. Stem cells express specific morphological, molecular (OCT4; Nanog; Sox2) and immunocytochemical markers (ALP; OCT4; SSEA4; TRA1-60; TRA1-81) confirming the successful reprogramming. Additionally, we evaluated their ability to differentiate into the three embryonic germ layers (ecto, endo and mesoderm) by immunocytochemical characterization and their ability to form teratomas *in vivo*. To date, this represents the first example of iPS cells derived from a very early extra-embryonic fetal tissues like chorionic villi (hIPS-CVS).

In this case prenatal diagnosis provided in the same time the opportunity to perform an early diagnosis of a genetic disease and to reprogram fetal cells to induced pluripotent ones.

hIPS-CVS/AF with hiPS-Fib can be considered a valid target for disease modeling and drug screening, and also an ideal source for autologous cell-replacement therapy in the later life of the fetus.

References

1. Trounson A, Shepard KA, DeWitt ND (2012) Human disease modeling with induced pluripotent stem cells. Curr Opin Genet Dev 22: 509-516.
2. Easley CA , Miki T, Castro CA, Ozolek JA, Minervini CF, et al. (2012) Human amniotic epithelial cells are reprogrammed more efficiently by induced pluripotency than adult fibroblasts. Cell Reprogram 14: 193-203.
3. Spitalieri P, Cortese G, Pietropolli A, Filareto A, Dolci S, et al. (2009) Identification of multipotent cytotrophoblast cells from human first trimester chorionic villi. Cloning Stem Cells 11: 535-556.
4. Somers A, Jean JC, Sommer CA, Omari A, Ford CC, et al. (2010) Generation of transgene-free lung disease-specific human induced pluripotent stem cells using a single excisable lentiviral stem cell cassette. Stem Cells 28: 1728-1740.

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Identification of the Molecular Events Underlying Liver Stem Cell Choices at the Branch of Epithelial/Mesenchymal Differentiation

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Beside the many promises and expectation, as yet current therapeutic applications of tissue engineering and regenerative medicine are restricted to a limited number of epithelial tissues (i.e. skin, cornea and heart valve) [1] where both 3D structure and instructive signals arising from cellular interplay show a low level of complexity. Regarding the liver, the challenge of the use of tissue engineering for the treatment of acute and chronic hepatic failure is still far from won; the organ, in fact, is composed by several histotypes that guarantee liver functions only when they correctly set up cell-cell interactions in the complex anatomical architecture of the hepatic lobule. Moreover, local micro-environmental elements, such as growth factors and both stiffness and composition of the extracellular matrix (ECM), provide important cues to determine cell fate. Particularly, mechanical properties of the ECM play a pivotal role in the regulation of many important cell processes that, eventually, decide cell fate and function.

Here, we present experimental data obtained with the use of a new cellular tool constituted by resident liver stem cells (RLSCs), derived from murine livers and established in lines [2]. These cells display a metastable phenotype, as underlined by i) the co-expression of epithelial and mesenchymal markers and ii) the ability to differentiate toward two mutually exclusive epithelial or mesenchymal derivatives: hepatocytes and hepatic stellate cells (HSCs). These progenitor cells, indeed, when transplanted in healthy growing livers (i.e. in murine newborns 1-2 days old) were found to contribute to the liver tissue with both hepatocytes and HSCs properly integrated in the hepatic architecture. Heterotopic transplantations, while confirm the dual differentiation potentiality of RLSCs, indicate as tissue local cues are necessary to drive a full hepatic differentiation [3].

One of the local elements mainly influencing the liver cell function is the elasticity/stiffness of the ECM. Liver tissue in physiological condition displays a liver stiffness (LS) lower than 6 KPa. LS increases during progression of liver fibrosis, a pathological state shared by several liver diseases and characterized by proliferation of collagen producing cells [4].

Our results showed that mechanical stimuli drive RLSCs differentiation choices within 24 hours: soft matrix (0,4 KPa) selectively induces epithelial differentiation while stiff matrix (80 KPa) induces mesenchymal differentiation.

We are currently characterizing, in this dynamic cellular model, the epigenetic events that anticipate liver-specific gene expression, focusing in particular on the promoter region of HNF4alpha, a transcriptional master regulator of hepatic differentiation. Our data would disclose new insight into the early events driving pivotal cellular choices at the branch of epithelial/mesenchymal differentiation.

References

1. Parenteau NL, Hardin-Young J (2002) The use of cells in reparative medicine. Ann N Y Acad Sci 961:27-39.
2. Conigliaro A, Colletti M, Cicchini C, Guerra MT, Manfredini R, et al. (2008) Isolation and characterization of a murine resident liver stem cell. Cell Death Differ 15: 123-133.
3. Amicone L, Costa V, De Santis Puzzonia M, Mancone C, Sacchetti B, et al. (2013) Evidence for a common progenitor of epithelial and mesenchymal components of the liver. Cell Death Differ 20: 1116–1123.
4. Mueller S, Sandrin L (2010) Liver stiffness: a novel parameter for the diagnosis of liver disease. Hepat Med 2: 49-67.

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New Insights about MSC Immunoregulation

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Mesenchymal stem cells (MSC) are a heterogeneous subset of stromal stem cells that can be isolated from many adult tissues. They can differentiate into cells of the mesodermal lineage, such as adipocytes, osteocytes and chondrocytes, as well as cells of other embryonic lineages. MSCs can interact with cells of both the innate and adaptive immune systems, leading to the modulation of several effector functions. Recent data have shown that MSC are licensed in their imunomodulatory activity by inflammatory signals such as interferon γ (IFNγ), thus supporting their use in clinical inflammatory conditions driven by an aberrant immune response such as in graft versus host disease and autoimmunity. In fact, upon in vivo administration in preclinical models of autoimmunity including experimental autoimmune encephalomyelitis (EAE), MSC induce peripheral tolerance inhibiting T and B cell responses to relevant self-antigens and T cell priming by dendritic cells. Moreover, MSC fostered tissue repair mainly by means of paracrine mechanisms and not through transdifferentiation in neural cells. In fact, recent studies have emphasized that MSC secretome may suffice to recapitulate many of the effects carried out by these cells on immune and tissue resident cells, thus suggesting that even engraftment in the target organ could be dispensable. This new paradigm predicts MSC clinical translation based on their ability to work mainly as drug-stores However, MSC priming by local cues, upon interaction with the host environment, represents a prerequisite to maximally enhance their therapeutic plasticity and may still support stem cells transplantation over the administration of their secreted factors. These results have been translated into appropriate clinical trials currently on-going.

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Bone Marrow-Derived Mesenchymal Stem Cells Promote Growth and Migration of Osteosarcoma and Hepatocarcinoma Cells through CXCR4 and AQP1

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Increasing evidences suggest that bone marrow-derived mesenchymal stem cells (BM-MSCs) are recruited into the stroma of developing tumors where they contribute to progression by enhancing tumor growth and metastasis, or by inducing anticancer-drug resistance. Although many experimental evidences exist supporting the therapeutic potential of MSCs, the mechanism of homing and recruitment of MSCs into tumors and their potential role in malignant tissue progression is still not well understood.

The aim of this study was to elucidate the role of BM-MSCs to promote tumor cell proliferation and invasion. Therefore, we analyzed whether chemokine receptor type 4 (CXCR4) and water channel molecule, aquaporin 1 (AQP1), both known to play a key role in cancer metastases, could affect MSCs mediated osteosarcoma and hepacarcinoma progression.

We used human mesenchymal stem cell isolated from bone marrow aspirates by gradient centrifugation and seeded in standard cell culture conditions. MSCs were grown for 48 h in medium with 1% fetal bovine serum to obtain conditioned medium (MSCs-CM) that was collected, filtered and stored at -20 °C. CXCR4 and AQP1 protein level was evaluated in human hepatocarcinoma (SNU-398) and osteosarcoma (U2OS) cells exposed to MSCs-CM. Proliferation of tumor cells co-cultured with MSCs or cultured in presence of MSCs-CM was evaluated by MTT assay. Tumor migration and invasion were assayed in 24-well transwell chambers using 8 um pore membrane pre-coated with collagen/fibronectin or matrigel, respectively. SNU-398 and U2OS cells, suspended in serum-free medium, were added to the upper chamber and incubated for 24-48 h at 37 °C in presence of MSCs-CM or MSCs (lower chamber) used as chemoattractants. Tumor cells pre-treated with a CXCR4 antagonist (AMD3100) or AQP1 antibody were also analyzed for proliferation, migration and invasion. Furthermore, involvement of Akt and/or Erk signalling pathways in tumor progression MSCs-mediated was examined.

Our results showed that CXCR4 and AQP1 expression is increased in human hepatocarcinoma (SNU-398) and osteosarcoma (U2OS) cells in presence of MSCs secreted factors. Conditioned medium from MSCs promoted proliferation, migration and invasion of tumor cells, whereas inhibition of CXCR4 and AQP1 significantly down-regulated these effects. Furthermore, SNU-398 and U2OS cells showed an enhancement of p-Akt and p-Erk levels when they were cultured in presence of MSCs-CM.

In conclusion these findings suggest that bone-marrow derived mesenchymal stem cells can promote the proliferation and invasion of osteosarcoma and hepatocarcinoma cells through CXCR4 and AQP1 overexpression. In addition, we found that MSCs may contribute to tumor progression by PI3K/Akt pathway and/or Ras/Erk cascade.

References

1. Torsvik A, Bjerkvig R (2013) Mesenchymal stem cell signaling in cancer progression. Cancer Treat Rev 39: 180-188.
2. Cuiffo BG, Karnoub AE (2012) Mesenchymal stem cells in tumor development: emerging roles and concepts. Cell Adh Migr 6: 220-230.
3. Xu WT, Bian ZY, Fan QM, Li G, Tang TT (2009) Human mesenchymal stem cells (hMSCs) target osteosarcoma and promote its growth and pulmonary metastasis. Cancer Lett 281: 32-41.
4. Ribatti D, Ranieri G, Annese T, Nico B (2014) Aquaporins in cancer. Biochim Biophys Acta 1840: 1550-1553.
5. Guo M, Cai C, Zhao G, Qiu X, Zhao H, et al. (2014) Hypoxia promotes migration and induces CXCR4 expression via HIF-1α activation in human osteosarcoma. PLoS One 9: e90518.