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Abstracts

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E Stoyanova et al., J Stem Cell Biol Transplant 2018, Volume 2
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Expression of pluripotency associated genes in human mesenchymal stem cells derived from umbilical cord and adipose tissue

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Human umbilical cord and adipose tissue are among the richest sources of mesenchymal stem cells (MSCs) with great promises in the field of regenerative medicine. Regardless of the intensive investigations of human MSCs from different tissues, the molecular mechanisms regulating the undifferentiated state and differentiation abilities are still unclear. The transcription factors OCT4, NANOG and SOX2 that are crucial for the efficient maintenance of the fine balance between self-renewal and differentiation in embryonic stem cells have been suggested to play a similar role also in mesenchymal stem cells. In the present work, the expression evaluation of OCT4A, OCT4B and OCT4B1 splice variants, NANOG and SOX2 were performed in MSC isolated from umbilical cord (UC), and adipose tissue (AT). UC-MSC displayed lower population doubling time than AT-MSC, (30.8±5h, 54.9±5h, respectively). Both cell types expressed the pluripotent markers OCT4A, NANOG and SOX2. The mRNA levels of OCT4B and NANOG were significantly higher in AT-MSC than UC-MSC ($p<0.05$). In addition, AT-MSC from different patients showed increased heterogeneity in mRNA levels of all analyzed genes compared to UC-MSC. In conclusion, higher expression of NANOG and OCT4B is not associated with better proliferative potential of AT-MSC. The UC-MSC from different samples showed lower variation in mRNA levels of OCT4A, OCT4B, OCT4B1, NANOG and SOX2 than AT-MSC, that make them a more appropriate candidate for clinical trials.

Biography

E Stoyanova pursued her PhD (2015) from the Institute of Biology and Immunology of Reproduction (IBIR) at the Bulgarian Academy of Sciences, Bulgaria. Currently, she is a Posdoctoral Researcher in the Department of Molecular biology at the same institute. Her research interests focuses on reprogramming of human soamtic cells, mesenchymal stem cells derived from bone marrow, adipose tissue, umbilical cord and cancer cells.

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Generation of mini lungs from human embryonic stem cells: analysis of the effects of vitamin D in the expression of DNA damage induced by bleomycin

Esmeralda Magro López, Alberto Zambrano Duarte and **Isabel Liste**
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The generation of organoids from human pluripotent stem cells allows deeper insight into human development, disease modeling, drug screening and regenerative medicine. Organoids are in vitro generated 3D structures containing multiple cell types that are organized similar to an organ, recapitulate some specific organ function and can mimic the responses of real tissues. Different human mini lungs (organoids) have been generated from both human embryonic and induced pluripotent stem cells (hESCs and hiPSCs respectively). We show here the generation of 2D and 3D mini lungs from two hESCs lines (AND-1 and AND-2) and have analyzed the effects of vitamin D and two non-calcemic vitamin D analogs in the expression of DNA damage induced by the antibiotic bleomycin, an agent widely used to induce lung fibrosis. The results indicate that bleomycin induced DNA double strand breaks in both types of structures and that vitamin D elevated its number. In addition, the two vitamin D analogs tested were able to reduce the DNA damage observed. The results are discussed in terms of the role of DNA damage in lung fibrosis and the potential use of vitamin D analogs in conditions characterized by DNA damage and senescence such as lung fibrosis.

Biography

Ms. Esmeralda Magro is a third year PhD student in Molecular Biosciences at the University Autonomous of Madrid. She is conducting a research project at the Institute of Health Carlos III, under the supervision of Dr. Alberto Zambrano. She did her M.Sc. thesis at Dr. Zambrano´s lab. This work was related to the role of vitamin D on DNA damage expression and cellular senescence in alveolar epithelial cells type II and human fibroblasts and its potential use as an antifibrogenic agent in Idiopathic Pulmonary Fibrosis (publication under review). She signs this work as co-first author. Currently, she is working on her thesis which is focused on the generation of two and three- dimensional human mini-lungs from human embryonic stem cells to model chronic respiratory diseases, preferably, the Idiopathic Pulmonary Fibrosis (IPF). She is also interested in studying the effect of different drugs and ligands for nuclear hormone receptors in DNA damage expression and cellular senescence in the context of lung fibrosis (*Magro-Lopez E et al., 2017, Viruses) and (Magro-Lopez E. et al., 2018, Stem Cell Res Ther*).

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Therapeutic effects of human mesenchymal stem cells with methylprednisolone treatment in rat spinal cord injury

Lee So Maeng[1], **Sang In Park**[2], **Ho Yong Jung**[2] and **Yong An Chung**[2]

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Methylprednisolone (MP), a glucocorticoid steroid, has an anti-inflammatory action and seems to inhibit the formation of oxygen free radicals produced during lipid peroxidation in a spinal cord injury (SCI). Currently MP is the standard therapy after acute SCI on reported neurological improvements. The combination therapeutic effect of human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) for transplantation time (1d, 7d, and 30d) after MP treatment on the axonal regeneration and on the behavioral improvement in SCI were studied in the rat. The spinal cord was injured by contusion using a weight-drop at the level of T9 and MP (30 mg/kg, i.m., 10 min and 4 h) was acutely administered after injury. hUCB-MSCs were labeled with GFP and our study performed the efficacy for transplantation time (1d, 7d, and 30 d) of hUCB-MSCs into the boundary zone of injured site. Efficacy was determined by histology, anterograde and retrograde tracing, and behavioral test. We found that hUCB-MSCs with MP treatment exerted a significant beneficial effect by neuroprotection and reducing cavity volume. Also the transplantation of hUCB-MSCs with MP treatment significantly improved functional recovery. Combined transplantation at 7d after SCI provided significantly greater efficiency than combined transplantation at 1d and 30d. These results suggest that transplantation time window of the hUCB-MSCs with MP treatment give rise to an earlier neuron protection strategy and effect of cell grafting in SCI. Thus our study may be considered as a therapeutic modality for SCI.

Biography

Lee So Maeng graduated from the College of Medicine, The Ewha Women's University as Medical Doctor with specialties in pathology from the The Catholic University of Korea (CUK), Republic of South Korea. She obtained her Postgraduation from the same university and worked on the topic entitled "Sequential changes in aberrant crypt foci and lectin expression in the early and late stages of DMH-induced colon carcinogenesis in rats". She is presently working at the Clinical Research Insitute of the Incheon St. Mary's Hospital at the College of Medicine (CUK).

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Syndecan-2: more than just a stromal cell marker

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The large-scale clinical expansion of MSCs to therapeutically relevant doses is a challenge to those in the cell therapy space phase. In addition, identifying donors that produce cells capable of withstanding the effect of extended passaging can be difficult. In order to assess whether cells are maintaining their activity and potency, we have focused developing assays around a novel cell-surface stromal cell marker, CD362/Syndecan-2 (SDC2). The heparan sulfate proteoglycan syndecans are transmembrane proteins involved in multiple physiological processes, including cell-matrix adhesion and inflammation. We found that the protein expression of SDC2 in umbilical cord-derived MSCs (ucMSCs) is lost with serial passaging and consecutive bioreactor expansions and this effect correlates with loss of IDO-1 activity/expression and reduced suppression of CD4+ T-cell proliferation. Furthermore, inflammation-induced loss of SDC2 by TNF-α or IL-1β promotes apoptosis, and increased CD54/ICAM-1 and MHC II expression in these cells and this phenotype is mirrored with adenoviral knock-down or siRNA directed against SDC2. This indicates that loss of stromal cell SDC2 has a negative impact on the potency of ucMSCs. Furthermore, certain donors with higher IFN -stimulated indoleamine 2, 3-dioxygenase IDO-1 and SDC2 expression, continue to express high levels through passaging and perform better in-vitro than those with lower starting expression, allowing the potential pre-selection of donors from as early a passage as P1-P2.

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Harnessing PU.1 and GATA-1 axis for priming human CD34+ stem cells towards myeloid lineages

Madhuri Joshi and **Gurudutta Gangenahalli**
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The active immunity in an immune compromised or radiation victim can be restored by generating functionally competent immune cells. Transcription factor like PU.1 plays a critical role in the differentiation of hematopoietic stem cells (HSCs) into immune cells. Another transcription factor GATA-1 (43 kDa) interacts with PU.1, which promotes differentiation of HSCs into erythroid lineages by blocking the binding of its coactivator c-Jun with β3/β4 region of PU.1. We have established an antagonizing mutation (Y244D) in β3/β4 domain by site-directed mutagenesis of PU.1, anticipating the enhancement of myelopoiesis if introduced into cells. We further assessed if it can really antagonize GATA-1 and promote myelopoiesis in cells upon transfection of native and mutant PU.1 protein formulated into nanoparticle structure. Approximately 30% of human bone-marrow CD34+ stem cells were transformed into myeloid lineage by using these NPs loaded with PLGA in vitro. In vivo studies by infusing primed CD34+ with these NPs and along with cytokines were conducted in irradiated nude mice. Results demonstrated higher survival rate (50-75%) in mice compared to control mice with a reasonable number of macrophages (43.11%) and neutrophils (50.44%) being observed on the 21st day. Further high-throughput microarray analysis in CD34+ cells whose selective differentiation was induced by PU.1 (Y244D) mutant transfection was performed to explore the myeloid specific gene-cluster responsible for myeloid development. Gene expression analysis demonstrated that a ≥2.5 fold change were observed in several genes like PRTN3, TLR4, EPAS1, FCER1A, NOD2, CBL, SMAD4, ANO6, TFRC, IL1R1 etc. Studies on establishing the role/mechanistic basis of these genes are presently under progress.

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Dental germs and palatal connective tissue stem cells: tools for oral regenerative therapy

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[2]The Oncology Institute Prof. Dr. Ion Chiricuţă, Romania
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Dental pulp, periodontal ligament, dental follicle, apical papilla, oral mucosa, periodontal granulation tissue, palatal tissue is considered easily accessible and important sources of mesenchymal stem cell (MSCs). In the current study, we isolated and assessed human dental germs and palatal connective tissue stem cells. MSCs were obtained from dental germs of wisdom teeth and palatal tissue samples were used from clinically healthy patients undergoing intervention due to the absence of space required for the eruption. After isolation the cells were cultured in propagation medium. Cells morphology, colony forming efficiency, population doubling capacities and multilineage differentiation potentials were investigated. After 1st passage, both cell lines possessed fibroblast like morphology, the frequency of colony forming efficiency for dental germs derived mesenchymal stem cells (dgMSCs) was significantly higher than that of palatal connective tissue derived mesenchymal stem cells (pMSCs). Significantly higher population doubling time was recorded for dgMSCs. The specific antigen makeup of the isolated MSCs were characterized in the 4th passage using a FACSCanto II flow cytometer (BD Biosciences, San Jose, CA, USA) and analyzed using the DIVA program. Both cell lines were positive for CD105, CD73, CD90, CD44, and CD49f and negative for CD34, CD45, and HLA-DR, but the levels of expression showed small differences. MSCs from both cell lines were successfully differentiated into osteogenic, adipogenic and chondrogenic lineages. Our preliminary results suggest that isolation, identification and immuno phenotyping of dgMSCs and pMSCs are feasible and may represent an easily available source for oral regenerative therapies.

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Therapeutic effects of human mesenchymal stem cells with methylprednisolone treatment in rat spinal cord injury

Lee So Maeng[1], Sang In Park[2], Ho Yong Jung[2] and **Yong An Chung[2]**
[1]St. Mary's Hospital – CUK, Republic of South Korea
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Dental pulpMethylprednisolone (MP), a glucocorticoid steroid, has an anti-inflammatory action and seems to inhibit the formation of oxygen free radicals produced during lipid peroxidation in a spinal cord injury (SCI). Currently MP is the standard therapy after acute SCI on reported neurological improvements. The combination therapeutic effect of human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) for transplantation time (1d, 7d, and 30d) after MP treatment on the axonal regeneration and on the behavioral improvement in SCI were studied in the rat. The spinal cord was injured by contusion using a weight-drop at the level of T9 and MP (30 mg/kg, i.m., 10 min and 4 h) was acutely administered after injury. hUCB-MSCs were labeled with GFP and our study performed the efficacy for transplantation time (1d, 7d, and 30 d) of hUCB-MSCs into the boundary zone of injured site. Efficacy was determined by histology, anterograde and retrograde tracing, and behavioral test. We found that hUCB-MSCs with MP treatment exerted a significant beneficial effect by neuroprotection and reducing cavity volume. Also the transplantation of hUCB-MSCs with MP treatment significantly improved functional recovery. Combined transplantation at 7d after SCI provided significantly greater efficiency than combined transplantation at 1d and 30d. These results suggest that transplantation time window of the hUCB-MSCs with MP treatment give rise to an earlier neuron protection strategy and effect of cell grafting in SCI. Thus our study may be considered as a therapeutic modality for SCI.

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Effect of triiodothyronine on remyelination enhancement after mesenchymal stem cell intraperitoneal injection in c57bl/6 mice cuprizone model of multiple sclerosis

Mohsen Marzban[1] and **Anahita Torkaman Boutorabi**[2]
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[2]Tehran University of Medical Sciences, Iran

Dental pulpMethylprednisolone (MP), a glucocorticoid steroid, has an anti-inflammatory action and seems to inhibit the formation of oxygen free radicals produced during lipid peroxidation in a spinal cord injury (SCI). Currently MP is the standard therapy after acute SCI on reported neurological improvements. The combination therapeutic effect of human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) for transplantation time (1d, 7d, and 30d) after MP treatment on the axonal regeneration and on the behavioral improvement in SCI were studied in the rat. The spinal cord was injured by contusion using a weight-drop at the level of T9 and MP (30 mg/kg, i.m., 10 min and 4 h) was acutely administered after injury. hUCB-MSCs were labeled with GFP and our study performed the efficacy for transplantation time (1d, 7d, and 30 d) of hUCB-MSCs into the boundary zone of injured site. Efficacy was determined by histology, anterograde and retrograde tracing, and behavioral test. We found that hUCB-MSCs with MP treatment exerted a significant beneficial effect by neuroprotection and reducing cavity volume. Also the transplantation of hUCB-MSCs with MP treatment significantly improved functional recovery. Combined transplantation at 7d after SCI provided significantly greater efficiency than combined transplantation at 1d and 30d. These results suggest that transplantation time window of the hUCB-MSCs with MP treatment give rise to an earlier neuron protection strategy and effect of cell grafting in SCI. Thus our study may be considered as a therapeutic modality for SCI.

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Peptide-polymer based system for targeting human stem cells to bone marrow

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Bone marrow transplantation (BMT) required for the treatment of hematopoietic diseases encounters challenges like limited availability of healthy cells, suboptimal homing, graft rejection etc. and therefore requires atleast 1-10x106 CD34+ stem cells/ kg body weight for higher chances of successful BMT. Dynamic nature of endothelial cells (ECs) not only allows it to control the traffic across the organ but also to regulate coagulation, angiogenesis, blood fluidity etc. which makes them potential target for therapeutics. As functional regulator EC express selective molecules to aid in functioning of the underlying tissue. For instance, vascular cell adhesion molecule-1 (VCAM1) which has been shown to control homing of infused stem cells and subsequent engraftment is constitutively expressed on BMECs only. In an attempt to exploit it as capturing ligand, we have designed a peptide-polymer based system that can target infused cells to BM thus enhancing targeted delivery and reducing the number of stem cells required for successful BMT. The system constitutes a peptide specific for VCAM1 conjugated to polymers (like chitosan, PAH, PSS, liposomes etc.) that can encapsulate cells shielding them from host immune system. To assess the potential of this system we conjugated it to dextran coated iron oxide nanoparticles (Dex-IOP) and observed an increase of nanoparticle delivery to BM by 22%, 46%, 51.5% and 43% for Dex-IOP and the peptide conjugated to liposome, chitosan and PSS and PAH respectively. Evaluation of this system to target cells to BM and its regeneration is being explored and will be discussed in detail during the conference.

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Expression of immunological markers in the lacrimal gland and conjunctive palpebral of dogs with keratoconjunctivitis sicca topically treated with mesenchymal stem cells

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Keratoconjunctivitis sicca (KCS) or dry eye syndrome, is predominantly immunomediated and dogs are an excellent model for understanding this disease due to immunomediated origin similar to that in humans. The objective is to compare the expression of immunological markers interleukin (IL), IL-1 and IL-6, tumor necrosis factor alpha (TNFα), and CD4T lymphocytes, before and after topical treatment with mesenchymal stem cells (MSC) in KCS in dogs. Twenty-two dogs, with a bilateral diagnosis of KCS, were treated topically with 50 µl (1x106 MSC) in the conjunctival sac in 4 applications at 7 day intervals, and evaluated monthly for 6 months. For analysis of the expression of IL-1, IL-6, TNFα, and CD4 markers, two collections were performed, one before and another at the end of the study, by fine-needle aspiration of the third eyelid gland and processed by immunocytochemistry and biopsy of the conjunctival palpebral and processed by immunohistochemistry. The results obtained through color density showed that at the pre KCS treatment moment there was a marked expression of all markers (IL-1, IL-6, TNFα, and CD4) and after 6 months there was a significant reduction in the marked area of all markers. These results demonstrated that these markers could be excellent tools for the diagnosis and progression of KCS and topical treatment of KCS with MSC was shown to promote a significant decrease in the expression of these markers after treatment which in future may represent another adjuvant therapy option in the treatment of KCS in dogs and humans.

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