Changing the Face of Modern Medicine:
Stem Cell and Gene Therapy
Organized Jointly by the
European Society of Gene & Cell Therapy (ESGCT),
International Society for Stem Cell Research (ISSCR)
and the French Society of Gene and Cell Therapy (SFTCG)
Lausanne, Switzerland
October 16–19, 2018
Abstracts

grade hESC line into a GMP facility. In parallel, we have established high quality standard to definite their characteristics, impurities and potency. This cells could be banked and maintain their phenotype and functionality when cultured on hAM. Indeed, when cultured for 1 month on denuded hAM, the RPE cells formed a typical retinal pigmented epithelium. Furthermore, hESC-derived RPE cells perform typical phagocytic activity and secrete VEGF in polarized manner. Finally, our TEP will be used for phase I/II clinical trials for the treatment of RP caused by a RPE defect.

P112

Comparative analysis of therapeutic efficacy of mesenchymal stromal cells isolated from different sources on rat model of thermal skin burn

I Eremin[1] I Korsakov[1] A Petrikina[1] T Chauzova[1] T Stupnikova[1] O Grinakovskaya[1] M Yasinovskiy[1] K Ustinov[1] A Pulin[1]

1: Federal State Budgetary Scientific Institution ''Institute of General Pathology and Pathophysiology''

Wide range of cellular products consisting of different types of cells obtained from various sources are used today in the regenerative medicine. Each source has its advantages and disadvantages. Comparative studies of cells isolated from various sources, on a single model are practically absent. The rat model of thermal skin burn was used. Following cellular products were investigated: allogeneic placenta derived multipotent mesenchymal stromal cells (MSCs) (single injection 5 mln or two injections 5 mln each with 1 week interval), adipose-derived stromal-vascular fraction (SVF) (isolated from 1 ml of fat), SVF (isolated from 1 ml of fat) + allogeneic adipose-derived MSCs (adMSCs) (1, 5 or 10 mln), SVF (isolated from 1 ml of fat) + autologous adMSCs (5 or 10 mln), autologous adMSCs (1, 5 or 10 mln), allogeneic adMSCs (5mln), allogeneic gingiva-derived MSCs (1 or 5 mln). Investigated product was administered subcutaneously at 24 points along the circumference of burn and under the wound bottom. Cells were administered at day 3 (single injection) or days 3 and 10 (two injections) after burn modeling. Control group received equal volume of saline. Primary endpoint was the time to complete epithelialization of the wound. Secondary endpoints were - reduction of dermal wound area at days 21 and 30 and the degree of regeneration according to histological examination. Greater therapeutic efficacy of single injection of allogeneic placenta-derived MSCs compared to all other cells was demonstrated. Second injection of placental MSCs significantly improved results. The study was funded by Russian Science Foundation (project #17-75-30066).

P113

Myocardial infarct repair with human adult muscle-derived stem cells ''MuStem''

A Rannou[1 2 4] G Toumaniantz[2 4] T Larcher[1] I Leroux[1] M Ledevin[1] A Hivonnait[2] S Menoret[3] I Anegon[3] F Charpentier[2] K Rouger[1] L Guével[1 4]

1: INRA/Oniris UMR 703 2: Inserm UMR 1087; CNRS UMR 6291- Institut du thorax 3: UMR 1064/facility TRIP/UMS3556, Center for Research in Transplantation and Immunology, Nantes, France 4: Université de Nantes

Heart failure is a major public health with no effective cure. Cell-based therapy represents a promising strategy although none of the cells investigated until now fulfils all the expected requirements. We isolated a population of skeletal muscle-derived stem cells (MDSCs) from healthy dog, that we named MuStem cells, and established a proof of efficacy of its use in dystrophic dogs. Recently, the human counterpart (hMuStem cells) was isolated and characterized by contribution to fibre formation after injection into injured mice muscle and high secretory activity. These data placed it as a potential advanced therapy medicinal product. In the heart failure context, beneficial tissue remodelling and modulation of contractile function were reported following administration of murine MDSCs. Similar effects mainly attributed to trophic factors were described after mesenchymal stem cell delivery. Considering the regenerative capacity and paracrine effect of hMuStem cells, we investigated whether they could be an interesting alternative for myocardial infarction treatment. For this, coronary ligation and intra-myocardial administration of hMuStem cells were performed in a new Rag1 and Il2Rg KO immunodeficient rat model. As opposed to hMuStem-treated rats, control rats presented lung atelectasia, cardiac atrophy-dilatation and hepatic congestion. Moreover histological and molecular analyses showed that hMuStem cells were implanted into the host cardiac tissue without generating arrhythmia 3 weeks post-injection. Echographic analyses highlighted functional and structural improvements of the infarcted heart with an increase of the left ventricle ejection fraction. In conclusion, hMuStem cells are able to implant into infarcted hearts and generate beneficial functional impact.

P114

Cell sheets as a platform for therapeutic delivery and tissue modelling

P I Makarevich[1 2] N A Alexandrushkina[1 2] P P Nimiristky[1 2] K V Dergilev[3] Y V Parfyonova[1 3] V A Tkachuk[1 2 3]

1: Faculty of Medicine, Lomonosov Moscow State University, Moscow, Russia 2: Institute of Regenerative Medicine, Medical Research and Education Centre, Lomonosov Moscow State University, Moscow, Russia 3: National Medical Research Center of Cardiology, Moscow, Russia,

Cell sheets have gained attention as a tissue-engineering tool for delivery of living cells along with their extracellular matrix to stimulate regeneration and tissue repair. Within last decade we have focused on cell sheets from MSC and other postnatal stem cell types to treat vascular disease (limb ischemia and myocardial infarction) and cutaneous wound healing in corresponding animal models. We used different approaches - from viral delivery to enhance growth factor production and efficacy to fabrication of decellularized matrices to make a cell-free therapeutic product or a feasible matrix for cell seeding. Thus, we consider cells sheets as a versatile platform for minimally-engineered tissue constructs for numerous potential application in cell therapy. However, cell sheets seem to go beyond just a way to deliver cells in a feasible manner. Using time-lapse microscopy we found MSC-based cell sheets to self-organize in a heterogenous ''hills and valleys'' pattern. Furthermore, our data supports the fact that such behaviour of MSC may be due to their attempts to recapitulate a ''niche-like'' environment which was supported by our data of increased ''stemness factor'' expression in cell sheets compared to monolayer. Furthermore, we have found that within cells sheets proliferation occurs despite obvious ''contact inhibition'' of division due to high thickness.

Histology studies have shown that cell matrix composition is significantly modulated over time of culture and cell sheets may have a more ''tissue-like'' condition that expected. The study was funded by RFBR Grant #17-04-01452 and partly by RSF grant #16-45-03007 (histology and animal experiments)

P115

Application of combined gene and cell therapy within an implantable therapeutic device for the treatment of severe haemophilia A

C Olgasi[1] S Merlin[1] C Borsotti[1] T Bergmann[2] D M Mazzucca[3] A Stolzing[4] M Zierau[5] P M Toleikis[3] J Braspenning[2] A Follenzi[1]

1: Department of Health Sciences, University of Piemonte Orientale, Novara. Italy 2: Department of Tissue Engineering and Regenerative Medicine, University Hospital Würzburg, Würzburg, Germany 3: Sernova Corp, London, Ontario, Canada 4: Centre for Biological Engineering, School of Mechanical, Electrical and Manufacturing Engineering, Loughborough University, UK 5: IMS Integrierte Management Systeme e. K., Heppenheim, Germany

Haemophilia A (HA) is an X-linked bleeding disease due to factor VIII (FVIII) deficiency and new regenerative medicine approaches to treat/cure haemophilia A require insights into cell compartments capable of producing (FVIII). We and others previously demonstrated that FVIII is produced specifically in endothelial cells. The aim of our work is to develop the technologies for a novel *ex vivo* cell-based therapy to treat HA that should lead to improved patient quality of life. We isolated blood outgrowth endothelial cells (BOECs) from healthy and patients' blood. BOECs were efficiently transduced with a lentiviral vector carrying the B domain deleted form of human FVIII under the Vascular Endothelial Cadherin promoter (LV-VEC.hFVIII). BOECs were characterized for endothelial phenotype and the number of integrated LV copies/cell was ~3. By FACS, we demonstrated that FVIII was expressed by 80-90% in LV-VEC.hFVIII transduced cells, and FVIII activity was evaluated by aPTT and ELISA. Ten million LV-VEC.hFVIII-BOECs were transplanted intraperitoneally in association with cytodex® 3 microcarrier beads in NOD/SCID g-null HA mice (n = 6). BOECs survived and secreted FVIII at therapeutic levels (12%) for up to 18 weeks and ameliorated the bleeding phenotype of the transplanted mice. As next steps, LV-transduced HA patient BOECs will be transplanted into an implanted prevascularized, scalable medical device (Cell Pouch™, Sernova Corp.) and optimized for sustained secretion of therapeutic FVIII in the NOD/SCID g-null HA mice. This is in preparation for future human clinical testing within the device in HA patients by transplantation of GMP produced autologous gene corrected BOECs.

P116

Mitochondrial genome mutations in induced pluripotent stem cells

J Park[1] Y Lee[1] S So[1] S Park[1] H Kim[1] R Lee[1] Y Han[1] D Kim[1] J Shin[1] J Hwang[1] B H Lee[2] E Kang[1]

1: Stem Cell Center, Asan Institute for Life Sciences, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea 2: Department of Pediatrics, Asan Medical Center Children's Hospital, University of Ulsan College of Medicine, Seoul, South Korea

Induced pluripotent stem cells (iPSCs) are important, potential sources for autologous cell replacement therapies to treat age-associated degenerative disease. However, it should be considered that somatic mitochondrial genome (mtDNA) mutations might be accumulated in human iPSCs from elderly individuals. In order to gain a further insight into the age-related progressive accumulation of mtDNA mutations in iPSCs, analysis of mtDNA mutation was performed with the iPSCs derived from young and elderly individuals, using Illumina MiSeq sequencer. We obtained 48 and 45 iPSCs lines from 31 elderly and 17 young subjects, respectively. The average number of mtDNA variants in an individual line was significantly higher in the group consisting of the elderly than that of a young group (1.90 ± 0.2 vs. 0.56 ± 0.1, $P < 0.0001$). Three percent of novel mutations were not reported in MitoMAP and 77% were non-synonymous or resided in RNA coding genes. Next, the selected iPSCs with certain mutations were differentiated into energy-demanding cells including hepatocytes, retinal pigment epithelium cells, and cardiomyocytes. The differentiated cells from mutant iPSCs displayed reduced mitochondrial functions. As demonstrated, the chance of occurrence of iPSCs carrying the pathogenic mtDNA mutations might be higher in the elderly subjects. Therefore, it is necessary to screen the mtDNA mutations in iPSC lines of such subjects prior to the application of cell therapy, disease modeling, or pharmacological screening.

P117

Generation of human iPSC-derived macrophages using a GMP-compliant process pipeline

A Rafiei Hashtchin[1] M Ackermann[1] C Halloin[2] H Kempf[2] A HH Nguyen[1] F Manstein[2] M Bickes[3] D Viemann[3] R Zweigerdt[2] N Lachmann[1]

1: Institute of Experimental Hematology, REBIRTH, Hannover Medical School (MHH), Hannover, Germany 2: Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Department of Cardiothoracic, Transplantation and Vascular Surgery, MHH, Hannover, Germany 3: Department of Pediatric Pneumology, Allergology and Neonatology, Hannover Medical School, Hannover, Germany

Macrophages are key components of the innate immune system with critical function in tissue homeostasis and host pathogen protection. Recently, transplantation of macrophages has been proposed as an effective and long-lasting therapy for different congenital disorders. Given the unique features of pluripotent stem cells, we aim to develop a scalable platform for the GMP-compliant generation of iPSC-derived macrophages. Considering our unique protocol to produce human iPSC-derived macrophages (iPSC-Mac) continuously, we established a suspension-based technique (4D), which is suitable for bioreactor mediated up-scaling. iPSC-Mac from the 4D-culture could be harvested continuously for 12 consecutive weeks. Of note, harvested cells showed a purity of >90% of CD45+ cells and stained positive for CD45+CD11b+CD14+CD163+. For future clinical application of iPSC-Mac, we propose to advance the protocol into a GMP-compatible ''all-in-one'' suspension cultivation and differentiation using stirred tank bioreactors. As a first step, we used GMP-compliant components to establish cultivation of iPSCs as pluripotent aggregates in suspension, which are suitable for bioreactor mediated up-scaling. After a phase of mesoderm priming for 7 days, aggregates were subjected to lineage instructive cytokines to induce the hematopoietic program. Production of iPSC-Mac started at day 14 of differentiation and continued for more than six-weeks with high efficiency. Generated iPSC-Mac exhibited classical