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ADVANCING SPINAL MUSCULAR ATROPHY INTERVENTIONS USING STEM CELL RESEARCH

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Stem cell research offers the ability both to study development and the nascent potential to develop therapeutics. By their very intractability, few areas of potential stem cell therapy have engendered more excitement and investment as neurological disorders. This presentation will review our recent findings that relate to spinal muscular atrophy, an autosomal recessive motor neuron disease caused by a genetic defect carried by as many as one in 75 people

A NOVEL EVIDENCE THAT A QUIESCENT MURINE BONE MARROW (BM)-RESIDING, DEVELOPMENTALLY EARLY, VERY SMALL SCA-1+LIN-CD45- CELLS ARE HIGHLY RESPONSIVE TO FOLLICLE STIMULATING HORMONE (FSH) AND LUTEINIZING HORMONE (LH) AND AFTER STIMULATION BY THESE SEX HORMONES PROLIFERATE AND DIFFERENTIATE INTO CD45+ HEMATOPOIETIC STEMPROGENITOR CELLS (HSPCS)

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Hypothesis. We hypothesized that BM-residng VSELs should similarly as ovarian VSELs respond to gonadothropin as well as to two major downstream effectors of this hormone that is FSH and LH.

Experimental strategies. Normal C57Bl/6 mice were injected marsh Gonadotropin or human FSH or LH for 10 days, and every day mice were injected with bromodeoxyuridine (BrdU) to label cells that are in the cell cycle. Subsequently mice were sacrificed and BM cells were flushed from bone marrow (BM) cavities and peripheral blood (PB) was obtained from vena cava (with a 25-gauge needle and 1-mL syringe containing 250U heparin). In BM and PB cell suspensions, we measured: i) the total number of Sca-1+Lin–CD45+ HSPCs and small Sca-1+Lin–CD45– VSELs by FACS and ii) the number of cycling BrdU+ HSPCs and VSELs iii) we evaluated the number of VSELs and HSPCs circulating in PB iv) as well as the number of clonogenic CFU-GM progenitors in BM and PB.

Salient results. We observed that the number of cycling BrdU+ VSELs increased from ~2 ± 0.05% (control) to ~13 ± 4% after FSH injection and ~20 ± 5% afer LH injection among BM cells derived from flushed bones. Furthermore, in comparison with control animals, we observed increase in number of VSELs and HSCs in BM after FSH and LH injection. We also noticed

increase in number of clonogenic CFU-GM and BFU-E progenitors after FSH and LH stimulation as compared to control mice.
Conclusions. Our data, obtained in an in vivo murine model strongly support that BM-residing VSELs similarly as their ovarian counterparts respond to stimulation by Gonadothropin, FSH and LH. This is another evidence that VSELs are migratory primordial germ cells-derived primitive stem cells that are deposited in adult tissue during development.

Keywords: VSELs, FSH, LH

A NOVEL VIEW OF BONE MARROW AS A "STEM CELL SENSOR" OF TISSUEORGAN DAMAGE-EVIDENCE THAT IN VIVO EXPOSURE TO THE NEUROTOXIN KAINIC ACID (KA) INDUCES PROLIFERATION AND NEURAL SPECIFICATION OF DEVELOPMENTALLY EARLY STEM CELLS DIRECTLY IN BONE MARROW BEFORE THEY ARE MOBILIZED INTO PERIPHERAL BLOOD

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We and others have demonstrated the presence of developmentally early stem cells in BM that we have named very small embryonic-like stem cells (VSELs). These cells are mobilized into peripheral blood, for example, during heart infarct, stroke, or skin burns however, the data on the immediate response of stem cells in BM during organ injuries are somewhat limited. C57Bl6 mice were injected with increasing doses of KA and at various time intervals mice were sacrificed to harvest BM, PB samples, and brains for analysis. Brain damage was confirmed by histological analysis. The number of Sca-1+Lin–CD45– VSELs and Sca-1+Lin–CD45+ HSPCs was evaluated in BM and PB by FACS. The cell cycle status of VSELs and HSPCs was evaluated by FACS in cells isolated from mice that received bromodeoxyuridine (BrdU) after KA injection. By employing RQ-PCR, we also measured the expression of genes that regulate stem cell pluripotency (Oct-4, Nanog, Sox2, and Rex1) and regulate neuronal development (Nestin, β3-tubulin, Olig1, Olig2, and GFAP). For the first time, we provide evidence that the compartment of developmentally early stem cells residing in BM responds robustly to brain damage induced by a neurotoxin. This effect seems to be specific for VSELs, as no significant changes were observed for HSPCs. The kinetics of changes in BM revealed that BM VSELs enter the cell cycle and, after they become specified into the neural lineage, egress from BM and enter the PB. Thus, our data provide novel evidence that developmentally early stem cells in BM "sense" the damage to brain tissue and respond to this type of organ injury. In parallel, we are studying the specificity of the response of BM-residing VSELs and HSPCs to other types of organ damage, such as heart infarct and acute limb ischemia.

Keywords: Very small embryonic-like stem cells (VSEL's), regeneration, brain damage.Comments: This work is supported by the EU Innovative Economy Operational Program POIG.01.01.02-00-109/09.

ARE MURINE MESENCHYMAL STROMAL CELLS A TRAP FOR HUMAN HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Background Humanized mice provide the opportunity to study human stem cell biology and immunology in vivo with less limitation by ethical and technical constraints. Thus, numerous new murine models were developed for the exploration of the human hematopoietic system. However, several limitations including functional failure are present. Understanding all molecular details underlying differences between the murine and human system is now urged to establish an ideal model.

Methods To get more insights into this issue, we have pioneered a co-culture system consisting of human CD34+ hematopoietic stem/progenitor cells (HSPCs) growing on human mesenchymal stromal cells (MSCs) as feeder cell layer (Freund et al. Stem Cells & Dev, 2006). In the current project, this established ex vivo system is applied to compare proliferation as well as morphological/physical characteristics of human CD34+ HSPCs cultured on either human or mouse MSCs. In both species, MSCs were isolated from bone marrow whereas CD34+ HSPCs were immuno-isolated from human peripheral blood upon G-CSF mobilization. MSCs were characterized by their morphology, the presence/absence of characteristic cell-surface molecules and their multipotential capacity.

Results As expected, we observed after 7 days of culture that human HSPC pools are expanding on MSCs, irrespective of their species origin, by comparison to fibronectin used as supporting matrix. Interestingly, although the number of CD34+CD133– HSPC cells increased in similar proportion, those harboring the more primitive CD133+ phenotypes (i.e. CD133+CD34+, CD133+CD34–) were significantly reduced on murine MSCs indicating a substantial difference between species. Next, we evaluated the migration behavior of HSPCs on MSCs by time-lapse video microscopy. While we observed a similar HSPC migration distance on both, human and murine MSCs, the number of HSPCs exhibiting morphologies associated with migrating phenotypes was significantly decreased after 7 days in culture with murine MSCs. These observations suggest that either soluble factors released by murine cells are reduced concomitant with the increased culture period – a phenomenon that will not be observed with human MSCs – or the adhesive properties, which influence the polarization of HSPCs will be distinct on murine MSCs by comparison to the human counterpart. To address the second hypothesis, we used atomic force microscopy (AFM)-based single-cell force spectroscopy. Unexpectedly, we found that detachment forces of either freshly isolated or 7-days cultured CD34+ HSPCs are threefold higher on murine MSCs by comparison to the human cells.

Conclusion Collectively, our results demonstrate that the adhesion properties of human CD34+ HSPCs differ on either human or murine MSCs. Such information raise some caution in data interpretation obtained when murine models are used to study the primitive properties of human HSPCs as well as their migration behaviour for instance in the homing process.

BRADYKININ FUNCTIONS IN NEUROGENESIS: FROM INDUCTION OF NEUROGENESIS TOWARDS PRECLINICAL MODELS OF PARKINSON´S DISEASE

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Bradykinin (BK) and related kinins are released into the plasma or interstitial fluid after proteolytic cleavage of kininogens. BK actions mediated through kinin-B2 receptors do not only participate in inflammation and blood pressure regulation, but have also been described for neurotransmission and neuromodulation. Novel functions for BK and its receptor were studied using neural stem and progenitor cells isolated from fetal rat or mouse brain (E 14 or E12.5, respectively) as in vitro models whose differentiation into neural and glial phenotypes closely resembles events occurring during cortex development. Functional kinin-B2 receptors, as studied by calcium imaging in the presence of bradykinin and liberation of BK into the culture medium suggested the existence of an autocrine loop participating in neural phenotype determination. Chronic inhibition of kinin-B2 receptor activity along differentiation resulted in down-regulation of muscarinic acetylcholine receptor activity and expression as well as purinergic receptor activity, but did not affect glutamatergic and GABAergic marker expression. Moreover, kinin-B2 receptor activity was also important for the determination whether progenitor cells originate neurons or glial cells. The presence of the kinin-B2 receptor antagonist HOE-140 resulted in reduced neurogenesis and enhanced gliogenesis, while addition of bradykinin increased neurogenesis together with a reduction of differentiation into glial cells.
As underlying mechanism for cell fate determination, Notch1 and Stat3 or NeuroD1 gene expression was induced following neurosphere treatment with HOE-140 or bradykinin. Moreover, that migration of neural progenitors as a prerequisite for differentiation into neurons was largely restricted in the presence of the inhibitor. These results were confirmed in migration and differentiation assays with neurospheres obtained from kinin-B2 receptor knockout mice. Neurogenic and neuroprotective features of BK in neurodegenerative diseases were further characterized in vitro and in vivo. 6 OH-dopamine-exerted cell death and inhibition of migration and neurogenesis of neurospheres induced to differentiation into dopaminergic neurons were abolished by BK. Moreover, degeneration of dopaminergic neurons and clinical symptoms in a rat model of Parkinson's disease were mostly reversed following a single bradykinin injection.

CHARACTERISATION OF NUCLEAR ARCHITECTURAL ALTERATIONS DURING IN VITRO DIFFERENTIATION OF HUMAN STEM CELLS OF MYOGENIC ORIGIN

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Cell differentiation is based on a synchronised orchestra of complex pathways of intrinsic and extrinsic signals that manifest in the induced expression of specific transcription factors and pivotal genes within the nucleus. One cannot ignore the epigenetic status of differentiating cells, comprising not only histones and DNA modifications but also the spatial and temporal intranuclear chromatin organisation, which is an important regulator of nuclear processes. In the present study, we investigated the nuclear architecture of human primary myoblasts and myocytes in an in vitro culture, with reference to global changes in genomic expression. Repositioning of the chromosomal centromeres, along with alterations in the nuclear shape and volume, was observed as a consequence of myotube formation. Moreover, the microarray data showed that during in vitro myogenesis cells tend to silence rather than induce gene expression. The creation of a chromosome map marked with gene expression changes that were at least 2-fold confirmed the observation. Additionally, almost all of the chromosomal centromeres in the differentiated cells preferentially localised near the nuclear periphery when compared to the undifferentiated cells. The exceptions were chromosomes 7 and 11, in which we were unable to confirm the centromere repositioning.
In our opinion, this is the first reported observation of the movement of chromosomal centromeres along differentiating myogenic cells. Based on these data we can conclude that the myogenic differentiation with global gene expression changes is accompanied by the spatial repositioning of chromosomes and chromatin remodelling, which are important processes that regulate cell differentiation.

Keywords: myoblasts, cell differentiation, chromosome positioning, nuclear architecure, gene expression

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CORD BLOOD DERIVED VERY SMALL EMBRYONIC-LIKE STEM CELLS (VSELS) OPTIMIZED ISOLATION PROTOCOLS AND IN-DEPTH CHARACTERIZATION

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The progress of research carried out since the last decade has brought focus on potential role of embryonic-like stem cells in adult tissue. The developmentally early Very Small Embryonic like Stem Cells (VSELs) isolated from murine bone marrow, human umbilical cord blood (UCB) and various other adult tissues have come forward as a promising candidate in these studies. VSELs are population of small non-hematopoietic pluripotent cells that are negative for Lineage markers and express Sca-1 in mice and CD133 in humans. Their embryonic-like characteristics include the expression of pluripotency markers and the ability of murine VSELs to give rise to cells of all three germ layers. However owing to their small size and density they are often lost during processing and also certain questions regarding the functional properties of UCB-VSELs remain unanswered raising a question on their existence. Thus optimized method for isolation and expansion of VSELs has been of concern for the scientific community.
Our work is focused on detection and enumeration of VSELs in UCB; developing isolation protocols to effectively preserve these cells during processing and comprehensively investigate their cellular properties. Using a combinational approach of RBC lysis and differential centrifugation we report that UCB-VSELs settle along with RBC during processing. These small cells in RBC fraction display unique morphology characterized by high nucleo-cytoplasmic ratio and express both at protein and transcript levels pluripotency markers like nuclear OCT-4,

Nanog, Sox 2 and cell surface markers like SSEA4. UCB-VSELs also express primitive hematopoietic markers CD34 and CD133 as well as primordial germ cell marker Stella and Fragillis. Flow cytometry carried out for enumeration and analysis of cellular DNA content demonstrates higher number of these cells in RBC fraction compared to MNC, their diploid nature and quiescent status characterized by their presence in G0/G1 phase of cell cycle. Using TRAP assay we also report high telomerase activity in CB-VSELs compared to other CB cells.
In order to establish efficient VSEL enrichment we are working on employing immunomagnetic separation techniques based on their expression of cell surface markers CD133 and SSEA4. Our results provide evidence for existence and pluripotent nature of VSELs in cord blood. The fact that VSELs are lost during banking necessitates better cord blood banking procedures and our work demonstrates one such efficient protocol. These findings may have significant potential to better understand biological role of VSELs in adult tissues and to advance VSEL based therapeutics.

CYTOMETRIC ASSESSMENT OF DNA DAMAGE- AND MTOR-SIGNALING, THE FACTORS CONTRIBUTING TO AGING AND SENESCENCE

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Two conceptual models are being promoted to explain the aging phenomenon. Cumulative DNA damage caused by reactive oxygen species (ROS), by-products of oxidative phosphorylation, is one of them (ROS concept). Constitutive stimulation of the mitogen- and nutrient- sensing mTOR/S6 signaling is the second mechanism (TOR concept). The flow- and laser scanning-cytometric methods were developed to measure the level of the constitutive DNA damage/ROS- as well as of mTOR/S6- signaling in individual cells. Specifically, persistent activation of ATM and expression of γH2AX in untreated cells detected with phospho-specific Abs reports constitutive DNA damage induced by endogenous ROS. The phosphorylation of Ser235/236-ribosomal protein (RP), of Ser2448-mTOR and of Ser65-4EBP1 informs on constitutive signaling along the mTOR/S6 pathway. The reported gero-preventive agents: rapamycin, metformin, 2-deoxyglucose, berberine, resveratrol, vitamin D3 and aspirin, all decreased the level of constitutive DNA damage signaling as seen by the reduced expression of γH2AX. They also decreased the level of intracellular ROS and mitochondrial trans-membrane potential ΔΨm, the marker of mitochondrial energizing. All these agents also reduced phosphorylation level of mTOR, RP-S6 and 4EBP1 in A549, TK6, WI-38 cells and in mitogenically stimulated human lymphocytes. The most effective was rapamycin. Although the primary target of each on these agents may be different the data are consistent with the downstream mechanism in which the reduction of translation rate through mTOR/S6K signaling is coupled with a decrease in the energy production through oxidative phosphorylation and leads to a decline in the level of ROS, mitochondrial potential and oxidative DNA damage. The decreased rate of translation induced by these agents may slow down cells hypertrophy and alleviate other features of cell aging/senescence. The reduced oxidative DNA damage is expected to lower predisposition to neoplastic transformation which may result from defective DNA repair at the sites coding for oncogenes or tumor suppressor genes. The data suggest that combined assessment of constitutive γH2AX expression, mitochondrial activity (ROS, ΔΨm) and mTOR signaling provides an adequate gamut of cell responses to evaluate effectiveness of suspected gero-preventive agents.

CYTOPROTECTIVE EFFECT OF AUTOPHAGY ON SURVIVAL OF ENDOTHELIAL PROGENITOR CELLS UNDER HYPOXIA CONDITIONS

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In recent years, more and more attention in cardiovascular regenerative medicine has focused on endothelial progenitor cell (EPC) transplantation to promote beneficial angiogenesis of the ischemic tissues. However, different approaches to cell-based therapies have been limited by poor survival of the engrafted cells. This investigation was designed to examine effects of autophagy on EPC survival in hypoxic conditions and infracted myocardium.

After EPC autophagy was inhibited with 3-MA under normoxic conditions, proliferation and viability of the cells was decreased, and the cells were failed to differentiate into endothelial cells. Under hypoxic conditions, Beclin-1 expression of the cells was upragulated and both MDC-labeled and LC3-positive punctas and autophagic ultrastructures in the cells increased significantly. When autophagy was inhibited with 3-MA under hypoxic conditions, the number of apoptotic cells increased. Conversely, apoptosis of the hypoxic EPCs was reduced when autophagy was induced by pretreatment with rapamycin. After transplantation into the infracted myocardium, the cells pretreated with hypoxia or rapamycin survived well. Density of the microvessels increased significantly. Cardiac functions were improved effectively.

These results demonstrate that autophagy is involved in proliferation and differentiation of EPCs. Furthermore, hypoxia activates autophagy, promoting EPC survival by inhibiting apoptosis. Enhancing autophagy with hypoxic preconditioning may be beneficial for survival of the transplanted EPCs in a local hypoxic environment.

EMBRYONIC STEM CELL-DERIVED MICROVESICLES AS EFFECTIVE CARRIERS OF MRNA TO TARGET CELLS

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Microvesicles (MVs) are membrane-enclosed sacs shed into the extracellular environment by direct budding from the cell plasma membrane or derived from the endosomal compartment. MVs are secreted by normal healthy as well as tumor and apoptotic cells and may contain mRNAs, miRNAs, proteins and lipids. MVs originated from a given cell type may act as mediators of cell-to-cell communication by transferring their bioactive contents from parent cells to cells of other origins.

Here, we studied transfer of selected mRNAs by MVs derived from mouse embryonic stem cell line ES-D3 (ESMVs) to fibroblastic cell line 3T3-L1. We compared the level of transcripts related to pluripotency, cardiomyogenesis and proliferation in ESMVs and ESCs by real-time RT-PCR method and further visualized reaction products on agarose gel. The results showed that ESMVs are enriched in several mRNAs compared to their parental cell line, including transcripts for Gata4 and Rasgrf1. Surprisingly, expression levels for pluripotency genes (Nanog, Oct4 and

Rex1) were significantly lower in MVs. Furthermore, we evaluated the expression of selected surface antigens by cytofluorometric analysis of ESMVs and revealed the presence of molecules expressed on the surface of ESCs, such as SSEA-1, Sca-1 and CD105. Lastly, we investigated the transfer of biologically active content of ESMVs by incubation of ESMVs with 3T3-L1 cell line and subsequent analysis of gene expression levels in target cells. The transfer assay demonstrated that MVs can mediate the transfer of mRNAs from ES-donor cells to 3T3-L1 recipient cells.
These findings suggests that ESMVs may be important mediators of signaling within stem cell niches and may be useful therapeutic tools for transferring mRNA and membrane molecules to target cells.

Keywords: microvesicles, embryonic stem cells, mRNA transfer

EXPRESSION OF PLURIPOTENCY MARKERS IN HUMAN PLACENTAL CELLS

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Objectives: The definitions pertaining to the pluripotent stem cells (SC) are based on a limited number of antigenic markers and do not allow for unambiguous determination of the homogeneity of each subpopulation. Moreover, the use of some crucial markers of the pluripotency such as SSEA-3 and SSEA-4 has been questioned. The aim of the study was an identification of SC that express known surface markers of pluripotency: SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81 in human placental tissues, their isolation and quantitative analysis based on the surface marker expression and co-expression under different culture media conditions.
Material and methods: Immunofluorescence techniques were used to identify and localize SC within the normal, term human placenta. A number of SSEA-4+, SSEA-3+, TRA-1-60+ and TRA-1-81+ cells and cells with co-expression of mentioned markers, cultured in media containing different protein supplements of animal origin was counted by flow cytometry.
Results and conclusions: We confirmed that cells with characteristics of embryonic SC could be identified in human placenta: in amniotic epithelium and chorion but not in decidua basalis. Disproportion in the number of SSEA-4+, SSEA-3+, TRA-1-60+ and TRA-1-81+ cells and cells characterized by co-expression of these antigens indicates, that amniotic epithelium is composed of SC being at different stages of development. The lack of quantitative differences observed between subpopulations cultured in different media indicates that the differences in the composition these media did not influence the number of cells expressing SC markers. It was concluded that human amnion might have attracted attention as an alternative source of SSEA-4+ SC.

Keywords: amniotic epithelium; placenta; pluripotent stem cells; markers of pluripotency

FGF SIGNALING PROMOTES PHYSIOLOGICAL BONE REMODELING AND STEM CELL SELF-RENEWAL

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FGF cytokines signaling activate many bone marrow cell types, including mesenchymal stem cells (MSC),osteoblasts, osteoclasts,hematopoietic stem cells and their dynamic microenvironment. Current results indicate that partial deficiencies in FGF signaling lead to only mild defects in steady state hematopoiesis and bone remodeling. However, in these studies, FGF signaling was shown to be crucial for stem cell self-renewal and for proper hematopoietic post-stress recovery. Activation of FGF signaling was shown to be important also for rapid AMD3100 or post 5-FU-induced hematopoietic stem and progenitor cell (HSPC) mobilization. In vivo FGF-2 administration successfully expanded both mesenchymal and hematopoietic stem and progenitor cells (MSPC and HSPC).FGF-2-induced expansion was characterized by enhanced HSC cycling without further differentiation or exhaustion of the stem cell pool due to ROS inhibition. In addition, FGF signaling manipulated the BM microenvironment by expanding and remodeling the supportive MSPC niche cells and decreasing BM endothelial cell barrier permeability. Mechanistically, FGF-2 signaling increased CXCR4 and cKit surface expression and membrane bound SCF while reducing CXCL12 levels and ROS signaling which induced MSPC, followed by HSPC expansion. Finally,FGF signaling is constitutively activated in many human leukemias which secrete this ligand, suggesting that malignant HSPC exploit this pathway for their constant expansion and to remodel cells in their microenvironment into malignant supportive and protective niche cells.Taken together, these results suggest that manipulation of FGF signaling can serve to improve current clinical stem cell mobilization and transplantation protocols. In addition, it may help to develop therapies specifically targeting leukemicstem cells and their supportive microenvironment.

GENERATION OF SKELETAL STRUCTURES BY HUMAN VERY SMALL EMBRYONIC-LIKE (VSEL) CELLS

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Human very small embryonic-like (hVSEL) are a resident population of multipotent stem cells in the bone marrow (BM) involved in the turnover and regeneration of tissues. The levels of VSEL cells in blood are greatly increased in response to injury, and they have been shown to repair injured tissues. Adult hVSELs, are SSEA-4+/CD133+/CXCR4+/Lin–/CD45–, express the pluripotency markers (Oct-4 and Nanog) and may be able to differentiate into cells from all three germ lineages. hVSELs isolated from blood by apheresis following G-CSF mobilization were fractionated and enriched by elutriation and FACS. Collagen sponge scaffolds containing 2000-30,000 hVSELs cells were implanted into cranial defects generated in SCID mice. Analysis by μCT showed that a cell population containing VSEL cells produced mineralized tissue within

the cranial defects compared to controls at 3 months. Histological studies show significant bone formation and cellular organization within the defects compared to cellular or scaffold controls alone. Antibodies to human leukocyte antigens demonstrated that the newly generated tissues were of human origin. Moreover, human osteocalcin was identified circulating in the peripheral blood. There was evidence that some level of hVSEL cells migrated away from the defect site using quantitative real-time PCR to detect for human specific Alu sequences. This study demonstrates that hVSEL cells can generate human bone tissue in a mouse model of skeletal repair. These studies lay the foundation for future cell-based regenerative therapies for osseous and connective tissue disorders including trauma and degenerative conditions such as osteoporosis, fracture and neoplastic repair.

IDENTIFICATION OF SSEA-4+ VERY SMALL EMBRYONIC-LIKE STEM CELLS IN HUMAN MYOCARDIUM

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Very small embryonic-like cells (VSELs) is rare population of cells expressing pluripotent stem markers (Oct-4, Nanog, SSEA-4). The adult bone marrow-derived VSELs may be expanded and differentiatiated into all three germ layers. VSELs were identified in murine bone marrow and solid organs, umbilical cord blood and peripheral blood in humans.
The aim was to assess the endogenous presence of VSELs in human hearts and their mobilization induced by cardiac surgery.
Patients with CAD undergoing CABG were enrolled. Fragments of right atrial appendage (RAA), internal mammary artery and BM samples were harvested. Blood samples (VSELs, EPCs, SDF-1, HGF, SCF, VEGF) were taken before, after weaning from cardiopulmonary bypass, after 24 hrs and 5-7 days. Fragments of RAA were cut (0.5-1.0 cm3), and enzymatically digested in collagenase I (2 mg/ml) for 30-45min in 37oC. Cell suspension was stained with antibodies against stem cell markers, CD34 (FITC), CD133 (APC), SSEA-4 (PE), and CD45 (APC-Cy7) for 30min. Cell were washed, fixed in 2% paraformaldehyde and resuspended in PBS. Nuclei were stained with 10mM of Hoechst 33342 10min before the analysis. Analyses were carried out on ImageStream X cytometer.
Cardiac tissue contains very small (4.8±0.6 μm) roundish (aspectratio 1:1) nucleated VSEL cells expressing embryonic antigens. ImageStream system showed the presence of early developmental marker SSEA-4 on the surfaceof these cells. The cells express CD34 and CD133 stem cell markers and lack CD45 indicating their non-hematopoietic origin.
We demonstrated for the first time the presence of VSEL cells expressing embryonic marker SSEA-4 in adult human myocardium.

Keywords: very small embryonic-like cells, human myocardium

IMPACT OF ALTERED GROWTH HORMONE SIGNALING ON AGING, AND LONGEVITY

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Mutant mice lacking growth hormone (GH) or GH receptors live longer than their normal siblings. Moreover various symptoms of aging are delayed and/or attenuated in these remarkably long-lived animals. In contrast, transgenic mice overexpressing GH are short lived with numerous signs of accelerated aging. Studies in Ames dwarf (Prop1^{df}) mice lacking GH along with prolactin and thyrotropin and in GH receptor deleted (GHRKO) mice identified multiple mechanisms that appear to link GH deficiency or resistance and the consequent reduction in the levels of insulin-like growth factor I (IGF-1) in the circulation with extension of health span and longevity. These mechanisms include reduced inflammation and increased levels of adiponectin, an anti-inflammatory adipokine, improved insulin sensitivity combined with reduced insulin levels, metabolic adaptations including increased reliance on oxidation of lipids and increased energy expenditure, enhanced activity of antioxidant enzymes and increased cellular resistance to various stressors. Results of studies in Dr. Ratajczak laboratory indicate that improved maintenance of stem cells likely represents another important mechanism of delayed aging and extended longevity in these animals. We conclude that physiological actions of GH paradoxically include effects with a negative impact on aging and life expectancy. In laboratory mice reduced GH signaling is linked to extended longevity by multiple, interacting mechanisms.

INHIBITION OF MUSCLE REGENERATION IS ASSOCIATED WITH LOWER ATROGIN 1 AND MURF 1 GENES ACTIVITY

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TNF-a stimulated mitogenicity in C2C12 myoblasts. Selected cytokines TNF-a, IFNa, or IFNg reduced the expression of myosin heavy chain (MyHC IIa). Together they also decreased the expression in the 8-days old myotubes but were unsuccessful when given alone. Molecular mechanisms of cytokine activities were controlled by NF-kB and JAK/STAT signaling pathways, as metabolic inhibitors of (curcumin) and (AG490) blocked some of TNF-a and IFNa/IFNg effects. Insulin was hardly antagonistic to TNF-a- and IFNa/IFN-g-dependent drop in MyHC IIa expression. Cytokines used individually or together also repressed myogenesis of C2C12 cells. Moreover, TNF-a- and IFNa/IFNg-dependent effects on C2C12 myotubes were associated with increased activity of Atrogin1 and MuRF1 genes, which code ubiquitin ligases. MyHC IIa gene activity was unaltered by cytokines. Inhibition of NF-kB or JAK/STAT with specific metabolic inhibitors decreased activity of Atrogin1 and MuRF1 but MyHC IIa gene. Overall, these results suggest cooperation between cytokines in the drop of MyHC IIa expression via NF-kB/JAK/STAT signaling pathways, and activation of Atrogin1 and MuRF1 genes as their molecular targets.

Keywords: muscle regeneration, TNF-a, IFN-a/g, insulin, NF-kB, STAT1-a, MyHC IIa

INTENSIVE PHYSICAL EXERCISE MOBILIZES STEM CELLS INTO PERIPHERAL BLOOD AND TRIGGERS PROLIFERATION OF VERY SMALL EMBRYONIC-LIKE STEM CELLS

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Bone marrow (BM) contains a variety of stem cells, including hematopoietic stem/progenitor cells (HSPCs), endothelial progenitor cells (EPCs), mesenchymal stem cells (MSCs), and the developmentally most primitive very small embryonic-like stem cells (VSELs). Evidence has accumulated that EPCs and HSPCs are mobilized into PB during strenuous exercise. Therefore, we became interested in whether a pool of bone marrow residing VSELs would respond to stimuli related to prolonged, strenuous exercise.

To test the effect of exercise on the mobilization and BM pool of stem cells, we employed C57Bl6 mice that were exposed to short (1- 14 days), and long- term (6 months) strenuous exercise in rotating wheels. These mice were subsequently sacrificed 1h after removal from the rotating wheels, and we i) measured changes in peripheral blood cell counts, ii) enumerated the number of VSELs and HSPCs, iii) performed in vitro clonogeneic assays, iv) employed the in vivo BrdU incorporation assay to evaluate the number of VSELs and HSPCs in BM undergoing cell division, and finally v) performed a molecular analysis of the expansion and mobilization of VSELs.

Our data confirmed that strenuous exercise mobilizes Sca-1+Lin-CD45+ HSPCs into PB, and, for the first time, we show that prolonged, enforced exercise of mice in rotating wheels is associated with expansion in BM and mobilization into PB of the most primitive population of stem cells, Sca-1+Lin-CD45- VSELs. The proliferation of VSELs has been confirmed by BrdU incorporation. In contrast to VSEL expansion, we did not observe significant changes in the number of BM-residing HSPCs. As expected, mice exposed to prolonged exercise exhibited a significant increase in skeletal muscle and a decrease in abdominal fat.

Keywords: stem cells, VSELs, hematopoietic stem cells, physical exercise. (Supported by NIA).

IN VIVO ISCHEMIC LIMB INJURY ACTIVATES PROLIFERATION AND MOBILIZATION OF PLURIPOTENT BONE MARROW- DERIVED VERY SMALL EMBRYONIC-LIKE STEM CELLS

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Very small embryonic-like stem cells (VSELs) were identified as rare quiescent population of nonhematopoietic cells in adult murine and human tissues including bone marrow (BM). VSELs may i) be mobilized in tissue injury and ii) participate in tissue regeneration including in heart

repair in mice. However, their activation and proliferation during such injury/ repair events in vivo have not been reported.
In this study, we examined if acute ischemic limb injury may stimulate both proliferation of quiescent VSELs in BM and their mobilization into peripheral blood (PB). Thus, 4-6 week old C57BL/6 mice underwent a hind limb ischemia by permanent proximal femoral artery occlusion. Mice were administrated with bromodeoxyuridine (BrdU) and scarified at 2, 7, 14 and 28 days following ischemia. PB and BM were collected from individual animals including contols. The presence of proliferating (BrdU+) VSELs (Sca-1+/Lin-/CD45-), endothelial progenitor cells (EPCs; Flk-1+/Sca-1+/Lin-/CD45-dim) and hematopoietic stem/progenitor cells (HSPCs; Sca-1+/ Lin-/ CD45+) in PB and BM was evaluated by flow cytometry and ImageStream system. The expression of genes related to the presence of VSEL and EPC fractions was examined by real-time RT-PCR. Moreover, we examined the change in expressionof 53 angiogenesis-related proteins.
We found that the number of non-proliferating VSELs was significantly increased in BM of ischemic mice at 7d post injury. Similar results were obtained for EPCs. Increased number of BrdU+ VSELs was accompanied with change in expression of genes guiding their proliferation. The data indicates vast impact of acute injury conditions on activation of VSEL proliferation in vivo.

Keywords: Very small embryonic-like stem cells (VSELs); ischemic limb injury; mobilization and proliferation of VSELs

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MARROW HEMATOPOIETIC STEM CELLS: A CONTINUUM OF CHANGE

Quesenberry P, Goldberg L, Dooner M

Current dogma indicates that long-term repopulating marrow stem cells are a stable dormant population with great renewal, differentiation and proliferation potential and that there is an orderly hierarchy of stem cells showing progressive restriction of lineage and loss of renewal and proliferative potential with differentiation. Work from 1998 (Hababian et al 1998) showing that purified lineage negative rhodamine low Hoechst low stem cells driven through a synchronized cell cycle transit lost capacity for long-term multilineage engraftment in late S/earlyG2 but then regained this is subsequent cycles, challenged this hierarchical model. Subsequent studies showed that with cycle passage there were stem/progenitor cell inversions. When purified murine marrow stem cells (lin-rhodamine low Hoechst low -LRH) was cultured in thrombopoietin (TPO), FLT-3 ligand (FLT-3L) and steel factor (TFS) the cells progressed synchronously through cell cycle. At 28-32 hours of culture (Early S phase) there were 13 major reversible increases in progenitor numbers from one time point to another and this was coupled with 11 major engraftable stem cell decreases while in 2 instances the engraftable stem cells were unchanged. Thus these primitive marrow stem cells reversibly shift from engraftable stem cells to progenitors without differentiation occurring. Homing of Lin-Sca-1+ stem cells to marrow also shifted with cycle progress as did "conversion to lung cells after transplantation into lethally irradiated mice. Gene expression also reversibly changed with cycle passage. There were dramatic differences in differentiation noted either with cytokine stimulated cell cycle passage or when purified marrow stem cells were separated into different phases of cell cycle using staining with Pyronin/Hoechst and fluorescent activated cell sorting. When LRH marrow cells were cultured in TFS and subcultured (1,000 cells) at varying cycle phases and differentiation determined, it was found that there were "differentiation "hotspots at early S-phase and in mid-S-phase for megakaryocytes and granulocytes, respectively. Further work done in the same

model at 8-14 hour time intervals but subculturing single cells and determining clonal progeny showed virtually total heterogeneity of outcomes at each cycle phase, although there were discernible patterns of differentiation. More recently studies on differentiation through cell cycle transit of LRH cells under TFS stimulation have been carried out in the time intervals of culture of 28-35 hours and 49-56 hours at ½ hour intervals. Dramatic changes in megakaryocyte and granulocyte differentiation were seen at virtually every ½ hour interval, lesser changes were seen with macrophages. These data suggest that very rapid changes in differentiation potential are occurring with cycle transit.
In a related area of work we have shown that lung or liver derived extracellular vesicle can transcriptionally alter the phenotype of bone marrow cells. We further demonstrated that such alterations induced by lung-derived vesicles changed dramatically with cycle phase and whether lung tissue was previously exposed to irradiation or not. These observations indicate that marrow cells/stem cells are on a continuum of change, possibly instantaneous in nature, which is then further influenced by extracellular vesicle exposure. Further work by Dr. Goldberg will be presented showing that long-term repopulating marrow stem cells are actively cycling and explaining the continuously changing phenotypes. This is truly a new stem cell biology.

MOBILIZATION OF VSELS AND HSCS POPULATIONS IN PATIENTS WITH ATRIAL FIBRILLATION UNDERGOING PERCUTANEOUS RF ABLATION

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In non-physiological circumstances such as tissue injury, amount of circulating stem cells (SC) may be elevated in peripheral blood (PB), due to inflammatory and mobilization response. Invasive medical procedures may also potentially trigger systemic reaction, which leads to mobilization of SC from their natural niches, especially from bone marrow (BM). It has been postulated that BM-derived stem cells may contribute to repair of damaged tissue, directly by differentiation, or more likely due to paracrine effects.
In our study, we analyzed appearance of selected SC fractions in PB of patients with atrial fibrillation undergoing percutaneous RF ablation in different time points after injury. We focused on population of very small embryonic-like stem cells (VSEL), which are rare, primitive SC sharing several features of embryonic SC. We also analyzed occurrence of hematopoietic stem cells (HSC).
By employing flow cytometry, we analyzed numbers of CD45-/Lin-/CD133+ and CD45-/Lin-/CD34+ fractions enriched in VSEL, as well as CD45+/Lin-/CD133+ and CD45+/Lin-/CD34+ HSC in PB aspirated from patients in different time points: before, immediately after (within 1h), 6 hrs and 18-24 hrs after procedure.
We observed distinct kinetics of mobilization of analyzed SC populations, with transient changes in amounts of circulating cells. In one subgroup of patients the number of CD45+/Lin-/CD133+ HSC decreased, while number of CD45-/Lin-/CD34+ VSEL was increased immediately after procedure. Moreover, 6 hrs after ablation, we observed elevated number of HSC in PB but depleted of VSEL. These results indicate that RF ablation may cause distinct response to tissue injury. Further analyses are necessary to understand the mechanisms of the observed diversity between patients' groups.

MOBILIZATION STUDIES IN C3-DEFICIENT MICE UNRAVEL THE INVOLVEMENT OF A NOVEL CROSSTALK BETWEEN THE COAGULATION AND COMPLEMENT CASCADES IN MOBILIZATION OF HEMATOPOIETIC STEMPROGENITOR CELLS

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The mobilization process has been postulated to be directed by (i) a decrease in SDF-1–CXCR4 and VLA-4–VCAM-1 interactions in bone marrow (BM) microenvironment (ii) release of neurotransmitters from the synapses of the nerves that innervate the BM (iii) reversal of the trans-endothelial chemotactic gradient between the BM microenvironment and plasma, (iv) activation of the plasminogen system, as recently proposed, and (v) activation of the complement cascade (ComC).

Mice defective in proximal component of complement cascade (ComC), for example C3-/- are easy mobilizers, which was explained by lack of C3a that increases retention signals of HSPCs to SDF-1 gradient (BM). In contrast mice defective in distal component of ComC (C5-/-) display defect in mobilization of HSPCs. In explanation we postulate that C5 cleavege fragments (C5a, C5b) are essential for egress of HSPCs into peripheral blood. Activation of ComC and proper cleavage of C3 is required to generation of C5 covertase (enzyme that cleaves C5 into C5a and C5b). Therefor we considered alternative pathways of proteolytic cleavage of C5 in C3-/- mice.

Activation of the coagulation cascade (CoaC) is a well-known side effect of granulocyte colony-stimulating factor (G-CSF). Herein, based on data that C5 can be activated/cleaved by thrombin, which exhibits C5 convertase-'like' activity, we evaluated the effect of specific, direct thrombin inhibitor, refludan, during G-CSF-induced mobilization in C3-/- mice.

The data presented in this work demonstrate, the existence of crosstalk between two evolutionarily ancient proteolytic cascades, the ComC and CoaC, in the mobilization of HSPCs. It is known that both cascades are activated in parallel in all situations, in which HSPCs are mobilized from BM into PB.

Keywords: mobilization, complement cascade, coagulation cascade, stem cells, hematopoietic stem cells, thrombin inhibitor

MURINE MARROW HEMATOPOIETIC STEM CELLS ARE ACTIVELY CYCLING AND EXIST ON A CONTINUUM OF CHANGE

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Prevailing wisdom holds that hematopoietic stem cells (HSCs) exist predominantly in a quiescent state. To date, however, most studies have examined only the cell cycle status of

highly purified populations of stem cells. In the studies presented here, we explored the cell cycle status of the total population of HSCs in unseparated whole bone marrow (WBM) and found a large population of actively cycling HSCs capable of long-term multilineage engraftment. WBM was separated into cell cycle-specific fractions using Hoechst 33342 alone or Hoechst 33342/Pyronin Y, or exposed to tritiated thymidine and then competitively engrafted into lethally irradiated mice. Percent donor chimerism was measured using flow cytometry to evaluate donor engraftment. Both approaches revealed a large percentage of actively cycling cells capable of long-term multi-lineage engraftment, with over 50% of the long-term engrafting cells in in S/G2/M in some studies. This is in stark contrast to studies showing that engraftment by highly purified long-term hematopoietic stem cells (LT-HSC) occurred almost exclusively when they were in G0. In addition, we studied the kinetics of LT-HSC through cell cycle in vivo using bromodeoxyuridine (BrdU). The percentage of LT-HSC that progressed through S-phase and returned to G0/G1 within 24 and 48 hours was 58% and 67%, respectively. This suggests that, although highly purified LT-HSC predominantly engraft while in a quiescent state, this same population of cells are actually actively cycling in vivo with relatively rapid kinetics. Given that marrow stem cells are actively cycling, they must be continually changing phenotype as they transit cell cycle. We hypothesized that, given such phenotypic lability, the cycling stem cells are lost during conventional stem cell isolation techniques that rely on stable epitope expression. To test this hypothesis, we harvested WBM from B6.SJL mice and performed standard isolation of a highly purified population of stem cells (Lineage negative c-kit+sca-1+CD150+CD41-CD48-). We collected the different cellular fractions created and routinely discarded during purification and competitively engrafted each fraction into lethally irradiated C57BL/6 mice. We found that over 75% of potential engraftment capacity in WBM was lost during conventional stem cell purification. Experiments utilizing tritiated thymidine suicide indicates that actively cycling cells within the lineage-positive fraction are among the discarded stem cells. Given the loss of a large pool of actively cycling HSC during standard stem cell isolation techniques, these data underscore the need to re-evaluate the total hematopoietic stem cell pool on a population level in order to provide a more comprehensive study of HSC biology.

NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS) DEFINE NOVEL ROLES FOR PROSTAGLANDIN E2 (PGE2) IN THE HEMATOPOIETIC NICHE AND A METHOD TO COLLECT HEMATOPOIETIC STEM AND PROGENITOR CELLS (HSPC) WITH IMPROVED FUNCTION

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The bone marrow microenvironment is intricately involved in stem cell maintenance and blood cell production. While advances have been made in our understanding of HSC/niche interactions, we do not fully understand regulatory function within the hematopoietic niche. For over 35 years we have known that PGE2 can regulate hematopoiesis having both positive and negative effects depending on time and cell lineage. While exploring endogenous PGE2 function, we observed HSC and HPC egress from the bone marrow after treatment of mice, non-human primates and normal volunteers following treatment with NSAIDs. This egress was, surprisingly, independent of the CXCR4/SDF1 axis and differed for HPC versus HSC, with HSC egress dependent on attenuation of the stromal niche and reduction in the niche retentive

molecule osteopontin. Enhanced NSAID-mediated HSPC egress was observed in combination with multiple mobilizers, including G-CSF, AMD3100 and GROβ suggesting an independent mechanism of action. NSAID enhanced grafts demonstrated superior repopulating ability and engraftment. PGE2 receptor knockout mice demonstrated that that HSPC egress resulted from inhibition of signaling through the E-prostanoid 4 (EP4) receptor. These studies redefine the role of PGE2 on hematopoiesis to include both cell intrinsic and cell extrinsic mechanisms differentially regulating HSC and HPC, and define a translational strategy to enhance collection of HSPC for hematopoietic transplant.

NOVEL MARKERS FOR THE PROSPECTIVE ISOLATION OF NEURAL STEM CELLS FROM THE ADULT MURINE HIPPOCAMPUS

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Neurogenesis in the adult mouse hippocampus is well characterized, however despite considerable effort there is a distinct lack of appropriate markers for the prospective isolation of the resident stem cells. In our search we have identified Prominin-1 and the lysophosphatidic acid receptor (LPA_1) as novel hippocampal precursor cell markers. Prominin-1 (CD133) is commonly used to isolate stem and progenitor cells from the developing and adult nervous system and to identify cancer stem cells in brain tumors. However, no information about the expression of Prominin-1 by precursor cells of the adult hippocampus was available. We have recently shown that Prominin-1 is expressed by a significant number of cells in the subgranular zone of adult mice in vivo and ex vivo, including postmitotic astrocytes. A small subset of Prominin-1^+ cells co-expressed the non-specific precursor cell marker Nestin as well as GFAP and Sox2. Upon fluorescence activated cell sorting (FACS), only Prominin-1/Nestin double-positive cells fulfilled the defining stem cell criteria of proliferation, self-renewal and multipotentiality as assessed by a neurosphere assay.

Using immunohistochemistry we have also found that LPA_1-GFP is expressed in the precursor cells in the adult dentate gyrus of the transgenic reporter mice. Lysophosphatidic acid (LPA) is an extracellular signaling lysophospholipid that binds to the G protein-coupled receptors LPA_{1-6} to regulate many important biological processes including neural cell development. FACS followed by neurosphere assays confirmed that the precursor activity was confined to the LPA_1+ cell population with >99% of total neurospheres being formed from the LPA_1-GFP+ cells. Surprisingly, the more commonly used precursor cell reporter line Nestin-GFP was a less specific marker of these cells with only 80% of the total neurosphere formation from the Nestin+ population. In addition, LPA_1-GFP can be used as a reliable marker of those cells in the dentate gyrus capable of being activated by physical activity, with a significant increase in the number of LPA_1-GFP+ cells following 10 days of voluntary running. Finally, by combining LPA_1-GFP expression with two cell surface markers, Prominin-1 and EGF-receptor, the purity of the isolated precursor population could be increased from 1/50 to 1/3 cells capable of forming a neurosphere. In summary, we show that the novel markers Prominin-1 and LPA_1-GFP can be used to enrich precursor cells from the adult murine dentate gyrus. In addition, we describe novel marker combinations (Nestin/Prominin-1, GFAP-GFP/Prominin-1/CD24, GFAP-GFP/EGFR/PSA-NCAM and LPA_1/Prominin-1/EGFR) for the simultaneous flow cytometric isolation of multiple cell types from the adult dentate gyrus. This approach will facilitate the

isolation and characterization of homogeneous cell populations of astrocytes, neural precursor cells and immature neurons in order to gain a deeper understanding of the underlying molecular regulation of hippocampal neurogenesis in response to activity or injury in individual cell types rather than at the level of the entire dentate gyrus.

NOVEL METHODOLOGY FOR SEPARATION OF OCT4+ VERY SMALL EMBRYONIC LIKE STEMCELLS (VSELS) FROM HUMAN UMBILICAL CORD BLOOD

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The fluorescence-activated cell sorting (FACS) and magnetic cell sorting (MACS) are the most effective isolating methods to purify rare cells. Recently, our group have identified a very rare population of umbilical cord blood (UCB)-derived very small embryonic like stem cells (VSELs) using marker-based sorting methodology. Isolated LIN-/CD45-/AC133+ cells are enriched for VSELs and express transcription factors Oct4, Nanog and Sox2 at mRNA and protein level, that are characteristic markers for embryonic stem cells (ESCs).
To better characterize VSELs at molecular level, we developed a novel three-step isolation procedure to isolate from human umbilical cord blood population highly enriched for VSELs. Accordingly, by using hypotonic lysis (step I), MACS separation (step II) and fluorescence-activated cell sorting based on intracellular staining for Oct-4 (step III), a well known major marker, which determine pluripotency of stem cells.
To prove that sorted cells truly express pluripotency marker Oct-4, we checked status of Oct-4 promoter methylation in UCB-derived VSELs and found that it has an open chromatin structure, what means that is actively transcribed. We have also used immunocytochemical staining to determine the presence and subcellular localization of Oct-4 in sorted VSELs.

PROMININ-1 (CD133) REVEALS NEW INSIGHTS INTO THE CELL BIOLOGY OF STEM AND PROGENITOR CELLS

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Prominin-1 (alias CD133) is a pentaspan membrane glycoprotein concentrated in various types of plasma membrane protrusions (e.g., microvillus, primary cilium, uropod). This cholesterol-binding protein is associated with membrane microdomains (lipid rafts), and several types of stem cells including those found in the neural and hematopoietic system express it. CD133 is widely used as a cell surface marker of cancer stem cells. Surprisingly, we demonstrated that in the developing embryonic mouse brain, CD133 is released from neuroepithelial progenitors into the lumen of the neural tube, concomitant with their differentiation, by means of plasma membrane-derived vesicles. These CD133-positive membrane vesicles are budding from the

tips of microvilli and primary cilia. Clinically, their level is up regulated in cerebrospinal fluid of glioblastoma patients. Do similar phenomena exist in other stem cell types or is it unique to those derived from the neural system? Is the release of CD133-positive membrane vesicles a read out of differentiation in general? We have investigated these questions using human CD133-positive hematopoietic stem and progenitor cells (HSPCs) growing on primary multipotent mesenchymal stromal cells (MSCs) as a feeder cell layer. We report here the following observations. First, CD133 is released from HSPCs into the culture medium in association with membrane vesicles that are sedimented after high-speed centrifugation. Second, these CD133-positive vesicles are enriched in membrane cholesterol, and contain Flotillin-1/2 and Syntenin-1 – a PDZ domain containing protein that interacts with the exosomal marker CD63 – raising the possibility that these vesicles originate not only from the plasma membrane protrusions, but also from intracellular structures. Within these vesicles, CD133 binds also directly to Syntenin-1. Third, the differential immunofluorescence revealed that CD133, in addition to its association with plasma membrane protrusions, is present in intracellular compartments, which at the electron microscopy level appear as multivesicular bodies demonstrating for the first time the association of CD133 with exosomes. Fourth, the amount of CD133-positive membrane vesicles found in the culture medium increases upon cultivation whereas the number of CD133-positive cells is decreasing, indicating a general link between the release of CD133-positive membrane vesicles and cellular differentiation. Finally, the association of CD133 with exosomes will be discussed.

SINGLE CELL LEVEL TRANSCRIPTOME ANALYSIS OF BONE MARROW-DERIVED OCT4+ VERY SMALL EMBRYONIC-LIKE STEM CELLS (VSELS) REVEALS THE MECHANISM FOR VSELS PLURIPOTENCY

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Recently, we identified a population of very small embryonic-like (VSEL) stem cells in adult bone marrow (BM), which are capable of differentiation in vitro into cells from all three germ lineages and in in vivo animal models. Employing gene-expression and epigenetic profiling studies we reported that VSELs in BM have germ-line stem cell like epigenetic features, i) open/active chromatin structure in Oct4 promoter, ii) parent-of-origin specific reprogramming of genomic imprinting (Leukemia 2009, 23, 2042-2051), and iii) that they share several markers with epiblast-derived primordial germ cells (PGCs), in particular with migratory PGCs (Leukemia 2010, 24, 1450-1461). However, it was not clear about how VSELs maintain pluripotent state. To address this issue we recently employed single cell-based genome-wide gene expression analysis and found that Ezh2, a polycomb group protein, is highly expressed in VSELs. This protein is well known to be involved in maintaining a bivalent domain (BD) at promoters of important homeodomain-containing developmental transcription factors. Of note a presence of BD is characteristic for pluripotent stem cells (e.g., ESCs) and as result of Ezh2 overexpression, VSELs, like ESCs, exhibit BD - bivalently modified nucleosomes (trimethylated H3K27 and H3K4) at promoters of important homeodomain-containing developmental transcription factors (Sox21 Nkx2.2 Dlx1 Zfpm2 Irx2 Lbx1h Hlxb9 Pax5 HoxA3). Of note, spontaneous (as seen during differentiation) or RNA interference-enforced down-regulation of Ezh2 removes BD what, results in lose of their plurioptentiality and de-repression of several BD-regulated genes that

control their tissue commitment. As another epigenetic factor, we found that VSELs highly express a transcript for transcription repressor CRTR-1, similarly to ESC. As result from in vitro differentiation of ESCs, we demonstrated that CRTR-1 could play a crucial role on the i) self-renewal, ii) Oct4 expression, iii) cell proliferation, and iv) differentiation of pluripotent stem cells. In conclusion, Our results show for first time that in addition to the expression of pluripotency core transcription factor Oct4, VSELs maintain their pluripotent state employing the epigenetic mechanisms similar to other pluripotent stem cell. Based on this our genome-wide gene expression study not only advances our understanding of biological processes that govern VSELs pluripotency, differentiation, and quiescence but will also help to develop better protocols for ex vivo expansion of these promising cells application in regenerative medicine.

STEM CELLS FOR REGENERATIVE MEDICINE IN AGING AND CANCER

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Autologous stem cells can be safely transplanted without being rejected by the body's immune system and they have unique characteristics compared to other sources of stem cells which makes them an increasing focus of research for clinical applications in regenerative medicine.
Autologous stem cell therapy is an established treatment for immune dysfunction of blood cancers. The lifetime likelihood of an individual in the U.S. needing a hemopoietic stem cell transplant, using either one's own stem cells or those from a donor is 1 in 200 which is much higher than previously reported estimates(Biology of Blood and Marrow Transplantation, March 2008). In the future it is believed that the need for autologous stem cell therapy will increase as it may offer remedies for strokes and neurodegenerative conditions such as Parkinson's and Alzheimer's diseases and chronic diseases such as heart disease as well as diabetes.
The function of stem cells decrease with age. Healthy adults can undergo a process known as adult stem cell collection where a person's autologous stem cells are taken from their blood after being mobilized from the bone marrow and the cells can be stored until potentially needed in the future.
This presentation will focus on clinical aspects of the use autologous stem cells for regenerative medicine with emphasis on strategies for optimizing their function.

STUDIES WITH DILUTED PLASMA REVEAL THE PRESENCE OF A REMARKABLY POTENT FACTOR THAT ENHANCES THE MOTILITY OF CANCER CELLS AND IS QUENTCHED BY FIBRINOGEN - A NOVEL VIEW OF CANCER METASTASIS

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Background. The most important problem in cancer therapy is the tendency of cancerous cells to leave a primary tumor and metastasize to different vital organs. Several candidate classes of molecules have been proposed to direct the metastatic process, including chemokines, growth factors, cytokines and bioactive lipids. Plasma and serum alone possess chemotactic activity, but all the factors responsible for this effect have not been well characterized. We found that highly diluted (1%) plasma possesses remarkable chemokinetic activity against cancer cell lines, which exceeds by several times the activity of optimal doses of SDF-1 or HGF/SF.
Aim of the study. Our aim was to better characterize this remarkable chemokinetic activity of normal plasma identified in plasma diluted to 1%.
Results. Diluted (1%) plasma possesses remarkable chemotactic activity against malignant tumor cells in Transwell migration assays, which rapidly decreases with increasing concentrations of plasma (>5%). In our cell lines, plasma diluted 1:100 activated phosphorylation of MAPKp42/44 and AKT, and the chemotactic response was inhibited by blocking Gai protein signaling by pertussis toxin (PTX) as well as by inhibition of MAPKp42/44 and AKT. This remarkable factor of molecular weight between 100 and 50 kD is sensitive to proteolytic treatment, is not removed from plasma by dialysis, and is temperature-sensitive, which collectively indicates a polypeptide structure. A similar effect has been observed with serum diluted 1:100, though chemotactic responsiveness was maintained at higher concentrations, what is probably connected to inhibitory effect of fibrinogen. Therefore, we hypothesise that fibrinogen in some manner affect this potent factor that enhances the motility of cancer cells.

Keywords: Metastasis, chemotaxis, chemokinesis, protein factor

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TESTICULAR VSELS MAY BE TARGETED FOR FERTILITY RESTORATION IN AZOOSPERMIC CANCER SURVIVORS

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Various adult body organs including gut, skin, bone marrow etc harbor quiescent and an actively dividing stem cell populations. A relatively quiescent population of stem cells termed 'Very Small Embryonic like Stem Cells (VSELs) has been shown to be present in many murine organs including brain, kidney, pancreas, testis, spleen etc. Our group reported presence of VSELs in adult mammalian testicular and ovarian tissue. Present study was undertaken to further characterize the mouse testicular VSELs and evaluate their potential to restore spermatogenesis in germ cell depleted testis.
VSELs were detected in azoospermic testicular biopsies of survivors of childhood cancer and also in chemo-ablated mice testis, possibly because VSELs resist oncotherapy due to their quiescent nature. VSELs were typically spherical in shape, of small size, high nucleo-cytoplasmic ratio, and expressed pluripotency (Oct-4A, Nanog, Sox-2), primordial germ cell (Stella) and VSEL specific (CD133) markers. Similarly in chemoablated mice testis, the VSELs were characterized to be Sca-1+ Lin- CD45- .
The somatic microenvironment 'niche' in chemo-ablated mice is compromised and may render VSELs non-functional. Thus attempts were made to restore somatic niche by transplantation of healthy Sertoli cells. Spermatogenesis was restored overcoming the meiotic block and producing functional sperm. Alternatively, transplantation of syngenic bone marrow derived mesenchymal stem cells also yielded similar results. Transplanted Sertoli or mesenchymal cells

possibly acted as a source of growth factors/ cytokines essential for VSELs differentiation. Since sperm formation occurred in vivo, various epigenetic concerns associated with 'synthetic gametes' are eliminated in our approach and thereby may offer an altogether new perspective to the field of oncofertility.

THE EFFECT OF LOW AND HIGH PLASMA LEVELS OF INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) ON THE MORPHOLOGY OF LIVER AND ADIPOSE TISSUE—STUDIES OF LARON DWARF AND BOVINE GROWTH HORMONE TRANSGENIC (BGHTG) MICE

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It is well known that somatotrophic/insulin signaling affects lifespan in experimental animals. To study the effects of insulin-like growth factor-1 (IGF-1) plasma level on the morphology of heart, liver and adipose tissue isolated from 2-year-old growth hormone receptor knockout (GHR-KO) Laron dwarf mice (with low circulating plasma levels of IGF-1) and 6-month-old bovine growth hormone transgenic (bGHTg) mice (with high circulating plasma levels of IGF-1). The ages of the two mutant strains employed in our studies were selected based on their overall ~50% survival (Laron dwarf mice live up to ~4 years and bGHTg mice up to ~1 year). Morphological analysis of the organs of long-living 2-year-old Laron dwarf mice revealed a lower biological age for their organs compared with normal littermates, with more brown adipose tissue (BAT) surrounding the main body organs, lower levels of steatosis in liver, and a lower incidence of leukocyte infiltration in different organs. By contrast, the organs of 6-month-old, short-living bGHTg mice displayed several abnormalities in liver including severe steatosis and inclusions in nuclei of hepatocytes also a reduced content of BAT around vital organs (i.e. heart and kidneys).

Keywords: Aging, brown adipose tissue, insulin-like growth factor-1 (IGF-1), growth hormone (GH), Laron dwarf mice

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THE HYPOXIA-SENSING PATHWAY IS A CRITICAL REGULATOR OF HEMATOPOIETIC STEM CELL QUIESCENCE, RESISTANCE TO RADIATION, AND TRAFFICKING

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Hematopoietic stem cells (HSC) comprise an active pool that divides regularly to regenerate the hematopoietic tissue, and a dormant pool that divides very infrequently in the adult bone marrow in steady-state (once every 25 weeks) and contains most of the serial reconstitution activity. This suggests that distinct HSC niches may co-exist to either maintain long-term HSC quiescence or promote division. Hypoxia is a defining characteristic of some of the HSC niches in the bone marrow. Indeed, we have previously found that quiescent HSC able to serially transplant preferentially reside in niches poorly perfused by the blood, whereas active HSC reside in more perfused niches and have more limited self-renewal potential in serial transplantations. Low perfusion results in decreased oxygen supply and hypoxia. Hematopoietic cells sense hypoxia via the transcription factor HIF-1α which is stable and active only in hypoxic environment below 2% oxygen. Indeed when oxygen concentration exceeds 2%, HIF-1α protein is hydroxylated on proline resides by prolylhydroxylase domain (PHD) enzymes and rapidly degraded by the proteasome. As deletion of the Hif1a gene impairs HSC self-renewal and serial reconstitution potential, we tested the in vivo effect of two small synthetic PHD inhibitors that stabilize HIF-1α protein independently of O_2 concentration. Mouse treatment with these PHD inhibitors stabilized HIF-1α protein in bone marrow cells in vivo. This resulted in increased proportion of HSC in quiescence in phase G0 and decreased proliferation as measured by bromodexoyuridine (BrdU) incorporation in vivo in the bone marrow. Furthermore, mouse pre-treatment with PHD inhibitors before 9.0 Gy sublethal irradiation accelerated blood cell recovery and increased 89-fold the survival of long-term reconstituting HSC. Therefore HIF-1α protein stabilization in hypoxic niches is an essential promoter of HSC quiescence. We then investigated the role of HIF-1α in therapeutic HSC mobilization in response to G-CSF. We have previously shown that G-CSF treatment increases hypoxia and stabilizes HIF-1α protein in the bone marrow. To demonstrate the role of HIF-1α in HSC mobilization, we generated mice in which the Hif1a gene can be specifically deleted in HSC in a tamoxifen-inducible manner and in which Hif1a gene deletion can be followed by concomitant induction of a yellow fluorescent protein reporter (SclCreER R26R$^{YFP/YFP}$ Hif1a$^{flox/flox}$ mice). Induction of Hif1a gene deletion in HSC with tamoxifen prevented their mobilization in response to G-CSF. Conversely, we found that additional stabilization of HIF-1α protein by administering PHD inhibitors in wild-type mice enhanced 20-fold HSC mobilization in response to G-CSF and Plerixafor, the most potent mobilizing combination used in the clinic. In conclusion our results demonstrate that niche hypoxia and HIF-1α are essential regulators of HSC quiescence and trafficking and that HIF-1α protein can be pharmacologically stabilized to promote HSC quiescence, radio-resistance and mobilization in vivo.

THE IMPORTANCE OF PRECISE CELLS ADMINISTRATION IN TERM OF THEIR FATE AFTER THE INJECTION INTO THE URETHRAL SPHINCTER

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Myogenic cells therapy is considered as an alternative method to treat urinary incontinence. The theoretical background for this approach is based on the concept that the transplantation of stem or progenitor cells can enhance urethral sphincter strength. The aim of this study was to follow the fate of muscle-derived cells (MDCs) autotransplanted into the uninjured porcine external urethral sphincter (EUS) with the use of endoscope. The experiment was performed on

9 female pigs. The cells isolated from skeletal muscle expressed desmin and were able to differentiate into myotubes in vitro, however the myogenic activity was decreasing during the culture. To verify the myogenic potential of cultured MDCs in vivo, the cells stained with PKH26 were first injected into the skeletal muscle (n=2). Next, 60x10(6) of MDCs were labeled with PKH26 and autotransplanted in three depots into the EUS using urethrocystoscopy (n=5). Two animals underwent sham operation. The tissues were collected after 28 days. To analyze the fate of injected cells, the PKH26 presence, the desmin expression and the distribution of acetylcholine receptors were evaluated in the tissue sections. Donor cells were localized within muscle layer, but also in other parts of urethral sections what suggested that the technique of injection was not precise enough. The immunohistochemical staining for desmin revealed that cells located in the muscle layer were integrated with muscle fibers which possessed acetylcholine receptors. However cells administered into non-muscle tissue did not express desmin. No unspecific red fluorescence was oberved in sham operated pigs. The experiments indicated the key importance of precise cells administration in term of their fate after the injection.

Keywords: autologous transplantation, porcine model, myogenic cells, urethral sphincter, urinary incontinence

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VERY SMALL EMBRYONIC-LIKE STEM CELLS FROM THE MURINE BONE MARROW DIFFERENTIATE INTO LUNG EPITHELIAL CELLS

Susannah H. Kassmer, Huiyan Jin, Ping-Xia Zhang, Emanuela M. Bruscia, Kartoosh Heydari, Joo-Hyeon Lee, Carla F. Kim and Diane S. Krause

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The view that adult stem cells are lineage restricted has been challenged by numerous reports of bone marrow (BM) derived cells giving rise to epithelial cells. Previously, we demonstrated that non-hematopoietic bone marrow cells are the primary source of BM derived lung epithelial cells. We have now tested the hypothesis that very small embryonic like cells (VSELs) are responsible for this engraftment. Using surfactant protein C knockout mice as transplant recipients, we directly compare VSELs to other BM subpopulations for their ability to produce type 2 pneumocytes in the lung. By transplanting VSELs from donor mice expressing H2B-GFP under a type 2 pneumocyte specific promoter, we demonstrate that this engraftment occurs by differentiation and not fusion. This is the first report of VSELs differentiating into an endodermal lineage in vivo, thereby potentially crossing germ layer lineages. Our data suggest that Oct4+ VSELs in the adult BM exhibit broad differentiation potential.

VSELS BIOLOGY IN ADULT MAMMALIAN GONADS

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Data from our laboratory shows that both mammalian testis and ovary houses a novel stem cell population termed VSELs, which are possibly the primordial germ cells that persist into adulthood. They are more primitive to the spermatogonial stem cells (SSCs), located on the basement membrane of the seminiferous tubules and maintain life-long testicular homeostasis.

These relatively quiescent and pluripotent VSELs (with nuclear OCT-4) possibly undergo asymmetric cell division to self-renew and give rise to the progenitors or 'immediate descendants' SSCs (with cytoplasmic OCT-4) which further differentiate and undergo meiosis to generate sperm. Similarly VSELs are located in the ovary surface epithelium (OSE) and are responsible for postnatal oogenesis. They undergo clonal expansion, form germ cell nests and meiosis resulting in primordial follicle assembly. Culture of scraped OSE cells from menopausal human ovary results in spontaneous differentiation of stem cells into oocyte-like structures, parthenotes and also ES-like colonies, demonstrating that VSELs persist in menopausal ovary but are unable to undergo oogenesis because of a compromised niche. Studies done in mice show that FSH stimulate the ovarian VSELs resulting in increased folliculogenesis.
VSELs have been implicated as the embryonic remnants that possibly result in tumors. It is interesting to note that OCT-4 (a specific marker of testicular VSELs) is considered as one of the most sensitive markers for testicular tumors. Also more than 90% of ovarian tumors originate from OSE (where VSELs are lodged). A discord between stem cells and their niche results in various pathologies including menopause and increased incidence of cancer with age. Restoring the somatic niche allows the VSELs to assume normal function.
A greater understanding of VSELs biology in the gonads will provide newer perspectives to infertility, fertility regulation, oncofertility, menopause and cancers. Gonads provide a good model to get insights into stem cell-niche interaction and hopefully our work will make significant contributions in the field of regenerative medicine.

BADANIA POPULACJI CD105+ I CD133+ USTALONYCH LINII RAKA NERKI W FUNKCJI STĘŻENIA TLENU

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WSTĘP Rak nerki jest oporny na radio- i chemioterapie, zatem istnieje pilna potrzeba poszukiwania nowych celów terapeutycznych (Bussolati, Bruno et al. 2008). Hipoksja pełni bardzo istotną rolę w progresji nowotworów litych (Qing and Simon 2009), niedotlenione środowisko występuje powszechnie w guzach nekrotycznych i zdolnych do przerzutowania (Rundqvist and Johnson 2010). Zgodnie z teorią występowania nowotworowych komórek macierzystych (CSCs), zidentyfikowano w raku nerki populacje komórek CD105+ oraz CD133+, które stanowią odpowiednio nowotworowe komórki macierzyste i progenitorowe.
MATERIAŁY I METODY Hodowle prowadzono w RPMI1640 (linie: 786-O, SMKT R2, RCC6), EMEM (linia ACHAN) lub McCOY (linia Caki-2) z dodatkiem 10 % FBS, w różnych stężeniach tlenu (1 % O2 - hipoksja; 20 % O2 - normoksja). Przeprowadzono analizę proliferacji za pomocą Alamar Blue oraz MTT. W analizie populacji CD105+ i CD133+ wykorzystano cytometr przepływowy Calibur.
WYNIKI Populacja CD105+ jest obecna w liniach 786-O, SMKT R2 i Caki-2. W 786-O istnieje istotna statystycznie różnica w ilości komórek CD105+ hodowanych w normoksji versus hipoksji. Tylko w hipoksji zaobserwowano istotny spadek ilości komórek CD105+ w czasie hodowli. W SMKT R2 udowodniono istotne statystycznie: zwiększoną liczbę komórek CD105+ hodowanych w normoksji aniżeli w hipoksji oraz spadek liczby komórek CD105+ w czasie hodowli. We wszystkich testowanych liniach nie potwierdzono obecności komórek CD133+. W testach Alamar Blue i MTT wykazano brak istotnego wpływ stężenia tlenu na proliferację i żywotność komórek dla większości linii.

Słowa kluczowe: nowotworowe komórki macierzyste, nowotworowe komórki progenitorowe, hipoksja, CD105, CD 133

KOMÓRKI MACIERZYSTE MOGĄ BYĆ ISTOTNYM PRZYSZŁOŚCIOWO SKŁADNIKIEM DERMATOLOGICZNYCH WYPEŁNIACZY TKANKOWYCH. WYNIKI WSTĘPNE BADAŃ NA MODELU SZCZURZYM

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Wstęp: Wypełniacze stosowane są w współczesnej dermatologii i medycynie estetycznej celem korekcji powierzchni skóry w przypadkach indywidualnej chęci pacjenta do poprawy jej wyglądu. Głównymi wskazaniami do zastosowania tego typu terapii jest próba możliwie w jak najlepszym stopniu zminimalizowania zmian związanych ze starzeniem takich jak zmarszczki, utrata elastyczności czy próba korekcji zmian o charakterze blizn lub powiązanych z powiększaniem ust i korekcją okolicy brwi. Najczęściej w tego typu zabiegach stosuje się wypełniacze syntetyczne które w niewielkiej liczbie przypadków mogą wiązać się z brakiem oczekiwanego efektu kosmetycznego lub powikłaniami. Istnieje bardzo mało prac związanych z możliwością stosowania preparatów zawierających autologiczne komórki macierzyste. A w opublikowanych pracach autorzy skupiają się głównie nad wpływem fibroblastów.

Cel pracy: Celem pracy było przedstawienie metodyki i wstępnych wyników badań dotyczących możliwości zastosowania komórek macierzystych jako składnika wypełniaczy tkankowych na eksperymentalnym modelu szczurzym.

Materiał i metody: W pracy wykorzystano 6, dziesięciotygodniowych szczurów rasy Wistar. Na wszystkie prowadzone badania uzyskano zgodę właściwej dla miejsca Komisji Bioetycznej, a prace były prowadzone w oparciu o zasady postepowania i pracy ze zwierzęcymi modelami doświadczalnymi zgodnymi z obowiązującymi dyrektywami UE. Wyodrębniono trzy grupy badawcze: (Grupa I), (Grupa II), (Kontrola). W grupie (Kontrola) zastosowano sam wypełniacz (kwas hialuronowy), w grupie (I) kwas hialuronowy wraz z komórkami macierzystymi MSC, w grupie (II) jako wypełniacz zastosowano materiał na bazie rybiego kolagenu wraz z komórkami macierzystymi MSC. Komórki macierzyste znakowano fluorochromem PKH 26. W badaniach stosowano technikę wszczepu ciągłego w obręb 3 okolic (czołowej, grzbietowej i klatki piersiowej).

Wyniki: Po uzyskaniu wstępnych wyników badań immuno-histopatologicznych nie wykazano żadnych zmian patologicznych i o charakterze zapalnym w obrębie miejsca injekcji. We wszystkich gupach udało uzyskać się oczekiwany efekt wypełnienia, który jednak najkrócej utrzymywał się w grupie (II). W grupie II stwoerdzono także trudniejszą możliwość aplikacji wypełniacza z wykorzystaniem techniki iniekcji ciągłej ze względu na mniej stabilną konsystencję preparatu w porównaniu do wypełniaczy z grupy (I) i Kontroli.

Wnioski: W badaniach wstępnych stwierdzono iż zastosowanie komórek macierzystych jako składnika wypełniaczy może być przyszłościowo istotnym elementem w postępowaniu w ramach procedur z zakresu dermatologii i medycyny estetycznej.

Słowa kluczowe: wypełniacze tkankowe, dermatologia, medycyna estetyczna, komórki macierzyste MSC

RYBI KOLAGEN - NOWY BIOMATERIAŁ DLA INŻYNIERII TKANKOWEJ I MEDYCYNY REGENERACYJNEJ

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Wprowadzenie: Kolagen jest jednym z najczęściej wykorzystywanych biomateriałów w inżynierii tkankowej i medycynie regeneracyjnej. Ryzyko przeniesienia gąbczastej encefalopatii bydła (Bovine Spongiform Encephalopathy, BSE) poprzez produkty lecznicze zaostrzyło przepisy dotyczące pozyskiwania i stosowania wołowego kolagenu. Nowym atrakcyjnym źródłem kolagenu wydaje się być rybia skóra.
Cel: Celem pracy była ocena tempa proliferacji linii komórkowej keratynocytów ludzkich (HaCaT) na rybim kolagenie.
Materiały i metody: Komórki HaCaT wysiano w gęstości 5000 komórek/cm2 na 24 dołkowe płytki polistyrenowe: pokryte rybim kolagenem (GI); ludzkim kolagenem typu IV (GII) oraz wolne od kolagenu (GIII). Komórki hodowano w pożywce hodowlanej (DMEM/ Ham's F-12, 10% FBS, antybiotyki) przez 7 dni. Ocenę proliferacji komórek wykonano przy pomocy testu MTT. Wykonano dokumentację fotograficzną.
Wyniki: Komórki HaCaT wysiane na kolagenie rybim wykazały prawidłowy wzrost oraz morfologię. Uzyskane wartości absorbancji potwierdzają znacznie wyższe tempo proliferacji komórek hodowanych na rybim kolagenie (GI) w porównaniu do komórek hodowanych na powierzchni polistyrenowej (GIII). Zaobserwowano również nieznaczny wzrost tempa proliferacji komórek na dołkach pokrytych kolagenem typu IV (GII) w porównaniu do komórek hodowanych na dołkach pokrytych rybim kolagenem (GI).
Wnioski: Rybi kolagen indukuje proliferację komórek HaCaT in vitro. Biomateriał ten może znaleźć szerokie zastosowanie w leczeniu oparzeń skóry z zastosowaniem technik inżynierii tkankowej.

Słowa kluczowe: inżynieria tkankowa, medycyna regeneracyjna, rybi kolagen, keratynocyty

WPŁYW AKTYWACJI LUB INHIBICJI RECEPTORA NIKOTYNOWEGO NA ZDOLNOŚĆ DO PROLIFERACJI I MIGRACJI MEZENCHYMALNYCH KOMÓREK MACIERZYSTYCH POZYSKANYCH Z GALARETY WHARTONA

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Rozpowszechnienie acetylocholiny, głównego przekaźnika układu cholinergicznego, jest znacznie większe niż do niedawna sądzono. W klasycznym ujęciu, układ cholinergiczny i jego mediator - acetylocholinę zwykło się wiązać z przekaźnictwem w układzie nerwowym. Prowadzone od wielu lat badania pozwoliły na potwierdzenie występowania większości elementów układu cholinergicznego także na innych (niż neurony) komórkach, w tym między innymi na komórkach odpornościowych, komórkach śródbłonka oraz komórkach macierzystych. Znaczącym jest fakt, że komórki te nie tylko odpowiadają na acetylocholinę, ale niejednokrotnie są zdolne także same produkować ten mediator.

Celem obecnej pracy badawczej była ocena wpływu podania wytypowanych agonistów i antagonistów cholinergicznego receptora nikotynowego na proliferację i zdolność do migracji komórek macierzystych pozyskanych z galarety Whartona ludzkiego sznura pępowinowego.

W badaniach in vitro zastosowano cztery związki chemiczne (agonistę i antagonistę receptora nikotynowego oraz selektywnego agonistę i selektywnego antagonistę podjednostki receptora nikotynowego) dodawane do hodowli komórkowych w czterech wzrastających stężeniach (10-7M, 10-6M, 10-5M, 10-4M). Stopień proliferacji komórek oceniono przy pomocy testów: Thiazolyl Blue (MTT), Alamar Blue, Czerwień neutralna (NR) i Sulforodamina B (SRB) a badanie migracji przeprowadzono z użyciem insertów w oparciu o metodę DilC12(3) pre-labeling.

Uzyskane wstępne wyniki wskazują na występowanie zarówno zaburzeń w proliferacji jak i zdolności do migracji komórek macierzystych poddanych działaniu wytypowanych stymulatorów bądź inhibitorów cholinergicznego receptora nikotynowego.

Słowa kluczowe: cholinergiczne receptory nikotynowe, mezenchymalne komórki macierzyste, nieneuronalny układ cholinergiczny