C7. Stem cells in science and medicine

Lectures

L16.1

Skeletal muscle stem cells: from classic to eclectic

Zipora Yablonka-Reuveni, Pascal Stuelsatz, Andrew Shearer
Department of Biological Structure, University of Washington School of Medicine, Seattle, WA, USA
e-mail: Zipora Yablonka-Reuveni <reuveni@u.washington.edu>

Our research focuses on defining the molecular and cellular signatures of myogenic progenitors in adult skeletal muscles. Satellite cells are recognized as the main source of myogenic progenitors in adult muscle. These cells, which reside underneath the myofiber basal lamina, contribute progeny for myofiber repair and can also self-renew, therefore meeting the functional definition of stem cells. Recent studies have suggested that pericytes (contractile cells engulfing the endothelium in the microvasculature) are also able to contribute to adult myogenesis. Specifically, we have been comparing satellite cells and pericytes in the somite-derived limb (LIMB) and diaphragm (DIA) muscles versus extraocular muscles (EOM) that derive from head mesenchyme and are not impacted in muscular dystrophies resulting from dystrophin (or associated protein) deficiency. Our view is that insights into the EOM progenitors may reveal mechanisms of potential benefit for combating muscle wasting in genetic disorders, disease and aging.

We have determined that LIMB, DIA and EOM satellite cells commonly express the nestin-GFP transgene, the Myf5nLacZ-knockin reporter, and the cell-surface antigen signature CD45-/CD31-/Sca1-/CD34+. Nevertheless, EOM satellite cells outperform their LIMB and DIA counterparts when compared in for the magnitude of progeny production in cell culture or efficiency of engraftment into host muscles. Surprisingly, we have found that EOM myogenic progenitors contain a subpopulation that shows anecstral expression of the smooth muscle marker myosin heavy chain based on Cre-Loxp system. The smMHC–Cre+ progenitors are myofiber-associated (hence representing bona fide satellite cells). The myofibers harboring these progenitors also display the smMHC-Cre driven reporter signal (indicating contribution of the smMHC–Cre+ progenitors to the formation or maintenance of their hosting myofibers). These smMHC–Cre+ myofibers constitute the EOM orbital domain, but are rare in the global domain and not present in LIMB and DIA muscles. Collectively, our data suggest that a subpopulation of the EOM satellite cells may derive from bona fide smooth muscle cells that express (or previously expressed) the smooth muscle specific transgene, smMHC–Cre. We are also developing the means for direct isolation of vascular smooth muscle cells from body and head muscles to determine whether these cells possess myogenic potential 

L16.2

How to fix broken heart — stem cells in experimental cardiology

Ewa Zuba-Surma
Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland
e-mail: Ewa.Zuba-Surma <ewa.zuba-surma@uj.edu.pl>

Ischemic heart disease (IHD) is a major cause of mortality and morbidity worldwide. Despite significant advances in medical therapy and interventional strategies, the prognosis of millions of patients with acute myocardial infarction (MI) and ischemic cardiomyopathy remains dismal. During the past decade, the attention of biomedical researchers has increasingly been directed to stem cells as potential mediators of effective tissue repair in injured organs. Various bone marrow (BM)- and other tissue-derived cell types have been employed for the repair of infarcted myocardium including freshly isolated and genetically modified BM mononuclear cells, auto- and allogeneic mesenchymal stromal cells and recently, various types of multi-/pluripotent stem cells (PSC). Although the variety of cell and predominantly stem/progenitor cells have been applied in experimental therapies of heart injury, there is still no agreement in scientific and clinical world which subpopulation/s of cells would be the most efficient in such treatment.

Thus, simultaneously to several ongoing clinical trials with BM-derived cells that are focused on safety and investigate the optimal cell delivery route, dose and target population of patients, great attention has been paid on new concepts to enhance the capability of BM cells to induce the functional recovery of the myocardium or identifying optimal and more potent cell populations suitable for heart repair. Unquestionably, successful applications of stem/progenitor cells in regenerative cardiovascular medicine would need to be safe, ethically acceptable and therapeutically efficient. Sources and application protocols for such optimal stem cell therapy are still being optimized and need scientific discussion.

Acknowledgements

Support: NIH (NIA, NIAMS), MDA.
The role of planar cell polarity (PCP) signaling pathway in wound healing

Tomasz Wilanowski

Nencki Institute of Experimental Biology, Laboratory of Signal Transduction, Warsaw, Poland
e-mail: Tomasz.Wilanowski@nencki.gov.pl

The planar cell polarity (PCP) pathway, also known as non-canonical frizzled pathway or Wnt-independent frizzled pathway, governs processes that require coordinated orientation and movement of cells within a plane of epithelium. In Drosophila PCP is necessary for the proper orientation of wing hairs, body bristles and ommatidia. PCP signaling occurs through the serpentine receptor frizzled (fz) and requires transmembrane proteins Van Gogh/Strabismus (Vang/Stbm) and starry night/flamingo (stan/fmi), and cytoplasmic factors dishevelled (dsh), prickle (pk) and diego (dgo), known collectively as the core PCP proteins. Downstream of these core factors are the small GTPases of the Rho family that provide links to the actin cytoskeleton. In vertebrates, PCP is active in the epidermis, as evidenced by the patterns of scales, feathers and hairs in different organisms. It is also crucial for the organization of internal tissues, such as the orientation of the sensory hair cells of the inner ear. Perturbed PCP signaling may also affect convergent extension and cause neural tube defects.

Mammalian wound healing also requires coordinated cell movement in the plane of epithelia. The link between PCP and wound healing was strengthened with the analysis of mice lacking the transcription factor-coding Grainyhead-like 3 (Grhl3) gene. Grhl3 is a mammalian orthologue of the Drosophila gene grainyhead, which is essential for the PCP pathway in the fly and is also involved in wound healing. Mice lacking Grhl3 exhibit severe neural tube defects and a marked disturbance in formation, maintenance and repair of the epidermal barrier.

Here we show that Grhl3 is a component of the mammalian PCP pathway, and that this pathway regulates wound healing in mammals. Mice carrying mutant alleles of PCP genes Vangl2, Celsr1, PTK7 and Scrib, and Grhl3, interact genetically, exhibiting failed wound healing, neural tube defects, and disordered cochlear polarity. Using phylogenetic analysis, chromatin immunoprecipitation and expression analyses in Grhl3-null mice, we identified RhoGEF19, a Rho activator involved in PCP signaling, as a direct target of GRHL3 regulation. Knockdown of Grhl3 or RhoGEF19 in cultured keratinocytes induces defects in actin polymerization, cellular polarity and wound healing, and reexpression of RhoGEF19 rescues these defects in Grhl3-knockdown cells. These results define a role for Grhl3 in PCP signaling and broadly implicate this pathway in epidermal repair.

Stem cell fate control by biomimetic cues

Leonora Buzanska, Ilona Szablowska-Gadomska, Marzena Zychowicz, Krystyna Domańska-Janik

NeuroRepair Department, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland
e-mail: Leonora.Buzanska@cmdik.pan.pl

Stem cells are characterized by two unique properties in one cell: their self-renewal capacity and their multilineage differentiation potential, which make them an ideal source for cellular therapy and regenerative medicine as well as for pharmaco-toxicological applications. However, to make such applications possible, the generation of appropriate stem cell models and the development of well-controlled procedures for in vitro stem cell expansion, differentiation and maintenance are required. Advancement in biotechnology provides tools to build up in vitro “biomimetic” micro-environments resembling a natural stem cell niche, where the cell is provided with diverse extracellular signals exerted by soluble and structural cues, mimicking those found in vivo. On the other hand, recent advancement in stem cell technology is providing tools to generate stem cells from any tissue of the body, so called induced Pluripotent Stem (iPS) cells, having properties to differentiate into all possible cell types, similarly to Embryonic Stem (ES) cells. We aim to combine this two emerging technologies in building up the methodology for the future personalized neurogenesis in vitro. Our preliminary data showing the influence of epigenetic control over reprogramming of human cord blood derived stem cells to pluripotency and their further differentiation into neuronal cells will be presented. The cellular response is linked to the defined in vitro micro-environment mimicking in vivo conditions. The focus will be paid to the content and architecture of the structural factors as well as the content of soluble factors and oxygen conditions in the in vitro stem cell niche.

Biotechnological progress in construction of 2D and 3D functional domains providing in vitro conditions for the successful stem cell fate control will be discussed.

Acknowledgements

Sponsored by grant from Polish Ministry of Scientific Research and Higher Education No 5978/B/PO1/2010/38 and No 2211/BP01/2010/38.
Oral presentations

O16.1

Differentiation of myoblasts and embryonic stem cells can be stimulated by IGF-1 and IL-2

Marta Przewoźniak1,2, Edyta Brzoska1, Lisa Mohamet2, Christopher M. Ward2, Maria A. Ciernyńch3

1University of Warsaw, Faculty of Biology, Department of Cytology, Warsaw, Poland; 2University of Manchester, Core Technology Facility, Manchester, United Kingdom

Myogenic precursor cells (MPCs) are essential for skeletal muscle regeneration. In damaged muscles they become activated, proliferate, differentiate, and migrate to the site of the injury where they reconstruct damaged muscle fibers. Regeneration is accompanied by secretion of various growth factors and cytokines, such as IGF-1 (insulin-like growth factor 1) and IL-2 (interleukin 2), which are mostly produced by immune cells invading damaged muscle. These factors are also secreted by MPCs and may regulate their proliferation and fusion during muscle regeneration. Growth factors, such as IGF-1, have been shown to be involved in lineage specific differentiation of embryonic stem cells (ESCs). Myoblast and ESCs differentiation requires adhesion proteins, such as integrin alpha3, alpha7 and beta1, M-cadherin and E-cadherin, tetraspanins CD9 or CD81, and also metalloclopease ADAM12 that is involved in extracellular matrix remodeling.

In this study we analyzed the effects of IGF-1 and IL-2 in vitro. We observed that IGF-1 and IL-2 stimulation of MPCs and ESCs results in increased expression of adhesion proteins that are involved in myoblast fusion. We showed that differentiation of MPCs is stimulated by IGF-1 or IL-2, as shown by an increased fusion index. Furthermore, these effects were not mediated by changes in expression of adhesion proteins crucial for myoblast fusion, i.e. integrin alpha3, ADAM12, CD81, and V-CAM 1. However, IGF-1 and IL-2 elevated the expression of CD9 and M-cadherin, which is a marker of myoblasts. We also show that IGF-1 or IL-2 accelerated, similar to MPCs, M-cadherin expression in differentiating ESCs. Thus, CD9 and C-madherin are involved in both MPC and ESC differentiation. These results suggest that IGF-1 and IL-2 can be used to modulate ESC differentiation of mesodermal lineages.

O16.2

Modulation of PPAR-gamma activity influences EPC functions

Jerzy Kotkiewski1, Anna Grochot-Przęczek1, Magdalena Kozakowska1, Ewa Zuba-Surma1, Rafał Derlacz2,3, Józef Dulak1, Alicja Józkowicz1

1Jagiellonian University, Faculty of Biochemistry, Biophysics and Biotechnology, Department of Medical Biotechnology, Poland; 2Adamed Ltd, R&D Department, Poland; 3University of Warsaw, Faculty of Biology, Department of Metabolic Regulation, Poland

E-mail: Jerzy Kotkiewski <j.kotkiewski@uj.edu.pl>

Thiazolidinediones (TZD) the commonly used insulin sensitizers are synthetic agonists of PPARγ nuclear receptor. By stimulation of PPARγ action, TZD improve insulin action and normalize glycemia in diabetic patients. Although TZD can stimulate some functions of endothelial progenitor cells (EPC) both in vitro and in vivo more experiments must be performed to demonstrate pathways involved in this actions. Our aim was to find expression changes in diabetic and healthy EPC and check how modulation of PPARγ activity affects EPC biology.

Experiments were performed on the 12-weeks old wild type (wt) and diabetic (db/db) mice. For in vitro studies bone marrow population enriched in EPC (approx. 600 times) was used, whereas in in vivo tests EPC were characterised as the CD45-/KDR+/Sca-1+ cells. Importantly, percentage of EPC was significantly reduced by 40-80% in the bone marrow of diabetic mice in comparison to wt (in db/db only 0.003% of all cells were CD45-/KDR+/Sca-1+). Reduction related to blood glucose levels suggested that diabetes influences the number of EPC according to its severity. These defect was partially or fully reversed by an oral administration of rosiglitazone 10 mg/kg, for 14 or 28 days, respectively. Similar trend for CXCR4+ cells in bone marrow was observed: a reduction in db/db animals and stimulation upon rosiglitazone treatment. We detected a reduction by 40% of EPC in blood of db/db mice but surprisingly the oral administration of rosiglitazone did not change it. Furthermore, in in vitro assays, EPC isolated from db/db mice displayed impaired migratory and angiogenic potential. Migration of db/db cells was decreased by 50%, whereas ability to form tubes on matrigel by 40%. Importantly, in both tests functions of diabetic EPC were restored by rosiglitazone (10 μmol/L, 24 h) in a PPARγ-dependent manner.

Next, based on the EPC transcriptome analysis we found that proteoglycan 4 (PRG4) was one of the strongest downregulated gene in db/db cells (7-fold decrease). Since PRG4 stimulates proliferation and survival of hemangioblasts we tried to induce its expression in vitro by various antidiabetic drugs. Only after rosiglitazone (10 μmol/L, 24 h) stimulation we observed 2.5 fold PPARγ-dependent induction in PRG4 expression both in wt and diabetic EPC. Thus, PPARγ is an important regulator of EPC biology and its induction may be used to improve the EPC functions.
Caffeine inhibits differentiation of lung cancer stem cells by modulating their respiratory metabolism

Marcin Serocki, Michal Sabisz, Andrzej Skladanowski
Department of Pharmaceutical Technology and Biochemistry, Gdansk University of Technology, Gdansk, Poland
E-mail: skladanowski <as@chem.pg.gda.pl>

It is well established that many tumor types contain a fraction of cells, with stem cell-like properties, called cancer stem cells (CSCs), that are resistant to apoptosis induced by therapeutic agents. The presence of CSCs may explain why a standard anticancer treatment, that eliminates only differentiated cancer cells, does not lead to cancer cure.

We previously showed the existence of a caffeine-sensitive mechanism that controls the number of cancer stem cells (CSCs) in lung tumor cell population. To characterize these molecular mechanisms, we have developed tumor A549-Coff cells which are able to grow in the continuous presence of 1 mM caffeine and become about 3-fold resistant to the drug. Caffeine-resistant cells have decreased cell doubling time and changed sensitivity to standard antitumor drugs. For some drugs (SN-38, cisplatin, etoposide) caffeine-resistant A549 cells showed increased sensitivity, for others (ICRF-187) these cells were about 3-fold cross-resistant.

We observed that caffeine transiently induces DNA damage pathway and production of reactive oxygen species (ROS) as revealed by the oxidation-sensitive fluorescent probe H2DCFDA. Production of ROS by caffeine was not observed in A549-Coff cells. We also noticed that the morphology and number of mitochondria were changed in caffeine-resistant cells. In addition, transmembrane mitochondrial potential was greatly increased in A549-Coff cells, however, total ATP production was similar for both cell types. Measurements of surface oxygen consumption showed increased activity of the glycolytic pathway in A549-Coff cells. Interestingly, the SP population in A549-Coff cells decreased to 2% of total cells compared to about 10% observed in A549 cells. This was accompanied by lower fractions of stem-like cells in caffeine-resistant cell populations.

In conclusion, we show here that exposure of A549 cells to caffeine leads to changes in the respiratory metabolism and decreased fraction of both SP-cells and CSCs. This can be related to the inhibition of CSC differentiation and possibly their self-renewal. We propose that caffeine or other compounds that mimic caffeine action may be used in innovative cancer therapies which are based on small molecular weight inhibitors of CSC differentiation and self-renewal.

Heme oxygenase-1 regulates mobilization of progenitor cells in response to hindlimb ischemia in diabetes and its crucial for their efficiency

Anna Grochot-Przęczek, Jerzy Kotlinowski, Katarzyna Starowicz, Jolanta Jagodzińska, Anna Stachurska, Magdalena Kozakowska, Agnieszka Jaźwa, Ewa Zuba-Surma, Krzysztof Szade, Józef Dulak, Alicja Józkowicz
Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland
E-mail: Anna Grochot-Przęczek <Anna Grochot-Przęczek - anna.grochot-przeczek@uj.edu.pl>

Heme oxygenase-1 (HO-1) has proangiogenic and cytoprotective effects, which may play a pivotal role in bone marrow-derived progenitor cells (BMPCs) function and influence their therapeutic efficiency. HO-1+/+ and HO-1+/− mice were injected i.p. with streptozotocin (STZ) to induce type I diabetes, four weeks later underwent femoral artery ligation. HO-1+/− diabetic mice exhibited impaired blood flow recovery in comparison to HO-1+/+ healthy and HO-1−/− diabetic mice. To investigate whether this phenomenon is related to BMPCs mobilization, the number of Sca-1+CXCR4+ cells in BM and peripheral blood was analysed. Interestingly, HO-1+/− diabetic and HO-1−/− healthy mice demonstrated the highest mobilization and the strongest proliferation of BMPCs in response to hindlimb ischemia, what was accompanied by the most potent increase in SDF-1 gradient between BM and ischemic muscles. The reason for the slower blood flow recovery despite of strong BMPCs mobilization might be the poor condition of BMPCs deficient in HO-1. In vitro and in vivo data indicate impaired function of BMPCs with targeted deletion of HO-1 gene. Such cells were less viable when exposed to H2O2 or hemin then wild type cells. Moreover, HO-1−/− BMPCs proliferated slower in response to VEGF treatment, did not migrate to SDF-1 neither to 10% FBS medium and produced lower amounts of proangiogenic factors VEGF, SDF-1 and KC in comparison to HO-1+/. In vitro angiogenesis assay revealed impaired ability of HO-1−/− cells to form tubes on matrigel and conditioned media from these cells were less potent in stimulation of tube formation by human umbilical vein endothelial cells in comparison to HO-1+/- cell media. Furthermore, HO-1−/− cells injected intradermally into skin cutaneous wounds exhibited low survival level in comparison to HO-1+/+.

In summary, the impaired blood flow recovery in HO-1+/− diabetic mice is accompanied by a strong BMPCs mobilization, however this cells are not efficient in blood perfusion restoration. As in vitro and wound experiment data indicate, it might be related to the poor function of BMPCs deficient in HO-1.
**P16.1**

**Various sources of somatic cells for direct reprogramming into iPS**

Maciej Sułkowski, Marcin Majka

Department of Transplantation, Polish-American Institute of Pediatrics, Jagiellonian University Medical College, Krakow, Poland

e-mail: Maciej Sułkowski <maciej.sulkowski@gmail.com>

**Introduction:** The use of induced pluripotent stem cells (iPS) in regenerative medicine is a promising perspective. Somatic cells reprogrammed into patient- and disease-specific iPS and their further differentiation could result in generation of autologous cell/tissue types of interest.

Source of somatic cells is one of many factors influencing yield of iPS production. Ideal cell source should be common, rich and widely available. Cells harvest should not involve invasive methods.

Among others, plucked hair and rodents’ tails tips are sources of cells (keratinocytes and fibroblasts respectively) which fulfill most of the requirements.

**Materials and Methods:** Murine fibroblasts were isolated from tail tips which were cut, peeled, minced into 0.5 cm fragments, placed on six-well plate until stuck, and incubated in DMEM with 10% FBS and antibiotics.

Human keratinocytes were isolated from volunteers’ plucked hair by two methods: enzymatic release (A) and direct outgrowth (B). In method A different enzymes (trypsin, liberases, collagenase, dispase), concentrations and incubation times were applied. In method B hair were stuck to plastic covered with matrigel and cultured until outgrowth.

**Results:** Virtually 100% of murine tail tips fragments gave rise to cells cultures (including tails from mice stably trans-fected with GFP). Fibroblast were visible since the first day post-isolation and on day 5 post-isolation around 10^4 cells could be calculated from 5 tails fragments. They could be serially subcultured and cryopreserved.

Isolation of keratinocytes proved to be more problematic. Method B showed poor efficiency — only about 10% of plucked hair gave outgrowth, which could be noticed since day 5 post-isolation. In method A, trypsin showed low efficacy and released highest proportion of dead cells. Among liberases most efficient was liberase 2 (on average 10^3–10^4 cells from one hair) while liberase 1 was least efficient. Liberase 4, dispase and collagenase effects were intermediate. Another obstacle was poor adherence of released keratinocytes despite their high viability.

**Conclusion:** Rodents’ tail tips is a satisfactory source of somatic cells for reprogramming. Protocols for isolation of human keratinocytes are more demanding and require further optimization. Nevertheless, plucked hair and tail tips are promising cell sources for reprogramming and may play a significant role in many studies, both clinical and basic.

---

**P16.2**

**Stem cells and their role in endometriosis**

Maria Woluń-Cholewa, Krzysztof Szymanowski, Ewa Nowak-Markwitz

Department of Cell Biology, Department of Mother’s and Child’s Health, Clinic of Gynecological Oncology Poznań University of Medical Sciences, Poznań, Poland

e-mail: Woluń-Cholewa <doskon@ump.edu.pl>

Similar to eutopic endometrium, endometriosis undergoes cyclical changes with each menstrual cycle. Adult progenitor stem cells are said to be responsible for this remarkable regenerative capacity. In this study we decided to determine whether adult stem cells are present in endometriosis, as they could contribute to recurrence of this disease. The studies were conducted in ten fragments of ovarian endometriosis obtained at the secretory phase of the menstrual cycle. The presence and location of mature stem cells was verified on the basis of immunohistochemical analysis of glycoprotein P. Glandular epithelial cells isolated from endometriosis were assessed for their clonogenicity and stained with rhodamine 123, a dye actively eliminated by glycoprotein P. Immunohistochemical studies demonstrated that glycoprotein P was present in the cell membrane of a few glandular epithelial as well as stromal cells. After 48 hours of in vitro culturing, epithelial cells were found to form two types of colonies. The first type of colonies and most frequently observed were characterized by irregular and asymmetrical shape. The second type had a symmetrical shape. It was also noted that after three days of incubation, symmetric colonies lost their regularity. On the basis of rhodamine 123 staining of epithelial cell cultures three populations of epithelial cells were observed: unstained, weakly and strongly stained cells. It should be noted that the unstained cells were the most “flattened” and located in the center of the colony. It was demonstrated that the existence of cells with properties resembling those of stem cells in ovarian endometriosis might be responsible for manifestation and cyclic regeneration of endometrial tissue.

**Acknowledgements**

The study was supported in the part by Polish MNISW grant: NN407084836.
The fate of mouse embryonic stem cells transplanted into growing muscle of pax7-deficient and wild-type mice
Karolina Archacka, Władysława Streminska, Marta Tarczyłuk, Maria A. Ciemerych, Jerzy Moraczewski
Department of Cytology, Faculty of Biology, University of Warsaw, Warsaw, Poland
e-mail: Karolina Archacka <kczaja@biol.uw.edu.pl>

During the first weeks of life the mass of mouse skeletal muscle increases several times due to intensive growth. The key role in this process is played by satellite cells, i.e. muscle stem cells, that serve as precursors of myoblasts fusing with growing myofibers. The crucial function of satellite cells in muscle growth is underlined by the phenotype of mice devoid of pax7 gene, which is the marker of satellite cells. Although development of skeletal muscle of pax7-deficient mice seems to be unperturbed, these mice are characterized by the rapid loss of satellite cells during the first three weeks of life. As a result skeletal muscle growth as well as regeneration of pax7 knockout mice is impaired.

The main aim of our experiments was to verify whether other types of cells, including mouse embryonic stem cells (ES cells), can substitute for satellite cells and participate in the growth of skeletal muscle. ES cells possess a myogenic potential as they can generate myoblasts both in vivo (teratoma development) and in vitro (embryoid bodies formation). ES cells were introduced into growing muscle of both wild-type and pax7-deficient mice. Next, we examined whether transplanted ES cells are able to survive, proliferate and undergo myogenic differentiation in the response to the signals coming from the surrounding tissue. We also assessed the influence of ES cells transplantation on skeletal muscle structure and checked whether these cells are able to colonize the satellite cells niche.

ES cells transplanted into the growing muscle survive and proliferate. However, these cells only rarely migrate out of the site of injection or undergo myogenic differentiation in response to the environmental cues. We have not detected any ES-derived cells in the niche of satellite cells. Moreover, the structure of the muscle injected with ES cells was perturbed due to presence of numerous mononuclear cells and connective tissue development. These results suggest that ES cells are not able to participate in the growth of skeletal muscle. ES cells cannot replace satellite cells, thus, they cannot counteract to the loss of satellite cells and impaired growth of skeletal muscle of pax7-deficient mice.

Reactive oxygen species level in imatinib treated CD34+ CML cells
Grażyna Hoser1, Tomasz Stokłosa2, Eliza Głodkowska-Mrówka2, Ilona Seferyńska3, Danuta Wasilewska1, Tomasz Skorski4
1Laboratory of Flow Cytometry, Medical Center of Postgraduate Education, Warsaw, Poland; 2Department of Immunology, Medical University of Warsaw, Warsaw, Poland; 3Department of Hematology, Institute of Hematology and Blood Transfusion, Warsaw, Poland; 4Department of Microbiology and Immunology, Temple University, School of Medicine, Philidelphia, PA, USA
e-mail: Grażyna.Hoser <graho@cmkp.edu.pl>

Genome instability is a serious problem in the anticancer therapy. It is the result of accumulation of reactive oxygen species in cancer cells, specially in cancer stem cells. Patients with chronic myeloid leukemia (CML) survive three stages of leukemia development: chronic phase, acceleration and blast crisis. Patients in chronic phase are successfully treated with imatinib, the specific inhibitor of p210 protein thyrso kinase responsible for development of the leukemia. Several patients receive the full cytogenetic response, part even the full molecular response. However, several patients retain few leukaemia stem cells in the organism. These cells do not proliferate and remain quiescent. The non-proliferating cells may accumulate mutations leading to next phases of the leukemia. Reactive oxygen species: superoxide anion, H2O2, reactive hydroxyl groups can damage DNA bases to produce oxo-derivatives, or to induce DNA double strand breaks and further chromosomeal aberrations.

The goal of the presented experiment was to compare the reactive oxygen species level in slow and often dividing CD34+ CML cells.

Isolated CD34+ positive cells from patients in chronic phase CML were labeled with fluorochromes allowing to recognize quiescent cell populations and proliferating cells. The cells cultured in vitro for 5 days were incubated 48h with imatinib and with cytometric method the reactive oxygen species level was assayed in both cell populations. In our preliminary study we detected an elevated level of ROS in CD34+ quiescent cells when compared to residual cells. Imatinib inhibited of O2-, H2O2 and .OH levels less effectively in CD34+ quiescent than in proliferating cells.
Mesenchymal stem cells (MSCs) are characterized as adherent, fibroblastoid, non-hematopoietic cells. They are multipotent, with the capability to differentiate into at least three lineages: osteocytes, chondrocytes and adipocytes. The main source of MSCs is the bone marrow. Although the existence of MSCs is nowadays unquestionable, the scientists agree that MSCs constitute a heterogeneous population. Usually, there are some surface antigens like CD105, CD90 or CD271 used for the isolation of cells with similar self-renewal or differentiation potential. However, no single specific marker has been identified up to now. Owing to that, the precise isolation and classification of cells as MSCs is often difficult.

We analysed the role of ecto-nucleotides in the biology of mesenchymal stem cells of mouse bone marrow and MSCs-derived differentiated cells. The ecto-nucleotides and ecto-nucleotidases constitute a part of purinergic signaling system. We detected some substantial differences in the activity of ecto-nucleotidases on the surface of undifferentiated MSCs and MSCs-derived mature cells (adipocytes and osteocytes), depending on the degree of cell differentiation. Our results indicate that undifferentiated MSCs have the high activity of 5’-nucleotidase (CD73) in the opposite to mature adipocytes and osteocytes. On the other hand, osteocytes and adipocytes effectively metabolize ADP according to the reaction: ADP→ATP + AMP. These results suggest the presence of active adenylate kinase on the surface of differentiated cells. Furthermore, the creation of ATP and AMP is much more effective in osteocytes. These results demand further experiments concerning the expression of enzymes and receptors engaged in the purinergic signalization. However, the changes in the enzymatic profile of cells suggest that 5’-nucleotidase may be an universal marker of mesenchymal stem cells and adenylate kinase may allow to distinguish differentiated mature cells.