Lecture

L10.1

The EU research agenda on regenerative medicine: opportunities and challenges

Arnd Hoeveler

European Commission, Directorate-General for Research and Innovation, Unit F4: Advanced Therapies and Systems Medicine
e-mail: Arnd.Hoeveler@ec.europa.eu

The theme Health under the Seventh Framework Programme of the European Union (2007-2013) is aligned with the fundamental objectives of EU research policies: improving the health of European citizens and increasing competitiveness of European health-related industries and services, as well as addressing the socio-economic dimension of health care and global health issues. Over 6 billion Euros are available for funding research activities in the Health area under the Seventh Framework Programme. One of the three main pillars of this Programme is Health Biotechnology, and it is within this part where a sub-activity dedicated to “Innovative Therapeutic Approaches and Interventions” focuses in particular on regenerative medicine. We presented an integrated strategy for the area of regenerative medicine, combining innovative approaches in cell, gene and immunotherapy. The aim is to develop advanced therapies and technologies with broad potential application, to promote translational research including in-patient studies/clinical trials, to further support the recent European regulation on Advanced Therapy Medicinal Products (ATMP), and last not least to encourage the involvement of SMEs which are important for supporting technology. This strategy will be continued in our future calls. Most approaches in this area build around (stem) cell therapy, integrating new biomaterials, gene therapy or immunotherapy. In the period covered so far, 35 projects were funded in regenerative medicine with more than 300 million Euros. The funded projects aim at developing new technologies such as improving cell culture conditions, creating new cell lines, or aim directly at regenerating damaged or diseased tissue or organs in pre-clinical and clinical settings. Targets thereby range from bone/cartilage, to heart, muscle, CNS, liver, kidney or skin regeneration. With the currently open sixth call for proposals (deadline 4 October 2011) the focus will be on transplantation. This 2012 work programme places a strong emphasis on small and medium sized enterprises(SMEs) and stimulate innovative ideas for research and SMEs via broad, bottom up topics to be implemented through a two-stage submission and evaluation procedure. Such activities are also envisioned to complement the ongoing public-private partnership with the pharmaceutical industry and the Innovative Medicine Initiative.

L10.2

Umbilical cord blood in prevention of premature birth complications

Bogusław Machaliński

Department of General Pathology, Pomeranian Medical University, Szczecin, Poland
e-mail: Boguslaw.Machalinski <machalinski@sci.pam.szczecin.pl>

The frequency of preterm labour has risen noticeably over the last few years. Hence, there is growing interest in the identification of markers that may facilitate prediction and prevention of premature birth complications. We sought to explore potential association between the number of circulating stem/progenitor cell populations and the level of chemokines/growth factors with the incidence of complications typical of prematurity. We found that the number of cord blood (CB) non-hematopoietic stem cells (non-HSCs) is inversely associated with the body mass of preterm infants. More notably, a high number of CB hematopoietic stem cells (HSCs) is strongly associated with a lower risk of prematurity complications. Moreover, we revealed that the number of circulating endothelial progenitor cells (EPCs) is inversely associated with the Apgar score of preterm infants. EPCs in full-term infants were maintained at constant, relatively low level over the 6-week follow up, whereas the population of circulating EPCs in preterm infants gradually decreased during first six weeks of postnatal life. Furthermore, the number of CB EPCs positively correlated with VEGF concentration. We conclude that CB HSCs are markedly associated with the development of premature birth complications. We speculate that the differences in the number of circulating EPCs may also contribute to impaired vascular growth and development of vascular-derived prematurity complications. With these issues in mind, we introduced stem cell-based therapy in the immature preterm infants to prevent development of premature complications.
Gene therapy for wound healing

Jozef Dulak
Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland
e-mail: Jozef Dulak <jozef.dulak@uj.edu.pl>

Gene therapy has been recently proven to be effective in treatment of inherited diseases, such as severe combined immunodeficiencies caused by mutation of adenosine deaminase (ADA) gene or a gene for the common γ-chain subunit of cytokine receptors (X-SCID). Promising results have been also achieved in treatment of Leber’s congenital amaurosis, thalassemia and in X-linked adrenoleukodystrophy. However, despite the progress in treatment of acquired diseases, there is still a place for improvement of the conditions which are resistant to pharmacological approaches and cause significant morbidity in humans. Among these are unhealing ulcers caused by the complications of diabetes.

Impaired wound healing in diabetes can result from impairment of migration and proliferation of cells as well as angiogenesis. Recently we have shown that simultaneous overexpression of pro-angiogenic VEGF-A and FGF-4 achieved by adeno-associated viral vectors (AAV) provided better effect than delivery of a single gene (Jazwa et al., 2010, Genetics Vaccine & Therapy). We have also demonstrated that delayed skin regeneration can happen when there is a lack of heme oxygenase-1 (HO-1) a stress inducible enzyme known to play cytoprotective, antioxidant and pro-angiogenic functions (Grochot-Przeczek et al., 2009, Plos One). Interestingly, delayed wound healing in leptin receptor deficient db/db mice was associated with the impaired induction of HO-1 after the injury. Accordingly, adenoviral gene transfer of HO-1 increased regeneration in those animals, the effect associated with the increased vascularisation. Our recent data indicate also that revascularization of ischemic hind-limbs is impaired by HO-1 deficiency. Overexpression of HO-1 using the hypoxia-regulated plasmid vectors accelerated the restoration of blood flow and decreased the incidence of toe necrosis in mice subjected to femoral artery ligation. The effect was associated with the reduction of inflammation and decreased cell death and modulation of microRNAs involved post-injury skeletal muscle regeneration.

Current studies indicates that amelioration of complications of wound healing can be achieved by combined treatments with several factors or overexpression of genes exerting the beneficial effects on multiple levels. Overexpression of microRNAs, which individually modulate the expression of numerous genes can be the next step in elaboration of the strategies for more effective treatment of chronic conditions.

Modulation of proliferation and invasiveness of cancer cells with silenced STAT3 gene

Ilona Bednarek, Anna Galilejczyk, Natalia Gawlak, Anna Kulczycka
Medical University of Silesia in Katowice, Faculty of Pharmacy and Division of Laboratory Medicine, Department of Biotechnology and Genetic Engineering, Sosnowiec, Poland
e-mail: Natalia Gawlak <nataliagawlak84@gmail.com>

In order to develop an effective anticancer therapy, there is a necessity to understand the molecular processes, which are responsible for conversion of normal cells into malignant ones. What is more, an effective therapeutic strategy should not harm the regular cells. Numerous data shows, that constitutive activation of Signal transducer and activator of transcription 3 (STAT3) is present in many human malignancies. Thereby, STAT3 seems to be a good target in the anticancer therapy. It has an influence on excessive proliferation of cancer cells, as it induces the expression of cyclin D1. This transcriptional factor regulates metases through the activation of metalloproteases (MMP), especially MMP-2 and MMP-9. It has also an effect on the process of angiogenesis, as it activates vascular endothelial growth factor (VEGF). It is consider that direct targeting STAT3 might be more effective than targeting single genes involved in malignant phenotype. In presented project, we suppressed the expression of STAT3 gene using RNA interference system (RNAi) and checked, how this process would influence on malignant phenotype of chosen cancer cell lines. The effect of STAT 3 silencing on extracellular matrix degradation as well as on cancer cell proliferation was indirectly evaluated by the estimation of the relative changes in MMP-9 and histone H3/a mRNA expression levels using quantitative Real Time PCR technique. A MMP-9 protein, zinc-dependent endoproteinase, participates in breaking down components of the extracellular matrix. Due to this fact, an estimation of mRNA levels of MMP-9 using gene-specific pair of primers was useful in assessment of invasiveness of cancer cells. In turn, the expression of H3/a was tightly associated with S phase of monitored cell cycle, (BrdU incorporation assay); finally we assumed, that changes in modulated cancer cell proliferation could be well mirrored by the results of histone H3/a expression level. Silencing of STAT3 expression by gene-specific RNA interference leded to the decrease in cancer cell proliferation and invasiveness, accompanied by changes in MMP-9 and H3/a mRNA levels.

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Improved blood flow recovery after femoral artery ligation and stimulation of skeletal muscle regeneration by HO-1 gene transfer

Agnieszka Jazwa, Jacek Stepniewski, Martin Zamykal, Alicja Jozkowicz, Jozef Dulak
Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland
e-mail: Agnieszka Jaźwa <agnieszka.jazwa@uj.edu.pl>

Heme oxygenase-1 (HO-1) is an inducible, cytoprotective enzyme that catabolizes heme to free iron, carbon monoxide (CO), and biliverdin, which is endogenously converted to bilirubin. Through these compounds, HO-1 mitigates cellular injury by exerting antioxidant, anti-apoptotic, and anti-inflammatory effects. Uncontrolled overexpression of HO-1 can, however, cause some detrimental effects. Here, we report the successful construction and use of plasmid vector expressing human HO-1 under the regulation of three hypoxia-responsive elements and a minimal cytomegalovirus promoter (pHRE-HO-1). Human microvascular endothelial cells (HMEC-1) transfected with pHRE-HO-1 and cultured in hypoxic conditions (0.5% oxygen) verexpressed HO-1 demonstrating that our construct is functional. Moreover, these cells survived better when exposed to a sublethal dose of \( \text{H}_2\text{O}_2 \) (500 \( \mu \text{M} \)) and had increased migratory potential than control HMEC-1 cells transfected with pHRE-empty vector and kept in hypoxia. More importantly, when delivered in vivo, pHRE-HO-1 significantly improved the post-ischemic foot blood flow and decreased the incidence of toe necrosis in C57BL/6 mice subjected to femoral artery ligation. These protective in vivo effects were associated with reduced levels of pro-inflammatory cytokines (IL-6 and CXCL1) and decreased expression of caspase-3. Moreover, HO-1 delivered into mouse skeletal muscles seems to influence the regenerative potential of myocytes as it significantly modulated the expression of transcriptional (myogenin, Pax 3 and Pax7) and post-transcriptional (miR-146a and miR-206) regulators of genes involved in satellite cell differentiation and skeletal muscle regeneration. Our results demonstrate that pHRE-HO-1 vector can be further investigated as a tool to provide therapeutic effects in critical limb ischemia.

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