TAKING ON HD

Huntington's disease (HD), spinal cord injury and melanoma represent main research programmes of the project EXAM, as Jan Motlik, head of IAPG's Laboratory of Cell Regeneration and Plasticity, details

Research project EXAM is supported by the European Union via the programme of regional development, 'Research and Development for Innovation'. The main objective of the EXAM project is to develop a centre for scientific research focused on the application of research outcomes obtained in the area of developmental and tumour biology, cell cycle regulation, research of neurodegenerative diseases and proteomics in practice, especially in biomedicine and biotechnologies. The researchers at the Institute of Animal Physiology and Genetics have been traditionally achieving internationally recognised results in the aforementioned areas of the basic research and the implementation of the current project will strengthen, as extensively as possible, co-operation with the application sphere and support the application of achieved results. Although the applied research has quite a long tradition in our institute, we have been striving to establish a completely new level of a direct interconnection between the basic and applied research.

Research Programme 1

Research programme 1 is oriented to proteomic research of spinal cord defects in the laboratory rat. It will focus on discovering the optimal combination of molecules that will characterise the type of spinal cord defect and will enable fast and reliable diagnosis of its scope. The results will furthermore serve to monitor medical application of transplantation of spine precursor cells to a defective spine tissue with the aim to obtain material for the development of an analytical test that will specifically determine survival and involvement of the transplanted cells to the spine tissue reparation. The main aim of the proposed research project is the characterisation and

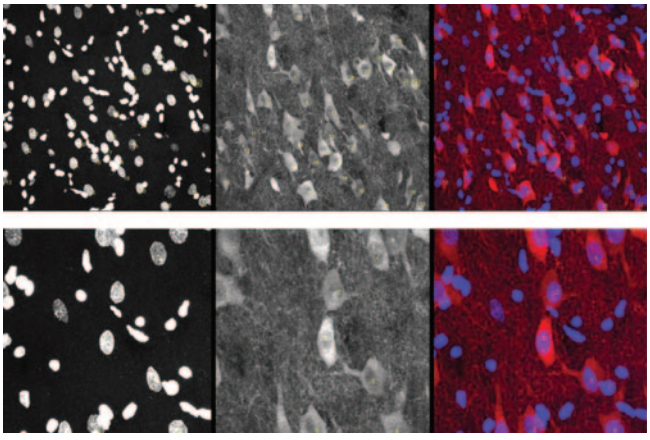

Fig. 1 Counting of DARPP-32+ neurons in HD transgenic and WT miniature pig brain slices from confocal 3D images by LAS AF Lite software

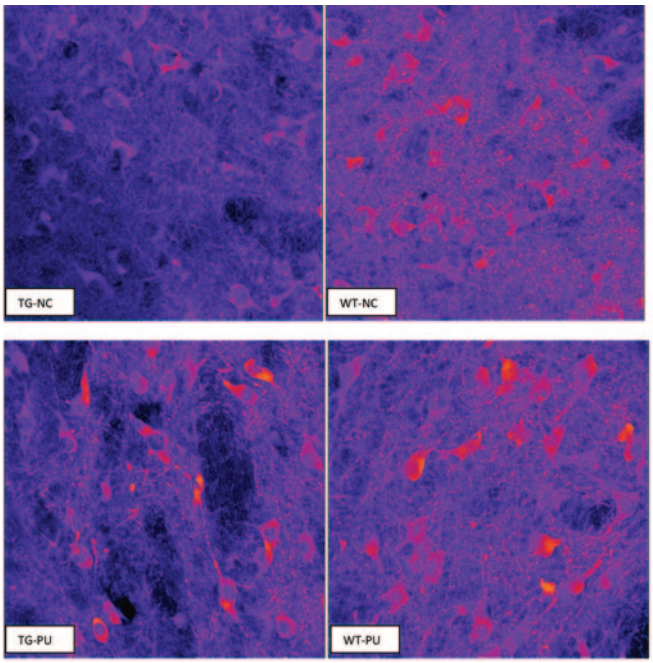

Fig. 2 Evaluation of DARPP-32+ neurons signal expression in HD transgenic and WT miniature pig brain slices from confocal Z-Stacks by Fiji software

selection of a set of human-specific cell surface, intracellular and extracellular (secreted) proteins/peptides that are being associated or released by human neural precursor cells or fully differentiated post-mitotic neurons and glial cells. These proteins or peptides will be analysed in model of human neural stem

cell and neural precursor cell differentiation, in vitro as well as in vivo, after human cell grafting to animals with spinal cord injury and amyotrophic lateral sclerosis. Provided that potential protein or peptide markers will be found and successfully verified, these markers will be then used for development of analytical methods for sorting cells suitable for transplantation and for objective in vivo identification of survival and differentiation of transplanted human neuronal and glial cells derived from human embryonic stem cells.

Recent advances in in vitro culture and differentiation of stem cells highlight the enormous potential of these cells and cell lines derived from stem cells in treatment of neurological dysfunctions. Human embryonic stem cells (hESC) offer the particular advantage of prolonged proliferative capacity and great versatility in the lineages that can be formed in culture. Translating these advantages into clinical benefits faces many challenges, including efficient differentiation into the desired cell type(s), maintaining genetic stability during long-term culture and, finally, ensuring the absence of potentially tumorigenic hESC from the final cell

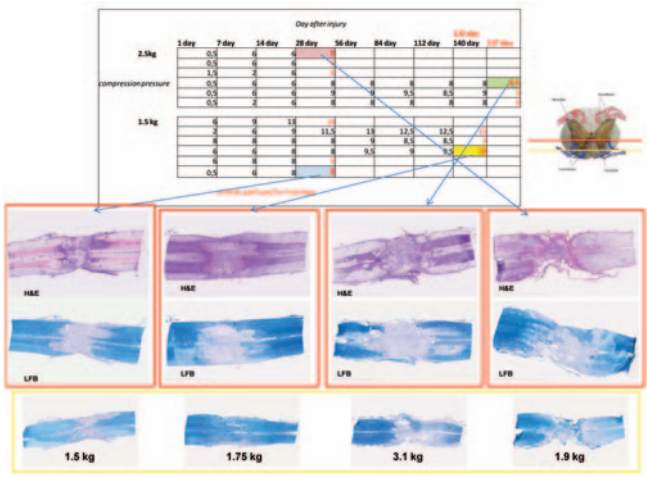

Fig. 3 Histological assesment (Hematoxylin-eosin and Luxol Fast Blue staining) of miniature pig L3-L4 spinal segments after spinal compression with porcine neurological motor score correlation

candidate. In the transition from the experimental to the practical, clinical use is very necessary to identify specific biomarkers that are either typical for surface of precursor cells and that can be used for sorting of specific cell population, or typical for differentiation to neurons and glial cells that can be used to distinguish various neuronal and glial subtypes. These demands urge us to search for more suitable and better accessible molecules that might be simply measured before therapeutic use of cells and that would predict therapeutic effective of transplanted cells.

Research programme 2

This programme will develop through application of viral vectors, a transgenic model of the Huntington's disease (HD) in minipigs that will be the starting point both for the testing of the newest pharmaceuticals and for the application of all methods of regenerative medicine in the treatment of neurodegenerative diseases. HD is a progressive neurodegenerative disorder characterised by motor, cognitive and behavioural dysfunction. There is currently no treatment to delay progression of the disease. Available pharmacological treatment is only able to moderate some of the symptoms, though reliable animal models are needed to progress further in research of HD and its treatment. Moreover, the use of large animal models of HD provides the benefit of human-like brain size and neuroanatomy as well as the opportunity of longitudinal studies with a slow disease progression. However, to provide patients with appropriate treatments in a reasonable time frame is a costly process. Therefore appropriate animal models are critical for the success of translational research.

This demanding, long-lasting research focused on the development of the transgenic model of Huntington's disease geared to mapping of endogenous huntingtin expression in miniature pigs, as well as on spectroscopic analysis of the brain basal ganglia. Lentiviral vectors for the mutated huntingtin are used for transfection of neural stem cells and striatal neurons under in vitro conditions. Until now our laboratory possesses vectors that code N-terminal part of huntingtin (540 bp) and they have either 23 Q (glutamine, control) or the prolonged glutamine stretch (145 Q, mutated). Using biochemical and immunocytochemical method (at the protein level) or molecular biology methods (DNA and RNA levels), striatal neurons and NSCs are characterised and dynamics of formation of nuclear inclusions and cytoplasmic aggregates will be characterised. Due to memberships in the European Huntington's Disease Network, medical doctors, researchers and PhD students are in a close contact with the international experimental laboratories but they also have a chance to present their results on common conferences and seminars.

Research programme 3

This programme will diversify the strain of melanoma miniature pigs (MeLiM) into two sub-strains with a different progress of a tumoural disease (the MeLiM sub-strain with progreding melanoma and the MeLiM sub-strain with a spontaneously regreding melanoma) to use these strains of miniature pigs for the development and the testing of new treatment methods, including pharmacological intervention against this type of tumoural diseases.

The unique MeLiM strain of miniature pigs with hereditary malignant melanoma has been bred in the Laboratory of Tumor Biology by intended long-term crossbreeding. Studies executed so far have clearly shown distinct similarities of this swine tumour with human melanoma. Therefore the MeLiM strain represents an excellent animal model for full-scale study of this tumour disease. More than two thirds of the affected animals show spontaneous tumour regression, whereas the remaining individuals (unless an appropriate immunotherapeutic treatment is performed) die of the progression of the tumour disease.

The objective of the research plan is to carry out – by a selection with the use of genetic, karyological and morphologic markers, diversification of the MeLiM strain into two sub-strains with a different course of tumour disease: a) sub-strain MeLiM with progressing melanoma, b) sub-strain MeLiM with spontaneously regressing melanoma.

Apart from that, various lines or clones of swine melanoma cells will be isolated from the tumours obtained from these sub-strains and these cells will be used for studies on cultivated cells. Overall, a globally unique animal model for malignant melanoma will be developed that will be applicable both in vivo and in vitro for a more efficient therapy of this problematically curable and globally increasing tumour disease.

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