Laboratory of Cell Differentiation

Haematopoietic and neural cell differentiation, zebrafish development, nuclear receptors, chemical biology

Petr Bartůněk

petr.bartunek@img.cas.cz

www.img.cas.cz/research-groups/petr-bartunek

The main interest of the laboratory lies in the molecular mechanism of cell fate determination. In the lab we have established in vitro systems to study the self-renewal and differentiation of haematopoietic, neural and mesenchymal stem cells. We use growth factors and small molecules as tools to manipulate these systems. More recently, we have initiated more systematic search for such tools using chemical biology/genetics approaches.

Nuclear receptors function as ligand-dependent transcription factors to regulate gene transcription in response to specific physiological stimuli such as steroids, retinoids, thyroid hormone and vitamin D. Thyroid hormone receptors, activated in response to thyroid hormone (T3), are known to modulate the level of serum cholesterol via complex regulatory pathways. By screening for T3-regulated genes we have identified Disp3, a sterol-sensing domain-containing protein. DISP3 is predominantly expressed in specific cell types of the brain, retina and testis and localizes within the endoplasmic reticulum, and was found to co-localize with cholesterol. Ectopic expression of DISP3 in fibroblasts resulted in elevated cholesterol levels combined with an altered cholesterol and lipid distribution. We proposed that DISP3 represents a new molecular link between thyroid hormone and cholesterol metabolism in the brain (Zikova et al. 2009). In addition, we have identified two neural stem cell lines that highly express Disp3. We have performed RNAi and overexpression studies and found out that Disp3 is able to modulate the cell fate of neural stem cells and their progeny. To better understand the role of Disp3 in vivo we have established mouse transgenic lines overexpressing Disp3 in astrocytes (GFAP promoter) and oligodendrocytes (PLP promoter). Analysis of these mice demonstrates the important role of Disp3 in lipid homeostasis in neural cells in vivo.

We have extended our studies on vertebrate haematopoietic development by introducing a new model organism in our laboratory – the zebrafish – and we have established ex vivo cultures of haematopoitic cells (Stachura et al. 2009). Recently, we have produced several recombinant zebrafish growth factors (Epo, Gcsf, Tpo) that allow us to establish, for the first time, zebrafish haematopoietic clonal assays in semisolid media (Stachura et al. 2011, Svoboda et al., submitted). Moreover, these tools enabled us to reveal the clonogenic and proliferation capacity of bi-potent thrombo/erythropoietic progenitors with respect to their mammalian hematopoietic counterparts. Despite obvious phenotypic differences between fish and mammalian thrombocytes and erythrocytes, our results strongly demonstrate the evolutionary conservation of basic processes and molecular mechanisms of erythro/thrombopoiesis in the vertebrates.

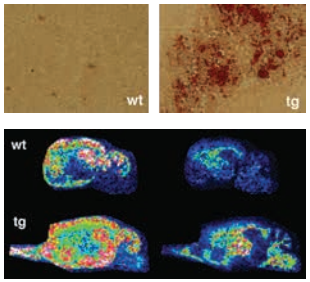

Fig. 1. Disp3 regulates lipid homeostasis in vivo
(A) Lipid accumulation in lipid droplets in PLP-Disp3 transgenic animals. Oil red staining of sagittal section of mouse cortex. (B) Distribution of various sfingolipids in wt and PLP-Disp3 transgenics as revealed by MALDI imaging of sagittal sections of mouse brain.

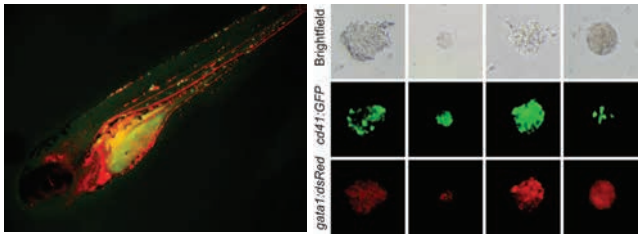

Fig. 2. Zebrafish as a model to study vertebrate hematopoiesis
(A) Double hemizygous transgenic zebrafish Tg(gata1::DsRed); Tg(cd41::EGFP) at 4 days post fertilization with single hematopoietic cells fluorescently labelled (red, erythroid cells, green, thrombocytes). (B) Hematopoietic cells derived from zebrafish whole kidney marrow (WKM) were cultivated ex vivo in semisolid media (methocel) in the presence of recombinant zebrafish thrombopoietin (TPO) and erythropoietin (Epo), giving rise to bi-potent thrombo/erythropoietic progenitors.

- Ministry of Education, Youth and Sports of the Czech Republic, LC06077 - Centre for Chemical Genetics, 2006-2011, P. Bartůněk
- FP6 EU, 18652 CRESCENDO - Consortium for Research into Nuclear Receptors in Development and Aging, 2006-2011, P. Bartůněk
- GA CR, GA310/08/0878 - The role of the cells prion protein in erythroid differentiation, 2008-2012, P. Bartůněk
- GA CR, GA204/09/1905 - Disp3: A potential role in the self renewal and differentiation of neural stem cells, 2009-2011, P. Bartůněk
- GA CR, GAP305/10/0953 - New regulators of megakaryocyte and erythroid lineage commitment, 2010-2013, P. Bartůněk
- FP7 EU, 261861, EU-OPENSCREEN - European Infrastructure of Open Screening Platforms for Chemical Biology, 2010-2013, P. Bartůněk
- Operational Programme Prague – Competitiveness, CZ.2.16/3.1.00/24020, CZ-OPENSCREEN - National Infrastructure for Chemical Biology, 2011-2013, P. Bartůněk
- Ministry of Education, Youth and Sports of the Czech Republic, LM2011022, CZ-OPENSCREEN - National Infrastructure for Chemical Biology, 2012-2015, P. Bartůněk
- Ministry of Industry and Trade of the Czech Republic, FR-TI4/802 - Development of new chemical compounds with anti-tumor activities or use in regenerative medicine, 2012-2015, P. Bartůněk
- TA CR, TA02010212 - ReceptorX: Integrated platform for drug discovery and development, 2012-2015, P. Bartůněk
- Operational Programme Prague – Competitiveness, CZ.2.16/3.1.00/28026 - Label-free Technology Platform, 2012-2013, P. Bartůněk
- GA CR, GAP301/12/1748 - The role of DISP3 protein in lipid metabolism, 2012-2015, P. Batůněk

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From the left:
António Pombinho, MSc / PhD Student · Jana Konířová, MSc / PhD Student, Ondřej Svoboda, MSc / PhD Student, David Sedlák, PhD / Postdoctoral Fellow · Tomáš Bartoň, MSc (since 2011) / PhD Student · Karolína Ditrychová / Diploma Student · Martina Zíková, PhD / Research Fellow · Petr Bartůněk, PhD / Head of Laboratory · Dita Franke-Kidorová, MSc / Project Manager · Jana Oltová / Diploma Student · Olga Machoňová, MSc / Research Assistant (since 2012) · Michaela Marešová, MSc / Research Assistant · Assoc Prof Jindřich Jindřich, PhD / Research Fellow

Not in the picture:
Assoc Prof Daniel Svozil, PhD / Research Fellow (since 2012) · Jana Bartůňková, MD / Research Assistant · Eva Mašínová, MSc / Research Assistant (maternity leave) · Tomáš Můller, MSc / Research Assistant (since 2012) · Ctibor Škuta, MSc / PhD Student (since 2011)