Laboratory of Cell Differentiation

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

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The main interest of the laboratory is study of the molecular mechanism of cell fate determination. We have established in vitro systems to get insight into 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 a more systematic search for such tools using chemical biology approaches.

Recently, we have identified Disp3, a sterol-sensing domain-containing protein. DISP3 (PTCHD2) is predominantly expressed in neural tissues. Ectopic expression of DISP3 in fibroblasts resulted in elevated cholesterol levels combined with an altered cholesterol and lipid distribution (Zikova et al. 2009). We have performed RNAi and overexpression studies of neural stem cell lines and found out that Disp3 is able to modulate the cell fate of neural stem and progenitor cells. We found that ectopically expressed DISP3 promotes cell proliferation and alters expression of genes that are involved in tumorigenesis. Finally, the differentiation profile of DISP3-expressing cells was altered, as evidenced by delayed expression of neural specific markers and a reduced capacity to undergo neural differentiation (Zikova et al. 2014).

We have extended our studies on vertebrate haematopoietic development to the zebrafish model and we have established ex vivo cultures of haematopoietic cells (Stachura et al. 2009). Recently, we have produced several recombinant zebrafish growth factors (Epo, Gcsfa/b, Tpo) that allow us to establish, for the first time, zebrafish haematopoietic clonal assays in semisolid media (Stachura et al. 2011). Granulocyte colony-stimulating factor (Gcsf) drives the proliferation and differentiation of granulocytes, monocytes, and macrophages. Analysis of the zebrafish genome indicates the presence of two Gcsfs, likely resulting from a duplication event in teleost evolution. We show that in addition to supporting myeloid differentiation, zebrafish Gcsf is required for the specification and proliferation of haematopoietic stem and progenitor cells. These findings may bring information on how haematopoietic cytokines had evolved following the diversification of teleosts and mammals from a common ancestor (Stachura et al. 2013). Moreover, these tools enabled us to reveal the clonogenic and proliferation capacity of bi-potent thrombo/erythropoietic progenitors with respect to their mammalian haematopoietic counterparts. Despite obvious phenotypic differences between fish and mammalian thrombocytes and erythrocytes, our results strongly demonstrate the evolutionary conservation of the basic processes and molecular mechanisms of erythro/thrombopoiesis in the vertebrates (Svoboda et al. 2014).

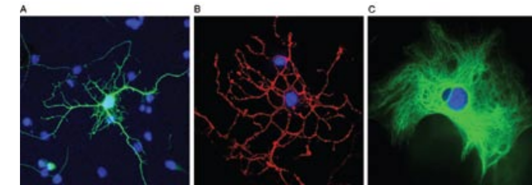

Fig. 1. Differentiation of neural stem cells in vitro
Differentiation of mouse neural stem cells (NS-5 cell line) into (A) neurons (betaIII-tubulin – green) at day 12, (B) oligodendrocytes (O4 - red) at day 9 and (C) astrocytes (GFAP – green) at day 4. Nuclei are stained with DAPI (blue).

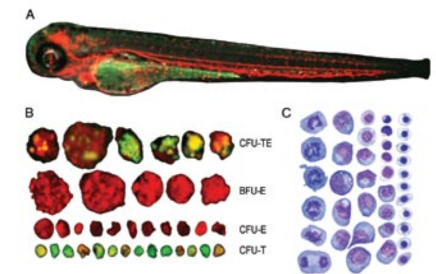

Fig. 2. Zebrafish as a model to study vertebrate haematopoiesis
(A) Double hemizygous transgenic zebrafish Tg(gata1:DsRed); Tg(cd41:EGFP) at 4 days post fertilization with single hematopoietic cells fluorescently labelled (red, erythroid cells, green, thrombocytes). (B) Colonies of hematopoietic cells derived from adult zebrafish whole kidney marrow 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. (C) Morphology of cells isolated from methylcellulose cultures (as in B) after six days in culture were stained with May-Grünwald Giemsa.

- GACR, GAP301/12/1478 – The role of DISP3 protein in lipid metabolism, 2012-2015, P. Bartůněk
- MEYS, LM2011022 – CZ-OPENSCREEN: National Infrastructure for Chemical Biology, 2012-2015, P. Bartůněk
- MIT, FR-TI4/802 – Development of new chemical compounds with anti-tumour activities or use in regenerative medicine, 2012-2015, P. Bartůněk, V. Kořínek
- TACR, TA02010212 – ReceptorX: Integrated platform for drug discovery and development, 2012-2015, P. Bartůněk
- MEYS, LO1220 - CZ-OPENSCREEN – National infrastructure for chemical biology, 2013-2018, P. Bartůněk
- OPPC, CZ.2.16/3.1.00/21547 – Centre for Model Organisms, 2014-2014, P. Bartůněk
- FP7 EU, 261861 EU-OPENSCREEN – European Infrastructure of Open Screening Platforms for Chemical Biology, 2010-2015, P. Bartůněk
- GACR, GAP305/10/0953 – New regulators of megakaryocyte and erythroid lineage commitment, 2010-2013, P. Bartůněk
- OPPC, CZ.2.16/3.1.00/24020 CZ-OPENSCREEN – National Infrastructure for Chemical Biology, 2011-2013, P. Batůněk
- OPPC, CZ.2.16/3.1.00/28026 – Label-free technology platform, 2012-2013, P. Batůněk

1. Svoboda O, Stachura DL, Machoňová O, Pajer P, Brynda J, Zon LI, Traver D, Bartůněk P: Dissection of vertebrate hematopoiesis using zebrafish thrombopoietin. **Blood 2014** 124(2): 220-8.
2. Tumova L, Pombinho AR, Vojtechova M, Stancikova J, Gradl D, Krausova M, Sloncova E, Horazna M, Kriz V, Machonova O, Jindrich J, Zdrahal Z, Bartunek P, Korinek V: Monensin inhibits canonical Wnt signaling in human colorectal cancer cells and suppresses tumor growth in multiple intestinal neoplasia mice. **Mol Cancer Ther 2014** 13(4): 812-22.
3. Ziková M, Konířová J, Ditrychová K, Corlett A, Kolář M, Bartůněk P: DISP3 promotes proliferation and delays differentiation of neural progenitor cells. **FEBS Lett 2014** 588(21): 4071–4077.
4. Kaspar P, Ilencikova K, Zikova M, Horvath O, Cermak V, Bartunek P, Strnad H: c-Myb inhibits myoblast fusion. **PLoS One 2013** 8(10): e76742.
5. Stachura DL, Svoboda O, Campbell CA, Espín-Palazón R, Lau RP, Zon LI, Bartunek P, Traver D: The zebrafish granulocyte colony-stimulating factors (Gcsfs): 2 paralogous cytokines and their roles in hematopoietic development and maintenance. **Blood 2013** 122(24): 3918-28.

From the left: Lucie Nencková, MBA / Research Assistant (until 2014), Petr Šálek, PhD / Project Manager (since 2014), Petr Bartůněk, PhD / Head of Laboratory, Jana Bražinová, MSc / Project Manager (since 2014), Zuzana Kotrbová / PhD Student (since 2014), Tomáš Müller, MSc / Research Assistant, Jana Oltová / PhD Student, Dita Franke-Kidorová, MSc / Project Manager (maternity leave), Kristýna Blažková / PhD Student (since 2014), Martina Zíková, PhD / Research Fellow, Michaela Marešová, MSc / Research Assistant (maternity leave), António Pombinho, MSc / PhD Student, Ctibor Škuta, MSc / PhD Student, Karolína Ditrychová, Bc / Diploma Student, David Sedlák, PhD / Postdoctoral Fellow, Martina Šnegoňová / PhD Student (since 2014), Olga Machoňová, MSc / Research Assistant, Jana Konířová, MSc / PhD Student, Martin Popr, MSc / Research Assistant (since 2014), Ivan Čmelo, MSc / PhD Student (since 2013), Ondřej Svoboda, MSc / PhD Student

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