# Session 5: Stem cells and cancer

## Lectures

## L5.1

### Fighting the hypoxic stress in cancer to restore antitumor immune response

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In $pO_2$-associated pathologies as cancer hypoxia turns on angiogenesis. Impaired $O_2$-delivery contributes to tumor growth, metastasis and hypoxia-selection of aggressive cancer stem-like cells. Consequently, the anticancer strategies directed to pathological angiogenesis and its destruction are being revisited towards normalization. This challenge is reached by several $pO_2$ control strategies. The latter fundamental potential is underlined by the resulting changes of the tumor microenvironment. Those appear deep enough to transform the cellular recruitment and humoral composition (chemokines and receptors) around tumor cells such as the immune suppression due to hypoxia, is no longer maintained and efficient immune response is favored. The level of cancer stem-like cells is drastically reduced.

Clinical strategies take advantage of the $pO_2$ increase that occurs in adapted antiangiogenic protocols to transiently regulate vessels structure and re-establish their function through the normalization process. Meanwhile, other cancer therapies can be beneficially applied. Success depends on the reliability of $O_2$ imaging and measurement. Normalization is an adjuvant to chemo- and radiotherapy as it allows immune tolerance reduction and helps efficient antitumor response. Thus stabilization of vasculature normalization is the challenge for efficacy of treatments. Stable normalization of vessels could be reached by several concordant approaches to increase stably the intra-tumor $pO_2$:

- directly enhancing $O_2$ delivery by red blood cells using an allosteric effector of hemoglobin also controls PI3K/AKT/mTOR pathway, allows stable angiogenesis normalization;
- target the tumor site by therapeutic gene expressed in a hypoxia-restricted manner: VEGF trap conditioned to hypoxia (soluble VEGF receptor-2);
- a high degree of tumor targeting is reached by precursor endothelial cells, incorporating the developing blood vessels, they normalize angiogenesis. They can carry vectors toward the tumor.

These methods allowed the demonstration that hypoxia compensation helps radiotherapy and/or chemotherapy, reduces efficiently the population of cancer cells expressing stemness markers and we show that by reverting pathologic angiogenesis, through stable normalization, immune tolerance is converted into efficient immune response.

**References:**

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# L5.2

## Leukemia stem cells – getting to the roots of the disease

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Leukemia stem cells (LSCs) were the first cancer stem cells proven experimentally to exist. More than 20 years ago, John Dicks' group from Toronto characterized phenotype of a single acute myeloid leukemia cell which was able to recapitulate frank leukemia in animal model. Since then, phenomenon of cancer stem cell has been described in majority of human malignancies including other types of leukemia, as well as solid tumors. Among them, chronic myeloid leukemia (CML) serves as a valuable model for studying the role of LSCs. This disease is characterized by almost uniform genetic aberration, the presence of Philadelphia chromosome (result of reciprocal translocation t(9;22)(q34;q11) and formation of fusion BCR-ABL1 encoding chimeric protein, BCR-ABL1, which is constitutively active oncogenic tyrosine kinase that phosphorylates a number of downstream target proteins and in effect, facilitates expansion of leukemic cells. Introduction of tyrosine kinase inhibitors (TKI), with imatinib as a model drug targeting BCR-ABL1, represents one of the major breakthroughs in oncology in XXI century, which changed dramatically the landscape of the CML therapy. In majority of CML patients, TKI induce long-lasting and durable therapeutic response. However, despite unquestionable success of TKI in the treatment of CML, they will not cure CML. This is caused by intrinsic resistance of LSCs to TKI through only partially understood mechanisms. Although LSCs harbor BCR-ABL1, they are not dependent on the oncogenic kinase. This is caused by the activation of alternative pathways (such as WNT/β-catenin or C-MYC), which one is crucial is not clear and is a matter of intense debate and research. Recently, advances in genetics, especially next-generation sequencing allows us to look with high resolution at the clonal architecture of leukemia, including LSCs and find additional genetic and epigenetic aberrations responsible for drug resistance. Hopefully these advances in our understanding of LSCs biology not only will help to understand CML pathogenesis but also other hematological malignancies.

# Oral presentations

# O5.1

## Heme oxygenase-1 in hematopoietic stem cells: an interplay between cell cycle and DNA damage

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Heme oxygenase-1 (HO-1) is a cytoprotective enzyme which regulates the response of hematopoietic stem cells (HSC) to acute stress. Classical enzymatic activity of HO-1, namely degradation of prooxidative heme to carbon monoxide, iron ions and biliverdin, is linked to its cytosolic localization. However, our findings show that HO-1 can be localized also in the nucleus what is especially pronounced in HSC. We hypothesize that nuclear HO-1 may protect stemness potential and genetic material of HSC.

We found that HO-1 localizes almost solely in the nucleus of HSC (LKS $CD150^{+}CD34^{-}$) where it forms visible foci. When HSC lack HO-1 they show impaired functions. HSC from HO-$1^{-/-}$ mice lose balance between quiescent state and activation as they enter cell cycle more often (27.6% *vs.* 16.2% in HO-$1^{+/+}$ in G1 phase, $p<0.05$). Moreover, their differentiation is biased towards myeloid population: we detected more mature myeloid cells in peripheral blood of HO-$1^{-/-}$ animals. Finally, when we transplanted HSC from HO-$1^{-/-}$ donor mice to HO-$1^{+/+}$ recipients reconstitution of blood was significantly diminished in comparison to HO-$1^{+/+}$ donors (52.3% *vs.* 89% chimerism among granulocytes, $p<0.01$).

RNA-Seq analysis of HSC isolated from HO-$1^{-/-}$ mice revealed difference in expression of 621 genes in young animals in comparison to HO-$1^{+/+}$ littermates - it resembled transcriptome from aged HO-$1^{+/+}$ mice. Among pathways affected the most were cell cycle and DNA replication pathway. Indeed, we observed that HO-$1^{-/-}$ HSC proliferate extensively already in young animals (1.5 months-old, 14.9% *vs.* 2.3% in HO-$1^{+/+}$ in S/G2/M phase, $p<0.05$). This leads to increased number of HSC (0.011% *vs.* 0.006% in HO-$1^{+/+}$, $p<0.01$) and premature exhaustion of HSC pool.

Proliferating HO-$1^{-/-}$ HSC accumulate more DNA damage as shown by higher signal intensity from phosphorylated γH2AX (16.9% *vs.* 8,2% in HO-$1^{+/+}$, $p<0.0001$). Accordingly, we observed increased expression of genes involved in DNA damage response and DNA repair pathways, especially homologous recombination pathway and Fanconi anemia as shown by RNA Seq. However, lack of HO 1 appears to negatively affect function of those proteins, leading to further accumulation of DNA damage.

Concluding, HO-1 plays a key role in maintaining HSC properties by supporting DNA repair and facilitating proper cell cycle. Lack of HO-1 leads to accumulation of DNA damage and premature exhaustion of HSC pool which lose their functionality.

# Posters

## P5.1

### TRIM28 multi-domain protein maintains Cancer Stem Cell population in breast tumor development

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Tripartite motif-containing protein 28 (TRIM28), also known as Krüppel-associated box(KRAB)-associated protein 1 (KAP1) is a transcriptional co-repressor that maintains the pluripotency of embryonic stem cells, mediates DNA damage response and controls chromatin organization, acting as a co-repressor for KRAB family zinc finger (KRAB-ZNF) proteins. It was previously shown that Trim28 downregulation in mouse embryonic stem cells (mESCs) resulted in decreased expression of specific pluripotency markers as well as spontaneous loss of stem cell phenotype. Recently, high *TRIM28* levels have been correlated with higher aggression and lower survival rates in breast cancer, suggesting TRIM28 role in breast cancer progression. Moreover, increased levels of TRIM28 protein have been observed in liver, gastric, lung, pancreatic and prostate cancer, and, in patients with gastric or pancreatic cancer, high levels of TRIM28 also correlate with a significantly lower survival rate. Therefore, we evaluated the role of TRIM28 protein in the regulation of breast cancer stem cells (CSC) populations and tumorigenesis *in vitro* and *in vivo*.Our data suggest that significant downregulation of *TRIM28* gene expression diminished the ability of breast CSCs to self-renew and consequently to accelerate tumor growth *in vivo*. Downregulation of *TRIM28* expression in triple-negative breast cancer (TNBC) xenografts led to reduced expression of pluripotency and mesenchymal markers as well as inhibition of signaling pathways previously shown to maintain self-renewal of normal and cancer stem cells. Moreover,*TRIM28* depletion reduced the ability of cancer cells to induce tumor growth when subcutaneously injected in limiting dilutions, suggesting inhibited self-renewal of cancer stem cells upon *TRIM28* knockdown. Furthermore,*TRIM28* reduction leads to dysregulation of cell cycle, cellular response to stress, cancer cell metabolism, especially to the inhibition of oxidative phosphorylation - recently described major mechanism regulating maintenance of CSC population. This report is the first to present the role of the TRIM28 in regulating the CSC population in breast cancer. Our findings may pave the way to novel and more effective therapies that target TRIM28 in breast tumors.

## P5.2

### Highly cytotoxic FGF2-conjugates

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Antibody-drug conjugates (ADCs) are new class of anticancer treatment agents. ADCs combine the selectivity of targeted treatment, ensured by monoclonal antibody (mAb), with the potency of cytotoxic agent. We applied similar approach but, instead of mAb, as a targeting molecule, we used a natural ligand of fibroblast growth factor receptor 1 (FGFR1), a tyrosine kinase receptor, which has been reported to be overexpressed in several types of tumors, including breast and lung cancer. Here, we described the development and characterization of novel cytotoxic conjugates based on fibroblast growth factor 2 (FGF2) molecule binding with high affinity to FGFR1. These conjugates contained dolastatin derivative monomethyl auristatin E (vcMMAE) or auristatin Y (vtAY), the cytotoxic drug that blocks tubulin polymerization and inhibit cell division. We demonstrated that FGF2 conjugates exhibited high and specific cytotoxicity towards cells overexpressing FGFR1.

# P5.3

## Heme oxygenase-1 affects melanoma clonogenic capacity and tumorigenic potential

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Heme oxygenase-1 (HO-1) is the cytoprotective enzyme, previously shown to enhance melanoma growth, metastasis and angiogenic properties. Melanoma initiating cells (MIC) have been described as a population with cancer stem cell (CSC) properties, although their role in melanoma remains controversial. We investigated the effect of HO-1 activity in B16(F10) murine melanoma cell line, in sorted subpopulations displaying MIC markers.
B16(F10) cells displayed high clonogenic capacity *in vitro*, additionally enhanced under hypoxic conditions (0.5% $O_2$), where it reached up to 30% ($p<0.001$) in comparison to normoxia We identified and studied several subpopulations within B16(F10) cell line: cells expressing CD20, CD24 or CD133 antigens (surface MIC markers), cells with high ALDH activity and slowly-dividing subpopulation (characterized by retention of PKH dye). Clonogenic *in vitro* tests and *in vivo* transplantations showed that these subpopulations are not enriched in CSCs and none of the studied surface proteins or functional features can be used as a MIC marker. Overexpression of HO-1 enhanced spheroid growth of melanoma cells in 3D culture in matrigel. In the same time it decreased clonogenic capacity of B16(F10) cells both in normoxic ($p<0.001$) and hypoxic ($p<0.01$) conditions, alough HO-1 overexpressing populations contained unchanged proportion of stem cell-like cells, as demonstrated by end-point dilution assay. Additionally, the HO-1 overexpressing cells formed smaller clones but showed higher migration capacities. This can be connected with increased expression of genes inoved in survival and tumor metastasis like Abcg2, Notch1 and Snail2. *In vivo* transplantation of 10 cells with further serial transplantations of 100 cells showed that HO-1 showed a tendency to improve the survival of primary transplants ($p=0.09$), but decreased the long term tumorigenic potential of B16(F10) cells in secondary and tertiary recipients.
To sum up, HO-1 increases melanoma cell growth and migration but decreases their clonogenic potential and long term tumor initiation capacities.

# P5.4

## Clinicopathologic implications of PIWIL1 and PIWIL2 aberrant expression in different types of cancer

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PIWI proteins, a subclade of the highly conserved Argonaute family proteins, bind to a newly discovered class of non-coding small RNAs called PIWI interacting RNAs (piRNA), which are 25–31 nucleotides in length. Complex of piRNA and PIWI proteins are predominantly expressed in germline cells where have been demonstrated to be involved in germline development, stem cells self-renewal and gametogenesis. The human PIWI subfamily consist of four members: PIWIL1/HIWI, PIWIL2/HILI, PIWIL3 and PIWIL4/HIWI2 (Meister G, 2013, *Nat Rev Genet* **14**: 447-459). A growing number of data have revealed the aberrant expression of PIWI genes and proteins level in various cancers suggesting that both PIWI proteins and selected piRNAs are involved in tumorigenesis (Siddigi S, Matushansky I, 2012, *J Cell Biochem* **113**: 373-380).
The aim of these studies was to evaluate the expression profiles of the PIWIL1 and PIWIL2 protein and their transcripts in various breast, colon and non-small lung cancer tissues obtained from cancer patients. We aimed to determine the expression pattern and possible prognostic significance of PIWIL1 and PIWIL2 in cancer development and progression.The expression level of PIWIL1 and PIWIL2 in paired cancerous and non-cancerous tissues was measured by real-time reverse transcription-polymerase chain reaction (RT-PCR) assay. Immunohistochemistry was performed to confirm the observed changes on mRNA level and detect tissue localization of PIWI proteins. To verify the results obtained for cancer patients, transcripts and protein of PIWIL1 and PIWIL2 were evaluated in a series of different cancer cell lines. We found significant changes in the expression of PIWIL1 and PIWIL2 in colon, breast and non-small lung cancer tissues compared to corresponding non-cancerous samples. Aberrant expression were also correlated with clinicopathological features of patients. Our results indicate a reciprocal regulation between PIWIL1 and PIWIL2 indicating also that PIWI proteins seems to be promising cancer stem cells markers.

**Acknowledgments:**

The study was supported by the Leading National Research Center (KNOW, 2014-2018) of Wroclaw Center for Biotechnology.