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ABSTRACT BOOK

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ABSTRACT BOOK

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CONTENTS

ABSTRACTS OF LECTURES (in alphabetical order of speakers)

BLANPAIN, Cèdric .................................................................................................................. 9
CLARKE, Michael F. .................................................................................................................. 10
DICK, John E. .......................................................................................................................... 11
FRENETTE, Paul ....................................................................................................................... 12
GIANCOTTI, Filippo G. ............................................................................................................. 13
GLIMM, Hanno ......................................................................................................................... 14
HANAHAN, Douglas ............................................................................................................... 15
JAMIESON, Catriona ............................................................................................................... 16
HUELSKEN, Joerg ..................................................................................................................... 17
KIM, Carla ............................................................................................................................... 18
LEVINE, Ross L. ....................................................................................................................... 19
LIU, Hai-Kun ........................................................................................................................... 20
MCKAY, Ronald ....................................................................................................................... 21
MILSOM, Michael ................................................................................................................... 22
NIEHRS, Christof .................................................................................................................... 23
PARADA, Luis F. ....................................................................................................................... 24
PLASS, Christoph ..................................................................................................................... 25
RICH, Jeremy N. ...................................................................................................................... 26
RUDOLPH, K. Lenhard ............................................................................................................ 27
DE SAUVAGE, Frederic .......................................................................................................... 28
TRUMPP, Andreas .................................................................................................................. 29
VALIENTE, Manuel ............................................................................................................... 30
<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABERGER, Fritz</td>
<td>35</td>
</tr>
<tr>
<td>ARNDT, Kathrin</td>
<td>36</td>
</tr>
<tr>
<td>AUGUSTIN, Iris</td>
<td>37</td>
</tr>
<tr>
<td>BASER, Avni</td>
<td>38</td>
</tr>
<tr>
<td>BELLVIS, Pablo</td>
<td>39</td>
</tr>
<tr>
<td>BEYELER, Sarah</td>
<td>40</td>
</tr>
<tr>
<td>BHAVASAR, Shefalee</td>
<td>41</td>
</tr>
<tr>
<td>BRECKWOLDT, Michael O.</td>
<td>42</td>
</tr>
<tr>
<td>CABEZAS-WALLSCHEID, Nina</td>
<td>43</td>
</tr>
<tr>
<td>DHAWAN, Abhishek</td>
<td>44</td>
</tr>
<tr>
<td>EDGAR, Bruce A.</td>
<td>45</td>
</tr>
<tr>
<td>EIBL, Robert H.</td>
<td>46</td>
</tr>
<tr>
<td>ESPINET HERNÁNDEZ, Elisa</td>
<td>47</td>
</tr>
<tr>
<td>GIACHINO, Claudio</td>
<td>48</td>
</tr>
<tr>
<td>GLAUCHE, Ingmar</td>
<td>49</td>
</tr>
<tr>
<td>GOIDTS, Violaine</td>
<td>50</td>
</tr>
<tr>
<td>GOLESTANEH, Azadeh Fahim</td>
<td>51</td>
</tr>
<tr>
<td>GRALLA, Robert</td>
<td>52</td>
</tr>
<tr>
<td>HAAS, Simon</td>
<td>53</td>
</tr>
<tr>
<td>HEILER, Sarah</td>
<td>54</td>
</tr>
<tr>
<td>KAEBISCH, Constanze</td>
<td>55</td>
</tr>
<tr>
<td>KIJONKA, Marek</td>
<td>56</td>
</tr>
<tr>
<td>KIM, Ella L.</td>
<td>57</td>
</tr>
<tr>
<td>KLIMMECK, Daniel</td>
<td>58</td>
</tr>
<tr>
<td>KNAPP, Silvana</td>
<td>59</td>
</tr>
<tr>
<td>KRITSCH, Daniel</td>
<td>60</td>
</tr>
<tr>
<td>LAURENTI, Elisa</td>
<td>61</td>
</tr>
<tr>
<td>MAURER, Jochen</td>
<td>62</td>
</tr>
<tr>
<td>MELE, Valentina</td>
<td>63</td>
</tr>
<tr>
<td>MENDE, Nicole</td>
<td>64</td>
</tr>
<tr>
<td>MULLINS, Christina Susanne</td>
<td>65</td>
</tr>
</tbody>
</table>
NOLL, Elisa M. .................................................................................................................. 66
NUNUKOVA, Alena ............................................................................................................ 67
OBOROTOVA, Marina ........................................................................................................ 68
PAPADIMITROPOULOS, Adam ........................................................................................ 69
PETERSSON, Monika ........................................................................................................ 70
PHILLIPS, Emma ............................................................................................................. 71
PRENDERGAST, Áine M. ................................................................................................. 72
RAHMIG, Susann ............................................................................................................ 73
SCHIPPER, Dorothee ....................................................................................................... 74
SCHMIDT, Christin ........................................................................................................... 75
SCHÜLE, Roland ............................................................................................................... 76
SCOGNAMIGLIO, Roberta ............................................................................................... 77
SKODA, Jan ....................................................................................................................... 78
UCKELMANN, Hannah .................................................................................................... 79
VON PALESKE, Lisa .......................................................................................................... 80
WASKOW, Claudia ............................................................................................................ 81
WERHEID, David ............................................................................................................. 82
WINKLER, Anna-Lena ...................................................................................................... 83
WOBUS, Manja ............................................................................................................... 84
WOLNYY, Damian ............................................................................................................ 85
ZERJATKE, Thomas ......................................................................................................... 86
ZICKGRAF, Franziska M. ............................................................................................... 87
ABSTRACTS OF LECTURES
Mechanisms regulating stemness in skin cancers

Cédric Blanpain

WELBIO, Université Libre de Bruxelles, Belgium

For the vast majority of cancers, the cell at the origin of tumor initiation is still unknown. Two epithelial skin cancers are frequent in human populations: the basal cell carcinoma and the squamous cell carcinoma. We developed genetic lineage tracing approaches to identify the cells at the origin of these two types of cancer in mice, isolate these oncogene targeted cells by flow cytometry and determined the molecular changes associated with tumor initiation (Youssef NCB 2010, Youssef NCB 2012, Lapouge PNAS 2011).

Cancer stem cells (CSCs) have been described in various cancers including skin squamous cell carcinoma. Using different approaches in mice, we and others have recently shown that mouse squamous skin tumors contain cancer stem cells characterized by their greater ability to be propagated long term upon transplantation into immunodeficient mice (Lapouge EMBO J 2012) or by their ability to fuel tumor growth using lineage tracing experiments (Driessens Nature 2012).

To define the mechanisms that regulate skin cancer stem cells, we transcriptionally profiled cutaneous CSCs at different stages of tumor progression and identified genes preferentially up-regulated in CSCs. Using state of the art genetic gain and loss of function in mice, we are defining how some of these genes regulate tumor stemness and malignant transition in vivo within their natural environment. I will discuss how the combination of extrinsic factors such as the vascular niche (Beck Nature 2011) and intrinsic factors, such as the expression of Sox2, a transcription factor expressed in a variety of developmental progenitors and adult stem cells, regulate tumor heterogeneity, renewal and invasive properties of CSCs during skin cancer progression (Boumhadi Nature 2014).

This work is supported by WELBIO, the FNRS, and the European Research Council (ERC).
Identification of a molecular switch between quiescent and migratory mammary stem cells

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Primary human tissue contains a complex mixture of cell types which can only be partially purified by cell sorting based on surface markers. To gain further insight into mammary gland and tumor biology, we performed highly parallel single cell multiplexed gene expression measurements on individual normal and malignant mammary cells. This approach enabled discovery of novel normal mammary stem cell markers and gave insights into normal and malignant tissue architecture. We identified two distinct normal mammary stem cell populations: a proliferating, stem cell population that expresses genes associated with EMT as well as quiescent normal stem cell population which does not express basal cell markers. Both populations had similar engraftment ability in vivo, demonstrating that there are likely two physiological stem cell states. Single cell genomic analyses of the two stem cell populations identified two transcription factors that are molecular switches controlling each cell fate. The implications of these two stem cell pathways for metastases and cancer treatment will be discussed.
The cellular and molecular basis for intra-tumoral heterogeneity is poorly understood. Tumor cells can be genetically diverse due to mutations and clonal evolution resulting in intra-tumoral functional heterogeneity. Often proposed as mutually exclusive, cancer stem cell (CSC) models postulate that tumors are cellular hierarchies created due to epigenetic programs that are sustained by CSC. I will focus on three lines of evidence showing these models are highly integrated. Gene signatures specific to either AML LSC or normal HSC are highly similar and define a common stemness program. Compared to non-stem cell transcriptional programs, only stem cell signatures were significant independent predictors of patient survival in 4 large clinical databases of >1000 samples. Thus, determinants of stemness influence clinical outcome of AML across a spectrum of mutations indicating that many genetic abnormalities coalesce around stem cell properties. Secondly, we have carried out a series of combined genetic and functional studies of the LSC from either B-ALL or AML that point to commonalities between clonal evolution and CSC models of cancer. LSC from diagnostic patient samples were genetically diverse and reconstruction of their genetic ancestry showed that multiple LSC subclones were related through a complex branching evolutionary process and specific genetic events influence L-IC frequency. Also study of paired diagnostic (Dx) and relapse (Rx) samples are revealing that individual subclones possess distinct functional growth properties and that rare Dx subclones are chemotherapy resistant and become enriched at Rx. Thus the clonal evolution models are highly relevant in cancer but need to be extended to adopt the concept that CSC are subject to clonal evolutionary forces. Finally, the combined genetic and functional analysis of AML is revealing fundamental insights into the cell of origin, nature and biological consequences of initiating lesions and order of subsequent mutations; concepts that demonstrate how highly integrated the CSC and genetic evolution models must be. Highly purified hematopoietic stem cells (HSC), progenitor and mature cell fractions from the blood of AML patients were found to contain recurrent DNMT3a mutations (DNMT3amut) at high allele frequency, but without coincident NPM1 mutations (NPM1c) present in AML blasts. DNMT3amut-bearing HSC exhibited multilineage repopulation advantage over non-mutated HSC in xenografts, establishing their identity as pre-leukemic-HSC (preL-HSC). preL-HSC were found in remission samples indicating that they survive chemotherapy. Thus DNMT3amut arises early in AML evolution, likely in HSC, leading to a clonally expanded pool of preL-HSC from which AML evolves. For therapy to be more effective, our findings indicate that each genetic subclones must be targeted and that any cells that possess stemness properties, whether they are LSC or ancestral preL-HSC, must also be eradicated.
The identification of niche cells in the bone marrow has proved to be a challenging undertaking due to the complexity of its cellular constituents, the paucity of specific markers to accurately separate stromal cells, and its poorly accessible location in calcified bone. Several recent studies have indicated that the vasculature of the bone marrow may form major niches for maintaining the delicate balance between self-renewal and differentiation of hematopoietic stem cells (HSCs). Our laboratory has previously found a key role for nerve fibers from the sympathetic nervous system (SNS) in regulating HSC migration. These nerves are closely associated with blood vessels in many tissues. Through investigations to characterize the stromal cell targeted by the SNS, we have identified Nestin+ mesenchymal stem cells as a putative niche cell owing to their significant physical association with HSCs, and high and regulated expression of “niche factors”. Further studies have revealed distinct Nestin+ cell subsets wherein the NG2+ pericyte-like arteriole-associated fraction harbors dormant HSCs whereas the reticular-like sinusoids-enriched LepR+ subset is associated with more proliferative HSCs. The complexity of the niche will be illustrated by recent data suggesting a contribution of a differentiated progeny, the megakaryocyte, in promoting HSC quiescence in a distinct niche via CXCL4-mediated feedback loop. Implications of the bone marrow microenvironment in malignancies such as acute myelogenous leukemia will also be discussed.
Dormancy and reactivation of metastatic stem cells

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Insights into the mechanisms that enable disseminated cancer cells to survive during dormancy and to subsequently outgrow into life-threatening lesions may lead to the identification of novel therapeutic targets for the prevention or treatment of metastatic disease. In principle, functional genetic screens can lead to the rapid identification of strong mediators of a selectable phenotype. However, practical barriers have so far prevented successful application of these methods to a process, such as metastasis, that can only be successfully modeled in vivo. We have developed flexible and high-throughput functional genetic screens, which enable the identification of single genetic entities that mediate metastatic reactivation of breast cancer in the mouse. By using this strategy, we have identified canonical genes and microRNAs that mediate metastatic reactivation in the lung. To identify genes that oppose reactivation, we have screened an expression library encoding shRNAs and have identified target genes that encode potential enforcers of dormancy. I will discuss mechanistic studies, which provide additional support to the hypothesis that the metastasis-initiating cells are cancer stem cells or revert to this state upon infiltrating a permissive niche or molding one in the target organ.
Clonal dynamics in gastrointestinal cancers

Hanno Glimm

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Tumor-initiating cells (TIC) are thought to drive progression and metastases formation of solid cancers. Our research focuses on functional and molecular characterization of heterogeneity within the TIC compartment of primary human colorectal and pancreatic tumors. Understanding dynamics of clonal activity and mechanisms driving TIC activation and metastasis formation in vivo will serve as a basis to develop targeting strategies directed against functional TIC activity in human solid cancers.
Cross-species analysis reveals subtypes of pancreatic neuroendocrine cancer with distinctive metastatic and metabolic phenotypes

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Thirty years ago I engineered a transgenic mouse model – RIP1-Tag2 – that has proved to be a powerful tool with which to study the parameters of multistage tumorigenesis, revealing and validating hallmarks of cancer, and in turn their promise as therapeutic targets. A persistent critique has been that this model of pancreatic neuroendocrine cancer (PanNET), driven by the SV40 T antigen oncoprotein (which abrogates the TP53 and RB tumor suppressor pathways) may not recapitulate its cognate human cancer, as are reflected in 2nd generation mouse models of other cancers that employ signature genes whose mutation in humans drives the human cancer. We have now performed parallel transcriptional profiling on PanNET from human and the mouse model. The analysis reveals two molecular subtypes common between mouse and human. One involves well-differentiated hormone-expressing islet tumors (IT). The second, dubbed met-like primarily (MLP) due to its association with most liver metastases and its poorly differentiated state, is found in human and mouse, with similar profiles. A third human subtype is associated with loss not of RB and TP53 but rather Men1, a subtype which is not reflected in the RIP-Tag2 model but likely in another mouse model involving loss of Men1. The IT and MLP subtypes differ in their expression of mature islet ß cell genes and genes that are expressed in developing islet progenitor cells. We infer that the cell-of-origin of IT is mature insulin-producing islet ß cells, whereas the MLP originates from an islet progenitor cell.
Niche-dependent malignant reprogramming of tissue-specific progenitors into self-renewing cancer stem cells (CSCs) contributes to therapeutic resistance, progression and relapse. In advanced therapy resistant human malignancies, such as lobular breast cancer, hepatocellular cancer, esophageal squamous cell cancer and blast crisis chronic myeloid leukemia (CML), RNA sequencing (RNA-seq) has uncovered RNA editome imbalances driven by deregulation of adenosine deaminase acting on RNA (ADAR) editases that promote cancer stem cell (CSC) generation. As an important paradigm for understanding the molecular evolution of therapy resistant CSCs, CML undergoes blast crisis (BC) transformation following aberrant acquisition of self-renewal capacity by progenitors harboring cytokine-responsive ADAR1 p150 overexpression. However, extrinsic and intrinsic oncogenic ADAR1 activators, downstream stem cell regulatory mRNA and microRNA targets and ADAR1’s niche-dependent role in malignant reprogramming of progenitors into CSCs had not been elucidated. In this study, RNA-seq and qRT-PCR revealed that FACS purified BC CML progenitors expressed higher levels of JAK2 dependent inflammatory cytokine receptors than normal and chronic phase (CP) progenitors. In a RAG2/−gc/− humanized mouse model of BC CML, selective JAK2 and BCR-ABL1 inhibition reduced CSC self-renewal commensurate with a significant diminution in BCR-ABL1 and ADAR1 p150 expression. Further phospho-JAK2 nanoproteomic and RNA editing qRT-PCR analyses revealed that lentiviral human JAK2 transduction of CD34+ progenitors enhanced ADAR1 editase activity. Co-transduction of progenitors with lentiviral human BCR-ABL1 and JAK2, increased LIN28B pluripotency gene expression and replating capacity, as an in vitro surrogate measure of self-renewal. Notably, lentiviral ADAR1 transduction reduced let-7 stem cell regulatory microRNA levels, increased LIN28B transcripts and enhanced chronic phase CML progenitor replating to a level similar to BC. In human CSC-supportive stromal co-culture assays, targeted ADAR1 and JAK2 inhibition reduced ADAR1, restored let-7 microRNA expression and prevented malignant progenitor self-renewal. These results provide the first demonstration that JAK2-mediated cytokine and intrinsic oncogenic signaling pathways converge on ADAR1, which drives CSC generation by impairing stem cell regulatory microRNA biogenesis. Moreover, targeted reversal of ADAR1-editase mediated malignant progenitor reprogramming may contribute to eradication of inflammatory niche resident CSCs in a broad spectrum of malignancies. Finally, RNA recoding events including RNA editing of GLI and splice isoform switching of CD44, ROR1, and BCL2 family members favoring embryonic splice isoforms rarely expressed in normal adult stem cells have emerged as prominent drivers of CSC survival and self-renewal thereby representing novel diagnostic and therapeutic targets.
Mechanisms of metastatic colonization and their therapeutic application

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Most deaths from cancer are a consequence of the metastatic spread of the disease. This demands to develop novel and more effective treatment strategies targeting in particular metastatic cancers. We have been working to understand the importance of the cancer stem cell population and the role of the metastasis-associated stroma in modulating the efficiency of colonization. We find that the ability of cancer stem cells to initiate growth and tumor-induced changes in the local stroma are key factors which determine metastatic spread. I will describe several examples of developmental signaling pathways including the TGFbeta, Wnt or Hedgehog pathways which enable tumors to grow at secondary sites such as the lung or the liver. Surprisingly, their output depends on the local environment and can be at times metastasis-supportive or -preventive illustrating a complex network of cellular interactions and locally adapted stroma cell populations. Within cancer stem cells, we identify a transcriptional regulator which prevents differentiation and allows to maintain this important population. I will describe our recent attempts to alter this program using drug-induced differentiation which can result in promising therapeutic responses.
Our long-term goal is to elucidate the role of stem cells in lung homeostasis as a prerequisite to the development of therapeutic strategies that can be used to prevent or attenuate lung disease and lung cancer. Our previous experience isolating the first stem cell population from the adult murine lung, termed bronchioalveolar stem cells (BASCs), and our demonstration of a role for these cells in lung cancer serves as a platform to address these questions. We have developed three-dimensional (3D) co-culture and subcutaneous co-injection assays that allow us to quantitatively assess the identity and the differentiation potential of lung stem cells. This approach has also led us to uncover mechanisms that regulate differentiation and the lung injury response in vivo. We defined a cross-talk between lung endothelial cells and lung stem cells via a novel signaling axis involving Bmp4, NFATc1 and Tsp1; this pathway drives BASCs to differentiate into the alveolar epithelial cell lineage (Lee et al, Cell, 2014). Our work in the intersection of stem cell biology and lung disease has expanded into new insights for understanding metastasis and non-small cell lung cancer (NSCLC). We previously showed the adenocarcinoma Kras/p53 mutant mouse model contains Sca1+ tumor-propagating cells (TPCs), the cells that recapitulate the tumor by transplantation. We recently showed multiple lung tumor sub-populations can give rise to metastatic disease, and that the Sca1+ CD24+ TPCs have the highest metastatic potential. We also showed the Hippo pathway mediators Yap/Taz are necessary and sufficient for lung cancer progression (Lau et al, EMBO, 2014). We collaborated with Kwok Wong (DFCI) to characterize the first mouse model of lung squamous cancer, the second most common NSCLC. In this model that utilized deletion of Lkb1 and Pten, we identified a TPC population defined by the markers Sca1 and NGFR and showed that TPCs may have unique immune evasion properties (Fillmore, Cancer Cell, 2014). Our ongoing work will further establish the mechanisms by which TPCs control lung cancer progression and metastasis.
Specific combinations of acute myeloid leukemia (AML) somatic mutations are associated with distinct clinical and biologic features. However, in vivo models do not exist for the majority of common, poor-prognosis genotypes. Concurrent mutations in FLT3 and TET2 are associated with adverse outcome in AML. We hypothesized that activating mutations in FLT3 would cooperate with inactivating mutations in TET2 to induce AML in vivo, and that we could investigate AML pathogenesis and therapeutic response using this model. We generated Tet2fl/flFlt3ITD mice, which resulted in fully penetrant, lethal AML. Multipotent progenitors (LSK CD48+CD150-) from Vav-cre+Tet2fl/fl-Flt3ITD mice propagated disease in secondary recipients and were refractory to chemotherapy and FLT3-targeted therapy consistent with leukemia stem cell (LSC) activity. We hypothesized that Tet2/Flt3-mutant LSCs possess a distinct epigenetic/ transcriptional signature that contributes to leukemic cell self-renewal and therapeutic resistance. RNA-seq analysis revealed that genes involved in normal myeloid differentiation, including GATA1, GATA2, and EPOR, were transcriptionally silenced and marked by increased promoter methylation. Flt3ITD mutations and Tet2 loss cooperatively remodeled DNA methylation and gene expression to an extent not seen with either mutant allele alone, including at the Gata2 locus. Re-expression of Gata transcription factors restored differentiation of AML stem cells and attenuated leukemogenesis. Our data demonstrate that TET and FLT3 mutations can cooperate to induce AML in vivo, with a defined LSC population that is resistant to targeted therapies and characterized by site-specific changes in DNA methylation and gene expression. Most importantly, we demonstrate that re-expression of specific epigenetic target genes is able to reprogram LSCs and restore hematopoietic differentiation, providing a rationale for the use of epigenetic therapies to reprogram AMLs characterized by mutations in epigenetic regulators.
Evidence and significance of a brain tumor stem cell *in vivo*

**Hai-Kun Liu**

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Cancer stem cells (CSCs) have been suggested as potential therapeutic targets for treating malignant tumors, but the in vivo supporting evidence is still missing. We established a mouse model that allows stem cell-specific gene targeting in unperturbed high-grade mouse brain tumors. Using a GFP reporter driven by the promoter of the nuclear receptor tailless (Tlx), a neural stem cell (NSC)-specific transcription factor, we demonstrate that Tlx-positive cells in primary brain tumors are mostly quiescent. Lineage tracing demonstrates that single Tlx-positive cells can self-renew and generate Tlx negative tumor cells in primary tumors, suggesting that they are brain tumor stem cells (BTSCs). After introducing a BTSC-specific knock-out of the Tlx gene in primary mouse tumors, we observed a loss of self-renewal of BTSCs, which leads to a significant prolongation of animal survival. Furthermore, we demonstrate that loss of Tlx in BTSCs leads to the induction of essential signaling pathways mediating cell cycle arrest, cell death and neural differentiation. Additionally we show that Tlx serve as a novel prognostic marker in human high-grade brain tumors indicating reduced survival. Our study provides a novel therapeutic target of targeting glioblastomas and demonstrates that BTSCs are suitable therapeutic targets, thus strongly supporting the CSC hypothesis and the importance of therapeutic approaches targeting CSCs.
Mammals are formed from a small group of founding stem cells that form the major organs and tissue types. A major research achievement of the stem cell field is the discovery of ex vivo strategies that generate many differentiated cell types. The hope of developing new therapies across a wide range of medical conditions has generated significant investment in the developmental potential of human induced pluripotent stem (iPS) cells.

In contrast to animal models that utilize inbred strains to reduce experimental variability, the successful use of human pluripotent stem cells would ideally embrace the genetic variation found in the human population. To this end, we have established a simple experimental condition where different human pluripotent stem cells can be precisely compared using RNA sequencing as they self-renew and differentiate. This analysis suggests that stable differences in the expression of hundreds of genes distinguish different pluripotent cell lines. This analysis also identifies differences in self-renewing cells that predict the differentiation efficiency of pluripotent lines.

Cell imaging combined with genetic or pharmacological perturbation demonstrated that differentiation was regulated by the dynamic spatial and temporal control of transcription factors predicted from the RNA sequencing data. In a new series of iPS lines chosen to control for race and gender, high content imaging of differentiation to neural or somatic fates showed clear differences between genomes. These results suggest powerful new strategies to understand the developmental and signaling mechanisms regulating somatic stem cells. They encourage a systematic analysis of how human genetic variation influences the origins of cancer risk and response to pharmacological intervention.
A hallmark of aging is the accumulation of DNA damage in hematopoietic stem cells (HSCs) and this is thought to drive age-related attrition of the hematopoietic system. In line with this hypothesis, accelerated aging disorders are frequently associated with defective DNA repair, such as the bone marrow failure (BMF) syndrome Fanconi anemia (FA). It has been shown that long-term quiescence is a feature of the most functionally potent long-term (LT)-HSCs and we hypothesize that this state of cell cycle dormancy protects the genome from the replication-induced DNA damage that drives functional decline. HSCs can be forced to enter cell cycle in vivo in response to stimuli that mimic physiologic stress such as infection or blood loss. Under these conditions of hematopoietic stress, we could observe that wild type (WT) murine LT-HSCs demonstrate a dramatic induction of de novo DNA damage, correlating with altered mitochondrial metabolism. Thus, upon LT-HSC exit from quiescence, mitochondria showed a shift towards energy production via oxidative phosphorylation and a concurrent increase in reactive oxygen species (ROS) production. DNA damage was rescued by overexpression of ectopic ROS scavenging enzymes, confirming this link. The FA DNA repair pathway is critical for resolving this stress-induced DNA damage, since FA knockout mice demonstrated extremely high levels of DNA damage compared to WT in response to stress stimuli, as well as a rapid depletion of functional LT-HSC resulting in eventual BMF. Although serial exposure to stress agonists did not provoke full BMF in WT mice, functional HSC numbers were reduced to >10% of age matched controls and a strong myeloid differentiation bias was evident, consistent with a premature aged phenotype. In summary, stress-induced proliferation can drive accelerated aging of LT-HSC and a functional FA pathway is critical for preserving genomic and functional integrity in the face of such attrition.
Mitotic Wnt signaling promotes protein stabilization and regulates cell size

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Canonical Wnt signaling is thought to regulate cell behavior mainly by inducing β-catenin-dependent transcription of target genes. In proliferating cells Wnt signaling peaks in the G2/M phase of the cell cycle, but the significance of this ‘mitotic Wnt signaling’ is unclear. Here we introduce Wnt-dependent stabilization of proteins (Wnt/STOP), which is independent of β-catenin and peaks during mitosis. We show that Wnt/STOP plays a critical role in protecting proteins, including c-MYC, from GSK3-dependent polyubiquitination and degradation. Wnt/STOP signaling increases cellular protein levels and cell size. Wnt/STOP rather than β-catenin signaling is the dominant mode of Wnt signaling in several cancer cell lines, where it is required for cell growth. We propose that Wnt/STOP signaling slows down protein degradation as cells prepare to divide.
Mouse models of malignant GBM: Cancer stem cells and beyond

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Glioblastoma Multiforme (GBM) is an incurable cancer with a rapid progression and a prognosis of month from the time of diagnosis. Given the resistance to all known therapies, new paradigms to understand this disease and identify novel therapeutic targets are sorely needed. We have used genetically engineered models to ablate GBM relevant tumor suppressors in brain cells. Our fully penetrant mouse models indicate that adult stem cells and progenitors are preferential sites of tumor initiation. As such, further study of these cells, and how they transform may provide unique insights into tumor development and progression. Our efforts to understand whether additional cell types can give rise to GBM indicate that fully differentiated brain cells are considerably more resistant to tumor suppressor mediated transformation than are stem cells, but in contrast, OPC progenitor cells are also able to give rise to GBM that, while pathologically similar to stem cell derived tumors, also have unique growth and molecular properties that distinguish them clearly. I will discuss the state of understanding of these tumors and the implications for cancer stem cells and therapeutic opportunities.
Hematopoietic commitment of adult hematopoietic stem cells (HSC) involves epigenetic remodeling. Recently, tagmentation-based WGBS (TWGBS) was developed which works reliably with 10-30 ng DNA. We generated comprehensive single CpG-resolution genome-wide DNA methylation maps detailing epigenetically regulated elements during early HSC commitment from FACS-sorted HSC (LSK/CD34-/CD48-/CD150+) and their immediate progeny (MPP1 [LSK/CD34+/CD48-/CD150+], MPP2 [LSK/CD34+/CD48+/CD150+], and MPP3/4 [LSK/CD34+/CD48+/CD150-]). We identified 15,987 differentially methylated regions (DMRs) across all populations of which >13,000 comprise novel putative regulatory regions. Globally, early hematopoietic commitment displays progressive methylation changes. While, initial hematopoietic commitment is characterized by loss of methylation with 71% of DMRs loosing methylation from HSC to MPP1, later commitment steps within the MPP compartment are characterized by an increase in methylation, so that for example 75% of DMRs show methylation gain from MPP2 to MPP3/4. DMRs are highly enriched in cis-regulatory elements. Integrating the methylation data with RNA-seq expression data, we observed an unprecedented correlation between changes in DNA methylation and gene expression. As a result, numerous strong candidate genes that might be involved in controlling the molecular programs directing self-renewal and early hematopoietic commitment have been identified.
Target identification in glioma initiating cells

Jeremy N. Rich

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Background: Gliomas display cellular hierarchies with tumor initiating cells (TICs) at the apex that are functionally defined by the ability to self renew and propagate tumors similar to the parental tumors from which they are derived. TICs remain controversial, but their clinical relevance has been supported by resistance to cytotoxic therapies (Bao et al. Nature 2006) and promotion of tumor angiogenesis (Bao et al. Cancer Research 2006). TICs reside in specific functional niches in perivascular and hypoxic niches (Li et al. Cancer Cell 2009) that may offer the ability to disrupt tumor maintenance and therapeutic resistance through targeting the niche. Investigating TICs has already yielded novel molecular targets and pathways that are amenable to therapeutic targeting (Kim et al. Genes Development 2012; Eyler et al. Cell 2011; Guryanova et al. Cancer Cell 2011).

Methods/Results: Using patient-derived tumor models, we interrogated the regulation of the TIC phenotype by both cell intrinsic and microenvironmental influences present in tumors. TICs are enriched under low nutrient conditions due to the cooption of the high affinity GLUT3 transporter normally expressed by neurons (Flavahan et al. Nature Neuroscience 2013). We have now extended these findings to demonstrate that cellular metabolism is differentially regulated within the tumor hierarchy at several levels to provide resources for sustained self-renewal and proliferation. We also recently found that TICs have basal genotoxic stress activating PARP permitting radiosensitization (Venere et al. Cell Death Differentiation 2014). To discover novel TIC targets, we are using several technologies, including aptamers (Kim et al. Cancer Research 2013), flow cytometry (Lathia et al. Cell Reports 2014), and phage display (Liu et al. Cell Death Diff. 2014), showing that TICs manifest nodes of fragility mediating cell survival and invasion (e.g. JAM-A, VAV3, and CD97). Combining TIC models from patients with non-neoplastic progenitors from epilepsy resections, we are interrogating additional molecular regulators of the cellular hierarchy that can be distinguished from normal stem cells to minimize toxicity.

Conclusions: The conventional pyramidal unidirectional differentiation cascade with TICs at the apex has been called into question by studies demonstrating plasticity of the TIC phenotype (Cheng et al. Cell 2013), thus suggesting that targeting only TICs will likely fail to cure patients and require simultaneous targeting of TICs and the bulk tumor. Although the field of TIC biology is relatively young, continued elucidation of the tumor hierarchy holds promise for development of novel patient therapies.
Increasing evidence indicates that stem cells are the cell type of origin of cancer formation. Molecular mechanisms that contribute to stem cell transformation need yet to be delineated. In previous work we showed that DNA damage limits the self renewal of lymphoid biased HSCs by inducing BATF-dependent lymphoid differentiation. During my talk I will present novel data on consequences of BATF-deletion on the accumulation of mutations and transformation of lymphoid biased HSCs in aging mice.
The Notch, Wnt and Hedgehog signaling pathways play critical roles during embryonic development through modulation of proliferation, cell migration and differentiation. In adults, these pathways remain important in regulating stem cell function during normal tissue homeostasis. Inappropriate activation either through mutation or misexpression can result in tumorigenesis. The gastrointestinal tract is an example of tissue that remains dependent on Notch, Wnt and Hedgehog signaling for normal homeostasis. Intestinal epithelium is turned over every 5 days on average and is very dependent on stem cell activity. Multiple stem cell compartments, including crypt based columnar cells, the “+4” cells or cells in other more committed compartments, have been identified and contribute to intestinal homeostasis and/or regeneration. However, the respective contribution and interplay between these cells and their contribution to tumorigenesis is only beginning to be understood. As Notch, Wnt and Hedgehog pathways regulate stem cell function, normal intestinal homeostasis, and promote tumorigenesis, they are attractive targets for the development of anti-cancer stem cell therapeutics. The development of inhibitors targeting these pathways is therefore of the highest interest. We will discuss the role these developmental pathways in regulating intestinal stem cell populations in normal and tumor tissues.
Somatic stem cells are the cellular components responsible for the life-long maintenance and repair of highly regenerative tissues such as the skin, the gastro-intestinal mucosa or the blood forming system. In addition stem cells are critical components of repair in response to tissue injury and infection. Moreover, genetic alterations of stem cells and their progeny can lead to the generation of “leukemic stem cells” (LSC) or solid “cancer stem cells” (CSCs) that drive tumorigenesis and metastasis in hierarchically organized cancer entities. Due to their remarkable resistance to chemotherapy and radiation, CSCs are thought to be responsible for tumor re-occurrence as well as initiation and maintenance of metastases (1). We have recently established novel patient derived animal models for Myelodysplastic Syndromes (MDS), in particular for the lower risk types. Most relevant, we show that the patient derived mutant MDS stem cells (lin-CD34+CD38-) re-program their microenvironmental niches and establish a cross talk, which establishes an “MDS-hematopoietic-niche unit”. The functional relevance of this unit for the development and progression of MDS in patients is demonstrated by its capacity to propagate MDS in immune-compromised mice (2). Finally, since patients are still alive at the time the models are established, this generates a platform for personalized oncology allowing assessment and possibly targeting of MDS pathology at the level of individual patients. In addition, we have established a program to functionally identify and isolate blood circulating “metastasis initiating cells” (MICs) directly from the peripheral blood of breast cancer patients. By transplantation into immuno-compromised mice we demonstrated their capacity to initiate new metastases. These studies revealed the identification of MICs, which show an EPCAM+CD44+MET+CD47+ phenotype and are able to initiate new bone and lung metastasis (3). Moreover, the number of these circulating MICs in the blood of patients correlated with overall survival and offers novel possibilities for the design of improved diagnostic and therapeutic tools for metastatic breast cancer.

Metastatic entities face critical challenges during the progression of cancer. Colonization of secondary organs stands out for being the most limiting of all steps within the metastatic process. Although this applies to all secondary organs affected by cancer, the brain is certainly the best example as shown by experimental models and clinical presentation in patients. By interrogating a variety of brain metastasis experimental models from breast and lung cancer we have identified molecular and cellular mechanisms allowing metastasis initiating cells to successfully progress in this unique microenvironment. Plasmin from the reactive brain stroma acts as a defense against metastatic infiltration while anti-plasminogen activator (anti-PA) serpins are a required shield for cancer cells to surpass this defense. When devoid of anti-PA serpins, cancer cells invading the brain suffer two challenges, killing by plasmin-mobilized FasL and defective co-option of brain capillaries by plasmin-mediated destruction of cancer cell L1CAM. Although part of these mechanisms apply specifically to colonization of the brain, our recent data shows that co-opting the preexisting vascular network is a phenomenon that can be found more broadly in extra-cranial metastasis. L1CAM mediated vascular co-option is necessary for the initiation of secondary tumors in bone and lung establishing a common trait for multi-organic metastatic disease. By deconstructing the limiting steps of metastasis and dissecting its molecular regulation we have identified new targets for therapeutic intervention on metastasis initiating cells.
KEYNOTE LECTURES

The critical role of breast micro-environment in initiation, promotion, dormancy and drug resistance

*Mina Bissell*

Skin stem cells and cancer

*Elaine Fuchs*
ABSTRACTS OF POSTERS
Hedgehog-interleukin-6 signal cooperation drives basal cell carcinoma

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Objective: Aberrant activation of the Hedgehog (HH)/GLI signaling plays a key etiologic role in various human malignancies, including basal cell carcinoma (BCC) of the skin, one of the most common human cancers. Targeting HH/GLI signaling provides an effective treatment for BCC, but severe side effects, rapid development of drug resistance and disease relapse call for improved strategies. Identifying synergistic pathway interactions driving oncogenesis is paramount for elucidating new rational combination treatments based on simultaneous inhibition of cooperative oncogenic signals.

Methods: We applied a candidate-based approach to screen for growth factor and cytokine pathways synergistically promoting the oncogenic activity of HH/GLI. 3D tumor sphere formation served as read-out for synergistic signal interactions. Using a combination of genetic and chemical perturbation experiments along with molecular studies addressing pathway interactions we studied the functional requirement of cooperative pathways promoting oncogenic HH/GLI signaling.

Results: The candidate-based approach identified the Interleukin-6 (IL6)/STAT3 signaling pathway as a novel cooperative signal synergizing with HH/GLI in oncogenic transformation of human keratinocytes in vitro. Human BCC samples and HH-induced mouse BCC-like lesions express IL6 key effectors, including IL6RA, and activated nuclear STAT3. Chemical and genetic disruption revealed JAK2 as the critical kinase of the JAK family and STAT3 as the IL6 regulated transcription factor accounting in combination with GLI for HH-IL6 target gene regulation and oncogenic transformation. We provide evidence that signal integration occurs at the level of common HH-IL6 target gene promoters. Of note, RNAi mediated perturbation of Stat3 reduced in vivo growth of mouse BCC, as did genetic deletion of Il6ra in the skin of Ptch-deficient mice supporting the in vivo relevance of our data.

Conclusion: The data reveal IL6/JAK2/STAT3 signaling as a novel positive modulator of HH/GLI signaling in cancer and suggest that rationale-based combination treatments relying on the simultaneous inhibition of both pathways may be a promising strategy to target HH-induced BCC.
CD133 is a modifier of hematopoietic progenitor frequencies but is dispensable for the maintenance of mouse hematopoietic stem cells

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Prominin-1 (CD133) is a pentatransmembrane glycoprotein expressed on a number of somatic stem and progenitor cells, and it is widely used as a cell surface marker for the isolation and characterization of human hematopoietic stem cells (HSCs) and cancer stem cells (CSC) from various blood diseases including acute and chronic myeloid leukemias. However, despite its prominent role in human blood cells, the physiological role of CD133 in mouse hematopoiesis remains unknown. Here we show that CD133 is expressed on murine HSCs and myeloid progenitor cells. However, loss of CD133 has no functional effect on HSC number and function during steady state hematopoiesis and after transplantation. Blood cell numbers in the periphery and mature myeloid cells in the spleen, lymph nodes and peritoneal cavity are normal, but in the absence of CD133 in vitro colony formation of growth-factor responsive myeloerythroid precursor cells is impaired under steady state conditions. Loss of CD133 delays the recovery of hematopoietic insult and mature red blood cells are reduced in the periphery of CD133 KO mice after hematopoietic stress induction using 5-fluorouracil (5-FU). These results provide experimental evidence that CD133 is not a critical regulator of hematopoietic stem cell function in mouse, demonstrating a substantial species difference between mouse and man. However, absence of CD133 modifies frequencies of growth-factor responsive hematopoietic progenitor cells during steady state and limits the functionality of progenitor cells in response to hematopoietic stress.
The Wnt signaling pathway plays an important role in the specification and maintenance of precursor cell and stem cell lineages in various tissues. Evi/Wls is an essential component of the Wnt secretion machinery and is involved in the secretion of all Wnt proteins. Consequently, the genetic modulation of Evi globally affects Wnt signaling providing a unique experimental tool to modulate Wnt signaling through regulation of Wnt ligand secretion. Transgenic mouse embryonic stem cells (ESC) either deficient for Evi (Evi-LOF) or overexpressing Evi (Evi-GOF) were investigated with respect to their in vitro and in vivo stemness characteristics. Evi-LOF ESCs revealed reduced self-renewal, whereas Evi-GOF ESCs had increased self-renewal capabilities following LIF-withdrawal, supporting the concept that Wnt secretion maintains ESCs in their undifferentiated state. Likewise, lineage differentiation was promoted to cardiomyocyte differentiation in Evi-GOF ESCs whereas multiple germ layer formation was impaired in Evi-LOF ESCs. Upon transplantation in mice, ESCs differentiated in an uncoordinated manner, forming teratomas that gave rise to multiple tumorous embryonic tissues. Evi-LOF teratomas showed impaired tumor growth with hemorrhagic tissue. Interestingly, expression profiling of Evi-GOF teratomas revealed a strong alteration in the expression of genes involved in immune response modulation. Corresponding, T cell infiltration was reduced in Evi-GOF tumors suggesting an altered immune cell recruitment and function. Taken together, our study identified Evi-mediated Wnt secretion as a tumor promoting factor that modulates the tumor microenvironment.
It is well known that epigenetic modifications as well as regulation of transcription factors are closely associated to cellular identity, highly distinguishing between the pluripotent and committed status of cells. However, little is known about the importance of changes at the posttranscriptional level. Recent studies on hematopoietic stem cells emphasize that protein translation is tightly controlled in the stem cell compartment and changes in both directions severely deregulate the system and deplete the stem cell pool. Similarly, it is known that differentiation is characterized by global up-regulation of translation driven by increased ribosomal loading. Here we look at changes of the translatome in neural stem cells both in paradigms of inflammation and differentiation.

We apply ribosome profiling on primary neural stem cells, a novel method based on the deep sequencing of ribosome-protected mRNA fragments as a measure of translation. Coupled to RNA-sequencing, this method allows the identification of translationally controlled genes. We expose stem cells to interferon, an inflammatory cytokine that is highly up regulated after brain injury and monitor acute translational changes upon exposure. Analysis of ribosomal density along the transcript will be used for identification of regulatory sites. Motive analysis of these sites will identify RNA-binding proteins (RBPs), which can specifically bind and control the translational efficiency of a subset of genes which are potentially involved in proliferation and differentiation of stem cells.

In a complementary study, we generated mouse lines, which express a HA-tagged variant of a ribosomal protein in different cell compartments along the differentiation lineage of neural stem cell. These mice allow isolation of translated mRNAs in specific cell populations in vivo. Deep sequencing of these mRNAs reveals the translatome of different populations. Comparing the translation-profiles at different stages of differentiation will uncover key regulatory components.
Glioblastoma multiforme is one of the most common primary malignant brain tumors, with poor prognosis in both adult and children. Approximately 70%–80% of pediatric gliomas are characterized by the same mutations in the histone variant H3.3. These amino acid mutations in H3.3 are involved in the pathogenesis of several forms of human pediatric gliomas, including diffuse intrinsic pontine glioma (DIPG).

In this study we used an efficient brain tumor model to characterize the function and importance of the H3.3K27M mutation. RCAS vector overexpressing oncogenes (PDGF, AKT) and wildtype/mutant version of H3.3 were injected into P0 Nestin TVA Nestin-Cre-ERT2 mice. The resulting tumor model for DIPG produces multiclonal glioblastoma and exhibit key features of the current disease. Tumor cells overexpressing H3.3K27M overgrowth other tumor cells showing the tumorigenic potential of the K27M mutation. Our model reproduces the loss of H3K27me3 observed in human DIPG carrying the K27M mutation in the H3.3. Tumors overexpressing the wildtype H3.3 show similar levels to the control tumors. Finally, glioblastomas overexpressing H3.3K27M display metastasis in the spinal cord as it has been showed to occur in around 20% of the DIPG cases.

We have efficiently integrated H3.3 expression in our brain tumor model. The overexpression of histone H3.3 originates an in vivo growth advantage for tumor cells. Tumors carrying K27M mutation reproduce key features found in human tumor like the decrease of H3K27me3 and metastasis following the neuraxis. Further on we will block the expression of K27M mutation using a Nestin-Cre-ERT2 mouse strain and also characterize the epigenomics of these tumors to compare it with the human DIPG.
Targeting the bHLH transcriptional networks by mutated E proteins in experimental glioma

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Glioblastomas (GB) are aggressive primary brain tumors. Helix-loop-helix (HLH, ID proteins) and basic HLH (bHLH, e.g. Olig2) proteins are transcription factors that regulate stem cell proliferation and differentiation throughout development and into adulthood. Their convergence on many oncogenic signaling pathways combined with the observation that their overexpression in GB correlates with poor clinical outcome identifies these transcription factors as promising therapeutic targets. Important dimerization partners of HLH/bHLH proteins are E proteins that are necessary for nuclear translocation and DNA binding. Here, we overexpressed a wildtype or a dominant negative form of E47 (dnE47) that lacks its nuclear localization signal thus preventing nuclear translocation of bHLH proteins in long-term glioma cell lines and in patient-derived glioma-initiating cell lines and analyzed the effects in vitro and in vivo. Whilst overexpression of E47 was sufficient to induce apoptosis in absence of bHLH proteins, dnE47 was necessary to prevent nuclear translocation of Olig2 and to achieve similar pro-apoptotic responses. Transcriptional analyses revealed down-regulation of the anti-apoptotic gene BCL2L1 and the pro-proliferative gene CDC25A as underlying mechanisms. Overexpression of dnE47 in patient-derived glioma-initiating cell lines with high HLH and bHLH protein levels reduced sphere formation capacities and expression levels of Nestin, BCL2L1 and CDC25A. Finally, the in vivo induction of dnE47 expression in established xenografts prolonged survival. In conclusion, our data introduce a novel approach to jointly neutralize HLH and bHLH transcriptional networks activities, and identify these transcription factors as potential targets in glioma.
Stimulation of hERG (the human ether-à-go-go-related gene) Kv11.1 potassium channel activity by janus kinase 2

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hERG - the human Ether-à-go-go-related gene, Kv11.1 potassium channel is reported to be expressed in leukemic cells and leukemic stem cells and has been associated with establishing and maintaining cancer-like features in leukemic cells. Inhibition of hERG channel is reported to decreases cell proliferation of tumor cells and hERG channel plays significant role in cell proliferation and apoptosis. hERG are reported to be functionally up-regulated in neoplastic hematopoietic cells. Mechanisms up-regulating hERG in tumor cells and hematopoietic neoplasms remains ill-defined. Candidates include the Janus kinase-2 (JAK2), which is expressed in a variety of tumor cells where JAK2 over activity contributes to cell proliferation and cell survival. The JAK2V617F mutant is found in the majority of the philadelphia chromosome-negative myeloproliferative neoplasms, polycythemia vera, essential thrombocythaemia and myelofibrosis.

The present study thus explored whether JAK2 regulates hERG, Kv11.1 channel activity. To this end, hERG was expressed in Xenopus oocytes with or without wild type JAK2, V617FJAK2 or inactive K882EJAK2 and the K+ channel activity determined by dual electrode voltage clamp. Expression of hERG was followed by a marked increase of cell membrane conductance. The conductance was significantly increased following co-expression of JAK2 or V617FJAK2, but not by co-expression of K882EJAK2. Exposure of the oocytes expressing HERG together with V617FJAK2 to the JAK2 inhibitor AG490 (40 µM) resulted in a gradual decrease of the conductance. According to chemiluminescence JAK2 increased the channel protein abundance in the cell membrane. Incubation of K562 myelogenous leukemia cells with JAK2 inhibitors resulted in significant reduction in the hERG protein in the cell membrane as analysed by flow cytometry. In addition, hERG activity measured by patch-clamp experiments with incubation with JAK2 inhibitor. The decline of conductance in hERG and V617FJAK2 co-expressing oocytes following inhibition of channel protein insertion by brefeldin A (5 µM) was similar in oocytes expressing hERG with V617FJAK2 and oocytes expressing hERG alone, indicating that V617FJAK2 might slow channel protein insertion into rather than accelerating channel protein retrieval from the cell membrane. In conclusion, JAK2 stimulates hERG activity and thus stimulates K+ entry, an effect which may impact on cell proliferation and tumor cell growth in JAK2V617F myeloproliferative neoplasms and solid tumors involving aberrant hyperactivation of JAK2.

Keywords: JAK2V617F, leptin, erythropoietin, tumor, hERG, Kv11.1, myelofibrosis
Multi-parametric optical analysis of mitochondrial redox signals during neuronal physiology and pathology in vivo

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Redox signals play a central role in neuronal physiology and pathology. Here, we describe a new optical approach to measure fast mitochondrial redox signals with single organelle resolution in living mice that express genetically-encoded redox biosensors (Grx1-roGFP2) in their neuronal mitochondria (1). Disease models of spinal cord injury (acute neurodegeneration) and amyotrophic lateral sclerosis (chronic neurodegeneration) are employed. We demonstrate how parallel measurements with several additional biosensors to measure calcium (mito-RGECO-1, mito-GCaMP-3, TnXXL), pH (mito-SypHer) and potential (TMRM) can integrate these redox signals into a comprehensive characterization of mitochondrial function in physiology and disease. This approach revealed that axonal mitochondria undergo spontaneous reversible “contractions” that are accompanied by an oxidative burst. These contractions are amplified by neuronal activity and acute or chronic neuronal insults. Genetic and pharmacological manipulations reveal that contractions constitute respiratory chain-dependent episodes of mitochondrial depolarization coinciding with short matrix alkalization, followed by uncoupling and long lasting matrix acidification. In contrast, permanent mitochondrial damage after spinal cord injury is driven by axonal calcium influx and leads to mitochondrial permeability pore transitioning. Thus, our approach allows identifying a heterogeneity amongst physiological and pathological redox signals. Correlating such signals to functional and structural organelle and neuronal dynamics is demonstrated and underlying mechanisms are dissected (1). Moreover, this multi-parametric imaging approach could be extended to study cellular and organelle dynamics during other pathological states like neoplastic disease (2). Here, a better understanding of the neuroinflammatory milieu in the cancer microenvironment could be achieved.

Hematopoietic stem cells (HSC) harbor the highest self-renewal capacity and generate a series of multipotent progenitors (MPP) that differentiate into lineage-committed progenitors and subsequently mature cells. Despite intense research over the last decades the molecular basis of essential HSC features such as self-renewal and quiescence remains poorly understood. To determine the molecular programs employed by HSCs and MPPs, we performed an extensive global analysis combining quantitative proteome and transcriptome (RNA-seq) analyses on five FACS-sorted HSC and MPP populations – HSC (Lin<sup>−</sup> Sca-1<sup>+</sup> cKit<sup>+</sup>, LSK, CD34<sup>−</sup> Flt3<sup>−</sup> CD150<sup>+</sup> CD48<sup>−</sup>), MPP1 (LSK CD34<sup>+</sup> Flt3<sup>−</sup> CD150<sup>+</sup> CD48<sup>−</sup>), MPP2 (LSK CD34<sup>+</sup> Flt3<sup>−</sup> CD150<sup>+</sup> CD48<sup>+</sup>), MPP3 (LSK CD34<sup>+</sup> Flt3<sup>−</sup> CD150<sup>−</sup> CD48<sup>+</sup>) and MPP4 (LSK CD34<sup>+</sup> Flt3<sup>+</sup> CD150<sup>+</sup> CD48<sup>+</sup>), and MPP4 (LSK CD34<sup>+</sup> Flt3<sup>+</sup> CD150<sup>+</sup> CD48<sup>+</sup>) – as previously described in our laboratory (Wilson et al., Cell, 2008) and linked these to whole genome DNA methylation data. Proteomics and RNA-seq analyses identified more than 6,000 proteins and 27,000 genes demonstrating stage-specific expression clusters including Wnt and Lin28-Hmga signaling, the imprinted-gene-network, Hox genes, retinoic acid metabolism and an unexpected number of splice variants as regulatory modules installed in HSCs. Our data uncover differential expression landscapes of 493 transcription factors and 682 IncRNAs. Further, expression of Hox clusters and IncRNAs such as H19 during HSC differentiation are controlled by a progressive gain of methylation. Multipotency associated with a cell cycle/DNA repair signature identifies MPP2 as transient-amplifiers, while MPP3/4 show lineage commitment. This study provides a comprehensive genome-wide resource for functional exploration of the molecular, cellular and epigenetic processes operational at the pinnacle of the hematopoietic hierarchy.
Interaction of tumor cells with the hematopoietic stem and progenitor cell niche

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Background and Objectives: The bone marrow microenvironment, with mesenchymal stromal cells (MSC) as major cellular component supports self renewal and differentiation of hematopoietic stem and progenitor cells (HSPC). The microenvironment becomes site of choice for HSPCs because of chemoattractants, immobilised growth factors and adhesion ligands. The supportive functions of the niche could also be involved in its neoplastic transformation. Dissemination of breast cancer cells into the bone marrow has been described even in early stages of the disease. The study focuses on the influence of breast cancer cells on the genetic and functional profile of mesenchymal and hematopoietic progenitor cells within the bone marrow niche.

Methods: In vitro co-culture models of breast cancer cell lines – MDA-MB 231, MCF-7 and primary MSCs derived from bone marrow of healthy donors were used in the study. Hypoxic and normoxic culture conditions were used for the co-culture models.

Results: The breast cancer cell lines caused a significant reduction in HSPC adhesion to primary MSCs (88% by MDA-MB 231 cells; p<0.0005 and 73% by MCF-7 cells; p<0.0005). Atomic-force microscopy based single-cell force spectroscopy studies also indicated a higher binding force between tumor cells and MSCs as compared to HSPCs. MCF-7 and MDA-MB231 cells express ICAM1, which has been shown to promote breast cancer metastasis (Rosette et al., 2005). ICAM1 knockdown studies showed that the reduction in HSPC adhesion to MSCs by tumor cells was mediated through ICAM1 signaling. A cytokine array was performed to investigate if the breast cancer cell lines affect cytokine profile of MSCs. The array showed altered expression of growth factors and inflammatory molecules – bFGF, PDGF-BB (2.2 fold up-regulation and 0.5 fold downregulation in tumor co-cultures, respectively). Based on the cytokine array, a bFGF mediated increase in the proliferation of MSCs and breast cancer cells in co-culture was observed. The bFGF upregulation also caused an increased migration of tumor cells towards MSCs in a transwell migration assay. Interestingly, breast cancer cells caused a reduction in osteoblastic differentiation of MSCs by downregulation of PDGF-BB. Long-term co-cultures of tumor cells, HSPCs and MSCs showed a reduced support for primitive HSPCs in the neoplastic niche.

Conclusions: These findings indicate a perturbed HSC niche upon tumor invasion. The possible role of altered cytokine expression and consecutive downstream signaling in niche activation and bone turnover will be further studied using in vitro and in vivo approaches to recapitulate tumor micrometastases in the HSC niche.
Tissue homeostasis requires the coordination of stem cell divisions with the differentiation of stem cell progeny. If this fails stem-like cells can over-proliferate, predisposing the tissue to cancer development. But how a differentiation defect might initiate tumorigenesis is not so clear. According to current models differentiation-defective stem cells should remain dependent upon growth and survival factors in the stem cell niche, and require secondary mutations to initiate the run-away growth characteristic tumors. While the importance of immune cells, fibroblasts, and vasculature recruited to the tumor microenvironment is appreciated, how stem-derived tumor initiating cells interact with the stem cell niche prior to forming a new microenvironment is poorly understood. Here we investigate intestinal stem cell (ISC) tumors generated in *Drosophila* by suppressing Notch signaling, which blocks differentiation. These differentiation-defective cells require stress-induced divisions and an autocrine EGFR ligand (Spitz), during early tumor growth. After achieving a critical mass, the tumors induce apoptosis, JNK and YAP/Yki activity and cytokine (Upd2, 3) expression in surrounding enterocytes (EC). These paracrine signals, normally used within the niche to support regenerative growth, propel tumor growth without the need for secondary mutations in growth signaling pathways. We propose that niche appropriation by differentiation-defective stem cells may be a common mechanism of tumor initiation.
Chemokine-integrin crosstalk at the molecular resolution on lymphoma stem cells

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Chemokine-mediated integrin activation with rapid arrest on endothelium can easily be observed on lymphocytes using a flow chamber. Pioneering work with AFM resolved this adhesion at the single molecule level between two living cells. The author became the very first to 1) transfer his metastasis model of rolling B16 melanoma cells to molecular resolution, i.e. measuring the molecular forces of individual VLA-4/VCAM-1 adhesion receptors between two living cells; 2) to detect similar adhesion events between living lymphoma and either endothelial cells, or immobilized VCAM-1 fusion protein, respectively; and 3) to detect with his system the immediate activation of VLA-4 integrin receptors by a chemokine (CXCL12/SDF-1) at the single-molecule level and at the same time on a living cell. This very unique tool of measuring the activation state of metastasis-supporting cell adhesion receptors, as well as metastasis inhibiting repellent molecules will be evaluated for its use in the clinical management of metastatic solid tumors and leukemias.


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Exploring the PDAC-subtype-associated microenvironment in PDX models and patients

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Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive disease with dismal prognosis. Despite extensive research and the discovery of several promising drug candidates, little progress in PDAC treatment has been reported in the last years. Two facts can be behind these disappointing results. In one hand, although PDAC is still clinically treated as a single disease, three distinct molecular subtypes (classical, quasi-mesenchymal and exocrine) have been described based on expression profiling of microdissected epithelial tumor cells of PDAC samples. Interestingly, in vitro studies revealed differences in drug response of two of the proposed subtypes for which cell lines were available. These results highlight the importance of considering PDAC as a heterogeneous disease and point to the stratification of patients as a possible way to improve PDAC treatment response. An additional reason behind the limited efficacy of PDAC treatment might be the tumor microenvironment. PDAC is the solid tumor with the highest stromal content, which can account for up to 90% of the total tumor mass. The PDAC microenvironment is known to actively affect tumorigenesis and to impair drug delivery. Thus, rendering the PDAC microenvironment as an appealing therapeutic target to improve PDAC care.

We have developed a novel workflow to efficiently generate patient-derived orthotopic xenografts (PDX) and serum-free cell cultures from primary resected PDAC tumors. The established primary cell lines comprise for first time all three described PDAC subtypes. Additionally, when re-injected into immunodeficient mice, they generate xenografts with high pathological similarity to the original patient tumor, including a prominent stromal presence. To explore the differences in the microenvironment associated to the individual PDAC subtypes we have now generated gene expression profiles for the stroma of a number of xenografts from our PDX model representing all three subtypes.

Besides, RNA sequencing from different sub-populations isolated from fresh primary human PDAC tumors (as separated by fluorescent activated cell sorting according to surface markers) may reveal interesting interactions between the different tumor compartments. We have developed a set of immunohistochemical markers to identify the PDAC-subtypes that can be used in patient paraffin sections. Hence, the RNAseq data of the different tumor populations can be also easily studied in the context of the PDAC-subtypes.

We believe that these approaches will shed some light on how different stromal expression patterns are interconnected with different epithelial expression profiles and vice-versa, and that this information can be ultimately exploited for patient stratification and therapy.
Inhibition of Notch signaling releases neural stem cells’ tumorigenic potential

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Gliomas are aggressive brain cancers with limited therapeutic options and poor prognosis for patients. Although it remains controversial, neural stem cells in the postnatal brain are believed to be one origin of gliomas. The Notch signaling pathway is required for neural stem cell maintenance and, accordingly, promotes a self-renewing stem cell-like state in glioma cells. Therefore, Notch signaling is believed to be oncogenic in glioma. Here, we addressed the cell autonomous role of Notch signaling in glioma using conditional genetics in multiple mouse models and discovered an unexpected tumor suppressor function for Notch in the brain. Genetic deletion of core Notch pathway components shows that neither glioma initiation from neural stem cells nor tumor progression depend on Notch signaling activity. In contrast, Notch signaling inhibition accelerates platelet-derived growth factor-driven glioma growth in mice. Notch cooperates with known tumor suppressors to control cell proliferation, and loss of Notch signaling promotes a premalignant state in adult neural stem cells. We characterized the progression of Notch signaling-deficient cells towards transformation in vivo and performed genome wide analysis of downstream targets of Notch in neural stem cells and glioma cells that may be associated with increased tumor formation. Our findings uncover fundamental differences in the molecular requirements of normal neural stem cells versus glioma stem cells and reveal a novel Notch tumor suppressor function.
Aging is most apparent on the tissue level as a progressive decline of functional ability. Although a wide range of molecular mechanisms has been identified that are closely connected to cellular aging, it is rarely understood how such intra-cellular aging processes translate on the phenotypic level of the overall tissue. The distinct age-related increase in the incidence of hematopoietic diseases, such as myeloproliferative neoplasms and leukemias, has long been associated with alterations of hematopoietic tissue structure with age. Novel methods for cell fate analysis, such as the use of genetic barcodes, allow assessing the clonal architecture of hematopoiesis even in in-vivo situations. However, it is still a challenging problem to quantify clonal contributions over time and, therefore, to estimate and predict future developments.

We developed a simple mathematical model of a self-stabilizing hematopoietic stem cell population to generate a wide range of possible clonal developments, reproducing typical, experimentally and clinically observed scenarios. We use the resulting model scenarios to suggest and test a set of statistical measures that should allow for an interpretation and classification of relevant clonal dynamics. In particular, we apply machine-learning approaches to identify measures for the reliable classification of clearly distinguishable scenarios, such as the early distinction between normal and potentially pathological developments. We report on our results to which extent these measures are suitable to prospectively predict atypical developments.

Additionally to the insights into structural principles of age-related tissue remodeling, our effort to establish a reliable classification of pathological and non-pathological clonal dynamics has a direct potential for clinical applications. Leukemogenesis is a well-known and severe problem in gene therapy patients. Based on the tight post-therapy monitoring of clonal developments in these patients our identified measures and the resulting categorization can aggregate time course data and provide estimates for the risk of atypical clonal developments and predict the manifestation of leukemia.
SRC, a key regulator of the mesenchymal phenotype of glioblastoma stem-like cells as uncovered by RNAi screening

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Glioblastoma (GBM) is the most common primary brain tumor and among the deadliest human cancers with a median survival of patients after diagnosis of less than 15 months. Due to its location, aggressiveness and diffuse growth pattern, its therapy is tremendously challenging and no significant improvement in survival has been achieved over the past 30 years. Many patients do not respond to currently available drugs. This is thought to be mainly due to a small sub-population of resistant cells, the so-called glioblastoma stem-like cells (GSCs), that can continuously self-renew and regenerate the tumor. In addition, recent genomic, transcriptomic and methylation profiling studies have highlighted a greater level of heterogeneity between GBMs than previously thought. Based on the expression of cell surface markers, GSCs from the proneural and mesenchymal subtypes were able to be isolated. Interestingly, it was previously shown that proneural tumors give rise to mesenchymal recurrences, suggesting a proneural to mesenchymal transition (PMT). Although the genomic profiling of both subtypes is well defined, the mechanisms responsible for their plasticity are poorly understood.

Our goal was to identify survival and proliferative mechanisms that are specific to GSCs from one or the other subtype. In that respect, we have performed a lentiviral-based silencing screen targeting the complete human kinome. We have identified SRC as impairing the cell cycle specifically in mesenchymal GSCs upon silencing. In addition, knockdown of SRC led to a shift of the mesenchymal expression signature towards a more proneural profile, suggesting a major implication of the kinase in the PMT. We showed that besides no difference of SRC expression between both subtypes, major differences can be observed at the level of post-translational modification, reflecting a higher activity of SRC in the mesenchymal subtype. The level of phospho-SRC also correlated with poor prognosis of glioblastoma patients, suggesting that a SRC inhibitor could be used on a defined subset of glioblastoma patients, reinforcing the need of a robust patient classification to allow subgroup-targeted therapy.
Migratory behavior of a breast cancer cell line towards Mesenchymal stem cells

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Mesenchymal stem/stromal cells (MSC) migrate towards cancer cells in vivo, suggesting that engineered MSC populations could be used as vehicles to deliver “death-signals” or other therapeutics to cancer cells. Such targeted therapy could potentially overcome the dose-limiting toxicities associated with systemic treatment. We assessed the migratory behavior of MSC and cancer cell lines using a novel multichannel cell migration device to characterize and quantify migration of different cell types towards each other in response to their respective paracrine signals. In this migration device, independent cell cultures are established in opposing reservoirs, connecting via a series of microchannels, creating a buffer continuum between the two reservoirs, thus allowing the microchannel array to maintain a paracrine factor gradient between the reservoirs. The multichannel device then functions as a simple source-sink device. We used this device to study the migration of bone marrow-derived MSC and MCF-7 breast cancer epithelial cells towards each other. We observed that MCF-7 cells actively migrated either towards higher concentrations of FBS or towards MSC populations, but MSC did not actively migrate towards MCF-7 populations. Based on this result, in the context of existing literature, the active migration of MSC towards MCF-7 cells in vivo likely results from a cancer associated inflammatory response.
Impact of substrate extensibility on hematopoietic stem cells

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Hematopoietic stem cells (HSC) play an important role in the regeneration of the blood and the function of the immune system as they possess the ability to differentiate into every cell type of the blood. However, a systematic multiplication of HSC is not possible yet with today’s cell culturing methods. Up to now, the maintenance of the HSC properties is only possible in their natural environment in the bone marrow – the stem cell niche. There, the fate of the HSC is controlled by biochemical (cell-cell-contacts, ECM, soluble factors) as well as physical information (nanostructure, material properties).

Recent papers show the mechanosensitivity of stem cells – they react to the mechanical properties of their environment (Engler 2006, Huebsch 2010, Gilbert 2010, Lee-Thedieck 2012). The proliferation of undifferentiated HSCs can be enhanced on Tropoelastin coated surfaces (Holst 2010). The extensibility of Tropoelastin – the most elastic known biomaterial – was shown to be the critical factor. Though for biomolecules, it is hard to separate between the influence of mechanical properties and biological activity. Thus, we aim for synthetic molecules with tunable extensibility for a systematic study of its influence on HSCs.

Inspired by the natural protein Elastin, extensible peptids are developed with domains that unfold reversible under tension. The so called elastin-like polypeptides (ELP) possess a repeated amino acid sequence similar to Elastin. With the number of repeating units we plan to adjust the extensibility of the polypeptide. The ELPs are investigated by atomic force microscopy concerning the elasticity, surface roughness and adhesion. In the future, the effect of the ELPs will be compared to the positive influence of Tropoelastin on the proliferation of HSC in order to establish ELP as a new material for the cultivation of HSC.

Holst, J. et al., (2010), Substrate elasticity provides mechanical signals for the expansion of hematopoietic stem and progenitor cells. Nature Biotechnology, 28: 1123
Inflammation-driven fast-track differentiation of HSCs into the megakaryocytic lineage

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During an acute stress situation, such as infection, distinct mature cell types are heavily consumed in order to combat invading pathogens. These include blood platelets— the immediate progeny of megakaryocytes (Mks). The coordination of the fast restoration of normal platelet levels, and the role of hematopoietic stem cells (HSCs) in this regulatory process, remain poorly characterized. While a high expression of certain Mk transcripts in HSCs has been suggested to be indicative of a platelet-primed HSC subpopulation, the functional role of such “Mk lineage priming” is not well understood. Here, we investigated the Mk differentiation from HSCs during homeostasis and inflammation. Single-cell transcriptomics revealed a stochastic bimodal expression of Mk transcripts in HSCs. Upon commitment towards the Mk lineage, a subset of HSCs switch to a highly coordinated unimodal Mk transcript expression program. Mk protein translation from Mk transcripts was largely silenced during homeostasis in HSCs, as inferred by proteome-wide quantitative mass spectrometry and ribosome profiling. In contrast, transcripts were efficiently translated upon inflammatory signaling, resulting in a striking increase of Mk proteins. This inflammation-induced translation of Mk transcripts in HSCs was mainly instructed by a cell-autonomous, non-canonical type I interferon-mTOR crosstalk signaling. Enhanced Mk protein production was accompanied by a commitment of HSCs towards the Mk lineage as well as by indications of megakaryocytic maturation, such as appearance of alpha-granular structures, increased cell size and cell cycle induction.

Our data reveal a highly efficient mechanism of inflammation-driven Mk maturation from HSCs, permitting rapid platelet recovery, demonstrating a functional role of Mk lineage priming in HSCs.
Claudin 7 promotes the epithelial-mesenchymal transition in human colorectal cancer

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Summary: Cancer initiating cells (CIC) are characterized by a set of markers, which includes in colorectal cancer (CoCa) EpCAM (EpC). In tumor tissue, EpCAM is frequently associated with claudin7 (cld7) and there is evidence that cld7 contributes to the stem cell features of EpC. To support the hypothesis, cld7 was knocked down (kd) in two human CoCa lines, HT29 and SW948, highly expressing EpC and cld7.

Methods: Experiments were performed with the human CoCa lines HT29 and SW948. Cld7 was knocked down (HT29-cld7kd, SW948-cld7kd). CIC features were evaluated in vitro and in vivo according to standard protocols. For in vitro experiments SCID mice were injected either intravenously or subcutaneously.

Results and Discussion: HT29-cld7kd and SW948-cld7kd cells displayed strongly decreased anchorage independent growth and the capacity for holoclone, respectively, sphere formation was significantly reduced. Nonetheless, tumorigenicity was not severely altered, but cld7kd cells poorly metastasize. In line with this, the migratory activity was strongly impaired and cld7wt cell migration was inhibited by cld7-associated anti CD49c, but not by anti-EpC. Furthermore, in cld7kd cells fibronectin and vimentin expression was reduced and EMT-associated Snail, Slug and Twist were poorly recovered in the nucleus of cld7kd cells. These findings correlated with cld7 recruiting EpC towards glycolipid-enriched membrane fractions, where it becomes accessible to cleavage by TACE and presenilin. In fact, mesenchymal proteins and EMT-related transcription factor expression were downregulated in cld7kd cells. Finally, uptake of HT29wt and SW948wt exosomes by the cld7kd lines sufficed to restore relocalization of EMT transcription factors to the nucleus and to restore motility of HT29-cld7kd and SW948-cld7kd cells.

Conclusion: The colocalization of cld7 with EpC in CoCa promotes a shift towards EMT, which requires phosphorylated cld7 promoted EpC cleavage, which initiates upregulation of EMT genes and translocation of phosphorylated cld7-associated EMT proteins to the plasma membrane. Notably, EMT features of CoCIC can be transferred via exosomes to non-CIC cells.
Ectomesenchymal stem cells are comprised of a higher portion of osteo-committed cells than mesenchymal stem cells

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Background and Objective(s): An increased lifespan in industrialized countries and a risky lifestyle of the young generation creates a strong need for bone reconstruction. Stem cells are discussed as alternative to common treatment strategies. However all stem cells include a risk for tumor formation. The tumor risk is closely linked to the differentiation potential of the stem cells e.g. embryonic stem cells have a higher risk for tumor formation than mesenchymal stem cells. Recently we could show that ectomesenchymal stem cells derived from the head region of the body have a reduced capacity to differentiate towards the mesenchymal lineages as compared to mesenchymal stem cells from liposuction material of other body regions. These stem cells which are embryonically derived from the branchial arches can’t differentiate towards the adipogenic lineage. They seem to be more committed towards hard tissues. In this study we wanted to address the question if the reduced potential of ectomesenchymal stem cells is only limited to the adipogenic lineage or if the potential to differentiate to other known mesenchymal stem cell lineages is reduced as well.

Methods: Ectomesenchymal stem cells were isolated from dental follicles and bone chips from the jawbone, and mesenchymal stem cells were isolated from liposuction material from belly. Both stem cell types were differentiated towards the adipogenic and osteogenic lineage. To test a differentiation lineage of another germ layer the cells were also differentiated towards the endothelial cell lineage. The differentiation towards the endothelial cell lineage was confirmed by the functional tests of Dil-ac-LDL uptake and endothelial cell sprouting in Matrigel® assay.

Results and Conclusion: As expected, dental follicle-derived, jawbone-derived and fat tissue-derived stem cells could be differentiated towards osteoblasts confirmed by Alizarin Red S staining of extracellular calcium deposits. Interestingly, both ectomesenchymal stem cells showed less Oilred O stained lipid droplets and less Dil-ac-LDL uptake. In addition, they displayed a decreased sprouting behavior in Matrigel® assay as compared to mesenchymal stem cells. The higher amount of osteo-committed cells reduces the risk of none or false differentiated stem cells in future application for bone regeneration and replacement and might thus reduce the risk of tumor formation.
Revealing the transcriptional network of the hematopoietic stem cell self-renewal regulator fubp1

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To understand the regulatory mechanisms that control self-renewal and differentiation of hematopoietic stem cells (HSCs), it is important to identify the molecules involved and to analyze their particular functions in detail.

We identified the transcriptional regulator FUSE Binding Protein 1 (FUBP1) as an important factor required for HSC self-renewal. The functional impairment of Fubp1 in mice led to largely reduced numbers of long-term repopulating (LT-)HSCs in the fetal liver and to embryonic lethality at day E15.5. Competitive transplantation experiments with Fubp1-impaired fetal liver cells or adult Fubp1 knockdown (kd) LT-HSCs resulted in significantly lower engraftment in primary and almost no engraftment in secondary transplanted mice. However, all hematopoietic lineages were produced in the absence of FUBP1, establishing it as an HSC-specific self-renewal factor in fetal and adult hematopoiesis.

To elucidate the involvement of FUBP1 in the transcriptional network that controls the self-renewal of LT-HSCs, we are investigating the upstream regulation of Fubp1 as well as its downstream signaling. Ex vivo cultured Fubp1 kd LT-HSCs displayed prolonged generation times and increased apoptosis, leading to reduced cell expansion. Transcriptome profiling and quantitative real-time PCR confirmed previous findings of FUBP1 as a regulator of the proto-oncogene c-myc and the tumor suppressor gene p21. In addition, we identified FUBP1 as a putative repressor of the pro-apoptotic gene Noxa. Transcription factors with known functions in HSCs like PU.1, C/EBPα, C/EBPβ and GATA2 up-regulated the Fubp1 promoter activity in Luciferase reporter assays. ChIP experiments demonstrated the binding of TAL1/SCL within the Fubp1 promoter region. Knockdown and overexpression analyses as well as mutations of potential binding sites will show whether any of these factors is indeed involved in the regulation of Fubp1 expression in HSCs.

The ongoing studies about the role of FUBP1 in the regulation of HSC self-renewal will further our knowledge about the development and maintenance of the hematopoietic system.
The propensity to undergo fusion as a characteristic property of stem-like glioma cells

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According to the cancer stem cells concept, a distinctive population of tumour cells called cancer stem cells (CSCs) is primarily responsible for the maintenance of malignant potential in some human cancers including glioblastoma multiformae (GBM). Due to their unique properties as unlimited self-renewal capacity, augmented DNA repair and inherent cytotoxic resistance, CSCs are thought to be the most clinically relevant target for cancer therapy. Recently, a new concept for malignant brain tumours has emerged based on the identification of a distinct population of cells (also termed as Brain Tumour Initiating Cells, BTICs) that exhibit fundamental properties of CSCs. While the BTIC paradigm is rapidly gaining widespread acceptance, there is still considerable uncertainty concerning the origin and roles of a small population of stem-like glioma cells (GSCs) implicated in the maintenance and malignant progression of GBM. In particular, it remains unclear if the low representation of GSCs reflects their lower proliferative potential compared to the rapidly proliferating bulk or a distinct mode of cell multiplication. The main objective of this study was to investigate the modes of cell multiplication in GSCs. To address this question, we compared the rates of cell duplication across a panel of six GSC lines with the known tumorigenic potential. In addition, glioblastoma cell line U87-MG was used as a model for non-stem-like glioma cells. The rate of cell proliferation was determined by using the BrdU incorporation assay. Cell division was investigated by using live cell imaging. While no apparent correlation between cell division rate and tumour growth rate was found across the six lines analyzed, our investigations established that GSCs are capable of utilizing different modes of cell division. Our study provides first evidence that GSCs are capable of fusing in a cell division-dependent manner. Our results indicate that there is a direct relationship between the fusing propensity of GSCs and their tumour growth promoting potential. Based on our data we propose a model that reconciles the seemingly contradictory relationship between high proliferative activity of GSCs and their low-level representation in GBM. Our findings urge a reconsideration of some of the key assumptions of the BTIC paradigm and underscore the importance of cell fusion as the distinctive characteristic of GSCs.
Transcriptome-wide profiling and post-transcriptional analysis of hematopoietic stem/progenitor cells during myeloid commitment

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Hematopoietic stem cells are critical due to their life-long self-renewal activity and generate multipotent progenitors that differentiate into lineage-committed and subsequently mature cells. In this study, we present a transcriptome-wide (RNA-seq) analysis of ex vivo isolated mouse multipotent hematopoietic stem/progenitor cells (Lin<sup>−</sup> Sca-1<sup>+</sup> c-Kit<sup>+</sup>) and myeloid committed precursors (Lin<sup>−</sup> Sca-1<sup>−</sup> c-Kit<sup>+</sup>), which covers coding and non-coding RNAs. Our data display the dynamic transcriptional networks and identify a novel stem/progenitor signature, which is characterised by cell adhesion and immune response components such as kallikrein-related proteases. We present a global signature of 498 IncRNAs which are potential regulators of self-renewal and/or multipotency. By integrating the transcriptome data with our recently reported proteome data (Klimmeck et al., 2012), we found post-transcriptional regulation of metabolism and response to oxidative stress. Finally, our study identifies a surprisingly high number of protein isoforms generated by alternative splicing upon lineage commitment. In summary, this in-depth molecular analysis outlines the enormous complexity of gene expression of coding and non-coding RNAs as well as posttranscriptional regulation during the early differentiation steps of HSCs.

Adipogenesis-mediated endothelial cell migration is not regulated via slit2

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Background and Objectives: Obesity is a common metabolic disorder in which people gain excessive adipose tissue which in addition increases the risk for cancer. In cancer metastasis angiogenesis plays a major role. The first step in angiogenesis is endothelial sprouting and thus endothelial cell migration. A signal transduction pathway that influences endothelial cell migration is the SLIT ligand/Roundabout (ROBO) receptor pathway. Interestingly it has been published that the SLIT2/ROBO4 pathway inhibits blood vessel development in both, cancer metastasis and endothelial cell migration. However, the role of SLIT proteins in angiogenesis is contradictory, implicating them to be involved in both pro- and anti-angiogenesis. The objective of this study was to discover the correlation between obesity and angiogenesis with respect to SLIT2.

Methods: Liposuction-derived mesenchymal stem cells were differentiated towards the adipogenic lineage for four weeks. After each week both, RNA and the supernatant of the differentiating stem cells, the so called conditioned medium (CM) containing adipokines, was collected. To investigate the influence of CM on endothelial cells (EC), primary ECs were isolated from bovine artery and exposed to CM for 24h. Subsequently the migration behavior of these cells was assessed in an in vitro scratch assay. In addition SLIT2 expression was investigated during the differentiation process.

Results: Here we show that CM containing adipokines secreted during adipogenesis from differentiating mesenchymal stem cells increases EC migration. Interestingly the RNA level of SLIT2 is not altered during the differentiation process and the ECs themselves are negative for SLIT2.

Conclusion: SLIT proteins have been described to be involved in angiogenesis inducing the sprouting of new vessels from existing ones. Our data indicates that adipokines secreted during the development of fat tissue leads to an activation of ECs as demonstrated by increased migration. However, this effect is not influenced by the SLIT/ROBO signal transduction pathway. Thus the inhibiting effect of SLIT2/ROBO4 shown in cancer metastasis is lacking in this model mimicking the effect of obesity on ECs.
Characterization of genes related to cisplatin-resistance in ovarian carcinoma cell lines

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Objective: Ovarian Cancer is the 7th most common cancer in women in Germany and the five year survival is only 38%. Most patients show a response to chemotherapy; however 80% relapse with a drug resistant phenotype. DNA hypermethylation may play a critical role during this process. The aim of this study is to characterize functionally relevant genes which acquire CpG hypermethylation in their promoter regions during cisplatin-resistance development in ovarian cancer cell lines.

Methods: For identification of hypermethylated marker genes genome wide methylation data were obtained by CpG microarrays for cisplatin-sensitive and –resistant ovarian carcinoma cell pairs. These data were validated by methylation-specific PCR and qRT-PCR. Epigenetically regulated marker genes were cloned into pBK-CMV and stably transfected in cisplatin-resistant cells. Cell viability of parental and transfected cells under cisplatin treatment was measured via MTT assay.

Results: Microarray analysis identified 37 genes associated with hypermethylated CpG islands in resistant compared to sensitive cells from 3 cell lines. The expression levels of four candidate genes were concordantly reduced. These genes were cloned into pBK-CMV vector and stably transfected in resistant cells. The transfected cultures showed in part increased sensivity to cisplatin treatment. Analyses of single cell clones (n>10 of each culture) are ongoing and will elucidate the effect of gene overexpression.

Conclusion: The development of cisplatin resistance in ovarian cancer cell lines correlates with the hypermethylation of specific CpG islands. Concordant transcriptional repression for some of these genes points to a functional involvement. Ectopic expression of these genes showed promising effects related to cisplatin-resistance. Knock down analyses of these genes (shRNA) should give further insight in resistance development and the relationship to the cisplatin resistant phenotype.
CDK6 coordinates hematopoietic stem cell pool dynamics through control of quiescence exit kinetics

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Blood formation is governed by a heterogeneous hematopoietic stem cell (HSC) pool. This pool is composed of a reservoir of deeply quiescent HSC with the longest repopulation capacity and of hierarchically organized subsets with more limited self-renewal and increased cell cycle activity. Control of HSC cell cycle activity plays an essential role in ensuring that blood demand can be met without HSC exhaustion or malignancy development. However the molecular mechanisms that limit HSC cell cycle progression while still permitting HSC activation are unclear. In addition it is unknown how differential cell cycle regulation is achieved in distinct HSC subsets. Here we show that functionally distinct human long-term (LT-) and short term (ST-) HSC are equally quiescent but display different quiescence (G0) exit kinetics that are governed by distinct post-transcriptional maintenance of CDK6 protein levels. Whereas LT-HSC exist in a canonical quiescent state marked by the absence of CDK6, ST-HSC reside in a primed G0 state characterized by high CDK6 levels that confer faster G0 exit kinetics. Enforced CDK6 expression in LT-HSC increases their proliferation by accelerating their G0 exit kinetics to that of ST-HSC. This results in a competitive advantage over wild-type LT-HSC, leading to an expansion of lineage-balanced LT-HSC, rather than HSC exhaustion that typically results from increased HSC cycling. Computational modeling using the cell cycle parameters calculated in this study shows that independent control of G0 exit and cell cycle transit inherently limits the number of LT-HSC divisions and restores normal hematopoiesis more efficiently upon perturbation. Collectively, we identify CDK6 as a master regulator of the latency of cell cycle entry of human long-term (LT-) and short-term (ST-) HSC. Functionally, we show that the differential modulation of the kinetics of exit from quiescence within the HSC pool serves as an important additional level of control that governs HSC maintenance and preserves LT-HSC by minimizing their number of divisions. Finally, the ability to modify G0 exit kinetics of LT-HSC without impairing repopulation and differentiation capacity has important implications for clinical HSC expansion.
Conventional chemotherapy attacks the bulk of the tumor indiscriminately with the aim to kill tumor cells. The notion that some of these cells are cancer stem cells, which are quiescent, more resistant, able to self-renew and give rise to differentiated progeny, challenges classical treatments. Differentiation therapy on cancer stem cells was first mentioned in the 1980s. The idea to push a cell stuck in a mutagen induced proliferative state towards its “natural” differentiation is very attractive. It would make a killing-agent obsolete since the differentiated cell would remain in a stable, non-proliferative state. Unable to sustain tumor growth the cancer would deteriorate over time.

We hypothesized that we could target a signaling pathway to terminally differentiate breast cancer stem cells, to acquire either a luminal or myoepithelial fate. Differentiated cells would be limited in their self-renewal capacity and would not be able to form tumors in vivo. To test this hypothesis, we treated an established triple negative breast cancer cell line (MDA-MB-468)(5) with a lentiviral shRNA library to knockdown individual kinases, using well established Keratin immunohistochemistry to identify differentiated cells. We identified over 30 kinases that can influence cancer stem cell fate.

Here we present a novel role for ALPK1 and ERN1 both working through the same pathway to elicit a differentiation response in primary breast cancer stem cells upon inactivation. ERN1 and ALKP1 knockdown cells form fewer tumors than control cells. The tumors that do form grow slower and show a more epithelial differentiated pattern. This finding could set the stage for new cancer treatments by targeting cancer stem cells for differentiation.
The receptor for hyaluronan acid mediator motility (RHAMM, CD168) expression in EMT-like cancer cells is a predictor of tumor progression in colorectal cancer

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Background/aim: The receptor for hyaluronan acid mediator motility (RHAMM) is a marker of tumor progression and decreased overall survival in patients with different solid tumor types. The aim of the present study was to investigate the functional and clinical role of RHAMM in colorectal cancer (CRC) using in vitro and in vivo models and to validate the results on a cohort (n=200) of CRC patients with full clinico-pathological features.

Material and methods: Two established CRC cell lines HT29 and HT116 (mismatch-repair proficient and deficient) expressing RHAMM by flow-cytometry were selected for proliferation, invasion and migration assays. For the in vivo analysis tumor cells were injected in immune-deficient NOD/SCID mice. RHAMM expression was additionally analyzed by immunohistochemistry on 200 CRC whole tissue sections, with particular focus on the single, detached and aggressive EMT-like cells at the invasion front and correlated to clinicopathological data and survival.

Results: In vitro, RHAMM silenced HT29 and HT116 cell lines showed a significant decrease in invasiveness and migration compared to untreated cells. In vivo, a lower tumorigenicity of HT29 and HT116 cells silenced for RHAMM compared to untreated cells was reflected by smaller tumor volumes in the injected immune-deficient NOD/SCID mice. In the immunohistochemical analysis RHAMM expression in EMT-like cancer cells was associated with lymphatic invasion (p=0.0013), higher tumor grade (p=0.021) and worse survival (p=0.034).

Conclusion: In vitro and in vivo findings as well as results on clinical patient data support the notion that over-expression of RHAMM is involved in tumor progression in CRC and highlight a promising basis for a therapeutic approach.
Balanced G1 phase regulation is crucial for cell fate decisions in human hematopoietic stem and progenitor cells

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Functionality of hematopoietic stem cells (HSCs) is maintained by fine-tuned fate decisions between quiescence or proliferation that can result in either differentiation or self-renewal. It remains completely unknown whether the transit time through cell cycle phases itself influences HSC fate decisions. We aimed at testing whether a shortened G1 phase affects fate choice of human HSCs. We reasoned that a shortened G1 phase may provide insufficient opportunity for the delivery of differentiation-inducing signals and therefore, the stem cell ‘favors’ self-renewing over differentiating proliferation and, as a consequence, the stem cell compartment expands in vivo. Human HSCs that overexpress early and late G1 phase regulators cyclin-dependent kinase 4 (Cdk4)-CyclinD1 (4D) or Cdk2-CyclinE (2E), respectively, in fact change their cell cycle profile in vitro, with increased frequencies of cells present in the S/G2/M-phase of the cell cycle. After transplantation into appropriate mouse recipients, 4D-transduced human hematopoietic stem and progenitor cells (HSPCs) show a much greater repopulation capacity compared to cotransplanted nontransduced cells, suggesting that a manipulation of G1 phase progression by 4D promotes the expansion of human HSPCs. Consistently, we find that 4D overexpressing donor bone marrow cells isolated from recipient mice more than 20 weeks after transplantation contain increased frequencies of growth factor-responsive cells compared to controls. Further, 4D-transduced HSPCs engraft secondary recipient mice more efficiently, suggesting that 4D promotes the expansion of human HSPCs in the primary recipients. In contrast, overexpression of 2E, a manipulation resulting in more pronounced changes of the cell cycle profile of HSPCs, results in rapid loss of functional HSPCs due to direct toxicity. Thus our data suggests that a balanced transit time through G1 cell cycle phase is crucial for the fate of human HSPCs.
From comprehensive human endogenous retrovirus expression in tumors to detailed specific analyses in colorectal carcinomas and a human stem cell model

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Expression of the HERV-H family has been previously associated with colorectal carcinomas (CRC) and most recently also with stem cells. Albeit the discovery of these relationships the characterization of individual HERV-H loci reactivation remains poorly achieved to date.

We recently extended the list of expressed HERV candidates using an Affymetrix custom microarray. In this study, we sought to validate the microarray findings by characterizing deeply HERV-H reactivations in clinical samples of CRC and integrating expression profiles, molecular patterns as well as clinical data on the one hand and apply this same approach to a human stem cell model on the other hand.

CRC relevant HERV-H sequences were selected from previous microarray results; locus specific primers were designed and meticulously validated. Then qRT-PCR analyses were performed towards two well-defined clinical cohorts (n=139 pairs of tumor and adjacent normal colon tissue). Additional samples from liver metastasis (n=16) as well as paired samples of normal/tumoral liver tissue (n=3) were analyzed. Sensitivity and specificity values of HERV-H reactivations were assessed and associations with clinical status were investigated. Correlations between HERV-H expression and microsatellite instable tumors (p<0.0001) and the presence of tumor cells in lymph nodes (p=0.0006) – representing a more aggressive tumor type – were established. In addition, HERV-H expression was maintained in the metastases but was absent from the liver tissues (normal and tumoral). Using the same platform, HERV-H elements were found differentially expressed between human embryonic stem cell and differentiated cells induced following two independent pathways. The absence of overlap between the subset of HERV-H elements identified in CRC and stem cell conditions will be discussed.

These results shed new insights on the basic behavior of the HERV repertoire on the one hand and on the other hand may support the search for alternative markers of CRC and human stem cells.
A novel mechanism as a mediator of drug resistance in the exocrine-like pancreatic ductal adenocarcinoma (PDAC) subtype

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PDAC is a highly aggressive disease with dismal prognosis. Despite extensive research and the discovery of several drug candidates, little progress has been reported since the approval of gemcitabine and erlotinib. For a number of other carcinomas, tumor subclasses have been uncovered that allow the use of targeted therapies. The mutational landscape of PDAC is complex and heterogeneous, raising the question whether subclasses also exist in PDAC. Collisson et al. described three PDAC subtypes that were identified based on their gene-expression profiles: The classical, the quasi-mesenchymal and the exocrine-like subtype. However, not all subtypes could be identified in the previously available model systems. We have established a novel patient-derived model system that allows the analysis of these three human PDAC subtypes in vitro and in vivo. Hence, we provide a systematic workflow to propagate human PDAC in orthotopic xenografts and to derive tumor-initiating primary cell lines of all three PDAC subtypes. HNF1A and Keratin 81 were identified as markers for subtype stratification by immunohistochemistry. Application of this two-marker set on a 258 large patient cohort confirmed a predominantly non-overlapping staining and revealed a significant difference in overall survival across the three subtypes. Furthermore, a drug screen uncovered subtype-specific drug sensitivities towards gemcitabine, erlotinib and dasatinib. Notably, the exocrine-like subtype was resistant towards all compounds tested. Thus, we aimed to identify the underlying cause of the observed drug resistance. Gene set enrichment analysis (GSEA) revealed an enrichment of several signatures within this subtype suggesting a novel mechanism of drug resistance. Analysis by qRT-PCR and western blot demonstrated the enhanced expression of several genes mediating this mechanism in the exocrine-like subtype in vitro and in vivo. These findings led to the identification of a novel protein target central to this mechanism. Additionally, retrospective immunohistochemical analysis of a large patient cohort confirmed that this target is predominantly found in those patient tumors classified as exocrine-like. Hence, we hypothesized that the observed strong activation of this mechanism in the exocrine-like PDAC subtype could be responsible for the drug resistance observed in this subclass. In line with this, functional inhibition of this mechanism resulted in increased drug sensitivity in the exocrine-like subtype. Taken together, these observations suggest that this novel mechanism has a role in mediating drug resistance in a subset of PDAC patients. Hence, our findings may ultimately advance personalized treatment by applying novel marker-based patient selection strategies in combination with tailored drug use.
Detection of cancer stem cell markers in rhabdomyosarcoma cell lines

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Rhabdomyosarcomas (RMS) are malignant mesenchymal tumors of the soft tissue and represent the third most common extracranial pediatric tumor with incidence 5 to 8 new cases diagnosed each year per 1 million children younger than 15 years. At present, in addition to the commonly known stem cell markers (Oct3/4, Sox2, Nanog), special attention is paid to specific markers that can be used for detection of cancer stem cells (CSCs) in RMS.

CD133 plasma membrane glycoprotein and especially its glycosylated epitope termed AC133, ABCG2 transporter, nestin, a intermediate filament protein and aldehyde dehydrogenase (ALDH1A1) represent the most frequently discussed putative markers of CSCs in RMS. The main aim of our study was to analyze expression of these CSCs markers in five in-house rhabdomyosarcoma cell lines.

Five rhabdomyosarcoma cell lines (NSTS-8, NSTS-9, NSTS-11, NSTS-22 and NSTS-28) derived from tumor tissue samples of patients surgically treated for RMS. The histogenetic origin of these cell lines was verified using detection of specific myogenic factor MyoD1. Expression of all five putative markers mentioned above CD133, AC133, nestin, ABCG2 and ALDH1A1 was analyzed using indirect immunofluorescence. Furthermore, RT-PCR was used for detection of NANOG, Oct3/4, Sox2 and CD133, ALDH1A1 and ABCG2 expressions at transcriptional levels.

The expression of the all CSCs markers mentioned above was showed in all five rhabdomyosarcoma cell lines used in this study. The proportion of cells positive for specific marker in cell lines range from 30- to 50% for nestin, from 40- to 60% for CD133, from 40- to 50% for AC133, from 20- to 30% for ALDH1A1, and from 40- to 50% for ABCG2 in the examined cell lines. Immunofluorescence also showed differences in the intensity as well as in the intracellular localization of the signals. Similarly, RT-PCR also showed differences in expression of selected markers among these five cell lines.

Our findings suggest that a subpopulation of cells with CSCs phenotype is present also in RMS cell lines.

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Antitumor drugs apoptosis induction

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One of the mechanisms of antitumor drugs action is the initiation of apoptosis by CD95/Fas signaling pathways activation. Antitumor drugs do not induce apoptosis in the absence of CD95/Fas receptor. Previously, we have shown that liposomal form of antitumor drugs has a cytotoxic effect on melanoma cells in the absence of CD95/Fas receptor.

The aim of this work was to determine forms of apoptosis caused by Aranose liposomal form. In examination of the liposomal antitumor drugs active mechanism were used cell lines of human melanoma mel-Mtp, mel-MtpX, mel-Kor and mel Z. These cells were characterized by the CD95/Fas receptor expression with flow cytofluorometry method and using monoclonal CD95-antibodies and by the presence of mRNA CD95 receptor. Cell lines Mel-Kor expressed CD95 antigen, while all others were negative. Gene mRNA of CD95/Fas was absent in mel-Mtp and mel-MtpX cells and attended in mel-Z and mel-Kor cells. Thus, mel-Z cells have mRNA of CD95/Fas antigen, but does not express the CD95/Fas receptor.

The aranose action on these cell lines was compared by MTT-test. Mel-Kor cells were sensitive to the cytotoxic effect of aranose, and mel-Mtp, mel-MtpX and mel-Z cells were sustainable (P<0.05). Liposomal aranose provided a dose-dependent cytotoxic action on all investigated cell lines. Freeze-dried aranose induced early and late apoptosis in mel-Kor cells at a concentration of 450 mg/ml after 24 h monitoring, at concentration of 900 mg/ml – late apoptosis and necrosis.

Liposomal aranose at a concentration of 450 mg/ml induced late apoptosis in these cells and at concentration of 900 mg/ml – late apoptosis and necrosis. Freeze-dried aranose didn’t induce apoptosis on mel-Z, mel-Mtp, mel-MtpX cells. Liposomal aranose induced early and late apoptosis in dose-dependent manner in mel-Z, mel-mtp, mel-MtpX cells. Unlike sensitive Mel-Kor cells liposomal aranose did not cause necrosis.
A three dimensional (3D) perfusion bioreactor-based tissue model of colorectal cancer

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Background: In this study we addressed the suitability of a perfused bioreactor to sustain 3D colorectal cancer cell growth and to test established treatment regimens in an in-vitro setting as compared to 2D cell culture or xenografts using the same cell line.

Materials and methods: HT29 colorectal cell line was dynamically seeded and cultured within 3D collagen sponges using a perfusion bioreactor device for 5 days. Chemotherapeutic treatment with 5-FU or in combination with an anti-apoptotic inhibitor, ABT-199, was performed for up to 4 days. Similarly, 3D static, 2D cell cultures and xenografts were performed and used as controls. DNA measurements, histology and cytofluorimetry/gene expression were performed to assess cell numbers, tissue characterization and proliferation/apoptosis, respectively. 5FU and BCL-2 expression in CRC in patients undergoing neo-adjuvant treatment prior to surgery were comparatively analysed.

Results: Upon perfusion, homogeneous seeding on scaffolds was obtained and significantly higher numbers of tumour cells were recovered, as compared to static cultures (13.7-fold increase). As compared to 2D-cultures, 3D-perfused constructs were characterized by tissue-like structures with heterogeneous pattern of proliferating and apoptotic cells and expression of CDX2 colorectal tumour marker, closely resembling xenografts’ tissue. 2-days treatment with clinically relevant concentrations of 5-FU had no effect in 3D-perfusion or in xenografts constructs, in contrast to a 55.8% inhibition in 2D cultures. The fraction of Ki67- cells was increased after a 2-days treatment in the 3D perfused and xenografts, but it reached again levels similar to untreated cells after 4 days. On the other hand, living cells in 2D cultures remained largely Ki67+. Importantly, in perfused cultures we could only observe a marginal effect on the expression of several apoptosis resistance genes, as compared to a significant down regulation of their expression in 2D and 3D static conditions. Interestingly, the combination of ABT-199 and 5-FU induced additional cytostatic and cytotoxic effects in 3D-perfusion but not in 2D cell cultures (83.3% inhibition vs. 39.8% p=0.003). Closely matching our data with perfused 3D cultures, we found that partially responsive (Dworak 2) cancers typically (10/11) express BCL-2, as compared with 0/3 highly (Dworak 3-4) responsive and 4/15 fully resistant CRC (Dworak 0/1, p=0.03) tumors.

Conclusions: Our data consistently indicate that 3D culture in perfused bioreactors efficiently mimic phenotypic and functional features observed in animal models and clinical specimens. These in vitro models will be of critical translational relevance to address fundamental issues in human tumor cell biology with diagnostic potential.
Investigating niche dependancy of early and late stem cell-derived tumors of the fly adult intestine

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The complex issue of how the microenvironment interacts with tumor cells to propel tumor progression is still not well understood. Here, we utilize a Drosophila intestinal stem cell (ISC)-derived tumor system as a tool to decipher signalling cues governing tumor and niche interactions during tumor progression. Previously, it has been shown that deficient Notch signalling in ISC lineages results in tumorous growth. Recent data from our lab strongly suggest that during early steps of tumor initiation, ISC-derived tumor cells secrete EGF ligands thereby activating EGF/MAPK signalling autonomously to promote their own growth. However, after achieving a critical mass, tumor cells stimulate neighboring cells (dying absorptive enterocytes) to secrete cytokines to additionally promote tumor outgrowth suggesting the requirement of a niche for tumor progression. Additionally, we found that Notch-deficient tumors fragments fail to progress to large tumor masses following transplantation. In contrast, we discovered that transplanted Notch deficient ISC-derived tumor cells expressing oncogenic RasV12 can grow outside their original stem cell niche. RasV12 transformation allows for transcriptional up-regulation of niche-associated factors such as EGF ligands, cytokines and JAK/STAT targets indicating that Notch-deficient tumors became niche-independent. Interestingly, transplanted Notch deficient RasV12 tumor cells seem to recruit a new microenvironment after progressing further. This involves the recruitment of trachea, which supply oxygen, indicating that ISC-derived tumor cells allocate factors to promote their growth. Intriguingly, we also observed a colonization of distal tissues. Future studies could reveal how late progressed and colonized ISC-derived tumors recruit factors or cell types to set up a novel niche.
Identification of novel therapeutics for glioblastoma from fungal natural products

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Glioblastoma is the most common and aggressive primary tumour in adults. The median survival of patients after diagnosis is around 15 months, which is the worst prognosis of all cancers. Much evidence has been gathered in the last decade to reveal a small sub-population of glioblastoma cells that are capable of self-renewal and tumour initiation. These are known as glioblastoma stem-like cells (GSCs). GSCs have been shown to be particularly drug resistant and can migrate; in this way they are thought to be responsible for patient relapse. Targeting these cells should cause the tumour to lose its ability to generate new cells, leading to complete tumour degeneration.

Natural products have historically formed the basis of medicine; indeed roughly 60% of therapeutics in oncology from the last 30 years are based on natural products. In particular, filamentous fungi produce a wealth of bioactive secondary metabolites, making the fungal kingdom an interesting hunting ground for potential lead compounds. The Technical University of Denmark (DTU) has an enormous fungal library, comprising land and marine fungi from all over the world. With the aim of identifying substances which induce apoptosis in GSCs, a screen of fractionated fungal extracts from this library was carried out on patient derived GSCs in culture, and cytotoxicity monitored. Extracts causing reduced cell viability were tested for cancer specificity and specific extracts further fractionated into semi-pure compounds. Dereplication has led to the identification of a certain class of compounds which are highly active against GSCs, but not against other normal cell types in vitro. The mechanism of action of this class of compounds is currently being investigated in vitro. Toxicity will be tested in vivo, with the aim of determining a suitable dose for experiments using a xenograft mouse model, in order to investigate whether the identified compound affects tumour development in vivo.
The role of the bone marrow stem cell niche in IFNα induced activation of hematopoietic stem cells

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The bone marrow hematopoietic-stem-cell niche is a cellular and molecular microenvironment in which hematopoietic stem cells (HSCs) reside in states of both quiescence and homeostasis. The niche regulates stem cell function, influences stem cell fate and is also responsible for the engagement of specific programs in response to stress, such as infection or inflammation. The primary response to infection involves synthesis of immune-modulatory interferon cytokines, such as interferon (IFN). We, and others, have clearly shown that in contrast to the anti-proliferative effect of IFNα on HSCs in vitro, in vivo, IFNα induces cell cycle entry of even the most dormant population of HSCs. Given the contrasting outcome of in vitro and in vivo exposure of HSCs to IFNα, it is probable that niche cells and molecular maintenance signals from the niche are required for activation. However, a comprehensive study has not been carried out on the role of the niche in the switch of HSCs into cell cycle by IFNα, or on the direct effect of IFNα on the niche. We can clearly show that interferon signaling on the niche side is not required for HSC activation. However, different niche components do respond to stimulation. A redistribution of niche cells in vivo following IFNα can be seen by staining these cells using specific antibodies in femur sections. We are currently further clarifying the role of the niche in the activation of hematopoietic stem cells, as well as the effect of stress on the stem cell niche.
Continuous de novo generation of human myeloid cells in a novel mouse model of human hematopoiesis

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Humanized mice represent a valuable tool to study human hematopoiesis and immune cell function. However, in currently available mouse models differentiation of human myeloid cell types is limited. To improve human myeloid cell engraftment we aimed at improving stable stem cell engraftment, which is the prerequisite for continuous myeloid cell differentiation. Further, myeloid progenitor cell expansion depends on Kit-mediated signaling. Thus, we hypothesized that introduction of a defective Kit receptor into mice would provide human Kit-proficient stem and progenitor cells an advantage over murine loss-of-function Kit-mutant stem and progenitor cells. Our novel mouse strain, NOD/SCID Il2rg-/- (NSG) KitW41/W41 (NSGW41) has a functionally impaired HSC compartment and therefore supports high and stable engraftment of human HSCs in the long-term without the need for previous irradiation. Transplanting limited numbers of human HSCs showed expansion of phenotypic human HSCs in these mice. Engrafted HSCs give rise to cells of the lymphoid, myeloid and erythroid lineages. Particularly, we observe improved reconstitution of myeloid cell types, even without addition of human growth factors specifically promoting differentiation of human myeloid lineages. Robust numbers of myeloid cells are found in the bone marrow and spleen of transplanted mice. We conclude that NSGW41 mice provide a new enhanced tool to study human HSC function including self-renewal and differentiation but also mechanisms of innate immunity.
The purinergic receptor P1A3 enhances the osteogenic differentiation

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Background and objective: Adenosine receptors, a conserved group of G-protein coupled receptors, are involved in the stem cell differentiation towards various lineages. Additionally, they have been found to play a crucial role in the growth inhibition of diverse cancer cell types. Especially the adenosine receptor subtype P1A3 has turned out to be a major influencing factor in these processes. An increased proliferation as found in cancer cells is generally accompanied by a decreasing differentiation. Since P1A3 is inhibiting cell proliferation we asked ourselves the question if P1A3 might play a role in stem cell differentiation. In this study, we therefore investigated the effects of P1A3 on the differentiation potential of human mesenchymal stem cells towards the osteogenic and adipogenic lineage.

Methods: Human mesenchymal stem cells were isolated, characterized and differentiated towards the osteogenic and adipogenic lineage. The P1A3 expression profile was analyzed on the RNA as well as on the protein level via semi-quantitative PCR and Western blot. Additionally, the functional role of the P1A3 receptor was investigated with a highly specific artificial P1A3 agonist and antagonist, respectively.

Results and conclusion: We could show that P1A3 plays a key role in determining MSC fate favoring the osteogenic and adipogenic over the endothelial and smooth muscle cell lineages. The functional role of this receptor was proven using a specific artificial agonist and antagonist. In addition, the specific agonist increased the osteogenic differentiation of MSCs. Since undifferentiated stem cells contain the risk of cancer development, the use of the P1A3 mechanism might improve the potential application of MSCs differentiated towards osteoblasts for future regenerative medicine approaches by reducing the risk of tumor formation.
Embryonal tumor with abundant neuropil and true rosettes (ETANTR, also known as ETMR) is a rare and highly aggressive embryonal CNS tumor and was first reported in 2000. The World Health Organization (WHO) defines ETANTR as a possibly unique variant of primitive neuroectodermal tumors (PNET). This tumor occurs primarily in infants and young children with a poor therapeutic outcome of < 1 year despite aggressive treatment. The histopathology combines features of CNS neuroblastoma and ependymoblastoma with a mixture of highly undifferentiated small cell areas. On the molecular level most ETANTR cases show focal amplification of the miRNA cluster 19q13.42 and high expression of the RNA-binding proteins LIN28A and LIN28B in almost all cases. Expression profiling of various CNS tumor entities identified LIN28A to be exclusively overexpressed in ETANTRs, making it a useful marker for diagnosis in the clinic. We hypothesize that LIN28A (and LIN28B) contribute to the aggressive outcome and histopathological features of ETANTRs and might be a potential target for future therapy. Therefore we are studying the role of LIN28A and LIN28B in ETANTR tumor development in different in vivo mouse models. With the primary human ETANTR cell line BT183 we are performing xenograft tumor injections into the brain of NOD/SCID mice. We are downregulating LIN28A and LIN28B expression via lentiviral transduction of shRNAs in these cells and study the changes in tumor growth and pathology in vivo. To test the oncogenic capacity of LIN28A and LIN28B we are currently establishing a mouse allograft model where we want to induce overexpression of LIN28A and LIN28B in combination with dominant-negative TP53 in primary mouse brain stem cells. Furthermore to study the role of LIN28A and LIN28B function during embroyogenesis we are currently performing in utero electroporation experiments to induce overexpression of LIN28A and LIN28B in combination with dominant-negative TP53 at embryonal stage E13.5 in different regions of the mouse brain. With all these mouse models we hope to understand the function of LIN28A and LIN28B in normal and tumor development and to identify better or even novel drug targets.
Lysine-specific demethylase 1 regulates differentiation onset and migration of trophoblast stem cells

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Propagation and differentiation of stem cell populations are tightly regulated to provide sufficient cell numbers for tissue formation while maintaining the stem cell pool. Embryonic parts of the mammalian placenta are generated from differentiating trophoblast stem cells (TSCs) invading the maternal decidua. Here we demonstrate that lysine-specific demethylase 1 (Lsd1) regulates differentiation onset of TSCs. Deletion of Lsd1 in mice results in the reduction of TSC number, diminished formation of trophectoderm tissues and early embryonic lethality. Lsd1-deficient TSCs display features of differentiation initiation, including alterations of cell morphology, and increased migration and invasion. We show that increased TSC motility is mediated by the premature expression of the transcription factor Ovol2 that is directly repressed by Lsd1 in undifferentiated cells. In summary, our data demonstrate that the epigenetic modifier Lsd1 functions as a gatekeeper for the differentiation onset of TSCs, whereby differentiation-associated cell migration is controlled by the transcription factor Ovol2.

Loss of Myc activity induces cellular dormancy in ES cells cultured in 2i mimicking the status of diapause embryos

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Mouse embryonic stem cells (ESCs) cultured in medium containing fetal calf serum (FCS) plus leukemia inhibitory factor (LIF) are metastable. Substitution of FCS by two inhibitors (2i) of the MAPK and GSK3b pathways reveals ESCs that more closely resemble the naïve state of pluripotency of the inner cell mass of the blastocyst. To genetically address the role of c-Myc and N-Myc in naïve ESCs, both genes were deleted using a Cre/loxP approach. Myc-dKO cells exit the cycle, arrest in a G0-G1 phase without signs of apoptosis and form smaller colonies remaining undifferentiated with unchanged expression of Oct4, Nanog and Sox2. Whole transcriptome analysis (RNA-seq) confirmed the expression of the core pluripotency network but revealed down-regulation of all metabolic and biosynthetic aspects of cellular physiology in dKO ESCs (cell cycle activity, DNA replication, ribosomal biogenesis and DNA/protein synthesis). The cellular and molecular phenotype of Myc-dKO ESCs is consistent with a status of “biosynthetic dormancy”. Strikingly, the signature of dKO ESCs is remarkably similar to an expression signature previously reported for diapause arrested pre-implantation embryos (Hamatani et al., 2004). In mice, diapause of early embryos is i.e. observed in mothers still feeding a litter and is critical for the isolation of ESCs. Very low expression of networks such as DNA synthesis, cell division, metabolic activity but high activity of the IGF pathway is observed in both diapause embryos and Myc-dKO ESCs. In summary, our data identify c/N-Myc activity as a key element controlling the entire biosynthetic and proliferative machinery of naïve ESCs without affecting the pluripotency network, thus separating the biological process of self-renewal into two distinctly regulated networks. Moreover, our data raise the possibility that Myc controls the reversible arrest of diapause embryos, suggesting that Myc regulates the spectrum of overall cellular activity ranging form dormancy to unleashed metastatic growth.
Detection of putative cancer stem cells markers in pancreatic ductal adeno-carcinomas

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Pancreatic ductal adenocarcinoma (PDAC) is highly lethal malignancy that represents the fourth most frequent cause of cancer-related deaths. The dismal prognosis of PDAC is mainly due to its late diagnosis often accompanied by metastatic disease and high chemo- and radio-therapy resistance of the primary tumour. Using detection of various individual markers, several research groups identified in PDAC a subpopulation of cancer stem cells (CSCs). Therefore, therapy approaches targeting these cells might be promising for the treatment of PDAC. Nevertheless, the specific combination of markers, which is characteristic for PDAC CSCs, is currently still uncertain.

In this study we focused on the detection of putative CSCs markers in three cell lines derived from PDAC tissue – named P6B, P28B and P34B. Expression of CD24, CD44, ESA (EpCAM), CD133 and nestin was evaluated using immunohistochemistry in the respective tumor samples, from which the cell lines were derived, and by indirect immunofluorescence, flow cytometry, immunoblotting and semiquantitative RT-PCR in the cell lines. At the protein levels, we detected the expression of all these CSCs markers in all three PDAC tumor samples and cell lines, although the cell positivity for each antigen varied among them. In cell lines, RT-PCR confirmed the expression of CSCs markers at the mRNA levels and revealed interesting expression pattern that was reflected also at the protein levels. While expression of CD24 was elevated in P28B cells, P6B and P34B cell lines expressed more CD133 and nestin in comparison with P28B cell line. In order to address possible differences in expression pattern between the cell lines, expression profiling was employed.

Taken together, we confirmed the expression of all five putative CSCs markers in all three PDAC cell lines as well as in the respective tumor tissues. Therefore, our further aim is to assess the overlap of expression of the individual CSCs markers using multicolour immunodetection and to perform functional assays on isolated subpopulation to validate their CSCs characteristics.

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The role of Matrilin-4 in stress-induced HSC activation and homeostasis

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The life-long maintenance of the blood system is accomplished by a pool of self-renewing and pluripotent hematopoietic stem cells (HSCs). Adult HSCs are found in a dormant state for most of their lifetime, entering cell cycle only to maintain homeostatic blood supply. This balance between dormancy and activation is controlled by external factors such as chemokines and cytokines as well as the interaction of HSCs with cells of the stem cell niche. Under stress conditions such as chemotherapy or bleeding, HSCs are stimulated to proliferate and give rise to various specialized blood and immune cells. The molecular mechanisms underlying the processes of activation of HSCs are still largely unknown.

Our group has previously shown that HSCs proliferation can be induced by in vivo IFNα treatment of mice. This response is dependent on signaling via the IFNα receptor (IFNAR) and STAT1 leading to downstream induction of IFNα target gene expression (Essers et al., 2009). To gain insight into the mechanism of activation, we compared the transcriptional response of HSCs of C57Bl/6 wildtype treated with PBS or IFNα by microarray expression analysis. Interestingly, we discovered several cell cycle inhibitors, such as p57 to be down regulated upon IFNα treatment, which might enable the cells to exit G₀ and start cycling. Even more striking is the downregulation of the extracellular matrix protein Matrilin-4. Matrilin-4 is a member of the von Willebrand factor A-containing family of extracellular adapter proteins, which form filamentous structures outside of cells. We found Matrilin-4 to be highly expressed in long-term HSCs compared to short-term HSCs or committed progenitors during homeostasis and it is almost completely depleted upon in vivo treatment with IFNα or other inflammatory cytokines. It is therefore possible that Matrilin-4 plays an essential role in niche remodeling following stress. Here we present our data on the investigation of the function of Matrilin-4 in HSC activation and homeostasis using KO mice and in vivo retroviral overexpression.
c-Myc expression in hematopoietic stem and progenitor cells is driven by a novel enhancer region 1.7Mb downstream of the coding region

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The c-Myc transcription factor is a central regulator of cellular proliferation, growth, metabolism and differentiation in many cell types including stem cells. Although it is known that c-myc expression is tightly controlled and can drive transformation if de-regulated, the mechanisms of its transcriptional regulation remain elusive. Here, we identified a cluster of enhancer-associated chromatin marks 1.7 Mb downstream of the mouse c-myc gene, present only in hematopoietic tissues. A LacZ reporter gene inserted next to this cluster showed specific expression in hematopoietic stem and progenitor cells (HSPCs). Mice homozygous for a deletion of this enhancer region presented with almost no myeloid and B cells, while HSPCs and megakaryocytes accumulated in the bone marrow, thereby closely mimicking the phenotype of mice in which the c-myc gene was conditionally deleted using Mx-Cre (1). Deletion of this enhancer region led to a dramatic reduction of c-myc expression in HSPCs. Importantly, compound heterozygous mice carrying one enhancer deletion allele and one c-myc null allele (deletion of the coding region) displayed a phenotype highly similar to the conditional knockout. Altogether, these data provide genetic evidence that this enhancer region directly controls, in cis, c-myc expression in HSPCs. Analysis of enhancer-associated H3K27ac marks by ChIP revealed the presence of individual modules within this enhancer which contribute differently to c-myc expression in either HSPCs or granulocytes. Strikingly, the enhancer region is highly conserved in human and focally amplified in a number of AML patients, suggesting that it may be a critical component driving high c-MYC expression in human leukemias. In summary, we identified a distant hematopoietic-specific enhancer region for c-myc and provide genetic data for its critical function as a key regulatory region in normal hematopoiesis and likely leukemia.

Stable human hematopoietic stem cell engraftment in mice supports sustained de novo generation of mature human blood cells

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To determine molecular control mechanisms regulating human hematopoietic stem cell function a surrogate environment that supports stem cell maintenance is necessary. We generated a novel mouse strain, Rag2−/− Il2rg−/− KitWv/Wv mice that carry a defective Kit receptor rendering endogenous mouse HSCs functionally impaired. We find that the mutant Kit receptor opens up the stem cell niches across species barriers and allows for robust and sustained engraftment of human HSCs after transfer into adult mice without the necessity for irradiation conditioning prior transplantation. Following stable engraftment in the mouse bone marrow niches, human HSCs give rise to lymphoid cells and to robust numbers of erythroid and myeloid lineage cells over long periods of time in primary and secondary recipient mice. Therefore, Kit-signaling regulates the competition between mouse hematopoietic stem and progenitor cells by a xenogenic blood stem cell graft.
Adipogenic-induced endothelial cell migration is linked to HOXC8 induction

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Background and objective: Among others, obesity can increase the risk of tumor formation. In various tumors HOXC8 was shown to be up-regulated, e.g. in breast cancer cells it was shown that HOXC8 is a key molecule in increased migration and metastasis. HOXC8 does so by binding to the cadherin-11 promoter. The increased cadherin-11 expression causes Rac1 up-regulation which rearranges the cytoskeleton. This rearrangement was shown to promote migration. We have shown recently that adipokines secreted during adipogenic differentiation promote endothelial migration. The aim of this study was to investigate the molecular basis of endothelial cell migration evoked via adipogenic conditioned medium with respect to HOXC8 expression.

Method: Bovine aortic endothelial cells (BAEC) were isolated from collagenase digested aortas. Their endothelial character was analyzed by specific markers such as PECAM-1. Scratch assays have been performed to investigate the migration of the endothelial cells under the influence of conditioned medium collected during adipogenesis. The migrated endothelial cells were compared to normally expanded BAEC for their HOXC8 gene expression using semi-quantitative PCR.

Results and conclusion: The expression of HOXC8 was up-regulated in migrating cells compared to normally expanded BAECs. Our data suggest a similarity between HOXC8 mediated migration of breast cell cancer cells and the migration of endothelial cells induced by adipokines secreted during adipogenic differentiation. Further investigations have to be performed to determine if the signaling pathway of HOXC8 is the same as in breast cancer cell migration.
Building of nanostructured surfaces to mimic the hematopoietic stem cell niche

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Hematopoietic stem cells (HSCs) are able to continuously form all types of blood cells. The transplantation of HSCs is increasingly gaining importance to treat a range of diseases. When using alternative sources for transplantable HSCs such as umbilical cord blood, the desired cell amount is frequently not sufficient to treat adult patients and at present there is a deficiency of methods and technologies to proliferate HSCs outside of their natural microenvironment – their niche – without loss of stem cell properties. In adults HSCs are located in their niche in the bone marrow where they maintain the ability to self-renew, because of the complex blend of different factors within this microenvironment. Besides soluble factors (such as cytokines) cellular and extracellular components, physical parameters including matrix stiffness and nanostructure play a role in the maintenance of HSCs. The aim of the presented project is to develop artificial nanostructured surfaces with combinatorial signal mixtures to mimic this aspect of the HSC niche. Therefore PEG-hydrogels with gold nanoparticles in an ordered structure with various distances is used to link different molecules of the niche and to study their influence on the HSC behavior. Human hematopoietic stem and progenitor cells, which were isolated from cord blood, are applied to nanopatterned, bio-functionalized gold nanoparticle PEG-hydrogel surfaces. We immobilized delta-like protein 1 (DLL1), a ligand triggering the notch pathway, to the gold nanoparticles and co-polymerized the adhesive peptide RGD homogeneously into the hydrogel. We could show that stimulation of HSC expansion by notch signaling depends on the nanoscale distance between presented DLL1 ligands. At smaller interligand distances around 20 nm HSC proliferation was more pronounced than on 80 nm spaced or homogeneously coated surfaces. This first result leads to the conclusion that the ligand density in the nanometer range is an important factor for HSC expansion and that notch signaling depicts a positive incentive. Therefore, more ligand pairs, their proportions and various distances will be evaluated with respect to their potential to foster HSC expansion.
Higher CD97 expression in leukemic stem and progenitor cells is associated with FLT3-ITD mutation

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**Background:** Normal hematopoietic stem cells (HSCs) and leukemia stem cells (LSCs) in acute myeloid leukemia (AML) are presumed to reside in specific niches in the bone marrow microenvironment. Targeting the niche is a promising strategy to eliminate persistent and drug-resistant LSCs. CD97 is the founding member of the EGF-TM7 molecule family, a subgroup of adhesion GPCRs, which are known to be expressed in lymphoid and myeloid cells but nothing is known about the expression and regulation in normal and malignant HSCs. Here, we investigated a possible implication of CD97 in AML and an association to clinically important mutations.

**Methods:** Different AML cell lines, MV4-11, MOLM-13, THP-1, EOL-1 and OCI-AML, were used to study CD97 mRNA by real-time PCR and protein expression by flow cytometry or Western blot. CD34+ HSPCs were isolated from apheresis of healthy donors. Moreover, 292 samples from patients with de novo acute leukemia were investigated using a 4-color immunophenotypic measurement. In addition, FLT3-ITD mutations and NPM1-mutations were detected.

**Results:** A quantitative real-time PCR analysis of AML cell lines with or without FLT3-ITD mutation revealed higher CD97 mRNA levels in cells carrying this mutation, as MV4-11, THP-1 and MOLM-13. Moreover, also OCI-AML3 cells which express wildtype FLT3 but mutated NPM1 showed a clearly higher CD97 expression than normal CD34+ HSPCs or EOL-1 cells, respectively. This result was confirmed at the protein level by flow cytometry. Treatment of MV4-11 cells with 0.5µM of the small molecule inhibitor PKC412 significantly decreased the CD97 expression. Another more specific FLT3 inhibitor SU5614 resulted in an even more decreased CD97 expression. In contrast, the low CD97 expression levels in EOL-1 cells and normal HSPCs were not affected by these inhibitors.

Transient transfection of CD97 siRNA resulted in reduction to 40% in expression and subsequently in inhibition of spontaneous migratory capacity of MV4-11 cells as well as of adhesion to a stromal layer. Our *in vitro* results were confirmed in a study of *de novo* AML patient samples. Elevated CD97 expression was associated with mutations in NPM1 (44% vs. 19%, p=0.002) and FLT3 genes (43% vs. 10%, p<0.001) as well as lower CD34 expression (52% vs. 83%, p<0.001).

**Conclusion:** We show for the first time that CD97 expression levels in malignant hematopoietic cells correlate with FLT3-ITD mutation. This mechanism may be used by leukemic cells to target and modulate the bone marrow niche and may serve as potential diagnostical or therapeutical target in AML.
Clonal heterogeneity within the exocrine pancreas

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The most abundant cell type in the mammalian pancreas are the acinar cells accounting for ~ 80% of all pancreatic cells. According to data from mouse models this cell type is the origin for the most common type of pancreatic cancer, pancreatic ductal adenocarcinoma (PDAC). Interestingly not all acinar cells transform to preneoplastic lesions upon KrasG12D expression indicating heterogeneity of the acinar cell compartment. This notion led to the hypothesis that there might be previously neglected hierarchy within the acinar cell compartment since stem/progenitor cells were proposed to be the cell of origin for many tumors. Here we use multicolor lineage tracing to study the clonal contribution of cells within the acinar cell population. We find substantial differences in the proliferation dynamics of single clones among acinar cells. As a complementary in vitro approach to assess clonal heterogeneity, we examine the organoid-forming capacity of these cells. In this assay, we identified a unique subpopulation of acinar cells with the ability to give rise to organoids. Thus, although the acinar population, similarly to the beta cells among the endocrine cells, is often considered as a homogeneous population we find clonally heterogeneous contribution to growth and maintenance of pancreas homeostasis.
Elucidating functional heterogeneity in haematopoietic progenitor cells: a combined experimental and modelling approach

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A detailed understanding of the mechanisms maintaining the hierarchical balance of cell types in haematopoiesis will be important for the therapeutic manipulation of normal and leukaemic cells. Mathematical modelling is expected to make an important contribution to this area, but the iterative development of increasingly accurate models will rely on repeated validation using experimental data of sufficient resolution to distinguish between alternative model scenarios.

The multipotent hematopoietic progenitor FDCP-Mix cells maintain a hierarchy from self-renewal to post-mitotic differentiation in vitro and are accessible to detailed analysis. Here, we report the development of a combined mathematical modelling and experimental approach to study the principles underlying heterogeneity in FDCP-Mix cultures. We adapt a single-cell based model of haematopoiesis to the conditions of cell culture and describe an association between proliferative history and phenotype of FDCP-Mix cells. While data derived from population studies are incapable of distinguishing between three mechanistically different model scenarios, statistical analysis of single cell tracking data provides a resolution sufficient to select one of them. This scenario favours differences between granulocytic and monocytic lineage with respect to their proliferative behaviour and death rates as a mechanistic explanation for the observed heterogeneity. Our results demonstrate the power of a combined experimental/modelling approach in which single cell fate analysis is the key to revealing regulatory principles at the cellular level.
Conventional long-term serum cultured ovarian cancer cell lines are used for drug screening and functional studies. Frequently, these cell lines do not resemble the original pathology of the primary malignancies and drug screens performed using cell lines might yield targets of limited clinical relevance. Furthermore, phenotypic heterogeneity between different tumors is lost in long-term serum cultured cell lines. This may include expression of putative novel drug targets and pathways. Here, we report the development of advanced xenograft and serum-free culture models to study ovarian cancer. This model enables functional tumor analysis and may help to dissect the molecular biology of serous ovarian carcinoma (SOC).
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