Abstract: 90
Trandifferenzierung von malignen B-Zellen in Makrophagen / Transdifferentiation of malignant B-cells into macrophages in a murine model of Burkitt´s lymphoma

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B-cell lymphomas, such as Burkitt's lymphoma, are malignant diseases of the hematopoietic system. They arise from transformed B-cells originating from diverse primary and secondary lymphatic tissues. Like in other cancers, the stroma of lymphomas is typically infiltrated by so-called tumor-associated macrophages (TAMs), which execute manifold tumor-specific functions. Interestingly it was shown, that mature B-cells could be efficiently re-programmed into macrophages by the overexpression of myeloid-specific transcription factors. Moreover, other studies observed in vitro that this lymphoid/myeloid plasticity might be also caused by oncogenes in cultured B-cells of murine lymphoma models. Therefore we consider, if lymphoma B-cells themselves might be a source of TAMs, besides the well-known infiltration of monocytic cells into the tumor environment. Based on the system of CD45.1/2 allotypes, a murine model of lymphoma was thus developed in this Master's thesis, which allows tracking of the conversion of lymphoma B-cells into TAMs. It could be shown, that some lymphoma B-cells of the established model in fact spontaneously switched into a macrophage-like phenotype. Accordingly, they start to express typical macrophage markers, but seem to perform a transition into a myeloid-like expression profile on the level of transcription as well. Furthermore, analysis of the recombined immunoglobulin heavy chain confirmed the clonal identity of lymphoma b-cells and TAMs. Although these cells do not exhibit an overt immunological phenotype, they show elementary functional properties of macrophages, such as phagocytosis as well as a post-mitotic character. Consequently, it could be shown that lymphoid/myeloid plasticity takes place in tumors and therefore in vivo, too. Intriguingly, this conversion is not only associated with phenotypical changes, but also with a profound change in the cellular biology of these cells. Which consequences the lymphoid/myeloid plasticity of lymphoma B-cells has for tumor development, progression, as well as for the outcome of therapy, and if these observations could be transferred to human tumors, will be object of future investigations.

Abstract: 180
Eine klonale Sukzession transient aktiver Tumor-initiierender Zellen treibt die in vivo Progression des humanen Pankreaskarzinoms / Clonal succession of transiently active TIC clones in human pancreatic cancer drives cancer progression in vivo

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Although tumor-initiating cells (TIC) seem to be critical for tumor progression and metastases formation of human pancreatic adenocarcinoma (PDAC), little is known about their clonal dynamics within tumors. To address this, genetically labeled primary PDAC cultures were used to study the clonal composition of PDAC TIC *in vivo*.

TIC were enriched from primary pancreatic cancer cultures in serum-free adherent culture as 3-dimensional epithelial colonies. Following transplantation they reliably induce serial tumors resembling the patient's tumor histology.

To monitor clonal kinetics, lentivirally marked TIC cultures (n=3) were serially transplanted. TIC specific lentiviral integration sites were identified by LAM-PCR and high throughput sequencing. Interestingly, very little clonal overlap was detected within serial xenografts (1°-3°) or pairs of 2° or 3° tumors. Clones contributing to primary tumor formation were replaced by previously not detectable TIC clones in subsequent generations. Mathematical modeling pointed to extensive variations in the proliferative rate of individual but otherwise homogenous TIC, mainly giving rise to non-tumorigenic progeny lacking self-renewal capacity. A limited number of acquired additional mutations identified by exome sequencing reveals an

exceptional genetic stability of xenografts during serial transplantation and underlines that clonal dynamics are based on changes in the functional activity state of TIC.

FBS induced cell differentiation resulted in monolayer formation and down regulation of described TIC or progenitor markers. Nevertheless, tumor initiation, self-renewal and TIC frequency in vivo remained unchanged. In addition, sorted CD133+ and CD133- fractions were both capable of forming tumors containing similar levels of CD133+, emphasizing that PDAC TIC display a pronounced phenotypic plasticity.

These data demonstrate that long-term tumor progression of serially transplanted PDAC is driven by a succession of transiently active TIC in temporally restricted bursts. The unprecedented functional and phenotypic plasticity of PDAC is underlined by the recruitment of inactive TIC in vivo and by the unchanged tumor initiating and self-renewal capacity under FBS induced differentiation. These results point out the need to develop new treatment approaches specifically targeting functional TIC activation in PDAC.

Abstract: 328
Hämatopoetische Differenzierung von humanen induziert-pluripotenten Stammzellen zu Granulozyten oder Makrophagen im "large-scale", als Basis für Zellersatz- und Genetische Therapien / Large-scale hematopoietic differentiation of human induced pluripotent stem cells provides granulocytes or macrophages for cell replacement- and genetic-therapies

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Hematopoietic differentiation of induced pluripotent stem cells (iPSC) holds great promise towards disease modeling, drug testing, and in particular the development of novel cell replacement- and gene therapy strategies. In the past, interest has been directed primarily at reconstituting stem cells, a cell type as of yet problematic to generate. Recently, however, also long-lived mature myeloid cells have been described and transplantation of those may open new therapeutic scenarios.

To prove this concept, we subjected human CD34+ cell-derived iPSC clones to an embryoid body (EB)-based myeloid differentiation protocol yielding so-called “myeloid cell forming complexes (MCFC)” within 7-10 days. Subsequent culture of MCFCs employed different cytokine combination including IL3, G-CSF or M-SCF for terminal differentiation. This resulted in continuous generation of >95% pure monocyte/macrophages (iPSC-MΦ) and/or granulocytes (iPSC-gra) from day 14 onwards over a period of 3-5 months at a quantity of 0.4-2.0 x 106 cells/week (cumulative 0.8-4.0 x 107 cells) per 3.5 cm well. Of note, production of myeloid cells was driven by a MCFC-resident CD34+ population of progenitor/stem cells giving rise to white, red, and mixed colonies.

Detailed characterization of mature myeloid cells demonstrated a typical monocyte/macrophage-morphology of iPSC-MΦ by cytospins and a surface-marker profile of CD45, CD11b, CD14, CD163, and CD68. In addition, iPSC-MΦ had the ability to phagocytose latex-coated beads similar to peripheral blood (PB) macrophages polarized to M2 and secreted MCP1, IL6, IL8, and IL10 upon LPS stimulation, whereas IFNy, IL1b, IL4, IL5, and IL12 were absent. iPSC-gra showed surface expression of CD45, CD11b, CD16, CD15, CD66b and a differential count containing pro-myelocyte (3%), myelocyte (5%), meta-myelocyte (30%), bands (22%), eosinophils (2%), basophils (1%), and segmented-neutrophils (37%) . Moreover, iPSC-gra were able to migrate towards an IL8 or fMLP gradient, form neutrophil extracellular traps, and up-regulate NADPH activity and ROS production upon PMA stimulation to a similar degree as PB granulocytes. In summary, we provide a novel hematopoietic differentiation protocol for iPSCs recapitulating key events of physiologic hematopoiesis and allowing for the prolonged large-scale production of myeloid lineage cells. Thus, this protocol appears particularly suited for cell-replacement strategies or to study hematopoietic development.

Abstract: 423
Aktivität von T-DM1 bei Patientinnen mit Hirn-metastasen von Her2-positivem Brustkrebs / T-DM1 is active in Her2-positive breast cancer brain metastases

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Background: Local therapy (radiotherapy, neurosurgery) remains the mainstay of brain metastases (BM) management. Recently, the LANDSCAPE identified lapatinib plus capecitabine (LapCap) as standard option for primary systemic treatment in oligosymptomatic patients (pts) with multiple Her2-positive BM. L is active in her2-positive breast cancer brain metastases
Limited evidence exists with regards to the potential activity of antibodies in BM. T-DM1 is an antibody-drug conjugate linking trastuzumab (T) to an anti-microtubule agent providing higher activity and lower toxicity as compared to LapCap. Here, we investigated the activity of T-DM1 in newly diagnosed or progressive BM.

Patients and Methods: Seven pts (median age 55 years) with Her2-positive BM were included. All pts had received prior T, four (57%) had received already LapCap, and two (28.6%) pertuzumab as well.
In two asymptomatic pts, T-DM1 was administered as primary therapy, while five pts had documented CNS progression upon prior local treatment. T-DM1 was administered every three weeks at a dose of 3.6 mg/kg.

Results: Median follow-up was 5.5 months and median brain metastases-free survival 11 months. Six pts (two with primary treatment and three receiving T-DM1 upon CNS progression) are currently assessable for CNS response. 3/6 pts (50%) had a partial remission, one patient progressing upon prior local therapy had stable disease lasting for fifteen cycles, and one patient had stable disease for 5 month. One patient had a minor response of BM on MRI but no reduction of brain oedema and increasing cortisol doses and was therefore deemed PD.

Conclusion: This prospective case series indicates that systemic therapy offers activity in Her2-positive BM. LapCap remains the standard of care but T-DM1 offers relevant clinical activity and should be investigated within the context of larger clinical studies.

Abstract: 628
Ptch2-/- aktiviert den kanonischen und alternativen Hedgehogsignalweg in der Stammzellnische, einhergehend mit Myeloproliferation, Stammzellverlust und Akzeleration von myeloproliferativen Erkankungen / Depletion of Ptch2 activates canonical and non-canonical Hedgehog signaling within the niche leading to myeloproliferation, stem cell exhaustion and accelerates Jak2V617F driven disease

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Aberrant Hedgehog ligand secretion, as shown in MPN and CML, not only induces constitutive Smoothened-dependent canonical HH-signaling causing GLI1 transcription, but also causes PTCH1-dependent non-canonical HH signaling leading to constitutive ERK activation.

Our investigations show, that depletion of the Ptch2 receptor in vitro and in vivo recapitulates overactivation of both pathways, while depletion of Ptch1 only reflects constitutive Gli1 activation pinpointing the important role of Ptch1 for Erk activation. Ptch2-/- mice develop a pronounced hematopoietic phenotype with leukocytosis driven by an increase in neutrophils, anemia, thrombocytopenia, loss of T-cells combined with a strong increase of cKit+ progenitors in the peripheral blood and increased extramedullary hematopoiesis causing splenomegaly reflecting a MPN phenotype. LKS (lin-cKit+Sca1+) cells residing within the BM (bone marrow) showed enhanced cycling properties causing exhaustion and loss of LKS cells over time, but improved stress hematopoiesis after 5-FU treatment. Niche change experiments show that cytopenias and loss of LKS cells are caused by overactivated HH signaling within the niche cells, causing depletion of osteoblasts and alterations of essential niche factors like CXCL12, Angiopoetin or Jagged1. In contrast, the hematopoietic Ptch2-/- is responsible for leukocytosis and even promotes LKS expansion and replating capacity in vitro.

Interestingly, depletion of Ptch2 in the niche or within hematopoietic cells dramatically altered Jak2V617F driven pathogenesis causing transformation of a non-lethal chronic myeloproliferative disease into an aggressive AML-like disease with up to 30% blasts in the peripheral blood.

In conclusion, combined constitutive canonical and non-canonical HH activation induced by depletion of Ptch2 causes a MPN phenotype driven by cell intrinsic, but mainly cell extrinsic mechanisms and accelerates myeloproliferative diseases caused by Jak2V617F.

Abstract: 647
NFATc1 reguliert Stammzellreifung und induziert sowohl eine myeloproliferative Erkrankung als auch unreife T-Zell Leukämien / Lymphome / NFATc1 regulates primitive stem cell maturation and induces myeloproliferation and immature T-cell lineage leukemias / lymphomas

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Introduction: NFATc1 is a member of the nuclear factor of activated T-cells (NFAT) transcription factor family. NFATc1 plays a central role in T-cell development and –activation. Overexpression of NFATc1 is seen in diverse types of solid cancers and was recently shown to mediate imatinib resistance in CML blast crisis cells. The calcineurin inhibitor cyclosporine A (CsA) blocks NFAT activity. Here we investigated NFATc1 expression and its functional consequences in AML, and modeled the biological effects of NFATc1 expression in early hematopoietic stem cells.

Methods: AML cell lines and primary AML samples were studied for NFATc1 expression. A mutant form of NFATc1 with constitutive transcriptional activity was used to test the role of active NFATc1 in cell line models. We generated an inducible transgenic mouse model (B6.HSC-SCL-CRE-NFATc1), in which expression of a constitutively active NFATc1 protein was targeted to the early hematopoietic stem cell compartment. Evolving changes in hematopoiesis were studied.

Results: We show that NFATc1 mRNA and protein is overexpressed in AML. In FLT3-ITD+ AML cells NFATc1 regulates sensitivity to sorafenib and expression of a constitutively active NFATc1-mutant induces potent FLT3-ITD-inhibitor resistance in vitro and in vivo. We hypothesized that NFATc1 expression may be involved in myeloid leukemogenesis. To provide genetic evidence for this, a tamoxifen-inducible, transgenic mouse model was developed, where an NFATc1 transgene

was inducibly targeted to be expressed in the primitive stem cell compartment. This induced two types of hematopoietic abnormalities, which co-existed and which were lethal in 30% of the mice: i) a CD4+/CD8+ double positive (immature) T-cell proliferative disorder, which was eventually associated with thymomas, and ii) a myeloproliferative disease, characterized by a CD11b/GR-1low, left-shifted myeloid compartment. Mice always showed significant splenomegaly, often leukocytosis, and always anemia and severe thrombocytopenia. Moreover, we found a severe B-cell developmental block.

Conclusion: These results provide first evidence for a regulatory role of NFATc1 in early hematopoiesis including malignant hematopoiesis by inducing T-cell leukemia / lymphomas and a myeloproliferative disorder. Findings also provide a therapeutic rationale for NFATc1 inhibition in myeloid and T-cell lymphatic disorders.