alterations of key metabolic enzymes as early events in cancer. These are particularly interesting as enzymatic activities can be targeted with great specificity and success. To understand the consequences of altered enzymatic activities on cancer metabolism in vivo, analytical methods for quantifying metabolites, and system approaches to analyse the genetic and metabolome data in model systems are required.
Materials and Methods: We have started to develop integrated maps of metabolic alterations at different stages of melanoma and glioma development using our zebrafish cancer models (Santoriello et al., 2010; Spitzner et al., 2014). We combined metabolome analysis (through NMR) and gene expression data (through RNA-Seq) at different stages of cancer development, in whole animals and dissected tumors.
Results and Discussion: We identified metabolic reprogramming in the choline pathway leading to decreased activity of the trans-sulphuration pathway and S-adenosyl-methionine availability, and increased glutamine /acetate production in glioma due to extensive reprogramming of the TCA cycle.
Conclusions: Changes in metabolites and enzyme expression leading to these metabolic reprogramming events were already detectable in transgenic larvae, thus suggesting that zebrafish cancer models may serve to study the evolution of metabolic reprogramming in cancer and provide an in vivo platform to screen for metabolic vulnerability in cancer.
No conflict of interest.

356 Non-canonical Hedgehog/AMPK-mediated control of polyamine metabolism is required for medulloblastoma growth

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Introduction: Developmental Hedgehog signaling controls proliferation of cerebellar granule cell precursors (GCPs) and its aberrant activation is a leading cause of Medulloblastoma (SHH-MB, Hedgehog molecular subgroup). Treatment of SHH-MB patients with the FDA-approved Hh inhibitor Vismodegib (which targets the transducer Smoothened) has been disappointing because of the occurrence of resistance, attributed to Smo inactivating mutations or to activation of downstream effectors. Thus, it is now believed that the identification and targeting of novel downstream components represents a preferable option for this disease.
Material and Methods: We used biochemical, cell biology and molecular biology approaches to characterize the molecular features of a novel mechanism involved in polyamine production upon Hh activation. The biological relevance of our findings has been evaluated in mice and patients with SHH MB.
Results and Discussion: We show here that Hedgehog promotes polyamine biosynthesis in GCPs by engaging a non-canonical axis leading to ODC translation. This process is regulated by AMPK, which phosphorylates threonine 173 of CNBP in response to Hedgehog activation. Phosphorylated CNBP increases its association with Sufu, followed by CNBP stabilization, ODC translation and polyamine biosynthesis. Notably, CNBP, ODC and polyamines are hallmarks Hedgehog-dependent Medulloblastoma (SHH-MB) and genetic or pharmacological inhibition of this axis efficiently blocks Hedgehog-dependent proliferation of Medulloblastoma cells in preclinical settings.
Conclusions: Together, these data illustrate a novel auxiliary mechanism of metabolic control by a morphogenic pathway with relevant implications in cancer.
No conflict of interest.

357 The impact of *Mycobacterium obuense* on innate and adaptive immunity

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Background: IMM-101 is an immunomodulatory treatment, comprising of heat killed whole cell *Mycobacterium obuense* (NCTC13365), currently under clinical investigation in a variety of cancers, including pancreatic cancer. At present, available pancreatic cancer treatments only provide 5% survival rate over 5 years, with a median overall survival rate of 6−12 months, highlighting the need for new therapies. A phase II clinical trial (NCT01303172) has shown that treatment with the immunomodulator IMM-101 in parallel with gemcitabine increases median survival to 7.0 months in patients with metastatic pancreatic cancer, compared to 4.4 months following treatment with gemcitabine alone (Dalgleish, A.G. and The IMAGE I Trial investigators, 2015). In light of the promising results of this combination treatment, elucidation of how IMM-101 induces its therapeutic effects is ongoing. Initial pre-clinical studies have suggested that antigen specific CD45RBlowCD44high cytotoxic CD8+ T cells are increased in IMM-101 treated animals and may play a key role.
Material and Methods: Dendritic Cell (DC) studies were conducted on IMM-101 (or control) stimulated murine bone marrow derived GMCSF DCs or FLT-3 DCs, or human monocyte derived DCs. DC phenotypic activation was assessed by flow cytometry, and cytokine secretion assessed in culture supernatants by ELISA or cytokine bead array. CD4+ T cells used in murine co-culture studies were isolated from the lymph nodes and spleens of transgenic OTII mice before being stained with CFSE to assess proliferation via flow cytometry.
Results: We have investigated the impact of different concentrations of IMM-101 on murine and human DC activation and function. GMCSF derived murine DCs displayed a dose dependent response to the immunomodulatory IMM-101, with elevated expression of activation markers and increased secretion of IL-6, IL-12p40 and NO, compared to controls. This dose dependent response to IMM-101 was also replicated in murine FLT-3 generated DCs (pDCs, CD11b+ cDCs and CD24+ cDCs) and human monocyte derived DCs. Using murine DC:T cell co-cultures, we have demonstrated that IMM-101 influences the processing and presentation of antigen by DCs to CD4+ T cells. We have also shown that IMM-101 activated DCs instigate a T cell specific IFN-gamma, and a non-T cell specific IL-17, response following re-stimulation of draining lymph node cell preparations obtained 7 days after adoptive transfer of DCs into naïve recipient mice.
Conclusion: Our demonstration that IMM-101 activates primary DCs, both murine and human, and promotes IFN-gamma and IL-17 production in vivo helps build a better understanding of the fundamental mechanisms that may bridge innate and adaptive immune responses and may help elucidate the mode of action of this promising cancer treatment.
No conflict of interest.

358 Novel methods for the isolation of tumor cells from human, mouse, and xenografted tumors

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Solid tumors are vascularized and infiltrated by stromal cells such as leukocytes, endothelial cells and fibroblasts. The amount and composition of those non-tumor cells depends on various factors including tumor entity and stage, treatment history, status of the host organism and site of tumor growth. This results in a widely unpredictable contamination of non-tumor cells within tumor samples frequently hampering downstream applications like cultivation and molecular analysis. However, the direct isolation of target cells is difficult as there is a lack of markers exclusively expressed by tumor cells in many cases. To overcome these hurdles, we have developed methods allowing for the isolation of tumor cells from human, mouse, and xenografted tumors by specifically depleting non-tumor cells from the respective primary material by utilizing magnetic cell sorting.
The combinations of antibodies specifically binding all non-tumor cells from human, mouse, and xenograft tumors have been identified by flow cytometry-based screening assays on multiple tissue samples as well as cell lines. The respective antibodies were coupled to superparamagnetic nanoparticles and optimized for efficient depletion utilizing MACS. Performance of non-tumor cell depletion was assessed by flow cytometry and cell culture followed by immunocytochemistry of separated cells. Additionally, whole exome sequencing on bulk tumor and separated samples was performed to assess improvement in downstream analysis.
Three different antibody cocktails were established for depletion of non-tumor cells from all common sources of tumor material. Using these optimized antibody combinations coupled to superparamagnetic nanoparticles allowed for the fast isolation of untouched tumor cells independent of tumor entity and origin. Typically, purities higher than 95% were achieved in less than 25 minutes. Even when starting with samples such as pleural effusions, containing only minor frequencies of tumor cells, purities of higher than 85% were reached by a single isolation step. By depletion of stromal cells, the downstream culture of tumor cells was significantly improved and standardized. In downstream analysis more SNPs were identified by next generation sequencing, and gene expression signatures of isolated tumor cells and cancer stem cells could be more reliably be interpreted upon removal of the bias caused by non-tumor cells.
Taken together, we have developed easy and fast procedures to isolate tumor cells independent of tumor specific markers or tumor entity from human, mouse, and xenografted tumors. This allows for increased sensitivity, specificity and reproducibility in downstream analysis of tumor cells and subpopulations thereof. The “untouched” isolation of tumor cells allows for the subsequent sorting and analyses of minor tumor subpopulations, such as cancer stem cells.
No conflict of interest.