death. The genomic characteristics of CTC and DTC have to be considered in order to better understand the biological properties and refine the clinical implications of these cells.
Material and Methods: CTC or DTC from metastatic breast cancer patients were identified by immunocytochemical (ICC) staining followed by light microscopy evaluation. Single cells were isolated by micromanipulation or micro dissection, including verification of the single cell end product. The DNA from each single cell was amplified by whole genome amplification (WGA) and the amplified product was applied to Agilent 44k or 244k Comparative Genomic Hybridization arrays.
Results: We adapted and established methods for micromanipulation, amplification and Single Cell array CGH (SCaCGH) at different sites.
Reproducibility and sensitivity of the methods were tested by analyzing the breast cancer cell line SKBR3. Genomic profiles of the different CTC and DTC were compared in relation to each other and among the patients. Our preliminary results show that genomic profiles of DTC/CTC present common breast cancer tumour aberrations, like gains and deletions at chromosome 1, 8, 11 and 17. Further, the profiles exhibit a high degree of concordance within the same patients and discordance among different patients.
Conclusions: Genomic analysis of single tumour cells is possible through micromanipulation, amplification and SCaCGH. This provides us with a powerful tool for in-depth studies of CTC and DTC in localized breast cancer, for identification of aberrations relevant for their metastatic potential and/or therapeutic susceptibility.
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368 The molecular switch NHERF1 induces tumour phenotypic changes associated with distinct metastatic organotropism in breast cancer via its PDZ domains

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Background: The mechanisms allowing disseminated cancer cells to colonize specific targets organs (organotropism) are unknown. However, organotropism is thought to emerge via acquisition of distinct sets of cellular capabilities, which are controlled by finely regulated signal transduction modules and result in specific tumour phenotypes. The PDZ domain-containing scaffolding protein NHERF1 recruits signaling protein partners and directs proteins to specific cellular locations thus regulating a range cellular behaviors. Moreover, NHERF1 is overexpressed in human breast cancer and its overexpresssion is associated with aggressive clinical characteristics and poor prognosis.
Material and Methods: To gain insight into the mechanisms and role of NHERF1 in tumour phenotypes and metastasis, we stably transfected a metastatic breast cell line, MDA-MB-231, with the pcDNA 3.1/Higro empty vector, with wildtype (wt) NHERF1 or with NHERF1 mutated in either the PDZ1 (HRF1) or PDZ2 (HRF2) domains and tested these clones both *in vitro* for their biological activities of invasion, migration, anchorage independent growth, invadopodia/podosome activation program, vasculogenic mimicry and angiogenic properties and *in vivo* for their tumourigenic ability and metastatic organotropism.
Results: Anchorage-independent growth and *in vivo* xenograft formation are reduced in wt-NHERF1 and HRF2-NHERF1 and increased in HRF1-NHERF1 with respect to pcDNA 3.1. Only HRF2-NHERF1 inhibited invadopodium formation and ECM degradation while podosomes, vasculogenic mimicry and neoangiogenesis were inhibited in wt-NHERF1 and HRF1-NHERF1. Intracardiac injection of BALB/c-nu/nu mice demonstrated that HRF1-NHERF1 produced visceral metastases and HRF2-NHERF1 favored bone metastasis. Therefore, NHERF1 overexpression can either (i) via its PDZ1 domain reduce tumour growth but increase *in vivo* osteotropism by inducing podosomes and vasculogenic mimicry in the tumour cells and neoangiogenesis in endothelial cells or (ii) via its PDZ2 domain promote invasion and *in vivo* visceral metastases by inducing invadopodia formation and ECM proteolysis.
Conclusions: We conclude that NHERF1 can differently reprogram the neoplastic phenotype and metastatic organotrophism by specific alteration of its PDZ domain function.

369 Dihydroartemisinin is a hypoxia active anticancer drug

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Background: Major aim of cancer therapy is the eradication of clonogenic tumour cells. Unfortunately, the microenvironment of solid tumours is mostly characterized by regions of acute or chronic hypoxia which are known to decrease tumour cell sensitivity to cell death induction by classical genotoxic treatments. Here, we propose a novel strategy to overcome therapy resistance of tumour cells under acute hypoxia by using the radical-forming endoperoxide Dihydroartemisinin (DHA). Aim of the present study was to evaluate the antineoplastic potential of DHA in colon carcinoma cells focusing on the role of hypoxia for its cytotoxic effects.
Methods: Sensitivity of colon cancer cells to cell death induction by DHA (12.5−50 μ?) was analyzed by fluorescence microscopy (cytochrome c release and Hoechst33342/propidium iodide-staining, PI), flow cytometry ($\Delta\psi$m, DNA fragmentation) and immunoblotting (caspase-activation, PARP-cleavage). To investigate the molecular mechanisms under normoxia (21% O_2) and hypoxia (0.2% O_2), HCT116 wild type (wt) and subclones with defined defects in apoptosis signalling (Bax−/−, Bak−/− or Bax/Bak−/−) were used. Clonogenic death was tested by colony formation assays. In addition, HCT116 wt xenograft experiments were performed with NMRI nu/nu mice.
Results: DHA induced concentration-dependent apoptosis in colon cancer cells under normoxic conditions. HCT116 wt cells were also highly sensitive to DHA-induced apoptosis under conditions of strong hypoxia (0.2% O_2), although absolute apoptosis levels were decreased compared to normoxia. Loss of Bax, Bak or Bax and Bak largely decreased DHA-induced apoptosis in normoxia and hypoxia. However, eradication of clonogenic tumour cells was only reduced in the Bax, Bak, or Bax/Bak-deficient HCT116 cells when treatment was performed under normoxic conditions; in contrast, the response of the different HCT116 subclones in strong hypoxia was almost similar. Finally, our preliminary data indicate *in vivo* activity of DHA in a HCT116 wt xenograft model.
Conclusions: DHA efficiently induces apoptosis in colon cancer cells under normoxic and hypoxic conditions in a Bax and Bak dependent manner. In contrast, loss of these two main effectors of apoptosis execution affected DHA-induced eradication of clonogenic tumour cells only in normoxia. Our findings suggest that DHA may be of particular value for the treatment of human solid tumours characterized by high levels of tissue hypoxia and apoptosis resistance.

370 The metastasis-promoting protein, S100A4, regulates mammary branching morphogenesis

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High levels of the S100 calcium binding protein A4 (S100A4), also called fibroblast specific protein 1 (FSP1), has been established as an inducer of metastasis and indicator of poor prognosis in breast cancer. The mechanism by which S100A4 leads to increased cancer aggressiveness has yet to be established; moreover, the function of this protein in normal mammary gland biology has not been investigated. To address the role of S100A4 in normal mammary gland, its spatial and temporal expression patterns and possible function in branching morphogenesis were investigated. We show that the protein is expressed mainly in cells of the stromal compartment during active ductal development, in pregnancy and in involution. In 3D culture models, topical addition of S1004 induced significant increase of the branching phenotype and a concomitant increase in expression of a previously identified branching morphogen, metalloproteinase-3 (MMP-3). These events were found to be dependent on MEK activation. Down-regulation of S100A4, using shRNA, significantly reduced TGFa induced branching and altered E-cadherin localization. These findings provide evidence that S100A4 is developmentally regulated and that it plays a functional role in mammary gland development by activating MMP-3, and in concert with MMP-3, acts as a morphogen required for invasion into the fat pad during branching. We suggest that S100A4-mediated effects during branching morphogenesis provide a plausible mechanism for how it may function in breast cancer progression.

371 Differences in the stroma of human ovarian carcinoma xenografts endowed with different angiogenic phenotypes

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Background: A fundamental characteristic of malignant cancers is the ability to surmount environmental controls by the host and induce changes in the neighboring tissue to favor local tumour growth, invasion, metastatic spreading, and perhaps contribute to drug response.
The over-expression of vascular endothelial growth factor (VEGF) is associated to poor prognosis and malignant progression. Importantly, VEGF, a major

angiogenic factor, is thought to play a decisive role in remodelling the tumour microenvironment.
The purpose of this study is to analyze, in an *in vivo* setting, the transcriptional response of the tumour stroma in reaction to angiogenic stimuli provided by the cancer cells.
Materials and Methods: Stroma was microdissected (PALM Microlaser System) from human ovarian carcinoma xenografts 1A9-VS1 (high VEGF, N = 5) and 1A9-VAS3 (low VEGF, N = 5), and processed for RNA isolation. Labeled cRNA was hybridized to GeneChip® Mouse Genome 430 2.0 Arrays (Affymetrix). Transcriptional differences of the stroma were evaluated by two approaches: a one-way, modified and error-weighted Analysis of Variance (ANOVA) with a P-value cut-off of 0.01 (Resolver SE System, Rosetta Biosoftware), and the GC_RMA stochastic algorithm (GeneSpring, Agilent). Validation of microarray data has been performed using Real Time RT-PCR and immunofluorescence.
Results: VEGF produced by the cancer cells induced the up-regulation of 294 and down-regulation of 162 genes in the tumour stroma. Among them, neuropilin-1, endoglin, CXCL2, and collagen IV were confirmed to be up-regulated, and this was seen not only in the same tumours used for microarray analysis but also in different biological samples. It has been described that neuropilin-1 is expressed by endothelial cells and is associated to VEGF signaling, endoglin and CXCL2 are expressed by endothelial cells and macrophages, respectively, and collagen IV is associated to the extracellular matrix. Altogether, these data demonstrate that the VEGF released from tumour cells alters the tumour microenvironment.
Conclusions: The gene expression differences found from microarray analysis are robust thus providing the confidence necessary for using the 1A9-VS1 and 1A9-VAS3 model to investigate the proteins altered in the tumour microenvironment. Studies are underway in order to study genes/proteins whose function and characteristics are not well known.
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372 Neurotrophin-3 production promotes human neuroblastoma cell survival by inhibiting the dependence receptor TrkC-induced apoptosis

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Background: Tropomyosin-related kinase receptor C (TrkC) is a neurotrophin receptor with tyrosine kinase activity that could behave as an oncogene. However, it has several characteristics of a tumour suppressor: its expression in tumours has often been associated with good prognosis; and it was recently demonstrated to be a dependence receptor, transducing survival signals in the presence of ligand and inducing apoptosis in the absence of ligand.
Material and Methods: We have screened human neuroblastomas (NB) tumours and measured NT-3 expression by RT-Q-PCR and immunohistochemistry. We have used NB cell lines *in vitro* and in an avian and murin model for tumour progression and investigated the proapoptotic effect of an antibody targetting NT-3/TrkC binding.
Results: Here we show that the TrkC ligand neurotrophin-3 (NT-3) is upregulated in a large fraction of aggressive human NBs and that it blocks TrkC-induced apoptosis of human NB cell lines, consistent with the idea that TrkC is a dependence receptor. Functionally, both siRNA knockdown of NT-3 expression and incubation with a TrkC-specific blocking antibody triggered apoptosis in human NB cell lines. Importantly, disruption of the NT-3 autocrine loop in malignant human neuroblasts triggered *in vitro* NB cell death and inhibited tumour growth and metastasis in both a chick and a mouse xenograft model.
Conclusions: Thus, our data suggest that NT-3/TrkC disruption is a putative alternative targeted therapeutic strategy for the treatment of NB.

373 Src family kinases in lung cancer

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Background: Lung cancer is the commonest cause of cancer-related mortality among both men and women, mostly due to the rapid development of drug resistance and early metastasis. In this study, we sought to evaluate the potential involvement of Src family kinases (SFK) in lung cancer biology and assess the possible benefits of their inhibition as a therapeutic approach for this disease.
Material and Methods: The following cell lines were used: A549, EKVX, HOP62, HOP92, H226, H23, H322M, H460, H522, HCC78, HCC95 NSCLC and U2OS osteosarcoma. Proliferation was assessed using crystal violet staining and Western Blotting for phospho-Retinoblastoma and p27. DNA synthesis was quantified by EdU proliferation assay. Induction of apoptosis was measured using caspase activity assay, FACS analysis (sub-G1 peak) and Western Blotting for activated caspases 3, 7 and 9, and PARP cleavage. Autophagy was monitored by fluorescent microscopy using LC3-GFP-expressiong cells and by Western Blotting for LC3.
Results: Here, we demonstrate that various Src family members, including Lyn and Lck, which were believed to be expressed solely in hematopoietic cells and neural tissues, are overexpressed and activated *in vitro* in a panel of SCLC and NSCLC cell lines and *in vivo* in lung cancer tissue microarrays, compared to normal lung tissue. Dasatinib (BMS-354825), a novel Src/Abl inhibitor, effectively blocks SFK activation at nanomolar concentrations which in turn result in significant decrease in cell numbers in the majority of lung cancer cell lines. However, we failed to detect differences in cell cycle progression upon dasatinib treatment. Also, we could only detect moderate induction of apoptosis. In contrast, we demonstrate that dasatinib as well as PP2, another SFK inhibitor, are strongly inducing autophagy. Last but not least, we show that combined treatment with dasatinib and etoposide or cisplatin, chemotherapeutic agents commonly used in lung cancer treatment has an additive effect.
Conclusions: Overall, our results suggest that inhibition of Src family kinases alone or in combination with chemotherapeutic treatment, may be a beneficial therapeutic strategy in the management of lung cancer patients.

374 New tools to evaluate genetic targets and therapeutic strategies against cancer: in vivo imaging and inducible systems to modify gene function

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Background: More predictive small animal models for compound and genetic target assessment are needed. We have used *in vivo* and *ex vivo* bioluminescent imaging technology to create oncology models to evaluate compound efficacy in mouse models of orthotopic tumour growth and spontaneous metastases. Orthotopic tumour models are more relevant with respect to host-tumour interactions, characteristic disease progression, metastatic potential and response to therapy than the currently used (subcutaneous) models for preclinical drug selection.
Materials and Methods: PC3-M-luc human prostate adenocarcinoma cells that were genetically modified to express firefly luciferase were inoculated orthotopically. *In vivo* bioluminescent imaging (BLI) was performed using an *In Vivo* Imaging System (IVIS®) on day 7 post tumour implantation. Taxotere® was then given at a dose of 20 mg/kg by i.v. injection on day 8. Subsequent tumour growth was monitored by BLI.
Results: Using this BLI-based model system, metastases were detected as early as 21 days post tumour implantation. After only 5 weeks, a majority of the mice (~75%) exhibited distant metastases *in vivo,* which were observed by shielding the photons emitted from the primary tumour. At the end of the study, *ex vivo* tissue BLI was performed on the lungs, diaphragms, liver, draining lymph nodes, brains and femurs of all of the animals. We were able to detect metastases in all of the animals imaged *ex vivo* (even those in which the tumours were too small to detect *in vivo*) in at least 2 of the evaluated tissues. Taxotere® effectively inhibited both the primary tumour growth and the development of metastases. A similar orthotopic/metastatic brain (U87-MG-luc2) model is in development.
Conclusions: Our platform is highly sensitive and facilitates the development of orthotopic and metastatic xenograft tumour models, which allow for the performance of quantitative and high throughput *in vivo* assessments of potential anti-neoplastic and/or anti-metastatic therapies. In addition, we are developing new oncology animal models that will combine inducible/reversible RNAi gene knockdown and KinaseSwitch technologies with the imaging technology to assess compound efficacy as well as to evaluate genetic targets *in vivo*. Preliminary data will be presented on two mouse models developed for target validation in breast cancer using inducible shRNA technology and on KinaseSwitch models which allow investigators to study the biological role of a specific kinase and possible side effects that result from its inhibition.