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.