dye labeled secondary antibodies. Finally, the protein expression levels of NSCLC tumors were associated with their response to specific drugs.
RPPA analysis was performed for 78 proteins in 53 xenograft models. Statistical analysis indicated significant associations between the expression of distinct proteins and the response rate to certain drugs (Erlotinib, Cetuximab, Paclitaxel, Carboplatin). We revealed an association between higher phospho-p38 expression and increased Paclitaxel response rate. ERK1/2 downregulation was observed upon Cetuximab treatment in responders.

334 POSTER

Tropomyosin Tm5NM1: A novel target for cancer therapy

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Background: The actin cytoskeleton is an important regulator of a variety of cellular functions including cell motility, adhesion, and proliferation. Changes in these processes are fundamental to cellular transformation making the actin cytoskeleton a long sought after chemotherapeutic target. Drugs developed to date have been unsuccessful due to their lack of specificity which ultimately causes unacceptable cardiac and respiratory toxicity. We have previously shown that tropomyosin (Tm), an integral component of the actin cytoskeleton, defines functionally distinct populations of actin filaments. We have identified a specific Tm isoform common to all tumour cells which regulates cell proliferation and have designed a new class of compounds to target this filament population.
Summary of results: The role of Tm5NM1, a ubiquitously expressed low molecular weight (LMW) Tm isoform, was investigated using both overexpression and knockdown neuroblastoma cell systems. Using clonogenic and proliferation assays we ascertained that elevated levels of Tm5NM1 accelerated cell proliferation. Conversely, siRNA knockdown of Tm5NM1 resulted in decreased cell growth. We have developed a novel class of anti-Tm compounds that target LMW Tm5NM1 containing filaments. Our lead compound, TR100 targets the actin cytoskeleton and is effective against a panel of neuroblastoma and melanoma cell lines (average LC50 ~2−3 uM). TR100 inhibited survival and growth in a 3D melanoma model, which simulates the tumour microenvironment, and significantly reduced tumour growth in the B16/F10 melanoma mouse model. In vivo data from the drug treated animals also showed no evidence of cardiac damage as measured by blood Troponin I levels and no obvious hypertrophy as measured by intraventricular septum thickness.
Conclusions: We have demonstrated for the first time a novel class of chemotherapeutic compounds which specifically target an actin filament population required for cell growth. This has enormous implications for the treatment of a variety of cancers.

335 POSTER

CD133+ or CD44+CD166+ cells from human colorectal cancer cell lines do not display cancer stem-cell features nor increased drug resistance

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Background: Tumour initiation and maintenance is caused by rare tumour cell subsets, defined as “cancer stem cells (CSCs)”, endowed with self-renewal and differentiation capacity. CSCs have a number of properties permitting them to survive conventional cancer chemotherapy and radiotherapy. The development of alternative therapeutic approaches specifically targeting CSCs is urgently needed. Primary screening of novel anti-cancer compounds is conventionally conducted on established tumour cell lines, easy to propagate in vitro and amenable to high throughput studies. However, whether they do actually comprise CSC populations resembling those of primary tumours remains highly debated.We performed phenotypic and functional characterization of putative CSC populations in established cell lines of human colorectal carcinoma (CRC) and evaluated their suitability for predicting efficacy of anti-cancer therapies.
Material and Methods: A panel of 10 established human CRC cell lines was studied. Expression of putative CSC markers, including CD133 or CD44/CD166 molecules, was evaluated by flow cytometry. CD133+ or CD44+ CD166+ cells were sorted from individual cell lines by flow cytometry and evaluated for CSC properties in comparison to their negative counterparts or to the parental cell line. Spheroid formation ability, clonogenicity, stemness-related gene expression, aldehyde-dehydrogenase (ALDH)-1 activity, side population (SP) phenotype, in vitro invasiveness, chemo-resistance and tumorigenicity upon injection in NOD/SCID mice were assessed.
Results: None of the putative CSC phenotypes analyzed was found to be significantly associated with functional features of CSC. Importantly, neither CD133+ nor CD44+ CD166+ cells showed significantly increased resistance to chemotherapeutic drugs currently in use for CRC treatment, as compared to their negative counterparts.
Conclusions: On established CRC cell lines, the expression of putative CSC markers does not correlate with CSC functional features. Our findings question the adequacy of established CRC cell lines for screening of CSC-specific therapies and underline the urgency to develop novel platforms for anti-cancer drug discovery.

336 POSTER

Splicing factors as novel therapeutic targets in ovarian and breast cancers

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We have reported earlier that two splicing factors, polypyrimidine tract binding protein (PTB) and SRp20 are overexpressed in ovarian tumors, compared to matched controls (*He et al, Clin Cancer Res 10:4652−60, 2004*). PTB is a widely-expressed RNA binding protein belonging to the heterogeneous nuclear ribonucleoprotein family (and is also known as hnRNP I) whose molecular functions include regulating internal ribosomal entry site (IRES)-mediated translation and, importantly, alternative splicing. SRp20 is a member of the serine/arginine-rich (SR) protein family with multiple functions in RNA processing such as polyadenylation and alternative splicing. Immunostaining of tissue microarrays revealed that both PTB and SRp20 are expressed differentially between benign tumors and invasive EOC, and between borderline/Low Malignant Potential tumors and invasive EOC. Our staining results reveal that expression of PTB and SRp20 is associated with malignancy of ovarian tumors but not with stage of invasive EOC (*He et al, ms submitted*). In addition to these clinical observations, we found that both of these splicing factors are highly expressed at the earliest stages of transformation in ovarian and breast tumor cell lines. Importantly, at least for PTB, there is little or no expression in normal ovarian surface epithelial cells and normal blood precursors. We established stable ovarian (A2780) and breast (MCF7) cell lines carrying doxycycline (Dox)-inducible PTBshRNA or SRp20shRNA. Knockdown by shRNA of either of these splicing factors in ovarian or breast tumor cell lines led to decreased cell growth. Moreover, we observed decreased colony formation and invasiveness in the A2780-PTBshRNA cells (*He et al, Oncogene 26:4961−8, 2007*). Further, knockdown of SRp20 in A2780 ovarian and MDA-MB-231 breast tumor cells by ~90% led to apoptosis that was associated with caspase-3, -7, and -9 activation and decreased expression of Bcl-2. Last, feeding Dox to mice bearing A2780 xenografts stably infected with Dox-inducible PTBshRNA led to suppression of tumor growth, compared to controls. Overall, our results suggest that both PTB and SRp20 may be novel therapeutic targets. Accordingly, we have begun to develop a cell-based two color assay to screen for small molecules that will inhibit these splicing factors, with the goal of bringing a new small molecule to the clinical treatment of ovarian and possibly breast cancer.
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337 POSTER

Differential action of ErbB kinase inhibitors on receptor oligomerization

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Background: ErbB tyrosine kinase receptors participate in several physiological processes and their deregulation is involved in the pathophysiology of cancer. Two main types of agents have been developed against them: monoclonal antibodies and small tyrosine kinase inhibitors (TKIs). We have studied the action of some TKI on ErbB activation and receptor interactions.
Material and Methods: Four breast cancer cell lines were used as models: MCF7 and T47D, BT474 and SKBR3. EGF and NRGb1 were employed as ErbB ligands.
Six TKI were used in this work, three reversible (erlotinib, gefitinib, and lapatinib), and three irreversible (canertinib, pelitinib and neratinib).