which provides a Dolichol Phosphate (DolP) substitute on regulation of Pgp expression in Doxorubicin resistant MCF-7 breast cancer cells.
Methods: Breast cancer cell lines, MCF-7 and MCF-7/ADR were used. Pol concentration in the culture medium made up 10^{-2}–10^{-6}. Immunohistochemical and Western blotting methods were used to detect the changes in the expression levels of MDR1 and DPAGT1 expression. Intermediates of DPC fractions were analysed by HPLC method.
Results: Overexpression of DPAGT1 was detected MCF-7/ADR cells, but not in MCF-7 cells. It is confirmed that plasmatic membranes of MCF-7 cells contain 5.6–6.4% of Pgp (the total protein amount) as a resistance marker. Resistant MCF-7/ADR cells differ from sensitive ones MCF-7 in Pgp content by 10−12 times. The study showed 8.5-fold DolP decrease in MCF-7/ADR cells. The investigations demonstrate that the situation can be changed by treatment with DolP and PP. The DolP concentration in MCF-7/ADR cells was returned to the normal level. It is established that DolP in the concentration 10^{-6} M aid 7−9-fold reducing Pgp in membranes of MCF-7/ADR cells. The MCF-7/ADR cells cultivation in medium with polyprenol proceeded to give lowered Pgp content in membranes no over 0.4−0.6%, which amount was consistent with the level of Pgp in MCF-7 cells. Overexpression of DPAGT1 was detected in MCF-7 and in MCF-7/ADR cells. It is established that Pol in the concentration 10^{-4} M aid 7−9-fold could overcome DPAGT1 overexpression which leads to regulation of Pgp N-glycosylation. Pol in concentration 10^{-2}−10^{-3} M induced apoptosis in MCF-7/ADR cells within 3−4 hours.
Conclusions: These results indicate that noncontrollable accumulation of Pgp, after MDR1 expression in MCF-7/ADR cells can be overcome using stimulation with dolichyl phosphate substitution. DPAGT1 overexpression in MCF-7/ADR can be overcome with Pol, which provides a DolP substitute for DPAGT1 normal expression.

Drug targets

331 POSTER DISCUSSION

Atrimers as a novel class of potent trimeric therapeutics inducing cancer cell death

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TRAIL death receptors DR4 and DR5 are attractive therapeutic targets in oncology as they are expressed in a wide variety of tumors, and DR4/DR5 agonists can induce p53-independent apoptosis. Agonistic monoclonal antibodies against DR4 or DR5, and recombinant TRAIL are currently being evaluated in clinical trials in combination with chemotherapy. However, these monoclonal antibodies target a single receptor type and, due to their bivalent nature, are not ideal to mimic the receptor-trimerizing effect of the potent natural trimeric ligand. The efficacy of recombinant trimeric TRAIL is hampered by its short half-life and its binding to decoy receptors that do not mediate apoptosis. We aimed to overcome these shortcomings of current therapeutics by developing stable trimeric death receptor agonists that do not cross-react with the decoy receptors and with half-lives expected to significantly exceed that of TRAIL. Potent DR4 and DR5 agonists were engineered using human tetranectin, a trimeric human serum protein of 60 kDa size, as a scaffold to generate Atrimers™. A panel of DR4 binders was selected from phage libraries displaying the C-type lectin domain (CTLD) of tetranectin with randomized loop sequences. Current lead DR4 Atrimers have sub-nanomolar affinity to DR4-Fc and show no detectable binding to DR5 or the decoy receptors. *In vitro*, they efficiently kill DR4-positive cancer cell lines with sub-nanomolar ED50, but do not kill DR4-negative cell lines. DR5 agonists were engineered by fusing phage-display-selected DR5 binding peptides to the N-terminus of tetranectin. Such DR5 agonists were equipotent to TRAIL in cell death assays with Colo-205 cells. Bi-specifics are currently being engineered by genetically fusing our most potent DR5 agonist peptide with our DR4 agonist Atrimers. Such a bi-specific Atrimer will target both DR4 and DR5 as stable trimer offering i) greater coverage due to differential expression with some patients expressing both DR4 and DR5 and ii) greater potency mediated in part by super-clustering via tumor cell specific binding on both ends of the molecule. In addition, improved tumor penetration (vs. antibodies) is expected due to smaller size (~70 kD). DR4/DR5 Atrimers therefore represent a novel class of cancer therapeutics selectively targeting the TRAIL receptors to efficiently induce apoptosis and provide many promises for the treatment of a broad range of cancer types.

332 POSTER

***POLQ* (DNA polyermase theta) as a novel therapeutic target: preclinical and clinical data**

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Background: We have previously shown that *POLQ* is upregulated in multiple different tumour cell lines and that *POLQ* depletion causes radiosensitisation. We have therefore conducted further studies to assess the suitability of POLQ as a therapeutic target.
Methods: Tumour cell lines were transfected with either non-targeting or *POLQ* siRNA. At 48h after transfection, cells were treated with cytotoxic agents prior to being assayed for clonogenic survival. Homologous recombination (HR) was assessed by quantifying RAD51 foci formation 4h after exposure to cytotoxic drugs. The I-Sce-I assay was also used to assess whether POLQ is involved in HR.
To assess the prognostic importance of *POLQ* we analysed tumour samples from two retrospective series of breast cancer patients (n = 279 in total) treated in Oxford, UK. *POLQ* mRNA expression was assessed by Affymetrix U133 array and compared with clinical outcomes. Published clinical series containing details of a further 537 breast cancer patients were accessed to confirm the findings seen in the Oxford cohorts.
Results: Tumour cells depleted of POLQ are sensitized to DNA damaging agents such as cisplatin, etoposide, doxorubicin, and mitomycin C. They are not sensitized to mitotic spindle poisons such as docetaxel. Cells depleted of POLQ have fewer RAD51 foci after exposure to cytotoxic drugs. POLQ knockdown also resulted in decreased HR efficiency as assessed by the I-Sce-I assay. All of these findings suggest that POLQ plays a role in HR.
The prognostic implications of *POLQ* overexpression were assessed in retrospective series of patients with early breast cancer. *POLQ* overexpression was associated with clinical features known to confer an adverse prognosis such as ER negative disease ($p = 0.047$) and high tumour grade ($p = 0.004$). Multivariate analysis showed that *POLQ* expression was associated with markedly worse relapse free survival rates independently of these other clinical features (HR 8.086; 95% CI 2.340 to 27.948; $p = 0.001$). Analysis of other published series supported these findings.
Conclusion: *POLQ* has limited normal tissue expression, but is overexpressed in a wide variety of different tumours. POLQ appears to be involved in HR with POLQ depletion rendering tumour cells sensitive to radiotherapy and multiple different DNA damaging agents. *POLQ* overexpression confers an extremely bad prognosis in breast cancer patients. This fact, combined with the above data make POLQ a highly appealing target for clinical exploitation.

333 POSTER

Identification of drug-associated proteins in NSCLC xenograft models by reverse phase protein arrays

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The treatment of non-small cell lung cancer (NSCLC) is currently limited by the fact that most therapies are not adapted to the individual response of a patient. The stratification of patients for the most efficient response to conventional chemotherapeutics and targeted therapies will improve established therapy schemes. Our project aims at unravelling the influence of specific signalling molecules on the response rate to common NSCLC drugs.
We quantified protein expression levels in patient derived NSCLC xenograft models. The tumor models are characterized by different response rates upon treatment with established chemotherapeutics (e.g. Gemcitabine, Paclitaxel, Carboplatin) and EGFR-targeted therapies (Cetuximab, Erlotinib). Protein expression was analyzed using the reverse phase protein array technology (RPPA). Protein lysates of 53 tumor samples were spotted in dilution series and replicates on nitrocellulose coated glass slides. Signalling proteins of cancer-relevant pathways (e.g. MAPK/Erk, JAK/STAT, PI3K/AKT) were detected by specific primary antibodies followed by IR-

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).