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-