patients with BRCA1-deficiency. The ability to identify these patients by gene expression profiling from FFPE derived breast tissue may also have significant clinical application.

631 POSTER

Mislocalization of the apoptosome protein Apaf-1 is a strong marker of drug resistance in B cell lymphomas

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Chemotherapy resistance remains a challenge in the clinical management of diffuse B-cell lymphomas despite aggressive treatment with CHOP and monoclonal CD20. We previously reported that sequestration of Apaf-1 to membrane lipid rafts was responsible for apoptosis resistance in B-cell lymphoma cell lines [1]. Here, we extended our studies to clinical biopsies from patients with B-cell lymphomas, T-cell lymphomas and reactive lymphadenopathy, to investigate if the resistance to drug-induced apoptosis was, indeed, a function of Apaf-1 mislocalization. Firstly, cells were separated from these biopsies and their sensitivity to a variety of apoptosis inducing agents was assessed. Whereas most T-cell lymphomas as well as reactive lymphadenopathy cells were sensitive to apoptotic stimuli, B-cell lymphomas exhibited strong resistance. We then investigated the expression of Apaf-1 and its intracellular localization in these clinical biopsies. To do so, cell fraction was performed to separate cytosol from membrane-enriched fractions. The latter were further subjected to density gradient centrifugation to obtain lipid raft fractions. We show that Apaf-1 was expressed in total cell lysates from B- and T-cell lymphomas, however upon fractionation the localization was strikingly different. In T-cell lymphoma samples as well as in cells derived from reactive lymphadenopathy biopsies, Apaf-1 expression was prominently detected in the cytosol, which correlated with the sensitivity of the cells to apoptotic stimuli. In contrast, whereas cytosolic Apaf-1 expression was significantly lower or absent in almost all B-cell lymphomas analyzed, increased localization of the protein was detected in membrane lipid rafts. The latter was confirmed by immunohistochemical analysis of tissues from the same biopsy specimens. Interestingly, the resistance of B-cell lymphomas to apoptotic execution (drug-induced or death receptor-mediated) was significantly bypassed upon incubation of cells with pharmacological agents that facilitated the dissociation of Apaf-1 from the lipid rafts to the cytosol. Taken together, our results implicate Apaf-1 mislocalization as a potential diagnostic marker for B-cell lymphomas as well as a predictor of response to therapeutic management. This work is supported by research grants to S.P. from the NMRC, Singapore, and the Singapore Cancer Syndicate.

References

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632 POSTER

Expression of a+β+ splice variant of human telomerase reverse transcriptase (hTERT) in cytokeratin 19 (CK-19) positive circulating tumor cells (CTCs) of breast cancer patients

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Introduction: Human telomerase reverse transcriptase (hTERT) is the catalytic subunit of telomerase. The α+β+ splice variant is the functional variant of hTERT. Circulating tumor cells (CTCs) have already been established as strong predictors of prognosis in patients with metastatic breast cancer. Our group has previously shown the prognostic significance of cytokeratin 19 (CK-19) mRNA-positive CTCs in early breast cancer. The aim of our study was to study the expression of hTERT α+β+ splice variant in CK-19 positive CTCs in breast cancer samples by qRT-PCR.
Materials and Methods: Peripheral blood (20 ml in EDTA) was obtained from 25 patients with early breast cancer before the administration of adjuvant chemotherapy, 14 patients with verified metastasis who were all tested positive for CK-19 expression by real time PCR (Stathopoulou et al, Int J Cancer 2006), and 17 female healthy volunteers. CTCs were isolated after ficoll density gradient centrifugation, following enrichment with immunomagnetic Ber-EP4 coated capture beads, mRNA was isolated using oligo $(dT)_{25}$ coated magnetic beads, followed by cDNA synthesis. The expression of hTERT α+β+ splice variant was tested in both CTCs and PBMCs fractions by quantitative real-time PCR in the LightCycler, (Mavroyiannou et al, Clin Chem, 2007).
Results: hTERT α+β+ splice variant was expressed in 4/14 (29%) of CK-19 mRNA positive CTCs samples from patients with metastasis and in 5/24 (21%) of CK-19 mRNA positive CTCs samples from patients with early breast cancer. None of the 17 female healthy volunteers CTCs fraction was tested positive for hTERT α+β+ splice variant, while the corresponding PBMCs fraction was positive in all cases.
Conclusions: To our knowledge this is the first report on the expression of hTERT α+β+ splice variant in CTCs of patients with early breast cancer and verified metastasis. Further studies are needed to evaluate and confirm our findings in a larger number of patients.

633 POSTER

MicroRNA-21 expression levels are accompanied by respective alterations in PDCD4 protein levels in non-small cell lung cancer

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Background: The aim of our study was to investigate the correlation between mature miR-21 expression levels and programmed cell death 4 (PDCD4) protein levels in non-small cell lung cancer.
Materials and Methods: Forty pairs of NSCLC fresh-frozen tissues and their corresponding noncancerous tissues were analyzed for the expression of mature miR-21 using quantitative real-time RT-PCR, as previously described (Markou et al., 2008). In parallel, PDCD4 protein levels were evaluated by immunohistochemistry. Deparaffinized sections cut from paraffin-embedded tissue samples were stained with a specific anti-PDCD4 antibody (1:100 dilution) (Ozaki et al., 2006) using HRP DAB kit (DAKO) for the detection. The tumor types and stages were determined according to the WHO classification. All samples were analyzed histologically to access the amount of tumor component (at least 70% of tumor cells) and the quality of material.
Results: Among the 40 NSCLC tissue specimens studied, suppresion of miR-21, in respect to their adjacent non-neoplastic tissues, was detected in 24 samples (60 %). In 15 out of these 24 patients (62.5%), we observed that the supression of miR-21 was accompanied by increase of PDCD4 protein levels. Mature miR-21 was overexpressed in 16 out of 40 patients (40%), and in 8 out of these 16 patients (50%) we detected reduced PDCD4 protein levels. Totally, in 23 out of 40 samples (57.5%), the altered miR-21 expression levels correlated with changed PDCD4 protein levels.
Conclusion: Our data indicate for the first time that PDCD4 protein expression levels are regulated by miR-21 in non-small cell lung cancer tissues.

634 POSTER

Patient-derived breast cancer xenografts: Molecular characteristics and growth properties

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Breast cancer is a heterogeneous disease for which several subtypes have been described according to histological and molecular determinants. Therapeutic decisions are dependent on tumor staging which includes molecular markers like estrogen receptor (treatment with anti-estrogens or aromatase inhibitors) or HER2 (treatment with HER2 targeting agents Trastuzumab or Lapatinib).
Here we present a panel of 12 patient-derived breast cancer xenografts, eleven of which have been established by Oncotest from primary patient material. Molecular profiling included expression analysis of receptors for estrogens (ER), androgens (AR) and progesterone (PR), HER2 and the E2F transcription factor 1 by quantitative RT-PCR and Affymetrix HG U133 plus2.0 array analysis. HER2 protein and phosphorylation were determined by ELISA and − for selected models − FISH analysis was performed to determine gene amplification. MAXF 713 and MAXF 1398 are ER positive, luminal B subtype tumors, whereas MAXF 1162 is a HER2-overexpressing, Lapatinib sensitive tumor. The high HER2 levels in MAXF 1162 are based on a gene amplification. MAXF 1322 is borderline HER2-positive, but insensitive towards Lapatinib and Herceptin. The majority of tumor models (8 out of 12) belong to the basal-like, triple negative breast cancer subtype.

In addition, the activity of Akt and MAPK pathways was analysed. Akt signaling is important in many breast cancers and 8 out of 12 tumor models depicted activated Akt, some of which also had highly activated p70S6K. The mutational status of PI3Kα and PTEN was assessed with PTEN protein expression also evaluated by Western Blot. Modifications in the PI3Kα activity and its negative effector PTEN modulate Akt kinase activity and downstream signaling.
Finally, the estrogen-dependency of the ER-positive models MAXF 713 and MAXF 1398 and their growth properties in dependence of subcutaneous versus orthotopic implantation were assessed. The xenograft models are routinely implanted subcutaneously, which sustains tumor growth. Nevertheless we found that of the 12 MAXF models as studied, 4 grew faster when implanted orthotopically into the mammary fat pad.
In summary, a unique collection of patient-derived mammary xenograft models has been characterised comprehensively in order to enable a pre-study selection of suitable tumors for in-vivo efficacy testing.

635 POSTER

Novel mechanisms of taxane and platinum resistance in Oesophagogastric Cancer Cells

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Background: Platinum analogues and taxanes are clinically effective in oesophagogastric (OG) cancer treatment. Nevertheless, resistance (primary or acquired) remains a major therapeutic problem. We developed platinum and docetaxel resistant cell line models and used gene expression profiling for hypothesis generation regarding predictive biomarkers for resistance and novel modes of action/resistance to facilitate target discovery and provide insight into mechanisms of cross resistance to inform optimal cytotoxic/targeted therapy strategies.
Material and Methods: Resistant daughter cell lines (n = 14) were generated from 3 parental OG cancer cell lines (OE33, OE21, AGS) by exposure to increasing concentrations of oxaliplatin, cisplatin and docetaxel. Cell viability was measured by the MTT assay. Drug resistance and cross resistance was determined. Gene expression analysis was performed on wild type and resistant daughter cell lines using Exon 1.0 Affymetrix microarrays. Gene expression data were normalized and statistically significant changes identified in GeneSpring GX v10.0.1. Ingenuity Pathway Analysis (IPA) software and GSEA gene sets were utilized to identify key biological pathways altered in resistant cells.
Results: Cisplatin and oxaliplatin cross resistance was observed in 5 out of the 6 platinum resistant cell lines. Docetaxel cross resistance was observed in only 1 out of 6 oxaliplatin and cisplatin resistant cell lines with 2 out of 6 lines showing increased sensitivity to docetaxel. Preliminary analysis demonstrates that the primary difference in gene expression between cell lines is tissue type (gastric vs. oesophageal) and histology (squamous vs. adenocarcinoma). Gene expression analysis of the oxaliplatin resistant lines identified 107 candidate genes involved in oxaliplatin resistance. Biological pathway analysis highlights DNA mismatch repair, down-regulation of cell cycling and anti-apoptotic response as potential mechanisms of oxaliplatin resistance.
Conclusions: Significant differences at the transcriptional level within the panel of OG cell lines are consistent with observed clinical heterogeneity of these tumours. No common mechanisms of resistance to all drugs were identified. We are currently processing further technical replicates in order to perform a comparative study (sensitive versus resistant) within each cell line and for each drug, to characterize gastric vs. oesophageal and adenocarcinoma vs. squamous cell carcinoma- dependent mechanisms of resistance and/or cross resistance.

636 POSTER

CD9 decreases adhesion and improves chemotactic response of B acute lymphoblastic cells: role in pathogenesis of TEL/AML1 leukemia?

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B lineage acute lymphoblastic leukemia (B-ALL) is the most common cancer in childhood and 25% displayed the *TEL/AML1* rearrangement. Previously, we have shown that this subset of B-ALL is characterized by five biological processes and 14 genes. We investigated the role of the *CD9* gene, which is involved in the motility process and is differentially down-regulated in *TEL/AML1* positive B-ALL. Indeed, the expression of CD9 has been correlated with the risk of metastases or a poor clinical outcome in various types of cancer. Because of a good overall prognosis of the *TEL/AML1* positive B-ALL and the special occurrence of very late relapses, we decided to study the impact of the expression of CD9 by motility assays in the context of *TEL/AML1* positive B-ALL.
Leukemic blasts isolated from bone marrow of patients and B-ALL cell lines that expressed or not CD9 (REH CD9+, Raji CD9− and Raji transfected with CD9) were used for the assays. For adhesion assay, 96-well plates were coated with superfibronectin (1 mg/ml). $5 \times 10e5$ cells/well were allowed to attach for 1h30 with or without blocking CD9-antibody (Ab). Adherent cells were quantified using MTS for 2h30 at 37ºC with absorbance measure at 540 nm. For migration assay, cells were seeded on 5mm transwell microporous polycarbonate membranes. $2 \times 10e6$ cells treated with different blocking Abs (CD9-Ab, CXCR4-Ab, IgG control) were loaded in the upper chamber while 100 ng/ml CXCL12 (B-ALL specific chemokine CXCR4 ligand) was added to the medium of the lower chamber. After 5 hours, the migrated cells were recovered from the lower chamber, numbered and analysed by FACS.
We showed that the expression of CD9 decreased the ability of lymphoblasts to attach to an extracellular matrix component both in B-ALL cell lines and in patients cells. This effect was reversed by CD9 blocking Ab. Conversely, migration was increased in CD9 positive cell lines whereas blocking CD9 reduced the chemotactic migration through membranes. A synergic effect with CXCR4 expression was also shown.
This study pointed out the importance of the CD9 expression on the ability of B-ALL cells to attach and migrate. Moreover, CD9 and CXCR4 seemed tightly linked in the chemotactic response to CXCL12. Because CXCL12-CXCR4 axis is known to facilitate metastasis and CD9 confers particular adhesion and migration properties to lymphoblasts, we raised the issue of a role of CD9 in the pathogenesis of the late extramedullary relapses of *TEL/AML1* B-ALL.

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Prediction of the human pharmacokinetics (PK) and pharmacodynamics (PD) of MLN9708, an investigational proteasome inhibitor

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The investigational drug MLN9708 is a modified dipeptidyl boronic acid that is a potent, reversible and specific inhibitor of the 20S proteasome. MLN9708 is currently being evaluated in first-in-human phase I trials of hematologic malignancies and solid tumors. The current work reports on the prediction of human blood MLN9708 PK and PD based on pre-clinical studies. In aqueous solutions MLN9708 is immediately and completely converted to MLN2238. Pre-clinical studies were performed using MLN2238 and human PK results were measured as MLN2238 concentration. The PK of MLN2238 in whole blood and plasma and the PD of proteasome inhibition in whole blood were characterized in immunocompromised mice, Sprague–Dawley rats, Beagle dogs and Cynomolgous monkeys. In all preclinical species tested, MLN2238 displayed a bi-exponential profile in whole blood and plasma following IV dosing, with a rapid initial disposition phase and a slow terminal disposition phase. Projection of human PK profiles was performed according to a modification of the method of Wajima et al. (Wajima T. et al. 2004. J Pharm Sci 93:1890−1900), using the experimental whole blood and plasma PK data from rat, dog and monkey. Projection of human whole blood PD profile (20S specific activity vs. time) was derived using the predicted human whole blood PK profile and in vitro proteasome inhibition parameters determined in monkey.
The human projected PK and PD were compared to the preliminary PK and PD results of the MTD cohort (1.76 mg/m^2) from the first-in-human IV study of MLN9708. A bi-exponential human plasma PK profile was predicted. For a dose of 1.76 mg/m^2, a C_{max} of 122 ng/mL an initial disposition phase $t_{1/2}$ of 0.25 hr, and a terminal disposition phase $t_{1/2}$ of 3.1 days were projected. In the clinical MTD cohort (n = 6), C_{max} was 319 ng/mL the initial disposition phase $t_{1/2}$ was 0.20 hr, and a terminal disposition phase $t_{1/2}$ was 1.8 days. Maximal whole blood proteasome inhibition was predicted to occur immediately following bolus injection of MLN9708, with 70% inhibition anticipated at a dose of 1.76 mg/m^2. At all doses maximum whole blood proteasome inhibition was observed in the first post dose sample (5 min), with 55% inhibition observed in the MTD cohort.
Conclusion: The projected PK and PD profiles of MLN9708 were similar to those observed in the MTD cohort of the FIH study of MLN9708.