We have assessed the phosphorylation status of AKT-473, MAPK and S6–240/4 at serial time points and compound dosages in both xenografts and mouse skin (surrogate tissue). Other relevant biomarkers like phosphorylation of PRAS40, nuclear translocation of FoxO3a or phosphorylation of the eiF4E/4G complex, together with the proliferation related Ki67 and Cyclin D1, were investigated. IHC/H-Score, IF/AQUA™ and RPPA/densitometry techniques were used in order to quantify these protein levels.
Results and Conclusions: GDC-0068 induced profound biomarker change along the PI3K pathway. Across the three platforms, the data significantly correlated at time points/doses for which GDC-0068 was active. As observed for other AKT catalytic inhibitors, AKT-473 was consistently hyperphosphorylated in a dose response manner and therefore useful for assessing the compound presence in the tumor. FoxO3a nuclear shift was persistent throughout the study, indicating constant AKT inhibition. Other biomarkers such as P-S6–240/4 and Ki67 were reduced upon acute AKT inhibition but their response was not maintained after long-term GDC-0068 administration. AQUA™ and RPPA are quantitative techniques that provide unbiased and reproducible biomarker evaluation.

628 POSTER

Stem cell marker expression profile in colon cancer xenografts and their corresponding cell lines

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The isolation and characterization of tumorigenic colon cancer stem cells may help to devise novel diagnostic and therapeutic procedures. In the present study we characterized a panel of 15 human colon carcinoma cell lines and their corresponding xenografts for their expression of 5 different potential stem cell markers: CD133, CDCP1, CXCR4, CD24 and CD44. Detection of the different surface markers was done by flow-cytometry (FACS). In 8 out of 15 models mRNA expression of the investigated markers was determined using a gene expression array (HU133 plus 2.0). Tumor growth behavior in correlation to CD133 expression was determined in SW620, when CD133+, CD133− and unsorted cells were injected subcutaneously (sc) into NOD/SCID mice. All five determined so called stem cell markers showed different but distinct expression patterns in the examined tumors. CD133 was highly expressed (>85% of positive cells) on 3 out of 5 patient-derived cell lines whereas in long-term culture based models CD133 expression ranged from 0−<20%. In 12/15 cell lines more then 80% of the cells were positive for CD24 and 13/15 were positive for CD44 to an extent of 70% and more. 11/15 cell lines expressed CDCP1 on $\geqslant$ 83% of their cells. CXCR4 was expressed exclusively on 3 cell lines (94L, SW480 and A293). Analyses of the corresponding xenografts revealed a significant reduction of cell numbers expressing the investigated surface markers. Gene expression analyses disclosed a strong correlation between CD133 and CD44 (0.952; p-value <0.005) and CD24 (0.81; p-value <0.005). Within the investigated xenografts small subpopulation of double (CD133/CD44 or CD133/CD24) and triple (CD133/CD24/CD44) positive cells could be described. In vivo growth behavior studies of SW620 revealed a CD133 dependent tumor growth in vivo, as CD133 positive subclones of SW620 showed significantly higher take rates and doubling times then the unsorted cell line. In contrast CD133 negative subclones induced significantly lower take rates and doubling times in comparison to the unsorted cell line. Tumors of all three cell types were analyzed as described above and showed the same expression pattern of the five investigated surface markers. Our data strongly recommend CD133 as a potential stem cell marker within the investigated colon carcinoma panel. Further studies will elucidate its role as a potential therapeutic target.

629 POSTER

Genetic polymorphisms of transforming growth factor-beta1 and estrogen metabolizing enzyme in estrogen receptor-positive and -negative infiltrating ductal breast carcinoma

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Background: Interactions between the transforming growth factor and estrogen metabolizing enzymes play important role in maintaining reproductive homeostasis. The aim of this study was to examine the effect of single nucleotide polymorphisms (SNPs) in the CYP1A1–6235T/C (rs4646903), SULT1A1–638G/A (rs9282861), TGFB1–509C>T (rs1800469) and TGFB1−29T>C (rs1982073) gene on the risk, progression and response to neoadjuvant chemotherapy in a cohort of estrogen receptor (ER)-positive and -negative infiltrating ductal breast carcinoma patients.
Material and Methods: This study included 178 women with ER-positive and negative histologically confirmed infiltrating ductal breast carcinoma, who received two-four cycles of neoadjuvant chemotherapy in the Tomsk Cancer Research Institute. The control group consisted of 290 unrelated women with benign breast pathologies. Genotyping was performed on genomic DNA using polymerase chain reaction and restriction fragment length polymorphism.
Results: Both ER-positive and -negative patients groups carrying SULT1A1(A/A) genotype were found to be significantly associated with increased risk of ductal breast carcinoma (OR = 2.02; p = 0.002 and OR = 1.88; p = 0.03, respectively). We showed the protective effect concerning this disease for the CYP1A1(T/T) and SULT1A1(G/G) genotypes independent of patients estrogen receptor status. In addition the ER-positive women with the TGFB1–509(T/T) genotype had a significantly lower risk of developing ductal breast carcinoma (p = 0.01) while a trend to the same association was observed among the ER-positive TGFB1−29(T/T) carriers (p = 0.051). There was also trend for association between the ER-positive TGFB1–509(C/C) genotype and large tumor size (p = 0.057). We found that the TGFB1–509(C/C) and SULT1A1(A/A) genotypes were non-statistically significant related with a poorer response to chemotherapy in ER-positive women (p = 0.09 and p = 0.06, respectively). Both ER-positive and negative the carriers of the CYP1A1(T/T) genotype showed an association with better response to neoadjuvant chemotherapy compared to the carriers of the other genotypes although the differences did not reach statistical significance (p = 0.09 and p = 0.06, respectively).
Conclusions: These findings suggest that genetic polymorphisms in TGFB1–509C>T and TGFB1−29T>C may modify individual susceptibility to ER-positive infiltrating ductal breast carcinoma. Further studies are needed to clarify the effect of SNPs analyzed in this work on prognosis and the efficacy to neoadjuvant chemotherapy in ER-positive and negative patients with infiltrating ductal breast carcinoma.

630 POSTER

Microarray based expression profiling of BRCA1 mutated breast tumours using a breast cancer specific array to identify a profile of BRCA1-deficiency

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Background: The BRCA1 tumour suppressor gene is mutated in a significant proportion of hereditary breast cancer cases. In addition, downregulation of BRCA1 mRNA and protein expression is reported in approximately one third of sporadic breast cancers. BRCA1 is strongly implicated in the maintenance of genomic stability by its involvement in multiple cellular pathways including DNA damage signalling, DNA repair, cell cycle regulation, protein ubiquitination, chromatin remodelling, transcriptional regulation and apoptosis. To date, gene expression profiling has identified: (1) at least five breast cancer subtypes and that (2) BRCA1 mutant tumours segregate with basal-like breast cancers. These studies also provide evidence that breast cancers with germline mutations in BRCA1 are different from non BRCA1-related tumours. The main aim of this study is to investigate the underlying biology of BRCA1-mutated breast cancer.
Methods: Extensive gene expression profiling and data analysis were performed on a cohort of 70 FFPE (Formalin Fixed Paraffin Embedded) derived BRCA1 mutated breast tumours and matched sporadic controls using the Almac Breast Cancer DSA™ research tool. Functional analysis was performed by DAVID and METACORE. Validation of gene targets was performed by both qRT-PCR and Western blotting.
Results: A list of differentially expressed transcripts has been derived from the comparison of these BRCA1 mutant breast tumours and matched sporadic controls. Functional analysis of this gene list has identified the key genes and molecular pathways that are deregulated in these tumours. BRCA1 deficiency was associated with deregulation of pathways involved in: (1) immune response, (2) metastasis and invasion, (3) cytoskeletal remodelling, (4) spindle assembly and chromosome separation, (5) apoptosis and survival. Validation of the key genes underlying this BRCA1-deficient breast cancer profile has been performed.
Conclusions: This approach has revealed a set of transcripts that could potentially be used to identify both hereditary and sporadic breast cancer

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.

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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.