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.

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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