Results: The first cohort consisted of 52 invasive breast cancers (M), 36 non-malignant (NM) and 10 normal (N) breast tissue samples. Median age of the patients was 60 years. Median size of breast cancer was 25 mm (IQR 20−40 mm). ER status was not significantly different between tissue types (p = 0.50). Every SKFM expression was quantified in all tissue samples. Fyn was the most expressed SKFM in normal tissue and Lyn in the NM breast tissue. Blk was the least expressed SKFM in all breast tissues. In malignant breast tissue Src and Lyn were most expressed. SKFMs Lck and Lyn were higher expressed in ER negative compared to ER positive tumours. c-Src (p = 0.01) and Fyn (p = 0.03) were expressed at higher levels in lobular compared to ductal carcinomas, whereas Yes (p = 0.006) was only expressed in ductal carcinomas.
Cohort two consisted of 320 patients with median follow-up of 6.3 years. Median age was 58 years (IQR 24−90). Median tumour size was 20 mm (IQR 15−30 mm). In both cohorts majority of the cancer specimens were pathologically graded as G2 and G3. 49% of the patients were axillary lymph node positive. High cytoplasmic Src and membrane Y419Src kinase expression levels were significantly associated with decreased disease specific survival (p = 0.03, p = 0.02). Lyn was not associated with survival at any cellular location. High membrane Lck expression was significantly associated with improved survival (p = 0.03).
Conclusions: All eight SKFM are expressed in different breast tissues. In invasive breast cancer Src kinase is highest expressed and seems to have a negative impact on disease specific survival. Whereas, high membrane expression of Lck provides better clinical outcome in those breast cancer patients. Further investigations are needed to determine underlying mechanisms for this observation.

1024 POSTER DISCUSSION

EGFR single nucleotid polymorphism R521K is a predictor for the occurrence of skin rash

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Background: Cetuximab, a monoclonal antibody targeting epidermal growth factor receptor (EGFR) is the first molecular targeting approach for the treatment of head and neck squamous-cell cancer (HNSCC) that demonstrated clinical efficacy with prolonged progression-free and overall survival. The most common side effect of cetuximab is moderate to severe skin rash. In the current study we analyzed whether cetuximab-induced skin rash is correlated with distinct genetic variations within the EGFR gene and focused our analyses on gene polymorphisms known to modulate EGFR expression levels, its capacity of ligand binding or its mitogenic signaling activity. Furthermore the intensity of skin rash and gene polymorphisms were correlated with progression free survival (PFS) and overall survival (OS).
Materials and Methods: 50 patients enrolled in a single-arm phase II multicenter study for second-line treatment of stage III/IV metastatic or recurrent SCCHN with cetuximab/docetaxel were genotyped for EGFR intron 1 CA-single sequence repeat (CA-SSR) polymorphism and the single nucleotide polymorphism R521K within EGFR exon 13. Association between genotypes and incidence/grade of skin rash classified by Common Toxicity Criteria (CTC) was assessed by Pearson's chi-square test. Survival analysis were performed by Kaplan Meier.
Results: The relative genotype distribution within our patient cohort was comparable to that reported by the HAPMAP consortium for a European reference population. Overall, thirty-eight patients (76%) developed skin rash within 6 weeks of treatment. For the CA repeat polymorphism (minor allelic sum 27−33 CA-SSR, major allelic sum 34−40 CA-SSR) we failed to observe an association with skin toxicity (p = 0.17), PFS (p = 0.18) and OS (p = 0.055) In contrast, the R521K variant (Lys allele) was significantly associated with reduced skin toxicity (p = 0.012). In fact, skin rash of grade >1 developed in only 7/27 (25%) of patients with homozygous Lys/Lys or heterozygous Lys/Arg genotypes but in 14/23 (60%) of patients with homozygous Arg/Arg genotype. PFS (p = 0.14) and OS (p = 0.10) were not associated with the SNP R521K. PFS (p = 0.015) and OS (p = 0.031) were, however, significantly associated with the occurrence of skin rash.
Conclusion: Our study suggests that the EGFR R521K but not the CA repeat polymorphism is a useful predictive marker for skin toxicity in HNSCC. Furthermore the occurrence of skin rash is positively associated with PFS and OS. The evaluation of its correlation with EGFR expression, ligand binding and signaling activity is currently ongoing.

1025 POSTER DISCUSSION

Gene expression profiling identifies Fibronectin 1 and CXCL9 as candidate biomarkers for breast cancer screening

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Background: There is a need to develop blood-based bioassays for breast cancer screening. In the present study, we have used differential gene expression between breast cancer samples and benign tumors to identify candidate biomarkers for blood-based screening.
Methods: Two candidate proteins (Fibronectin 1, CXCL9) were identified from a gene expression dataset that included 120 breast cancer samples and 45 benign lesions. These candidate proteins were selected as follow: a. differential gene expression between cancer and benign lesion, b. protein released in the extracellular medium (SwissProt) and stable in the serum, c. commercially available ELISA kit, d. Accuracy of the ELISA assay in a feasibility study (n = 23). Concentrations of these two proteins were determined in blood samples by ELISA. Blood samples were from normal volunteers (n = 119) and early breast cancer patients (n = 133). Normal volunteers were blood donors.
Results: Seventy-three percent of the patients presented a cT1-T2 tumour. CA15.3 was within normal range (<30 IU/ml) in 114 patients (86%). Blood concentrations of CXCL9 and Fibronectin 1 were higher in cancer patients as compared to normal volunteers. Mean concentration for CXCL9 was 851 pg/ml (range: 121–3941) and 635 pg/ml (range: 12–4327) in cancer patients and normal volunteers respectively (p = 0.013). CXCL9 concentration was significantly higher in patients with ER-negative breast cancer (mean: 999 pg/ml) as compared to normal volunteers (p = 0.003), a data consistent with gene expression profile. Meanwhile, Fibronectin 1 mean concentration was 190 μg/ml (range) for cancer patients and 125 μg/ml (range) for normal volunteers (p < 0.001). AUC for breast cancer diagnosis were 0.78 and 0.62 for Fibronectin 1 and CXCL9 respectively. A combined score including Fibronectin 1 and CXCL9 dosages presented a sensitivity of 53% and a 98% specificity. Similar performances were observed for ER-negative tumors.
Conclusion: This study suggests that Fibronectin 1/CXCL9 dosage in serum could screen a significant rate of breast cancer, including ER-negative breast cancer. These data suggest that analysis of differential gene expression is a good approach to select candidate biomarker to set-up blood assays cancer screening.

Poster presentations (Mon, 21 Sep, 14:00−17:00)

Basic science

1026 POSTER

Mechanisms involved in increased sensitivity of cisplatin resistant human laryngeal carcinoma cells to lovastatin

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Background: Cisplatin (cDDP) is a widely used anticancer agent in the treatment of many solid tumors, but development of cDDP resistance limits its efficacy. In comparison to parental human laryngeal carcinoma HEp-2 cells, sublines resistant to cDDP, $CA3_{ST}$ and CK2, have altered cell morphology, adhesion and cytoskeleton organization, suggesting alterations in Rho GTPases activity. Isoprenylation of Rho GTPases is crucial for their targeting to cell membrane, the process which is inhibited with HMG-CoA reductase inhibitor lovastatin. We have found that cDDP-resistant cells are sensitive to lovastatin.
The aim of the present study was to examine possible mechanisms involved in this phenomenon.
Material and Methods: To examine the mechanisms involved in sensitivity of $CA3_{ST}$ and CK2 cells to lovastatin, we used cytotoxicity assay, semiquantitative RT-PCR, Western blot analysis and transient transfection.
Results: Lovastatin treatment increased the expression of RhoB in all cell lines tested, and reduced the expression of Rac1 and Cdc42 (more in cDDP-resistant sublines). The toxicity of lovastatin and its effect on Rho GTPases was inhibited by addition of geranylgeranyl pyrophosphate, and to less extent farnesyl pyrophosphate. We found recently that RhoB downregulation confers resistance to cDDP and hypothesized that decreased RhoB expression could cause sensitivity to lovastatin. However, silencing of RhoB in HEp-2 cells with specific siRNA did not