Wednesday, 24 March 2010 18:15–19:15

POSTER SESSION

Tumour biology and immunology

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Perioperative assessment of the kinetics of circulating tumour cells in patients with operable breast cancer based on cytokeratin-19 mRNA detection

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Background: The studies on circulating tumor cells (CTCs) provide new insight into the biology of metastasis. The aim of this study was to evaluate the effect of surgery on the kinetics of CTCs in patients with surgically resectable breast cancer.

Materials and Methods: The detection of CK-19 mRNA-positive CTCs by real-time reverse transcriptase polymerase chain reaction in the blood was analysed in 104 stage 0−IIIA breast cancer patients at four different times: prior to surgery, upon completion, 24 hours after surgery, and 15 days after surgery. Furthermore, a late sample was assessed prior to initiation of adjuvant chemotherapy in a subgroup of 53 patients. As negative controls, peripheral blood was obtained from 50 female patients undergoing excision of benign lesions and from 11 female patients receiving surgery for early stage colorectal cancer.

Results: No significant difference was noted between samples of breast cancer patients at different time intervals with respect to the median CK-19 mRNA-positive cells. The overall percentage of breast cancer patients who were CK-19 mRNA-negative before surgery and turned positive at any time point postoperatively was 14.9%. There was no significant correlation between CK-19 mRNA-positivity and classical prognostic factors. A significant increase in CK-19 mRNA-positivity (32.1%) was observed in the late sample of the subgroup of 53 patients after a median of 54 days.

Conclusions: CTCs identified perioperatively based on CK-19 mRNA detection in patients undergoing surgery for early breast cancer are independent of the surgical procedure. There is no clear correlation to indicate which patients are more likely to have detectable CTCs. Although in the perioperative period CTCs are detected in a low proportion of patients, the detection rate may increase over time and with longer follow-up.

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The role of suppressors of cytokine signaling in human breast cancer

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Background: Suppressors of cytokine signaling (SOCS) are important negative feedback regulators of the JAK/STAT signaling pathway, and have been recently investigated for their role in the development of different cancers. In this study, we examined the expression of SOCS1−7 genes in normal and breast cancer tissue and correlated this with several clinico-pathological and prognostic factors.

Materials and Methods: SOCS1−7 mRNA extraction and reverse transcription were performed on fresh frozen breast cancer tissue samples (n = 127)and normal background breast tissue (n = 31). Transcript levels of expression were determined using real-time PCR and analysed against TNM stage, tumour grade, and clinical outcome over a 10 year follow-up period.

Results: SOCS 1, 4, 5, 6 and 7 expression decreased with increased TNM stage (TNM1 vs. TNM3 p = 0.039, TNM1 vs. TNM4 p = 0.016, TNM1 vs. TNM3 p = 0.012, and TNM1 vs. TNM3 p = 0.044 respectively). SOCS 2 and 3 expression decreased with increased Nottingham Prognostic Index (NPI) (NPI1 vs. NPI3 p = 0.033, and NPI2 vs. NPI3 p = 0.041 respectively). SOCS7 expression decreased with higher tumour grade (Grade 3 vs. Grade 2 p = 0.037). After a median follow-up period of 10 years, we foundhigher levels of SOCS1, 2, and 7 expression among those patients who remained disease-free compared to those who developed local recurrence (p = 0.0073, p = 0.021, and p = 0.039 respectively). Similarly, we found higher levels of SOCS 2, 4, and 7 expression in those who remained disease-free compared to those who developed distant recurrence (p = 0.022, p = 0.024, and p = 0.033 respectively). Patients who remained disease-free had higher levels of SOCS 1 and 3 expression compared to those who died from breast cancer (p = 0.02, and p = 0.033 respectively). The disease free survival (DFS) and overall survival (OS) curves showed that higher levels of SOCS 1, 3 and 7 were significant predictors of better DFS (p = 0.015, p = 0.024, and p = 0.03 respectively) and OS (p = 0.005, p = 0.013, and p = 0.035 respectively). Higher levels of SOCS 4 were significant in predicting better OS (p = 0.007) but not DFS.

Conclusions: Higher mRNA expression levels of SOCS 1, 3, 4, and 7 are significantly associated with earlier tumour stage and better clinical outcome in human breast cancer.

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Prediction of lymph node involvement in breast cancer from primary tumour tissue using gene expression profiling

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Lymph node involvement is the most important prognostic factor in breast cancer, but yet little is known about the underlying molecular mechanisms. Whether a "lymphatic metastasis signature" can be defined for breast cancer is currently unclear. Here, to identify a molecular signature associated with nodal metastasis, gene expression analysis was performed on a very homogeneous group of primary breast tumors (postmenopausal, ER+, HER2-, grade 3 invasive ductal cancer).

The datasets considered in this study are two own datasets (prospective dataset containing 48 node negative and 48 node positive samples; independent dataset containing 20 samples) and 5 publicly available datasets on breast cancer for which the lymph node status was provided (106 samples). For our datasets, RNA was isolated and hybridized to the Affymetrix Human U133 Plus 2.0 microarray chip. All datasets were preprocessed with MAS 5.0. We used the annotation provided by Dai for the conversion of probes to genes. The 5000 most varying genes were included. Finally, our prospective dataset was standardized per sample across all genes. The other datasets were first reduced to the subset of genes included in our final model before standardization. A model was built by weighted Least-Squares Support Vector Machines at a significance level for gene inclusion of 0.05. The Spearman correlation coefficient was used to identify genes that gradually increase or decrease with changing lymph node ratio (number of positive lymph nodes divided by total number of lymph nodes). A 10-fold cross-validation strategy was followed to train a model on our 96 patients. The model that has been validated on the internal and the external datasets contained those genes that were selected in at least half of the cross-validation iterations at the significance level 0.05 (241 genes). The area under the ROC curve for the internal dataset is 0.647 and 0.651 for the external datasets. The model includes a high number of apoptosis related (26) and zinc ion binding (43) genes. Pathway analysis using the Molecular Signatures Database revealed multiple relevant gene sets i.e. BRCA, BAF57, Van 't Veer.

In conclusion, our model provides evidence that lymph node involvement in breast cancer is not a random process. Whether the model is a general predictor for lymph node involvement will be evaluated in a next step.

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Strategy to augment the efficacy of immunotherapy for refractory breast cancer: a pilot clinical study of adoptive cell therapy combined with trastuzumab

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Background: Cancer targeting mAb Trastuzumab as a single agent has activity in metastatic breast cancer; however, the mechanism of action for this clinical activity is not fully understood. Whereas interruption of HER family member signaling occurs, trastuzumab also could interact with host immune-cells via its Fc domain. Based on these data, a clinical trial was performed to test whether trastuzumab, when combined to adoptive cell therapy in patients with HER2+ tumor, can increase the therapeutic efficacy, and be safely given.

Materials and Methods: 14 patients with recurrent breast cancer (HER2+ vs. HER2−: 7pts vs. 7pts) who had failed the conventional chemo-radiotherapy were enrolled. Autologous-tumor cell stimulated lymphocytes

(AuTL) was administrated into recurrent tumor site biweekly, and additionally trastuzumab (2 mg/kg) was infused systemically every week in the 7 HER2+ patients. Patients continued on therapy until disease progression.

Furthermore, to assess the mechanism of trastuzumab-effects in the therapy, dendritic cells (DC) from peripheral monocytes of several healthy donors were generated in IL-4/GM-CSF in vitro, and fed with trastuzumab-treated/-untreated HER2+ tumor cells or tumor cell lysate. These antigen-loaded DCs were examined in the phenotype, cytokine productions, and the ability to induce HER2 specific T cells in vitro.

Results: In the clinical trial, one patient of PR was observed in the HER2+ group, which showed marked regression in the injected field of metastatic lymph node, but none of PR in the HER2−. SD/PD was 3/3 or 3/4 pts in the HER2+ or HER2− group, respectively. The carcinomatous pleural effusion was disappeared and/or well controlled in 6 pts (HER2+ vs. HER2−; 4 vs. 2 pts), and the tumor marker proteins (CEA, CA15−3, TPA) were decreased significantly in 5 pts (HER2+ vs. HER2−; 4 vs. 1 pts). Adverse effects were tolerable in all the patients.

In DC experiments in vitro, trastuzumab-opsonized antigen-loaded DC showed significant enhancement of the ability to induce CD8+ T cells specific for HER2-peptides with the higher production of IL-12p70.

Conclusions: Adoptive cell therapy combined with trastuzumab is a well-tolerated regimen. Our preliminary data suggest that this strategy may benefit heavily pretreated HER2+ metastatic breast cancer patients. It might be in part due to the involvement of mAb in the ability of DC cross-presentation followed by the enhancement of antitumor cellular immunity.

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BRCA1 mutation is strongly associated with a triple negative phenotype in breast cancer patients

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Introduction: Our aim was to compare the differences in clinical presentation and tumor pathology features among breast cancer patients with BRCA1 and BRCA2 mutations and non-BRCA carriers.

Material and Methods: Tumor pathologic features (histology, hormone receptor and Her2 expression) and clinical characteristics (age and clinical stage at diagnosis, gender, bilaterality, and BRCA mutation status) were examined in 188 breast cancer patients who underwent BRCA germline genetic testing between 2002 and 2008 through a retrospective review of our hereditary cancer database.

Results: Of the 188 patients, 129 (69%) were non-BRCA carriers, 28 (15%) were BRCA1, and 31 were BRCA2 (16%). Age at diagnosis was similar among the three groups (40, 42 and 46 respectively, non-statistical different). Among male breast cancer patients (7), none was a BRCA1 carrier and three were BRCA2 carriers. Bilaterality was more frequent in BRCA1 and BRCA2 carriers compared to non-carriers (25%, 24%, and 14%, respectively, non-statistically different). Triple-negative breast cancer (estrogen receptor, progesterone receptor, and HER-2/neu negative) was diagnosed in 85% (18/21) of the BRCA1 carriers, 21% (4/19) of the BRCA2 patients, and 29% (27/93) of the non-BRCA patients ($p < 0.01$). We did not observe any patient with a BRCA1 mutation and HER2 overexpression ($p < 0.01$ compared with BRCA-2 and non-BRCA carriers), while HER2 overexpression was similar between BRCA2 (15%) and non-BRCA carriers (23%) ($p = 0.47$).

Conclusions: Breast cancer in BRCA1 mutation carriers is more frequently triple negative while patients with BRCA2 mutations have a similar clinical and pathologic phenotype than non-BRCA patients. These differences may have therapeutic implications.

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Ductal carcinoma of the breast with morphologic and inmunohistochemical features like columnar cells

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Background: Columnar cell lesions (CCL) of the breast are detected with increasing frecuency in routine practice. The frequent coexistence of CCL with low grade DCIS in the same breast and overlapping morphologic features with ADH and DCIS provides evidence for CCL being a candidate precursor in the progression to low grade DCIS and invasive carcinoma. This hypothesis has been supported by the similar cytologic appearance of cells within atypical cystic lobules and low grade DCIS of the same specimens or cells within CCL and cells comprinsing coexisting DCIS or tubular carcinoma.

Material and Methods: In 500 cases of breast cancer in our routine practice, we identified four cases of ductal carcinoma with morphologic features like columnar cells. We assessed inmunohistochemistry (IHC) studies for estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR), HER-2/neu, cytokeratin 19 (CK 19), cytokeratin 5/6 (CK5/6), cytokeratin 34bE-12 (CK34bE-12), Bcl-2, cyclin D-1 (CD1), Ki67 MIB1. Results of hormonal receptors were scored by H-Score previously described. HER-2/neu were scored positive with score 3. Ki67MIB1 was scored by percent of the positive tumor cells.

Results: The characteristics of the patients and IHC are shown in the table. Microscopically the tumors showed irregular ducts lined with one or two layers columnar cells with prominent apical cytoplasmic snouts and intraluminal secretion. Nuclear/cytoplasmic ratio was increased. Nuclei round to ovoid, hyperchromatic nuclei with inconspicuous nucleoli. In other areas there were solid pattern and complex architecture with micropapillae, fronds, arcades, rigid bridges. We observed in three cases in some areas intraluminal calcifications.

Gender/Age	Tumor size (cm)	Axilla status	Symptom duration	ER	PR	AR	HER2neu	CK19	CK5/6	CK34β E 12	CD1	Bcl2	Ki-67
Female/94	3×3	1 node +	1 year	200+	180+	+	−	+	−	+	+	+	30%
Female/47	2.5×2	-	2 years	200+	170+	+	−	+	−	+	+	+	20%
Female/80	2×2	-	2 years	150+	100+	+	−	+	−	+	+	+	20%
Female/43	1.5×1	3 nodes +	1 year	200+	170+	+	−	+	−	+	+	+	30%

Conclusion: The morphologic and inmunohistochemical features of these carcinomas are similar with columnar cells. These carcinomas could be the malignant form of the CCL. We need further studies for categorize these tumors.

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Molecular biology, markers

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The prognostic value of angiogenesis genes polymorphisms in women with infiltrating ductal breast carcinoma

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Background: Angiogenesis is an important step in the development of infiltrating ductal carcinoma which is the most common histologic type of breast cancer. Polymorphisms in genes encoding angiogenic factors or their receptors are known to predispose to breast cancer [Schneider et al., 2008; Clar et al., 2009]. The aim of our study was to investigate the association of functional polymorphisms in the *VEGF-2578C*/A (rs699947), *FGFR2A*/G (rs1219648), *TGFB1–509C>T* (rs1800469) and *IL10–592C>A* (rs1800872) genes with infiltrating ductal breast carcinoma risk, progression and response to neoadjuvant chemotherapy.

Material and Methods: Two hundred sixteen patients with operable primary infiltrating ductal breast carcinoma ($T_{1-4}N_{0-2}M_0$; age from 20 to 77 years) who received two-four cycles of neoadjuvant chemotherapy in the Tomsk Cancer Research Institute were included in the present study. The healthy women ($n = 286$; age from 30 to 75 years) from Western Siberian region were used as the control group. DNA was extracted from peripheral blood and the genotypes were analyzed using PCR-restriction fragment length polymorphism protocols.

Results: The frequencies of VEGF-2578A/A, FGFR2G/G and IL10–592A/A variants were significantly higher in the patient group when compared with controls (OR = 2.3, $p = 0.002$; OR = 2.3, $p = 0.002$ and OR = 3.2, $p = 0.008$ respectively). Significantly lower frequencies of FGFR2A/A and