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molecular biology (pre-clinical)

96O TRACING TUMOR LINEAGE AND PROGRESSION THROUGH GENOMIC COPY NUMBER PROFILING AT THE SINGLE CELL LEVEL

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Background: Genomic analysis by microarray, and more recently DNA sequencing, has provided important insights into the role of copy number variation in human cancer. However, these methods can yield only approximate results when applied to mixed populations of rapidly evolving cells. In such cases our understanding would be improved by dissecting genetic events at the single cell level.

Methods: We have developed a method of single nucleus sequencing (SNS) to quantify the genomic copy number of individual tumor cells. We have shown that a single lane of sequence reads are sufficiently distributed across the genome to measure copy number at a resolution of about 50kb. We have used SNS along with other methods for genomic profiling to analyze tumor segments and more than 100 single cells isolated from macrodissected primary tumors and metastases.

Results: From two heterogeneous Basal-like breast carcinomas we constructed a detailed phylogenetic lineage, showing that the majority of cells belong to one of several major subpopulations that have clonally expanded to form the mass of the tumor. In both cases the earliest detectable evolutionary stage was a hypodiploid clone with a characteristic sawtooth pattern. A geographically adjacent segment contained cells carrying the identical genomic markers that had apparently undergone endoreduplication to generate a pseudo-triploid genome that in subsequent steps had acquired many additional focal amplifications and deletions of cancer genes including in one cases, KRAS, EFNA5 and COL4A5.

Conclusions: Single cell copy number profiling confirmed that the vast majority of genomic events characteristic of the clones were present in each individual cell and that complex aneuploid patterns are not the result of mixed populations of tumor cells, but rather represent single tumor cells that have clonally expanded. Our data strongly support a polyclonal evolution model for tumor progression in which the majority of tumor cells, perhaps arising from an originally unstable precursor, continues to expand and proliferate to form the bulk of the tumor.

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97O MOLECULAR AND CLINICALLY DISTINCT PHENOTYPES IN HER2-OVEREXPRESSING BREAST CANCER (HER2+ BC) CORRESPOND TO ESTROGEN RECEPTOR STATUS (ER) STATUS

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Background: HER2 overexpression predicts response to anti-HER2 agents. Molecular profiling often suggests that ER+/HER2+ (double positive-DP) BC is a distinct entity from ER-/HER2+ BC. We investigated these differences and their potential clinical implications.

Methods: HER2 overexpresion was defined by FISH ratio >2.0 or mRNA. ER status was defined by ESR1 mRNA. We used 11 datasets of BC samples with gene expression (GE) and clinical outcome data (n=1110, 17% HER2+); 2 array CGH datasets (n=309, 27%) & 74 HER2+ neoadjuvantly trastuzumab-treated patients. HER2+ BC cell lines treated with anti-HER2 agents were used to investigate in silico hypotheses.

Results: In systemically untreated BC patients, the DP had the worse outcome compared with the ER+/HER2- (p=.001); ER-/HER2- (p=.02) but also the ER-/HER2+ (p=.02) subgroups. There were significant molecular differences in HER2+BC according to ER status- DP vs ER-/HER2+; 6.2% of whole genome GE & 1.1% copy number changes, FDR<1%). In HER2+ BC, ESR1 gene expression was significantly inversely correlated with ERBB2 (R= -0.3; p=0.02), EGFR (-0.6; p<0.001) and gene sets of RAS (-0.4; p=0.01), RAF (-0.8; p<0.001), MAPK (-0.7; p<0.001) and MEK (-0.4; p<0.001) pathway activation. However, there were positive correlations between ESR1 and ERBB3 (0.7; p<0.001) and AKT1 (0.4; p<0.001). A gene set of PI3K/AKT pathway activation could predict pCR in trastuzumab-chemotherapy patients in DP (AUC=0.8; p=0.005) but not in the ER-/HER2+ (AUC=0.6) group. HER2+ BC cell lines were treated with trastuzumab and lapatinib. BT474, ZR7530, SKBR3 (DP) showed decreased proliferation corresponding to decreased pAKT and pS6, whilst HCC1954 and HCC1569 (ER-) had no such association of pAKT and pS6 reduction and less growth inhibition. Furthermore, BT474 lines treated with fulvestrant to inhibit ER signaling were no longer sensitive to an AKT inhibitor (p=.001).

Conclusions: In HER2+ BC patients, ER status defines distinct molecular and clinical phenotypes. ER signaling may antagonize EGFR/RAS/MAPK signaling, leading to increased PI3K/AKT output in the DP. Future clinical trials in HER2+ BC should therefore be stratified for ER status.

Disclosure: All authors have declared no conflicts of interest.

98O ROBUSTNESS OF BREAST CANCER MOLECULAR SUBTYPES IDENTIFICATION

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Background: Gene expression studies classify breast cancer (BC) into at least three clinically relevant molecular subtypes: basal-like, HER2-positive, and luminal-like tumors, with each subtype exhibiting different prognosis and response/resistance to anticancer therapies. Perou et al. have published several iterations of the Single Sample Predictor (SSP), each of which have different sets of "intrinsic" genes. Recently, Sotiriou et al. introduced a fuzzy computational approach, Subtype Clustering Model (SCM), which uses only ER, HER2 and proliferation related gene modules (two versions of these lists have been published), to estimate probabilities of belonging to each BC molecular subtype. Demonstration of molecular heterogeneity of BC has changed the way clinicians perceive the disease and has dramatically impacted the design of new clinical trials. However, despite these important implications and the vast amount of publicly available microarray datasets, the robustness of this classification remains an open question.

Materials and methods: We statistically compared the robustness of existing classification models using prediction strength statistics in 24 datasets with over 4000 BC patients. Furthermore, we studied their concordance with respect to molecular subtypes and their clinical relevance through survival analysis of 1471 untreated node-negative patients.

Results: SCM models were significantly more robust than all three SSPs in identification of three and four subtypes ($p < 0.001$). Although all models were concordant (Cramer's V = 0.58-0.83, $p < 10\text{-}16$), with basal subtype being particularly well defined (83-93%), SCM models yielded stronger concordance than SSP models. Importantly, survival analysis of SCM yielded better discrimination of low-risk patients (low proliferative ER+/HER2- tumors).

Conclusions: We highlight significant disparities in robustness of BC molecular subtype classification models. SCMs outperformed SSPs and consistently identified molecular subtypes in numerous datasets from various microarray technologies and different laboratories. Demonstration of SCM robustness is a significant step towards acceptance and translation of these classification models into the clinic.

Disclosure: All authors have declared no conflicts of interest.

99P GENE EXPRESSION PROFILING IN CIRCULATING CELLS (CTCS) OF BREAST CARCINOMA PATIENTS

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Background: Based on the molecular gene expression profile of circulating tumour cells (CTCs) at the mRNA level, we have tried to characterize the algorithms of the early dissemination process and gene expression changes caused by chemotherapy (CHT) in primary and metastatic breast cancers (MBC). Czech and German CTC samples of MBC patients have been compared within the study.

Method: Blood samples (5ml) of 87 primary BC and 77 MBC patients were analyzed for CTCs using the AdnaTest BreastCancer (AdnaGen AG, Germany) for the detection of EpCAM, MUC-1 and HER-2 transcripts. RNA from formalin fixed paraffin embedded (FFPE)-tumour tissue (n=85) has been isolated by RecoverAllIM (Ambion). Obtained cDNA molecules have been gene-specifically pre-amplified for multimarker qPCR analysis measured on BiomarkIM Fludigm, USA) microfluidic chip for 48 samples and 48 testing positions (2034 rxn in total, 35 tumour specific-genes in total). qPCR results have been analyzed by GENEX v.s. 5.0 software (MultiD, SE).

Result: 286 CTC samples have been analyzed in total. CTCs were detected in 29/87 (33,3%) patients with primary BC before starting CHT and in 10/87 (11,5 %) after 2 CHT cycles. The Czech CTC positivity rate for MBC 10/16 (62,5%) has been comparable with the CTC detection rate in the group of German metastatic patients (38/56, 68%). The analysis has shown that the gene expression profiles of CTCs in primary breast cancer patients relate to the gene expression profiles of primary tumour. In opposite, the gene expression profiles of CTC in MBC differ from the primary tumour significantly. Analyzing the gene expression data from CTC-positive (Adnatest positive) patients in comparison to CTC negative patients and FFPE samples, we have revealed 20 genes that were differentially expressed ($p<0,05$) (e.g. progesterone receptor, MLF1lP -myeloid leukemia factor 1-interacting protein, and H2AFZ - H2A histone family, member Z). The predictive value of CTC expression profiles will be prospectively evaluated.

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101P RECEPTOR ACTIVATOR OF NF-KB (RANK) EXPRESSION ASSOCIATES WITH BONE METASTASIS IN BREAST CARCINOMAS

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Background: recently it has been demonstrated that RANK is overexpressed in about 65% of primary breast tumours. Here we explored the clinical significance of RANK expression in early breast cancer. For this purpose we included in the study a series of early breast cancer primary tissues and correlated IHC RANK expression with skeletal clinical outcome.

Material and methods: This study included 93 breast cancer patients with complete clinicopathological information and up to 2 years of follow-up. Samples contained 15 lobular carcinomas and 77 ductal carcinomas; N0-1 versus N2-3: 63/29; HER2/neu +/-/unknown: 25/55/13; sites of metastases: 16 patients only skeletal, 16 only visceral, 29 skeletal plus visceral, 32 without metastases. No patient was treated with neoadjuvant therapy. RANK protein expression was determined using immunohistochemistry. We considered RANK positive patients when more than 50% of tumoral tissues was scored as grade 2 (intensity higher than internal control -macrophages-) or 3 (intensity much higher than internal control).

Results: RANK was expressed in 38 (41%) of the primary tumor samples. RANK expression was independent from histotype (p:0.23), HER2/neu expression (p:0.47) and grading (p:0.39) but was dependent on nodes status (p:0.05). RANK positive patients showed a higher risk to develop skeletal metastases (p:0.023). Moreover, RANK expression was associated with accelerated bone metastasis formation ($P = 0.034$). Multivariate analysis confirmed that RANK is an independent prognostic indicator for early bone metastasis development (P 0.037).

Conclusions: RANK is clearly associated with bone metastasis formation and thus might have clinical utility in identification of patients with increased risk of bone metastasis and with increased probability to respond to anti-RANKL monoclonal therapy (denosumab). This is the first time that RANK has been linked to the bone metastasis process in breast cancer.

Disclosure: All authors have declared no conflicts of interest.

102P MICRORNAS REGULATE THE STEMNESS OF BREAST TUMOR INITIATING CELLS

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Cancers may arise from rare self-renewing tumour-initiating cells (T-IC) but it remains obscured how self-renewal capacity for multipotent differentiation and tumorigenicity of T-IC are maintained. Because miRNAs regulate cell fate, we compared miRNA expression in self-renewing and differentiated cells from the breast cancer cell lines, in breast T-IC (BT-IC), and non-BT-IC from primary breast cancers. We found that: (1) let-7 miRNAs and miR-128 were markedly reduced in BT-IC and increased with differentiation. In parallel, their target proteins, ha-ras, HMGA2, and hTERT, were elevated in BT-IC but were reduced after differentiation. (2) Infecting BT-IC with let-7-lentivirus or miR-128 lentivirus reduced proliferation and mammosphere formation in vitro and ability to form metastatic tumours, while antagonizing let-7 or miR-128 by antisense oligonucleotides enhanced self-renewal of non-T-IC. (3) Infecting BT-IC with let-7-lentivirus enhanced differentiation of the cancer stem cells via HMGA2 pathway, while with miR-128 induced apoptosis via inhibiting the activity of telomerase. (4) Targeting delivery of let-7 miRNA into BT-IC with a non-viral vector, HA-liposme, successfully inhibited mammosphere formation and tumorigenesis of breast cancer stem cells. Thus, self-renewal of breast cancer stem cells are tightly regulated by microRNAs, and targeting delivery of tumour suppressing microRNAs into BT-ICs emerges to be a novel approach in the breast cancer treatment.

Disclosure: The author has declared no conflicts of interest.

103P HUMAN CD4+ T CELLS INFILTRATING BREAST TUMORS EXHIBIT CRITICAL ALTERATIONS IN CELLULAR SIGNALING PATHWAYS COMPARED WITH THEIR COUNTERPARTS FROM THE LYMPH NODE AND PERIPHERAL BLOOD

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Flow cytometric analyses of fresh breast tumor cell homogenates revealed that CD4+ T cells constitute the major infiltrating CD45+ subpopulation, present at twice the frequency found in the patient's normal breast tissue. We used gene expression arrays to assess differences in the molecular profiles of CD4+ T cells from the primary tumor (TIL), axillary lymph node (LN) and peripheral blood (PB) of patients with ER+ and ER- invasive ductal breast carcinoma. Unsupervised analysis revealed that the greatest differences characterized their tissue origin (TIL, LN or PB) rather than the individual patient. Gene expression profiles of CD4+ TIL were distinctly different from their PB and LN counterparts while CD4+ from patient and age- and sex-matched control PB were remarkably similar. A significant increase in the number of differentiated CD4+ T cells was detected in the TIL (>90% CD45RO+) compared with the PB (40-60% RO+) but this was not characterized by a dramatic skew in any individual effector cell subpopulation (Th1, Th2, Th17, Treg). The major changes in gene expression observed in the TIL compared to the LN and/or PB are characterized by altered cellular signaling pathways, including: 1) significant suppression of the T cell receptor/CD3 complex and numerous downstream signaling molecules, 2) suppression of TGFβ/Activin-directed signaling in favor of the BMP signaling pathway, 3) increased expression of inhibitory receptors and adaptors and a subset of co-stimulatory receptors, 4) a restricted pattern of Th chemokine and cytokine expression, and 5) a specific pattern of adhesion receptor expression. Interestingly, very few differences in these cellular signaling pathways were observed in a direct comparison of CD4+ TIL from ER+ and ER- tumors. These data suggest that the tumor microenvironment rather than the tumor grade/phenotype is the driving force influencing the expression pattern of genes involved in regulating CD4+ T cell-mediated immune functions. This work was supported by grants from the FRS-FNRS, Télévie, Amis de l'Institut Bordet, and the European Union.

Disclosure: All authors have declared no conflicts of interest.

104P THE PROGNOSTIC IMPACT OF MOLECULAR SUBTYPES REMAINS SIGNIFICANT IN PATIENTS WITH DIFFERENT AGE GROUPS

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Introduction: Breast cancer (BC) molecular subgroups carry a significant prognostic impact among patients with BC. Similarly, young age at diagnosis is significantly associated with an unfavorable prognosis. We analyze the association between BC molecular subtypes and age at diagnosis.

Methods: Publically available gene expression data (Affymetrix U133A) of 1772 BC patients was pooled into a single database. Molecular subgroups were defined using the method by Hugh et al. (J Clin Oncol 2008). The dataset was analyzed using MAS5.0.

Results: Patients <40 years were significantly more often diagnosed with triple negative BC compared to patients 40-50 and >50 (34.8 vs. 25.4 vs. 17.3%, respectively),

whereas the luminal A subtype was diagnosed less often (20.6 vs. 31.9 vs. 41.1%, respectively). Among patients in ER positive (luminal A and B) but not triple negative and HER2 positive disease a continuous increase in estrogen receptor (ESR1_205225_at) expression with patient age was observed. No such effect was observed for expression of progesterone receptor (PGR_208305_at) or HER2 (ERBB2_216836_at). Patients with BC of the luminal A subtype showed a mean event-free survival (EFS) of 104 months (95% confidence interval (CI) 101-107) compared to 85 months (95% CI 80-92) for triple negative BC. Patients with age <40 years showed significantly more unfavorable prognosis (81 months (95%CI 72-90)) compared to patients with 40-50 or > 50 years (95 months (95%CI 90-99) vs. 93 months (95%CI 90-96), respectively). EFS differences between patients of distinct age were modest when patients were stratified for molecular subtype.

Conclusion: The prognostic impact of molecular subtypes remains significant in patients with different age groups, whereas the frequency of molecular subgroups may differ. The latter may contribute, though not exclusively, to the significantly lower EFS rates observed among patients with < 40 years compared to patients with higher age.

Disclosure: All authors have declared no conflicts of interest.

105P UPREGULATION OF ADAM17 PROTEASE AND HER LIGANDS THROUGH A PKB NEGATIVE FEEDBACK LOOP MEDIATES ACQUIRED RESISTANCE TO TRASTUZUMAB IN HER2 OVER-EXPRESSED BREAST CANCER

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It is still poorly understood how Herceptin (Trastuzumab) exerts its tumour inhibition effect and the reports on its effect on HER2 phosphorylation have also been variable among different investigators. Its acquired resistance mechanisms are also not yet fully determined. We have found the molecular mechanisms of why Herceptin fails to abolish HER2 phosphorylation despite being an anti-HER2 monoclonal antibody. HER2 phosphorylation was maintained by activation of the other HER receptors via their dimerisation with HER2. The activation of alternative HER receptors was due to an upregulation of HER ligands including heregulin and betacellulin, which in turn were mediated by a negative feedback loop. This feedback loop was activated because of the inhibition of PKB by Herceptin treatment since a PKB inhibitor (Akt inhibitor VIII, Akti-1/2) which decreases PKB phosphorylation in a different mechanism to Herceptin, also activated the loop. However, a panHER inhibitor JNJ-26483327 in combination with Herceptin was able to abrogate the feedback loop and decrease HER2 phosphorylation. Furthermore, the combination of drugs was synergistic in tumour inhibition in a BT474 xenograft model. Our data provides evidence that Herceptin resistance can be mediated by activation of HER family ligands as a result of a PKB negative feedback loop. This offers treatment opportunities for overcoming resistance in these patients, including approaches to target all HER receptors in combination with Herceptin treatment.

Disclosure: P. King: The author is an employee of Johnson and Johnson; T. Perera: The author is an employee and a small shareholder of Johnson and Johnson. All other authors have declared no conflicts of interest.

106P BREAST CANCER RESISTANCE TO ANTHRACYCLINES AND TAXANES UNDER A MAGNIFYNG LENS: EMERGING ROLE OF CIRCULATING TUMOR CELLS

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Purpose: The prognostic value associated with the count of circulating tumour cells (CTCs) in metastatic breast cancer (MBC) raise additional issues regarding the biological value of this information. A prospective study was conducted to investigate whether a baseline drug resistance profile of CTCs may predict response to systemic chemotherapy in patients with MBC. The drug resistance profile of CTCs was performed through the analysis of the expression of multidrug resistance related proteins (MRPs), belonging to ATP binding cassette transporters, which mediate the extrusion of chemotherapics out of cells in a selective manner.

Patients and methods: Forty-two (42) patients with diagnosis of MBC treated with anthracyclines / taxanes based regimens were enrolled in a prospective trial. CTCs were isolated from 10 cc of peripheral blood by CELLection™ Dynabeads® coated with the monoclonal antibody towards the human Epithelial Cell Adhesion Molecule (EpCam). CTCs positive samples were evaluated by RT-PCR assay for the expression of MRP1 and MRP2 (resistance to anthracyclines) and MRP7 (resistance to taxanes). The drug resistance profile was correlated to progression free survival (PFS) in a 24 months follow up by Kaplan-Meier product-limit method.

Results: A statistically significant difference in PFS was found between CTCs-positive and CTCs-negative patients ($p<0.0001$). PFS was found significantly shorter in patients with CTCs showing a ''drug resistance'' profile at the chemotherapeutic delivered regimen ($p>0.0001$). Of note is that the expression of MRP1-MRP2 on CTCs was not associated to resistance to non pegylated liposomal doxorubicin (Myocet®), suggesting that liposomal formulation could escape MRPs-mediated drug resistance.

Conclusions: In MBC, the presence of CTCs expressing MRPs is predictive of response to chemotherapy. The validation of the method here described may allow the definition of the utility of CTCs molecular signature as a further predictive tool in the treatment of breast cancer patients.

Disclosure: All authors have declared no conflicts of interest.

107P INVADING CHEMORESISTANCE SANCTUARY IN METASTATIC BREAST CANCER: A BIOCHARACTERIZATION OF ''STEM-LIKE'' DRUG-RESISTANT CIRCULATING TUMOR CELLS

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Purpose: Despite the increasing number of studies focusing on the prognostic impact of circulating tumor cells (CTCs) count in metastatic breast cancer (MBC) patients, the nature of these cells is still poorly understood. It has been suggested that more aggressive CTCs may share similar genotypic characteristics with so-called breast cancer stem cells, characterized by quiescence, self-renewal ability, and multidrug resistance, which allow these cells to drive tumour growth and to evade conventional therapy. Recent evidence suggests that aldheyde dehydrogenase 1 (ALDH1) expressing cells may have stem/progenitor cell features, and retain the essential property of self-protection through the enhanced activity of ATP binding cassette transporters, which may provide an explanation of their intrinsic drug resistance. Aim of the study was to correlate ALDH1 and multidrug resistance related proteins (MRPs) expression on CTCs from MBC patients.

Patients and methods: Forty-two (42) MBC patients were enrolled. CTCs were isolated from 10 cc of peripheral blood by CELLection™ Dynabeads® coated with the monoclonal antibody towards the human Epithelial Cell Adhesion Molecule (EpCam). The expression of ALDH1, investigated by RT-PCR, was correlated to the number of MRP transporters expressed on CTCs by the Pearson's correlation test.

Results: 28/42 (67%) patients were found positive for the presence of CTCs. ALDH1-positive CTCs were detected in 17 (60.7%) out of 28 CTCs-positive patients. The correlation between the number of different MRPs expressed in CTCs and ALDH1 was found statistically significant ($p<0.0001$).

Discussion: Recent evidence suggests that ALDH1-positive cancer cells serve as a significant and independent predictor of resistance to chemotherapy. Consistently with this hypothesis, we found in CTCs a significant correlation between ALDH1 and the number of MRP drug transporters expressed. We thus suggest that this subset of ALDH1/MRP expressing CTCs may represent a cell population with greater tendency to intrinsic drug resistance. Whether these cells may have stemness properties is currently under investigation.

Disclosure: All authors have declared no conflicts of interest.

108P HER2-SCFV-PROTAMINE MEDIATION IN DELIVERY OF PLK1 SIRNA TO HER2 OVEREXPRESSED BREAST CANCER

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Background: Breast cancer is the most frequent type of cancer in women, with a global annual incidence of approximately 200,000. Despite the improvement in detection and treatment, around 30% of newly diagnosed patients will die from the malignancy. We now know that the prognosis of the disease varies among different tumour subtypes with distinct gene expression profile. Among them, 20-35% of the invasive breast cancers overexpress human epidermal growth factor receptor 2 (Her-2/neu/c-ErbB2), which correlates with more aggressive tumour behavior and poor clinical outcome. Activation of Her-2 receptor results in phosphorylation of the intracellular catalytic

domains, and subsequently transduces signals of cellular proliferation and survival along various intracellular molecular pathways. Therapy inhibiting dimerisation and activation of Her2 receptor has been shown to be highly effective in the treatment of Her2-overexpressed breast cancers. In the present study, we investigated the specificity and efficiency of a fusion protein with ScFv against Her-2 and truncated protamine to deliver siRNAs into Her-2 expressing breast cancer cells *in vitro* and *in vivo*. Furthermore, we evaluated whether Her2-ScFv mediated delivery of PLK1 siRNA may inhibit tumour growth and metastasis of Her2 expressing breast cancer cells in cell cultures and to be implanted in immunocompromised mice.

Methods: The pACgp67B-Her2-ScFv-protamine plasmid has been constructed by gene clone. F5-P fusion protein was expressed and purified from insect cell baculovirus expression system and further identified. The capacity of F5-P fusion protein delivering FITC-siRNA to Her2-overexpressed breast cancer cells was determined by flow cytometry and confocal microscope *in vitro*. Then, small animal imaging system and tumour frozen section were performed to detect the distribution of fluorescence in the body after injection of the complexes of F5-P and siRNA to tail vein of mices. Western blot and Real time PCR were performed to evaluate target gene expression of Her2 overexpressed cells after siRNA delivery by F5-P. The proliferation and apoptosis of Her2 overexpressed cells treated with siRNA complex with F5-P were determined by 3H-thymidine, MTT, cloning-forming assay and a series of apoptosis assays such as Annxin V / PI and TUNEL assay. To evaluate *in vivo* delivery of siRNA, Her2 overexpressed breast cancer cells were inoculated in mammary fat pad or injected through tail vein of mice and were treated with F5-P and PLK1 siRNA through injection of tail vein. Tumours were analysed by growth curves, survival curves, immunohistochemical staining, as well as small animal imaging systems and real time PCR.

Results: The pACgp67B-Her2-ScFv-protamine plasmid has been constructed successfully, and could be expressed and purified by insect cell baculovirus expression system. F5-P fusion proteins expressed from vector can be best eluted with 250 mM imidazole. F5-P can bind siRNA and the ability of F5-P binding siRNA was increased in a dose-dependent manner. Flow cytometry and confocal microscopy showed that F5-P has delivered siRNA into Her2 overexpressed breast cancer cells but not into Her2 negative cells. FAM-siRNA complex with F5-P, or not injected into tail vein of mice, indicated that F5-P specifically delivers FAM-siRNA only into the tumour formed by Her2 overexpressed breast cancer cells. PLK1 siRNA delivered by F5-P inhibits cell proliferation and mammosphere formation of Her2 overexpressed breast cancer cells and induces G2/M phase arrest and apoptosis of SKBR3 and BT 474 cells *in vitro*. Injection of PLK1 siRNA complex with F5-P to tumour-bearing mice suppresses tumour growth of Her2 overexpressed BT 474 cells implanted into mammary fat pad and inhibits tumour metastasis of the same cell line implanted in nude mice.

Conclusion: The expressed F5-P proteins have the capacity of binding nucleic acid. F5-P can selectively deliver PLK1 siRNA to Her2 overexpressed breast cancer cells *in vitro* and *in vivo*. PLK1 siRNA delivered by F5-P inhibits cell proliferation and induces cell cycle arrest and apoptosis in Her2 overexpressed breast cancer cells *in vitro*. Furthermore, PLK1 siRNA delivery by F5-P inhibits Her2 overexpressed breast cancer tumours growth and metastasis *in vivo*.

Disclosure: All authors have declared no conflicts of interest.

109P DYNAMIC CHANGES IN BREAST CANCER GENE EXPRESSION IN VIVO PREDICT RESPONSE TO TAMOXIFEN THERAPY IN PATIENTS WITH BREAST CANCER

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Introduction: Tamoxifen is the most widely prescribed anti-estrogen treatment for patients with ER-positive breast cancer. However, there is still a need for biomarkers that reliably predict endocrine sensitivity in breast cancers and these may well be expressed in a dynamic manner.

Methods: In this study we assessed gene expression changes at multiple time points (days 1, 2, 4, 7, 14) after tamoxifen treatment in the ER-positive ZR-75-1 xenograft model that displays significant changes in apoptosis, proliferation and angiogenesis within 2 days of therapy.

Results: Hierarchical clustering identified six time-related gene expression patterns, which separated into three groups: two with early/transient responses, two with continuous/late responses and two with variable response patterns. The early/transient response represented reductions in many genes that are involved in cell cycle and proliferation (e.g. BUB1B, CCNA2, CDKN3, MKI67, UBE2C), whereas the continuous/late changed genes represented the more classical estrogen response genes (e.g.TFF1, TFF3, IGFBP5). Genes and the proteins they encode were confirmed to have similar temporal patterns of expression in vitro and in vivo and correlated with reduction in tumour volume in primary breast cancer. The profiles of genes that were most differentially expressed on days 2, 4 and 7 following treatment were able to predict prognosis, whereas those most changed on days 1 and 14 were not, in four tamoxifen treated datasets representing a total of 404 patients.

Conclusion: Both early/transient/proliferation response genes and continuous/late/ estrogen-response genes are able to predict prognosis of primary breast tumours in a dynamic manner. Temporal expression of therapy-response genes is clearly an important factor in the response to endocrine therapy in breast tumours which has significant implications for the timing of biopsies in neoadjuvant biomarker studies.

Disclosure: The author has declared no conflicts of interest.

110P ABERRANT METHYLATION OF ESR1 IN BLOOD AND ASSOCIATION WITH ESR1 NEGATIVE IN TUMOR OF BREAST CANCER PATIENTS

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Background: The methylation as impact factor on tumour progression and potential predictive implications remain relatively unknown.

Objective: To correlate methylation levels of promoter ESR1 with association to known prognostic factors in breast cancer and their correlation with Estrogen Receptor (ER) in tumour and luminal phenotype.

Material and methods: We quantified methylation levels of promoter ERS1 gene in 107 women with breast cancer by Real Time QMS-PCR SYBR green (methylation-specific PCR). Tumours were classified as phenotype basal, luminal A, Luminal B and phenotype HER2+.

Results: An inverse correlation between aberrant methylation promoter region ESR1 and ER expression was observed in breast cancer cells. Presence of methylated ESR1 in serum of breast cancer patients was associated with ER-negative phenotype ($p=0.0179$). We observed that methylated ERS1 was preferably associated with phenotype Basal Like and worse interval progression-free and survival ($p>0.05$). The amplification of HER2+ was correlated with significantly more frequent methylation of ERS1 gene ($p<0.05$).

Conclusions: This study identifies the presence of variations in global levels of methylation promoters genes in healthy controls and breast cancer with different phenotype classes and shows that these differences have clinical significance. Our results show that frequent methylation had a strong association with molecular phenotype of breast cancer and perhaps in the future can explain therapy resistance related to ER and HER2/neu status in breast cancer patients.

Disclosure: All authors have declared no conflicts of interest.

111P THE PENTANUCLEOTIDE (TAAAA)N REPEAT OF SEX HORMONE-BINDING GLOBULIN (SHBG) GENE PROMOTER: BREAST CANCER PATIENT PROFILE AND RELATION TO ESTROGEN-SENSITIVITY

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Sex Hormone-Binding Globulin (SHBG) is a serum glycoprotein regulating estrogen free fraction and cross-talking with estradiol pathways in breast cancer cells. The final SHBG effect on estrogenic signalling is reducing breast cancer cell proliferation. The presence of D327N single nucleotide polymorphism (SNP) in SHBG gene exon 8 amplifies its protective role. A pentanucleotide repeat polymorphism [PNRP $(TAAAA)_n$] within SHBG gene promoter, characterized by a number of repeats from 6 to 11, was also described and studied in several conditions, like PCOS, CAD, osteroporosis, where SHBG and estrogenic balance are important factors. Thus far, no data are available about $(TAAAA)_n$ polymorphism and breast cancer. In the present study, we evaluated $(TAAAA)_n$ polymorphism in 198 breast cancer patients (age 57 ± 13 yrs) and 61 healthy women (age 45 ± 18 yrs), already studied in our laboratory for D327N SNP (Becchis et al. BCRT 1999; Costantino et al. BCRT 2008). TAAAA repeat region was amplified from genomic DNA with PCR (forward 5'-GCTTGAACTCGAG AGGCAG; reverse 5'CAGGGCCTAAA CAGTCTAGCAGT); amplified products were analyzed by PAGE to determine the number of TAAAA repeats; results were confirmed by DNA sequencing. Frequencies for the different alleles were estimated by direct gene counting and compared with the chi_2 test. With respect to healthy controls, in breast cancer patients we observed a significantly higher frequency of $(TAAAA)_8$ allele (40% vs 24%; $p<0.05$).The higher frequency of $(TAAAA)_8$ was also observed in tumours positive for estrogen and progesterone receptors (ER+/PR+) [38%], but not in ER-/PR- tumours [20%]. Strong linkage disequilibrium with D327N SNP was detected as well. A group of patients who developed breast cancer after hormone replacement therapy for menopause was also studied; they did not present any increase in $(TAAAA)_8$ repeat as well as no association with D327N SNP. In conclusion, $(TAAAA)_8$ together with D327N SNP are strongly associated to estrogen sensitivity of breast cancer but are not characteristics of breast

cancer developing after HRT. SHBG genetic profile is a potential useful tool in the evaluation of breast cancer patients.

Disclosure: All authors have declared no conflicts of interest.

112P THE SECRETORY GTPASE RAB27B DRIVES POOR PROGNOSIS IN ERα-POSITIVE BREAST CANCER

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Vesicle exocytosis, controlled by secretory GTPases such as Rab27B, delivers pro-invasive growth regulators into the tumor microenvironment. The biological role and expression status of Rab27B in breast cancer was unknown. We studied Rab27B in ERα-positive breast cancer cells using GFP-fusion constructs of wild type Rab3D, Rab27A, Rab27B and Rab27B point mutants defective in GTP-binding or geranylgeranylation. In cell culture, cell-cycle progression was evaluated by flow cytometry and Western blot, and invasion was assessed using Matrigel and native collagen type I substrates. Orthotopic tumor growth, local invasion and metastasis were analyzed in mouse xenograft models. Mass spectrometry was performed to identify Rab27B-secreted pro-invasive growth regulators. In clinical breast cancer, Rab3D, Rab27A and Rab27B mRNA and/or protein were analyzed by q-RT-PCR (n=20) and immunohistochemistry (n=60). Rab27B-upregulation promoted G1/S phase cell cycle transition and increased proliferation, F-actin reorganization and invasion in cell culture, and invasive tumor growth and haemorrhagic ascites in a xenograft mouse model. Proteomics of purified Rab27B-secretory vesicles and the secretome of exogenous Rab27B-expressing breast cancer cells identified HSP90α as key pro-invasive growth regulator. HSP90α secretion occurred in a Rab27B-dependent manner and was required for MMP-2 activation. All Rab27B-mediated functional responses were GTP- and geranylgeranyl-dependent. Endogenous Rab27B mRNA and protein, but not Rab3D and Rab27A mRNA, associated with lymph node metastasis (P=0.0002) and differentiation grade (P=0.0014) in ERα-positive breast cancers. Rab27B regulates invasive growth and metastasis in ERα-positive breast cancer.

Disclosure: All authors have declared no conflicts of interest.

113P AN INTEGRATIVE ANALYSIS TO IDENTIFY EPIGENETIC ABERRATIONS IN BASAL-LIKE BREAST CANCER CELL LINES

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Introduction: Breast cancer is a heterogeneous disease characterised by multiple genetic, epigenetic and genomic alterations. Discrete genomic and gene expression changes have been associated with basal-like breast cancers. Breast cancer cell lines (BCCLs) are commonly used as laboratory models to identify and/or validate breast cancer subtype-specific 'drivers'. Genomic and epigenetic analyses of both breast tumours and BCCLs have shown that methylation events occurring in BCCLs reflect changes seen in primary tumours. We have extended these studies by integrating expression profiles with genomic and array-based methylation data with the aim to identify epigenetic-driven changes specific to basal-like BCCLs.

Methods: Simultaneous extraction of genomic DNA and RNA was performed on 25 BCCLs from the same passage. Illumina Golden Gate Cancer Panel methylation microarray data were generated and overlayed with their Illumina HumanWG-6v2.0 gene expression and genomic profiles based on 32k aCGH BAC and Illumina SNP 370CNV arrays. Genes with higher expression and unmethylated CpG profiles in basal-like BCCLs were selected and validated by qRT-PCR and methylation-specific PCR to confirm their expression and methylation status.

Results: Genes whose level of expression is dependent on their DNA copy number or methylation status were identified. 87 genes showed a significant inverse correlation between their expression and DNA methylation status with ACVR1, EPHA2, ETS1, GSTP1, MET, PRKCDBP and VIM having higher expression and lower methylation in basal as compared to luminal BCCLs and vice versa. PRKCDBP and GSTP1 were both less methylated and showed a DNA copy number loss in the 11p15 genomic region containing these genes specifically within the basal-like BCCLs (MDA-MB157, MDA-MB436, SUM1315 and SUM190). Genes reported to be preferentially expressed in luminal cancers (eg ESR1, TFF1, TGFB3, RARA) were hypermethylated and had a concordant lower gene expression in basal-like BCCLs.

Conclusion: The combined effects of DNA copy number and promoter methylation on the expression of subtype-specific genes suggest that epigenetic mechanisms may influence the establishment of different breast cancer phenotypes.

Disclosure: All authors have declared no conflicts of interest.

114P INFLUENCE OF ESTROGEN RECEPTORS GENES VARIANTS ON PROSTATE-SPECIFIC ANTIGEN EXPRESSION IN BREAST CANCER

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Background: It was suggested that PSA (prostate-specific antigen) could be a marker of endogenous balance between androgens and estrogens, but the relationships between their expressions in breast cancer are not well understood. In this context, we proposed us to investigate relationships between polymorphic tandem repeats (CAG, TA and CA) in AR (androgen receptor), ERα (estrogen receptor alpha) and ERβ (estrogen receptor beta) genes and the immunoexpression of PSA. We assessed also influences of CAG, TA, and CA repeats and other available prognostic factors (ER, PR, AR, HER2/neu, PSA expression, and nodal status) on disease-free survival.

Subjects and methods: We assessed polymorphic tandem repeats lengths by genotyping, followed by high-resolution denaturing polyacrylamide gel electrophoresis in 163 breast cancers. Immunohistochemistry was performed to assess the expressions of AR, PSA, ER, PR and HER2/neu proteins.

Results: PSA expression was correlated with shorter CA repeats in the 3'-untranslated region of ERβ (p=0.03). AR immunoexpression was correlated with CAG repeats on AR gene, higher number of repeats being linked to a higher AR immunoexpression (p=0.04). Performing logistic regression to investigate relationships with prognosis, we observed that PSA immunoexpression (p=0.004), the nodal status (p<0.001) and marginally, longer TA repeats (p=0.05) were correlated with increased disease-free survival. AR expression presented a low statistical value (p=0.054) in predicting evolution and was not entered into the multivariate regression analysis.

Conclusion: Our findings support the hypothesis that estrogens, through the beta-receptors variants influence the PSA expression in breast cancers.

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Disclosure: All authors have declared no conflicts of interest.

115P STROMA-DERIVED MARKERS PREDICTING BREAST CANCER PROGNOSIS AND TREATMENT RESPONSE

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Recent developments in tumor biology emphasize the importance of the tumor microenvironment. There is thus an increasing interest in the tumor stroma as a source for prognostic and predicative markers. PDGF receptors are important regulators of tumor stroma through stimulatory effects on cancer-associated fibroblasts (CAFs) and pericytes. A tissue-micro-array (TMA) based analysis of more than 500 breast cancers has been performed (Paulsson, Am J Path, 2009). The study revealed that high stromal PDGF-β-receptor expression significantly correlates with high histopathological grade, ER negativity and high HER2 expression. High stromal PDGF β-receptor expression was also correlated with significantly shorter recurrence-free and breast cancer specific survival. This was particularly prominent in pre-menopausal women. The potential response-predicative significance of stromal PDGF-β-receptors is now analyzed. A TMA-based study is analyzing more than 400 cases from a randomized study of adjuvant tamoxifen treatment. Strong indications have been obtained for interactions between benefit of tamoxifen and stromal PDGF-receptor status. Experimental studies are ongoing that investigates the effects of co-cultured PDFR-positive fibroblasts on tamoxifen-sensitivity of ER+ breast cancer cells. Finally, we are exploring the role of CAF-derived paracrine signals for trastuzumb-sensitivity of HER2+ breast cancer. In vitro studies indicate that activation of PDGFR in fibroblasts leads to a paracrine signaling that reduces the inhibitory effects of trastuzumab on HER2+ breast cancer cells. Fibroblasts were

shown to provide cancer cells with a HER2-independent activation of AKT. Clinical significance is evaluated by TMA studies analyzing associations between stromal PDGFR status and trastuzumab-response in HER2+ breast cancer. Our studies thus provide findings which, in general terms, confirms the role of the tumor stroma as a major determinant of breast cancer prognosis and response to treatment. The studies also provide a set of specific findings prompting mechanistic studies on how PDGFR signaling in tumor fibroblasts influence the functional properties of tumor epithelial cells.

Disclosure: All authors have declared no conflicts of interest.

116P FOUR POLYMORPHISMS IN CYTOCHROME P450 1B1 (CYP1B1) GENE AND BREAST CANCER RISK: A META-ANALYSIS

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Cytochrome P450 1B1 (CYP1B1) is a P450 enzyme implicated in the metabolism of exogenous and endogenous substrates. The metabolism of polycyclic aromatic hydrocarbons and other procarginogens through CYP1B1 may well lead to their activation. Four single nucleotide polymorphisms in CYP1B1 have been studied concerning their potential implication in terms of breast cancer risk: Leu432Val, Arg48Gly, Ala119Ser and Asn453Ser. This meta-analysis aims to examine whether the four aforementioned polymorphisms are associated with breast cancer risk. Eligible articles were identified by a search of MEDLINE bibliographical database for the period up to December 2009. Concerning Leu432Val polymorphism thirty studies were eligible (19,767 cases and 22,283 controls); ten studies were eligible for Arg48Gly polymorphism (11,321 cases and 13,379 controls); eleven studies were eligible for Ala119Ser (10,715 cases and 11,678 controls); and twelve studies were eligible regarding Asn453Ser (11,630 cases and 14,053 controls). Pooled odds ratios (OR) were appropriately derived from fixed-effects or random-effects models. Sensitivity analysis excluding studies whose genotype frequencies in controls significantly deviated from Hardy-Weinberg equilibrium was performed. Concerning Leu432Val, the pooled ORs (95%CI) were 1.021 (0.941-1.109) for heterozygous and 1.034 (0.930-1.150) for homozygous Val subjects. Subanalysis on African subjects demonstrated that heterozygous subjects were associated with increased breast cancer risk (pooled OR=1.918, 95% CI: 1.011-3.638). Concerning Arg48Gly, the pooled ORs (95%CI) were 0.933 (0.808-1.078) for heterozygous and 0.819 (0.610-1.100) for homozygous Gly subjects. Regarding Ala119Ser, the pooled ORs were 0.992 (0.896-1.097) for heterozygous and 0.935 (0.729-1.198) for homozygous Ser subjects. With respect to Asn453Ser, the pooled ORs were 0.961 (0.906-1.019) for heterozygous and 0.984 (0.846-1.144) for homozygous Ser subjects. In conclusion, this meta-analysis suggests that CYP1B1 Arg48Gly, Ala119Ser and Asn453Ser polymorphisms are not associated with breast cancer risk. Leu432Val may represent a risk factor for breast cancer in African women.

Disclosure: All authors have declared no conflicts of interest.

117P ESTROGEN METABOLISM GENES AND TRANSFORMING GROWTH FACTOR-BETA1 GENE POLYMORPHISMS IN ESTROGEN RECEPTOR-POSITIVE AND -NEGATIVE INFILTRATING DUCTAL BREAST CARCINOMA

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Introduction: The aim of our study was to determine the possible association between estrogen receptor status and CYP1A1-6235T/C (rs4646903), SULT1A1-638G/A (rs9282861) and TGFB1-509C>T (rs1800469) genes polymorphism with infiltrating ductal breast carcinoma risk and response to neoadjuvant chemotherapy.

Subjects and methods: One hundred sixteen patients with operable primary infiltrating ductal breast carcinoma who received two-four cycles of neoadjuvant chemotherapy in the Tomsk Cancer Research Institute were included in the present study. The healthy women (n=269) from Western Siberian region were used as the control group. Patient characteristics such as estrogen receptor status were evaluated. The genotypes were determined by RFLP-PCR.

Results: The ER-positive patients with TGFB1(T/T) genotype had a significantly decreased risk for infiltrating ductal breast carcinoma (p=0,01) than the ER-negative patients. The CYP1A1(T/T) and SULT1A1(G/G) genotypes significantly reduced risk of infiltrating ductal breast carcinoma among the ER-positive patients (p=0,008 and p=0,001, respectively) and the ER-negative patients (p=0,01 and p=0,01, respectively). In addition, both patients groups carrying SULT1A1(A/A) genotype had higher risk for ductal breast carcinoma development (OR=2,02;p=0,002 and OR=1,88;p=0,03, respectively). The ER-positive patients with the TGFB1(C/C) genotype responded more frequently to treatment as compared with negative estrogen receptor status patients (p = 0,09). The CYP1A1(T/T) genotype was found to be associated with a non-statistically significant better response to neoadjuvant chemotherapy among the ER-positive patients (p=0,09) and the ER-negative patients (p=0,06). Furthermore, ER-positive homozygous for the SULT1A1(A/A) genotype had a poorer response to chemotherapy than patients with ER-negative status (p=0,05).

Conclusion: Our study suggested that CYP1A1-6235T/C, SULT1A1-638G/A, TGFB1-509C>T genotypes may be associated with infiltrating ductal breast carcinoma risk and the efficacy neoadjuvant chemotherapy in patients with different estrogen status.

Disclosure: All authors have declared no conflicts of interest.

118P TRYPTASE AND PROTEASE-ACTIVATED RECEPTOR-2 EXPRESSION PARALLELED WITH MICROVASCULAR DENSITY IN BREAST CANCER PATIENTS

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Background: Tryptase, a serine protease stored and released from mast cells (MCs) granules has been identified as a new non-classical angiogenetic factor and it is an agonist of the proteinase-activated receptor-2 (PAR-2). We have evaluated the correlations between the number of MCs positive to tryptase (MCDPT), the number of breast cancer cells positive to PAR-2 (BC-PAR-2) and microvascular density (MVD) in a series of 97 primary T1-3, N0-2 M0 female breast cancer by means of immunohistochemistry and image analysis methods.

Materials and methods: Six-micrometers thick serial sections of formalin-fixed and paraffin-embedded bioptic tumor samples were microwaved at 500 W for 10 min. and treated with a 3% hydrogen peroxide solution. Sections were incubated with primary antibodies: monoclonal anti-tryptase (AA1; Dako, Glostrup, Denmark), polyclonal anti PAR-2 (N-19; sc-8206 Santa Cruz Biotecnology), and monoclonal anti-CD34 (QB-END 10; Bio-Optica Milan, Italy). Biotinylated secondary antibody, avidin-biotin peroxidase complex, and 3-amino-9-ethylcarbazole were in turn utilised. In serial sections "hot spots" were selected and individual vessels, single tryptase-positive MCs and breast cancer cells positive to PAR-2 were counted by means of image analysis at x400.

Results: Data demonstrated a significantly (r= ranging from 0.71 to 0.87; p: ranging from 0.001 to 0.003 by Pearson's analysis respectively) correlation between MCDPT, BC-PAR-2 and MVD to each other. No correlation concerning MCDPT, BC-PAR-2, MVD and the main clinical pathological features was found.

Conclusions: Published in vitro data suggest that tryptase may increase capillary growth and endothelial cell proliferation by activation of PAR-2. According to these data we shown that MCDPT, PAR-2 and MVD paralleled to each other suggesting a role in in vivo breast cancer angiogenesis. In this context several tryptase inhibitors such as gabexate mesilate and nafamostat mesilate might be evaluated in clinical trials as a new antiangiogenetic drugs.

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Disclosure: All authors have declared no conflicts of interest.

119P THE ROLE OF OESTROGEN SIGNALLING IN BREAST CANCER

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Breast cancer is a leading cause of malignancy in women worldwide. Oestrogen receptor alpha (ESR1), forkhead box A1 (FOXA1) and GATA binding protein 3 (GATA3) have been shown to be co-expressed in oestrogen receptor (ER) positive breast cancers and are markers of good prognosis. The aims are to determine how these three genes may function within the oestrogen signalling pathway in vitro and in tumours. We undertook a meta-analysis of over 800 samples from published breast cancer microarray datasets (including GSE3494, GSE7390, GSE6532 and GSE1456). Features of the data related to oestrogen signalling were analysed using bioinformatic techniques such as Bayesian network analysis, statistical meta-analysis and traditional linear models. Conclusions were evaluated in an independent breast cancer microarray dataset consisting of 30 breast tumour samples collected from New Zealand women (Muthukaruppan et. al., unpublished). The mRNA abundances of ESR1, FOXA1 and GATA3 were individually reduced in MCF7 breast cancer cells using small interfering RNA (siRNA), and the extracted RNA was hybridised onto Affymetrix Human Genome U133 Plus 2.0 arrays. The meta-analysis revealed that the mRNA expression level of ESR1 is a continuous variable across all breast tumours, regardless of their ER status as assessed by immunohistochemistry (IHC). Many genes differentially regulated between ER positive and ER negative tumours were downstream target genes of FOXA1, with subsets of those genes also being target genes of ESR1. Analysis of this microarray data revealed that the knockdown of either ESR1 or FOXA1 in MCF7 cells reduced the expression levels of many oestrogen target genes, including CCND1 and MYC, and reduced the activation state of many oestrogen target pathways. The level of ESR1 mRNA expression and oestrogen pathway activity indicated by the analysis of mRNA may be an important clinical parameter to consider in patients, in addition to the ER status determined by IHC. The transcriptional relationship between ESR1, FOXA1 and GATA3 appears to be complex, with many levels of cross-talk. Understanding the details of cross-regulation between these genes in oestrogen signalling may be pivotal in fully understanding the role of endocrine-related therapy in breast cancer.

Disclosure: All authors have declared no conflicts of interest.

120P IMMUNOHISTOCHEMICAL DETECTION OF THE CANCER STEM CELL PHENOTYPE IN PRE- AND POST-CHEMOTHERAPY BREAST TUMOURS

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Human breast cancer is a complex disease with large inter-tumoural and intra-tumoural heterogeneity. A population of CD44+/CD24$^{-/low}$/ESA+/ALD+ cells has been demonstrated to have tumour-initiating properties in breast cancer. The aim of this study was to develop the methodology of detection of this tumour cell phenotype immunohistochemically. Paraffin embedded tumour tissue was investigated from women who had given informed consent to a trial involving sequential neoadjuvant chemotherapy (FEC- Taxotere or Taxotere- FEC) for locally advanced breast cancer. Breast core biopsies were taken prior to administration of chemotherapy and at surgical excision of tumour. Identification of the putative cancer stem cell markers of CD44s, CD24 and aldehyde dehydrogenase (ALD) was achieved using direct immunofluorescence with monoclonal antibodies and a DAPI counterstain. Method development involved checkerboard antibody titrations after which the optimal antibody dilutions were used on samples derived from 6 patients. The putative cancer stem cell biomarker expression was quantitated by counting 1000 cells over 10 fields of view where the percentage of positive cells was validated by three independent assessors. This methodological approach proved to be a time effective means of rapidly quantitating breast cancer stem cells which expressed CD44, CD24 and ALD. Pilot studies on the first 6 patients have been completed and work is underway on a larger prospective cohort which will be presented.

Disclosure: All authors have declared no conflicts of interest.

121P P53 GENE COULD BE A NEW EFFECTIVE THERAPEUTIC TARGET IN TRIPLE-NEGATIVE BREAST CANCER: A META-ANALYSIS

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Purpose: To explore the relationship between P53 gene and triple-negative breast cancer, and determine whether P53 gene could be a new effective therapeutic target.

Materials and methods: We identified studies with quantitative data on the relation of P53 gene and triple-negative breast cancer (TNBC) through searching 12 databases online (Oct 1999 - Oct 2009) and reviewing the references written in English or Chinese. Summary estimates odds ratio calculated by using the fixed-effects model or the random-effects model as appropriate.

Results: We identified 12 eligible studies with 1532 cases of TNBC patients and 6329 controls of non-TNBC patients. The test for homogeneity resulted in $\div 2 = 200.16$ ($P<0.05$), it showed significant heterogeneity so a random effect model was applied. Our results showed that the expression of P53 gene could be much stronger in the TNBC group than that in the non-TNBC group (OR=2.10, 95%CI=1.21-3.65). In ethnicity-subgroup analysis, we found that in the Caucasian group, the expression of P53 gene were stronger in the TNBC group (OR=2.60, 95%CI=1.21-5.57), but there was no statistical significance in the Asian group (OR=1.69, 95%CI=0.83-3.45).

Conclusions: P53 gene could be an effective predictor and a good therapeutic target for TNBC patients in the future, especially in Caucasians. Further researches focusing on P53 gene would gain a breakthrough in the treatment of TNBC.

Disclosure: All authors have declared no conflicts of interest.

122P TOP2A STATUS IN BREAST CANCER DETERMINED BY NEWLY DEVELOPED PCR-BASED METHOD COMPARED WITH FISH AND IHC STAINING TECHNIQUES

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Objective: Topoisomerase II alpha (TOP2A) is the target of the anticancer agents from a group of anthracyclines. TOP2A status is considered to be a prognostic and a predictive factor for breast cancer patients when anthracycline-based chemotherapy is considered. Investigation of the prognostic or predictive significance of TOP2A requires a reliable and sensitive method for the measurement of gene copy number in tumor samples. Therefore, the objective of this study was to compare a newly developed PCR-based method for quantitative detection of TOP2A aberrations in frozen breast cancer sections with standard fluorescent in situ hybridization (FISH) and immunohistochemical staining (IHC).

Materials and methods: The study group included 169 consecutive breast cancer patients. TOP2A gene dosage was measured by real time PCR with dually labelled hydrolysis probes. Independently, TOP2A gene dosage and protein level were measured by FISH using TOP2A FISH pharmDx^{TM} Kit and IHC with monoclonal antibody (Ki-S1), respectively.

Results: PCR-based method revealed TOP2A amplification and deletion in 28% (48/169) and 11% (18/169) of cases, respectively, whereas FISH revealed these alterations in 19% (33/169) and 6% (10/169) of cases, respectively. IHC expression was scored according to the percentage of tumor cells with positive staining (0=no positive cells; 1=1-25%; 2=26-50%; 3=51-75%; 4=>75%). The 4 score of TOP2A was found in 28% (47/169) of cases. The results obtained by means of PCR correlated with those identified by FISH. Protein expression as defined by IHC did not correlate with the results of techniques performed at the gene level. TOP2A amplification (as determined by PCR) occurred more frequently in HER-2 positive breast carcinomas ($p<0.05$) and correlated with shorter disease free survival ($p=0.01$).

Conclusions: The newly developed real time PCR-based assay is an efficient method to perform TOP2A gene copy number analysis, with results comparable to FISH technique. Amplification of TOP2A seems to be an indicator of poor prognosis in breast cancer. However, the prognostic role of TOP2A deletions and predictive role of TOP2A status warrants further study.

Disclosure: All authors have declared no conflicts of interest.

123P ANALYSIS OF MIRNA – MICROARRAY DATA OF BREAST CANCER FOR THE PREDICTION OF LYMPH NODE INVASION

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The goal of this analysis is to investigate the loss of miRNA binding to its targets in this paired microRNA-mRNA data set. Microarray (17527 genes) and microRNA (229 microRNAs of which 189 are known microRNAs) data are present from 83 ER positive, HER-2 negative, poorly differentiated invasive ductal carcinoma of the breast. The primary outcome is lymph node invasion where 41 patients are lymph node positive and 42 are lymph node negative. Initial analysis showed that no microRNAs are differentially expressed between lymph node positive and lymph node negative patients. Next, we used the microRNA.org database of computationally predicted targets to assign genes to their corresponding miRNA. Spearman rho correlation coefficient was calculated between each miRNA and its target. Next, the correlation was calculated between each miRNA and its targets. A one-sided hypothesis test was used to determine the significance of inverse correlation between a miRNA and its target, the significance threshold was set at a pvalue of 0.05. This resulted in 26 microRNAs that were significantly inversely correlated with their computationally predicted targets. Of these 26 microRNAs 18 were outcome specific. In addition, 23 other microRNAs are only significant in one of the two outcome groups and not in the complete data set. Overall microRNAs were more active in the lymph node negative group. Finally, 8 microRNAs were significantly active in the lymph node negative group and at the same time significantly inactive in the lymph node positive group (hsa-let-7i,hsa-miR-143, 16, 200c, 27a, 375, 519a, 519b-3p). These miRNA's can be hypothesized as suppressing lymph node invasion. Conversely, there is only one microRNA which is significantly active in the lymph node positive group and inactive in the lymph node negative group, hsa-miR-361-5p. This microRNA and its targets can be hypothesized as promoting lymph node invasion. Due to the absence of large scale tissue specific expression profiles of miRNAs, it is currently not possible to assess if the rather low number of targets that is actually regulated by a miRNA according in our data is due to tissue specificity of a miRNA or that computational prediction of miRNA targets has a low sensitivity.

Disclosure: All authors have declared no conflicts of interest.

124P IDENTIFICATION OF NOVEL BIOMARKERS FOR BREAST CANCER, INCLUDING BRCA1-ASSOCIATED BREAST CANCER

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One of the major challenges in the management of breast cancer is predicting how the disease will progress and how well it will respond to a particular therapy. Molecular studies continue to address this issue, with notable progress including the identification of coding transcript signatures that help differentiate between subtypes of breast tumours and are associated with certain clinical characteristics. Our group is interested in identifying novel clinically-useful biomarkers that are DNA or miRNA based and therefore have the potential to be more stable and more easily and reliably detected in biospecimens, including blood. Biomarkers under investigation include gene regulatory elements and factors that are subject to disease-associated genetic or epigenetic changes, and downstream effectors of mutations in known breast cancer-associated genes, including BRCA1. Our studies have lead to the identification of regulatory sequences mapping to promoter, intronic, UTR and extragenic sequences of BRCA1, p16, AR and a number of miRNAs, and for which genetic and epigenetic changes affect gene expression. We have also identified a number of molecules, including miRNAs, that are differentially expressed in the pre-malignant mammary glands of a mouse model of BRCA1-associated breast cancer, and which have the potential to be useful biomarkers of disease progression in BRCA1 mutation carriers. We are currently investigating associations between these potential biomarkers and breast cancer phenotype, using biospecimens from kConFab, BreastCFR and the Princess Alexandra Hospital Breast Tumour Bank in Brisbane. This presentation will report on the identification, characterization and preliminary validation of these biomarkers.

Disclosure: All authors have declared no conflicts of interest.

125P COMPUTATIONAL DISSECTION OF TUMOR EXPRESSION PROFILES

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Gene expression profiling has the potential to improve clinical cancer treatment efficacy by predicting tumor sensitivity or resistance to particular drugs. However, it is difficult to collect a specimen of pure tumor cells; thus, microarray measurements usually reflect the contribution of tumor cells as well as stromal and other normal cells. We applied unsupervised matrix factorization methods to gene expression data to derive several sets of co-expressed genes, or modules, whose signatures together comprise a set of independent descriptors of breast tumors. Some of these modules correspond to specific cell types (adipocytes, lymphocytes, fibroblasts), while others reflect well-known tumor-intrinsic expression programs (ER, ERBB2, proliferation). We confirmed the specificity of the modules using expression data from purified normal cells and tumor cell lines, microdissected tumors, and bulk tumors with corresponding histological cellularity estimates. We examined several large gene expression data sets and found that the cell-type modules were highly variable and anticorrelated with tumor-intrinsic modules, confirming that variability in normal cell content is a potential source of measurement bias. Overall, these results provide an intuitive framework for the interpetation of tumor expression profiles that may improve accuracy in molecular characterization and drug response prediction.

Disclosure: All authors have declared no conflicts of interest.

126P HETEROZYGOUS FRAMESHIFT MUTATIONS OF TP53 IN HUMAN BREAST CANCER CAUSE DOMINANT-NEGATIVE AND GAIN-OF-FUNCTION EFFECTS THROUGH PROTEIN AGGREGATION

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The frameshift mutation of p53 tumor suppressor is frequently found in breast cancers. The completely different transcription caused by frameshift mutation is normally considered as non-functional, but whether the novel protein sequence can gain new functions in the cellular context has not been thoroughly studied. Here we show that the frameshift mutations of TP53 gene cause aggregation of the protein in the tissues of aggressive human breast cancer. The aggregates of p53 frameshift mutants accumulate in the cytoplasm, sequestering the wild-type allele and its homologue p73. The transient overexpression of these frameshift mutants under a CMV promoter in a p53-null cell line also resulted in cytoplasmic aggregates, and the co-expressed wild-type p53 and its homologues (p63, p73) were also found in the aggregates. Concomitantly, the transactivation functions of wild-type p53 and its homologues were interfered by the frameshift p53 mutants, suggesting both dominant-negative and gain-of-function effects mediated by protein co-aggregation. Our findings suggest that frameshift mutants may not only lose function, but also acquire new function by protein aggregation.

Disclosure: All authors have declared no conflicts of interest.

127P BREAST CANCER BIOMARKERS IN THE THREE ETHNIC GROUPS OF SARAWAK, MALAYSIA

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Introduction: The Malaysian state of Sarawak has a mixed ethnic population of Natives, Chinese and Malays. Breast cancer (BC) is the most common cancer. We present preliminary results obtained on a case series of 850 female BC patients diagnosed between 2001 and 2008, representative of the Sarawak patient population (as discussed in our previous publication: Devi et al. 2007).

Methods: Tumor markers were assessed using immuno-histochemistry, Her2 2+ and Her2 3+ were confirmed using FISH test. Patients were classified as follow: Luminal A : ER+ PR+ and Fish - Luminal B : ER+, PR+ and Fish + Her2-overexpressing : ER-, PR- and Fish + Triple-negative: ER-, PR- and Fish – Chi2 comparisons and multivariate logistic regressions were performed using SAS statistical software. The OR presented are adjusted on age and ethnic groups.

Results: BC incidence was higher among Chinese (ASR=30.6) than among Malay (ASR=21.2) or Natives (ASR=7.8). Compared to luminal A patients, triple negative patients were more likely to be Malay (OR=1.97, 95%CI =[1.33-2.2]) or Natives (OR=1.87 [1.25-2.78]) than Chinese, to have older age at menopause (OR for menopause >50 years = 1.68 95%CI = [1.00-2.83]) and less likely to have familial history of BC (OR = 0.50 [0.30-0.82]) or to have breast fed (OR ever vs. never = 0.61 [0.38-0.98]). Postmenopausal triple negative patients were less likely to be obese (BMI>=30) than postmenopausal Luminal A patients (OR=0.46 [0.22-0.6]). Compared to luminal A patients, Her2-overexpressing patients were more likely to be premenopausal (OR=2.80 [1.21-6.48]), to have children (OR=1.95 [1.03-3.68]) and less likely to have familial history of BC

(OR=0.49 [0.24-0.99]). Compared to luminal A patients, Luminal B patients were more likely to be Malay (OR=1.71 [1.01-2.88] and to be over-weighted (OR for BMI 25-29 vs. BMI<25 = 1.7 [1.05-2.75]) especially when premenopausal (OR = 2.03 [1.01-4.08]). Surprisingly, age distribution did not differ between Luminal A, B, Her2-overexpressing and triple-negative patients, however 80% of our patients were <60.

Conclusions: Our results did not conflict with those reported in the literature for Caucasian populations however some specific patterns were observed that may be specific to Asian populations.

Disclosure: All authors have declared no conflicts of interest.

128P DEVELOPMENT AND EVALUATION OF CLINICAL SIGNIFICANCE OF A REAL-TIME PCR ASSAY FOR TOP2A COPY NUMBER DETERMINATION IN BREAST CANCER

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Topoisomerase II alpha (TOP2A) is a target of the most potent anticancer agents from a group of anthracyclines. Amplification of TOP2A in breast cancer has been shown to result in poor prognosis and increased sensitivity to anthracyclines. The technique predominantly used for measuring TOP2A gene level is fluorescent in situ hybridization (FISH). Even though it is thought to be a golden standard, it is in many aspects limited. Therefore, the objective of this study was to establish a new real-time PCR-based method with hydrolysis probes for quantitative detection of TOP2A aberrations in frozen breast cancer sections. The study group consisted of 83 consecutive breast cancer patients. Tumour samples were taken by surgical excision or fine needle biopsy before any systemic treatment was ordered, and were immediately frozen in liquid nitrogen and stored in -80 °C for further analysis. TOP2A gene dosage was measured by real-time PCR with dually labelled hydrolysis probes. Gene copy number was calculated based on Pfaffl quantification method with a use of amyloid precursor protein (APP) as a reference gene. Parameters of the standard curves obtained for TOP2A and APP indicated good reaction performance, efficiencies of the reactions were calculated to be 102.5% for TOP2A and 98.2% for APP. In the examined tumours samples median TOP2A gene dosage was 1.08 and it ranged from 0.34 to 7.55. TOP2A amplification was found in 12 tumours (14.5%), no deletion was detected. Statistically significant positive correlation of TOP2A gene dosage with nodal status (p=0.042), tumour grade (p=0.012), and HER2 protein status (p=0.0018) was found. TOP2A status also correlated with disease free survival (p = 0.01724). Recurrence of the disease in the TOP2A-amplified group occurred in 3 out of 12 (25%) cases, in patients with normal TOP2A level in 2 out of 52 (3.8%) cases. The newly developed real-time PCR assay showed to be fast and easy to perform, which makes it possible alternative to FISH. Determined by the method TOP2A gene dosage was shown to be a potent predictive factor in breast cancer.

Disclosure: All authors have declared no conflicts of interest.

129P OVEREXPRESSION OF THE NOVEL HUMAN GENE, UBE2Q2, IN BREAST CANCER

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The ubiquitin-proteasome pathway facilitates the degradation of damaged proteins and regulates growth and stress response. This pathway is activated in various cancers, including breast cancer. We have previously reported that the novel human gene, UBE2Q2, is a putative ubiquitin-conjugating enzyme that is located on chromosome 15 and is overexpressed in tumor mass and invasive epithelium in head and neck squamous-cell carcinoma. Here, real-time polymerase chain reaction was used to investigate the expression levels of UBE2Q2 gene in a collection of 21 breast cancer tissues matched with normal adjacent counterparts. Immunohistochemistry and Western blot testing were also performed on formalin-fixed, paraffin-embedded tissue sections by using a rabbit polyclonal antibody that we generated against an amino acid sequence predicted from the DNA sequence of UBE2Q2 gene. In the 21 cases investigated, a high increase in the expression of UBE2Q2 mRNA was found in 8 breast cancers (38.1%), a moderately increased UBE2Q2 expression was observed in 7 cases (33.3%), and no significant changes were detected in 6 cases (28.6%) of tumor samples when compared with corresponding normal tissues. Consistently, a higher level of immunoreactivity for UBE2Q2 protein was detected in invasive epithelium of cancerous tissues when compared with that in the normal epithelium. Our data suggest that the novel human gene UBE2Q2 may have implications for pathogenesis of breast cancer and could be used in molecular diagnosis purposes in the future.

Disclosure: All authors have declared no conflicts of interest.

130P ANGIOTENSIN-REGULATING AMINOPEPTIDASES IN BREAST CANCER

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Angiotensins have been involved in breast cancer pathogenesis in both human and animal models. These peptide hormones show long-term trophic effects in the carcinogenic process, and angiogenic effects in the metastatic pathway. To date, three angiotensins, called angiotensin II (AngII), angiotensin III (AngIII) and angiotensin IV (AngIV) have been related to this disease. Due to the methodological difficulties to analyse these peptide hormones, one approach consists in to assay the specific activity of the proteolytic enzymes which regulates them, which could reflect the specific conditions brought about by the tumour process. In fact, AngIII and AngIV are the catabolic products obtained from the degradation of AngII. Four proteolytic regulatory enzymes of aminopeptidase type, aspartyl aminopeptidase (ASAP) and glutamyl aminopeptidase (GluAP), called together aminopeptidase A (APA), aminopeptidase N (APN) and aminopeptidase B (APB), participates in angiotensins catabolism. Here, we show the changes observed in the specific APA, APN and APB activities in the serum of both women with breast cancer and rats with mammary tumours induced chemically, to analyse the similarities and/or the differences found and their possible role in the carcinogenic process. In women with breast cancer, we have found significant increases in serum levels of APN and APB, independently of their hormonal status. On the contrary, we have found a significant decrease in ASAP activity in premenopausal women but not in postmenopausal women with breast cancer, although no changes are found in GluAP activity either in premenopausal or postmenopausal women with breast cancer. By other hand, in rats with mammary tumours induced by N-methyl nitrosourea, no changes have been observed in APN and ASAP activities, although significant increases occur in APB and GluAP activities. These results suggest that the proteolytic regulatory enzymes of angiotensins act differently in women and rodents on the mechanisms involved in breast cancer, but strongly suggest the importance of these enzymes in the carcinogenic processes and as targets in breast cancer therapy.

Disclosure: All authors have declared no conflicts of interest.

131P EXPRESSION OF METADHERIN MRNA IN PERIPHERAL BLOOD OF BREAST CANCER PATIENTS AND ITS CLINICAL SIGNIFICANCE

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Objective: To detect the expression level of MTDH mRNA in peripheral blood of the breast cancer patients and to explore the relationship between MTDH mRNA expression and the clinicopathological features in breast cancer patients.

Methods: Total RNA was extracted from peripheral blood of breast cancer patients by centrifuge column method, and reverse transcribed into cDNA. Real-time fluorescence quantitative reverse transcription polymerase chain reaction was performed for detecting the MTDH mRNA expression and then correlation between MTDH mRNA expression and different clinicopathological features was analyzed.

Results: The MTDH mRNA expression in peripheral blood of breast cancer patients was related to lymph node metastasis (LNM) and TNM stages and its expression in LNM patients was 4.317 times of Non-LNM ones. Statistical significance was found between1,2 stage and 3,4 stage (P <0.05). The MTDH mRNA expression of patients at 4 stage was 6.774 times of patients at 1 stage, and was 3.387 times that of 2 stage while its expression of patients at 3 stgae was 4.563 times of patients at 3 stage. The MTDH mRNA expression in peripheral blood of breast cancer patients was independent of

the factors such as age, ER,PR andHER-2(P value was 0.784,0.164,0.154,0.577 respectively.)

Conclusion: Expression level of MTDH mRNA in peripheral blood is closely related to poor prognostic factor and it maybe used as a predictive marker.

Disclosure: All authors have declared no conflicts of interest.

132P MIXED INVASIVE BREAST CARCINOMA ASSOCIATED WITH IN SITU BREAST CARCINOMA (IDC+DCIS), CORRELATES WITH INCREASED BREAST DENSITY (BD), CALCITONIN-GENE-RELATED-PEPTIDE (CGRP), 99MTC-(V)DMSA UPTAKE, PROLIFERATION INDEX KI-67, ESTROGEN RECEPTOR (ER) NEGATIVITY AND LOWER HISTOLOGICAL GRADE

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Background: We evaluated the variation of CGRP in patients with IDC+DCIS and pure IDC, in relation with BD, proliferation-seeking radiotracer 99mTc-(V)DMSA (scintimammography-SMM), Ki-67 and ER status. We assessed CGRP expression with histological grade.

Methods: We studied 24 women who were evaluated preoperatively with SMM. Histology revealed 12 IDC (grade II: 8 and grade III: 4 patients, mean size±SD: 2.6±1.3, mean age±SD=66.5±13.1 years) and 12 IDC+DCIS (grade II: 6 and grade III: 6 patients, DCIS component mean size±SD: 5.3±1.8 cm,IDC component mean size±SD: 2.5±1.1, mean age±SD=58.5±15.1 years). Immunohistochemistry for CGRP, Ki-67 and ER status was performed in all 24 surgical specimens. BD and SMM were calculated by computer-assisted methods and were statistically correlated with CGRP expression. BD, SMM, Ki-67 and ER were statistically compared between IDC and IDC+DCIS, while CGRP, Ki-67 and ER between patients with BD>25% and <25%. CGRP was also compared (t test) with grade II and grade III in both groups.

Results: Overall positive correlation was found between BD and CGRP (r=0.577, P<0.001). Positive correlation was established between SMM and CGRP only in IDC+DCIS (rSMM(IDC+DCIS)-CGRP=0.634,P<0.05). CGRP and Ki-67 were significantly higher in patients with BD>25% compared to <25%BD patients (P=0.00008 and P=0.014, respectively). BD and SMM were significantly higher in CGRP(+) than in CGRP(-) patients as well as in IDC+DCIS compared to IDC. Ki-67 was significantly higher, whereas ER significantly lower in IDC+DCIS than in IDC. In all patients, CGRP was significantly higher in grade II as compared to grade III (P=0.005). In the mixed group (IDC+DCIS) grade II cancers had significantly higher CGRP as compared to grade III ones (P=0.004).

Conclusions: CGRP, BD, SMM and Ki-67 were significantly increased, whereas ER significantly decreased in IDC+DCIS as compared to IDC, indicating that the IDC+DCIS is an entity more aggressive, ER-independent and possibly associated with a pathway linked to stromal involvement and CGRP activity.

Disclosure: All authors have declared no conflicts of interest.

133P STEROID RECEPTOR AND HER2/NEU EXPRESSION IN INFLAMMATORY BREAST CANCER COMPARED TO NON-INFLAMMATORY LOCALLY ADVANCED BREAST CANCER

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Inflammatory breast cancer was defined as particular clinico-pathologic entity due to aggressive behaviour and poor outcome. While oedema is the most common feature for breast cancer to consider it as locally advanced, the incidence of inflammatory breast cancer is infrequent. In our institute, we classify breast cancer as inflammatory type when the oedema and infiltration involves more than a half of the affected breast skin. The aim of the study was to evaluate and compare the expression and co-expression of steroid receptors (SR) (estrogen (ER) and progesterone (PR)) and Her2/neu both in inflammatory breast cancer (T4dN1-3M0) and non-inflammatory locally advanced breast cancer featured by skin oedema (T4bN1-3M0). Immunohistochemical analysis and *in situ* hybridization were performed using formalin-fixed or paraffin-embedded tissues of 51 T4dN1-3M0 and 49 T4bN1-3M0 breast cancer samples. In T4dN1-3M0 group, 23/51 (45,1%) tumours were SR+, and 26/51 (50,9%) were Her2+, while in T4bN1-3M0 group 35/49 (71,4%) tumours were SR+ and only 14/49 (28,6%) were Her2+. Interestingly ER+PR+ tumours, perceived to be more responsive to the hormonal treatment than ER+PR, were much common present in T4bN1-3M0 than in T4dN1-3M0 tumours: 16/49 (32,7%) versus 10/51 (19,6%), but the difference was not statistically significant (p=0,139). Literature data suggest that co-expression of ER/PR/Her2 corresponds to different molecular breast cancer subtypes. Among 51 T4dN1-3M0 patients, 22 (43,1%) had SR/Her2+ tumour profile, 19 (37,2%) were SR+/Her2, 4 (7,8%) were SR+/Her2+, and 6 (11,8%) patients had SR/Her2 tumours. In the group of 49 T4bN1-3M0 patients, the majority 28 (57,1%) had SR+/Her2 tumour profile, 7 (14,3%) were SR/Her2+, 7 (14,3%) were SR+/Her2+ and 7 (14,3%) patients hadSR/Her2 tumours. We conclude from our study that inflammatory breast cancer comparing with non-inflammatory oedematous locally-advanced breast cancer is characterized by high frequency of Her2-positive (p=0,037) and SR-negative (0,013) tumour profiles. Most of inflammatory breast cancers are SR negative and Her2 overexpressing while non-inflammatory oedematous locally-advanced breast cancers express SR positive and Her2 negative phenotype.

Disclosure: All authors have declared no conflicts of interest.

134P IMPROVEMENT OF HYPERICIN PROPERTIES AS AN ANTICANCER AGENT

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Photodynamic therapy (PDT) is an anticancer approach that depends on the retention of photosensitizer in tumor and its subsequent activation by light to generate, in the presence of oxygen, cytotoxic reactive oxygen species (ROS), which cause tumor ablation [1]. Hypericin (HY), a perylenequinone with potent photosensitizing properties induces in vivo and in vitro photocytotoxic activity and considered as therapeutic drug in medicine on important areas as cancer detection and as photosensitizer in PDT of cancer [2]. HY is active against murine models of breast cancer and squamous cell carcinoma, and it may be useful against metastatic cancer [3]. The mechanism of photodynamic actions of HY is thought to involve two types of reaction - type I (by generation of ROS) and type II (by generation of $_1O_2$) [2]. HY triggers the photooxidation of cells and subcellular components, e.g. red blood cells [4], which could be used as a model system to evaluate and improve the therapeutic potential of HY. In our study we have used low concentrations of HY (0.25-1.25 μM) that lead to type II photosensitization reaction, leading to apoptotic cell death. While generation of ROS is believed to be harmful and kills cancer cells, it was shown that HY possesses a dose- and exposure time dependent photohemolytic effect [4]. This ''side effect'' on erythrocytes could be lowered by joint application of HY with antioxidants, such as ascorbic acid, tryptophan and quercetin. Quercetin, a plant-derived flavonoid, is a promising antioxidant and anticancer drug, exerts its anticancer role through either diminishing or promoting ROS generation under different conditions. It was shown that above mentioned antioxidants at certain concentrations could significantly increase the erythrocyte resistance to HY photodynamic action. Thus combination of HY with water-soluble antioxidants could be a useful strategy that opens attractive avenues to improve the therapeutic efficacy of hypericin-based PDT. Dolmans DE, Fukumura D, Jain RK. Nat Rev Cancer, 3(5), p. 380-387, 2003. Miskovsky P. Current Drug Targets, 3, 55-84, 2002. Blank M, Lavie G. et al. Int. J. Cancer, 111, 596–603, 2004. Vardapetyan HR, Tiratsuyan SG et al. Proc. of SPIE, 608706, 1-8, 2006.

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135P AUTOCRINE GROWTH HORMONE EXPRESSION ENHANCES SDF-1-CXCR-4 AXIS ACTIVITY TO INCREASE AGGRESSIVE PHENOTYPE OF BREAST CANCER CELLS

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Autocrine growth hormone is one of the important factors in breast cancer tumorogenesis process and tumor growth. A large number of documents demonstrate the role of this hormone in increase of tumor cells invasive phenotype. However, autocrine growth hormone role in metastasis process of breast cancer remains to be investigated. In this experiment, we have focus on evaluation of autocrine growth hormone effects on molecular mechanisms underlying tumor metastasis. By using of a breast adenocarcinoma cell line, MCF-7, an autocrine growth hormone expression cellular model has been made and its effects on expression level of CXCR-4 gene, and also its effects on cellular motility and cell migration ability induced by SDF-1 has been evaluated. Our results indicate that autocrine growth hormone may enhances metastasis via over activation of CXCR-4/SDF-1 pathway. According to this experiment results, autocrine expression of growth hormone leads to upregulation

of CXCR-4 expression in MCF-7 cells and as a result, synergizes SDF-1 induced cell motility and invasion. According to our results, it seems that determination of autocrine growth hormone expression in breast tumor cells may be helpful to estimation of disease prognosis and metastasis possibility.

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136P CHROMOSOMAL ABERRATIONS IN BREAST CANCER PATIENTS IN SOUTH INDIAN REGION

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Breast cancer (BC) is the most common cancer in women and accounts for between 18-25% of all female malignancies world-wide. In India, the incidence of BC is increasing, with an estimated 80,000 new cases diagnosed annually. The frequency of chromosome instability in peripheral blood lymphocytes is relevant biomarker for cancer risk in humans. The focal aim of the present study was to identify the chromosomal alterations in BC patients in stage wise manner. In the present study 25 BC subjects were selected on the basis of CA15.3 marker which is the most widely used serum biochemical tumor marker in BC and equal number of controls were selected and confirmed by CA53 level. In the present study experimental subjects were categorized based on the stage wise manner. The work was carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. After signing a consent form, both cases (experimentals and controls) provided a blood sample (5 ml) to establish the 72hrs cell cultures. In the present study deletion, satellite formation and translocation were frequently observed in chromosome 1, 3, 11, 13 and 17. (46, XX, del ($1p^-$); 46, XX, del ($13s^+$); 46, XX, del ($17q^-$). 46, XX, t ($11q^{+}$;$17q^-$). Statistically significant results were obtained in experimenta subjects compared to control subjects, moreover stage IV and III subjetcs showed higher degree of chromosomal damages compare to stage I and II . In the near future, we can look forward to the identification of novel BC predisposing genes due to rapid advancement of gene discovery technologies.

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