molecular biology (pre-clinical)

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

J. Hicks, N. Navin, J. Kendall, D. Levy, M. Wigler
Department of Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, USA

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 only yield approximate results when applied to mixed populations of rapidly evolving cells. In such cases our understanding would be improved by disentangling genetic events at the single cell level.

Methods: We have developed a method of single nucleotide sequencing (SNS) to quantify the genomic copy number of individual tumor cells. We have shown that a single lane of sequencing reads can be distributed across the genome to measure copy number at a resolution of about 90kb. 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 B-Cell like basal-like breast carcinoma 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 clonal expansion to generate a pseudo-triploid genome that in subsequent steps had acquired many additional focal amplifications and deletions of cancer genes including in one case, KRAS, EFN3 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 aeneploid 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.

Disclosure: All authors have declared no conflicts of interest.

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

S. Loi1, B. Haibe-Kains1, N.D. Brown1, J. Metcalfe1, S. Majaj1, C. Desmedt2, C. Piccart4, F.J. Esteva5, C. Sotiriou6

Background: ER signaling is necessary for breast cancer cell survival and proliferation. Breast cancer cells with high ER expression are sensitive to endocrine therapy, whereas ER negative breast cancer cells are resistant to agonist or antagonist estrogen. ER status is the best-replicated clinical predictor for breast cancer outcome in several large clinical trials.

Methods: A total of 11 datasets of BC samples with gene expression data were used to identify co-expressed genes from the ER status correlation, with the aim to identify subtypes with distinct clinical and molecular characteristics.

Results: In systematic analysis of untreated breast cancer tumors, the DP had the worse outcome compared with the ER+/HER2+ (p<0.001) ER+/HER2- (p<0.02) but also the ER+/HER2+ with 2% (p<0.02) 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<0.01%. In HER2+BC, ER1 gene expression was significantly inversely correlated with ERBB2 (R=-0.3; p<0.002). EGFR (-0.6; p<0.001) and gene sets of BAS (-0.8; p<0.001), 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 ER1 and ERBB2 (0.7; p<0.001) and AKTi (0.4; p<0.001). A gene set of PI3K/AKT pathway activation could predict CIR in taxantrum-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. RT4/74, ZR7530, SKBR3 (DP) showed decreased proliferation corresponding to decreased pAKT and p65, whilst HCC1954 and HCC1395 (ER-) had no such association of pAKT and p65 reduction and less growth inhibition. Furthermore, RT4/74 lines treated with fulvestrant to inhibit ER signaling were no longer sensitive to an AKT inhibitor (p<0.001).

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.

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

K. Kolaitová1, D. Pintirová2, P. Tesařová2, V. Bobek2, V. Mikulová4, M. Kuběcová1, M. Bychová1, V. Rusnáková3, S. Kasem-Bauer1, M. Kubista3

1Tumor Biology, Third Faculty of Medicine, Prague/CZECH REPUBLIC, 2Tumor Biology, Third Faculty of Medicine, Prague/CZECH REPUBLIC, 3Oncologic Clinic, General University Hospital and First Faculty of Medicine Charles University in Prague, Prague/CZECH REPUBLIC, 4Department of Clinical Biochemistry and Laboratory Diagnostics, General Hospital and First Faculty of Medicine, Prague/CZECH REPUBLIC.

Background: Circulating tumor cells (CTCs) have been described as the most valuable tool for early detection of cancer. The number of CTCs correlates with a worse prognosis and tumor progression. However, these studies are only based on mRNA levels and do not consider the protein expression and signaling pathways which take place in human cancers. 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.

Disclosure: All authors have declared no conflicts of interest.
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 RecoverAll (Ambion). Obtained CDNA molecules have been gene-specifically pre-amplified for multimarker qPCR analysis measured on BiomarkHID (Fluidigm, USA) microfluidic chip for 48 samples and 48 testing positions (2034 run in total, 35 tumour-specific genes in total). qPCR results have been analyzed by GENEX v.s. 5.0 software (MultiD, SE).

Results: 286 CTC samples have been analyzed in total. CTCs were detected in 29/87 (35.3%) patients with primary BC before starting CHT and in 10/87 (11.5%) after 2 CHT cycles. The CTC CTC positive 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. progestoestrogen receptor, MLIPII - myeloid leukemia factor 1-interacting protein, and H2AFZ - H2A histone family, member Z). The predictive value of CTC expression profiles will be prospectively evaluated.

Supported by MZ CR IGA NS- 9976.

Disclosure: K. Kolosťová: I am declaring that I am shareholder of TATAA Molecular Diagnostics - the first academic spin-off company in Czech Republic, a company supplying the Czech Rep. with CTC-M. Kubista: I am the shareholder in TATAA Molecular Diagnostics - the first academic spin off company in Czech Republic, a company supplying the Czech Rep. with CTC-detection kits (AdnaGen). All other authors have declared no conflicts of interest.

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

D. Santini, B. Vrcer, A. Ruscio, C. Oterga, C. Porta, S. Galuzzo, N. La Verde, C. Carollo, A. Addià, G. Torini

1) Medical Oncology, University Campus Bio-Medico, Rome/ITALY; 2) Medical Oncology, University of Palermo, Palermo/ITALY; 3) University Division of Medical Oncology and Clinical Research Institute, Policlinico di Torino Foundation IRCCS (Policlinico Torino), Italy; 4) Internal Med Oncol, IRCCS San Matteo Univ Hosp, Pavia/ITALY; 5) Medical Oncology, University Campus Biomedico, Rome/ITALY; 6) Medical Oncology, O.O. Orselli-Gallara, Genova/ITALY; 7) Medical Oncology, S. Giovanni Di Dio Hospital, Naples, Naples/ITALY; 8) Medical Oncology, University Campus Biomedico, Rome/ITALY

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 tumour samples. RANK expression was independent from histotype (p=0.25), 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=0.032). 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

E. Song

Breast Surgery, No.2 Affiliated Hospital, Sun Yat-Sen University, Guangzhou/CHINA

Cancers may arise from rare self-renewing tumor-initiating cells (TICs) but it remains obscure how self-renewal capacity for multipotent differentiation and tumorigenicity of TICs are maintained. Because microRNAs regulate cell fate, we compared miRNA expression in self-renewing and differentiated cells from the breast cancer cell lines, in breast cancer (T-IC), and non-BC from primary breast cancers. We found that: (1) let-7 miRNAs and miR-128 were markedly reduced in BC-T and increased with differentiation. In parallel, their target proteins, ha-ras, HMG2, and STERT, were elevated in BC-T but were reduced after differentiation. (2) Infecting BC-T 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/miR-128 by antisense oligonucleotides enhanced self-renewal of non-T-IC. (3) Infecting BC-T with let-7/lentivirus enhanced differentiation of the cancer stem cells via HMG2 pathway, with miR-128 induced apoptosis via inhibiting the activity of telomerase. (4) Targeting delivery of let-7/miRNA into BC-T with a non-viral vector, HA-liposome, 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 BC-T 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


1) Institut Jules Bordet, Université Libre de Bruxelles, Brussels/BELGIUM, 2) Hôpital Erasme, Université Libre de Bruxelles, Brussels/BELGIUM, 3) Medical Oncology Clinic, Institut Jules Bordet, Brussels/BELGIUM, 4) Functional Genomics Lab, Institute Jules Bordet, Brussels/BELGIUM, 5) Pathology Department, Institut Jules Bordet, Brussels/BELGIUM, 6) Medicine Department, Institut Jules Bordet, Brussels/BELGIUM, 7) Medical Oncology, Institut Jules Bordet, Brussels/BELGIUM

Flow cytometric analyses of fresh breast tumor cell homogenates revealed that CD4+ T cells constitute the major infiltrating CD4+ subset, present at twice the frequency found in the patient’s normal breast tissue. We use 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), by array 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 the PB and LN counterparts while CD4+ from patient 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% CD45Ro+) 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 TGFß/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


1) Klinik und Poliklinik für Frauenheilkunde und Geburtshilfe, Universität Münster, Münster/GERMANY, 2) Gynäkologie und Obstetrics, University of Frankfurt, Frankfurt/GERMANY, 3) Seminology, West German Study Group/Breast Clinics Niederrhein, Mönchengladbach/ GERMANY

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 byHugh et al. (1 Clin Oncol 2008). The dataset was analyzed using MAS5.

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),
Annals of Oncology

Volume 21 | Supplement 4 | May 2010
doi:10.1093/annonc/mdq145 | iv

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 CELLectionTM Dynabeads® coated with the monoclonal antibody towards the human Epithelial Cell Adhesion Molecule (EpCAM).The expression of progesterone receptor were evaluated by RT-PCR assay for the expression of MRPs and MRB2 (resistance to anthracyclines) and MRJ (resistance to taxanes). The drug resistance profile was correlated to progression free survival (PFS) in a 24 months (95% CI 90-92) 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), 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 observed lower EFS rates observed among patients with < 40 years compared to patients with higher age.

Disclosure: All authors have declared no conflicts of interest.

1UPREGULATION OF ADAM17 PROTEASE AND HER LIGANDS THROUGH A PKB NEGATIVE FEEDBACK LOOP MEDIATES ACQUIRED RESISTANCE TO TRASTUZUMAB IN HER2 OVEREXPRESSED BREAST CANCER

M. Gielen1, P. King2, T. Perera3, P. Parker4, B. Larjani5, A. Koning6, A. Harris7

Weatherall Institute of Molecular Medicine, University of Oxford, Oxford/UNITED KINGDOM; 1Johnson & Johnson Pharmaceutical Research & Development, Turnhoutseweg/BELGIUM; 2London Research Institute, Cancer Research UK, London/UNITED KINGDOM; 3Molecular Oncology Lab, Weatherall Institute of Molecular Medicine, Oxford/UNITED KINGDOM; 4C210 Clinical Oncology Unit, Oxford Radcliffe Hospital NHS Trust, Oxford/UNITED KINGDOM

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 [N-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 employee of Johnson and Johnson; T. Perera: The author is an employee of Johnson and Johnson; P. Parker: B. Larjani: A. Koning: A. Harris:

2BREAST CANCER RESISTANCE TO ANTHRACYCLINES AND TAXANES UNDER A MAGNIFYING LENS: EMERGING ROLE OF CIRCULATING TUMOR CELLS

A. Petracca1, C. Nicolazzio1, A. Palazzolo1, A. Tuini2, A. Passaro4, A. Altavilla6, I. Cortesi5, G. Naso1, G. Ramondi1, P. Gazzaniga1

1Experimental Medicine, Sapienza University of Rome, Rome/ITALY, 2Experimental Medicine, Medical Oncology B, Sapienza University of Rome, Rome/ITALY; 3Oncología Médica B, Policlínico Umberto I, Rome/ITALY; 4Oncología Médica B, Policlínico Umberto I, Rome/ITALY; 5Medical Oncology, Policlínico Umberto I, Rome/ITALY; 6Experimental Medicine, Medical Oncology B, Sapienza University of Rome, Rome/ITALY; 7Experimental Medicine, Medical Oncology B, Sapienza University of Rome, Rome/ITALY

Purpose: The prognostic value associated with the count of circulating tumour cells (CTCs) in metastatic breast cancer (MBC) raise some 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 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.

Methods and results: 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). The expression of progesterone receptor were evaluated by RT-PCR assay for the expression of MRPs and MRB2 (resistance to anthracyclines) and MRJ (resistance to taxanes). The drug resistance profile was correlated to progression free survival (PFS) in a 24 months (95% CI 90-92) 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), 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 observed lower EFS rates observed among patients with < 40 years compared to patients with higher age.

Disclosure: All authors have declared no conflicts of interest.

3HER2-SCFV/PROTAMINE MEDIATION IN DELIVERY OF PLK1 SIRNA TO HER2 OVEREXPRESSIOND BREAST CANCER

E. Song, Y. Yao

Breast Surgery, No.2 Affiliated Hospital, Sun Yat-sen University, Guangzhou/CHINA

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 profiles. 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

Purpose: The prognostic value associated with the count of circulating tumour cells (CTCs) in metastatic breast cancer (MBC) raise some 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 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.

Methods and results: 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). The expression of progesterone receptor were evaluated by RT-PCR assay for the expression of MRPs and MRB2 (resistance to anthracyclines) and MRJ (resistance to taxanes). The drug resistance profile was correlated to progression free survival (PFS) in a 24 months (95% CI 90-92) 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), 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 observed lower EFS rates observed among patients with < 40 years compared to patients with higher age.

Disclosure: All authors have declared no conflicts of interest.
domains, and subsequently transduces signals of cellular proliferation and survival along various intracellular molecular pathways. Therapy inhibiting dimeterization 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 Her2 expressing breast cancer cells in cell cultures and to be implanted in immunocompromised mice.

Methods: The pMg67B-Her2-ScFv-prolamine 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. F5-P fusion protein was expressed and purified from insect cell baculovirus expression system: F5-P fusion proteins expressed from vector can be best eluted with 250 mM sucrose. F5-P fusion proteins expressed from vector can be best eluted with 250 mM sucrose. F5-P fusion proteins expressed from vector can be best eluted with 250 mM sucrose. F5-P fusion proteins expressed from vector can be best eluted with 250 mM sucrose. F5-P fusion proteins expressed from vector can be best eluted with 250 mM sucrose. F5-P fusion proteins expressed from vector can be best eluted with 250 mM sucrose. F5-P fusion proteins expressed from vector can be best eluted with 250 mM sucrose.

Results: The pMg67B-Her2-ScFv-prolamine plasmid has been constructed by gene clone. F5-P fusion protein was expressed and purified from 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 mammaformation of Her2 overexpressed breast cancer cells and induces G2/M phase arrest and apoptosis of SKBR3 and BT 474 cells in vitro. Infection of PLK1 siRNA complex with F5-P to tumour-bearing mice suppresses tumour growth of Her2 overexpressed RT 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 nuclear 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 inhibit breast cancer cells in vivo. 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.

110P ABDERR METHYLLATION OF ESR1 IN BLOOD AND ASSOCIATION WITH ESR1 NEGATIVE TUMOR OF BREAST CANCER PATIENTS

J. Martinez-Galán1, B. Torres-Torres2, R. del Moral3, M.I. Núñez1, J. Valdivia1, R. Luque1, J. Pehalver2, S. Rios3, M. Ruiz De Almodóvar2, J.R. Delgado2

1Medical Oncology, Hospital Universitario Virgen de las Nieves, Granada/SPAIN; 2Molecular Biology, Centro de Investigaciones Biomédicas, Granada/SPAIN; 3Radiation Oncology, Hospital Carlos Haya, Granada/SPAIN

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) tumour and luminal B 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=0.017). We observed that methylated ERS1 was preferably associated with phenotype Basal Like and worse interval progression-free and survival (p<0.05). The presence of methylation ERS1+ and ERexpress 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/new status in breast cancer patients.

Disclosure: All authors have declared no conflicts of interest.

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

N. Fortunato1, C. Piccion1, M.G. Catalano2, G. Boccazzi3

1Oncological Endocrinology, AOU San Giovanni Battista Torino, Torino/ITALY; 2Department of Clinical Pathophysiology, University of Torino, Torino/ITALY; 3Department of Clinical Pathophysiology & Oncological Endocrinology, University of Torino & AOU San Giovanni Battista Torino, Torino/ITALY

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 D237N single nucleotide polymorphism (SNP) in SHBG gene exon 8 amplifies its protective role. A pentanucleotide repeat polymorphism (PNNP) [TAAAT]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, osteoporosis, where SHBG and estrogenic balance are important factors. Thus far, no data are available about (TAAAT)n polymorphism and breast cancer. In the present study, we evaluated (TAAAT)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 (Rechci et al. BCRF1999; Costantino et al. BCRT2008). TAAAT repeat region was amplified from genomic DNA with PCR (forward 5’-GCTTGAACGTGAG-3’ and reverse 5’CAGGGCTAAA CAGTGTAGCAG-3’); amplified products were analyzed by PAGE to determine the number of TAAAT repeats; results were confirmed by DNA sequencing. Frequencies for the different alleles were estimated by direct gene counting and compared with the chi² test. With respect to healthy controls, in breast cancer patients we observed a significantly higher frequency of (TAAAT)6 allele (40% vs 24%; p<0.05). The higher frequency of (TAAAT)6 size 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 (TAAAT)6, repeat as well as no association with D327N SNP. In conclusion, (TAAAT)n together with D327N SNP are strongly associated to estrogen sensitivity of breast cancer but are not characteristics of breast cancer.
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.

11DQ THE SECRETORY GTPASE RAB27B DRIVES POOR PROGNOSIS IN ER+POSITIVE BREAST CANCER

A. Hendrix1, H. Deryn1, G. Braems2, P. Pauwels3, R. Van den Broecke2, V. Cocquyt2, W. Westbroek4, M. Bracke3, O. De Wever2
1Medical Oncology, Ghent University Hospital, Ghent/BELGIUM, 2Gynaecology, Ghent University Hospital, Ghent/BELGIUM, 3Pathology, Ghent University Hospital, Ghent/BELGIUM, 4Nghi, NIH, Bethesda/USA, 5Laboratory of Experimental Cancer Research, Ghent University Hospital, Ghent/BELGIUM

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 ER+ 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 protein were associated with secretome of exogenous Rab27B-expressing breast cancer cells identified HSP90 as a xenograft mouse model. Proteomics of purified Rab27B-secretory vesicles and the invasion in cell culture, and invasive tumor growth and hemorrhagic ascites in a xenograft mouse model. Proteomics of purified Rab27B-secretory vesicles and the secretome of exogenous Rab27B-expressing breast cancer cells identified HSP90a as key pro-invasive growth regulator. HSP90a 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=0.0012) and differentiation grade (P=0.0014) in ER+ positive breast cancer. Rab27B regulates invasive growth and metastasis in ER+ positive breast cancer.

Disclosure: All authors have declared no conflicts of interest.

11DQ AN INTEGRATIVE ANALYSIS TO IDENTIFY EPIDEMIC GENETIC ABERRATIONS IN BASAL-LIKE BREAST CANCER CELL LINES

A. Giogrioscki1, E. Noel1, A. Mackay2, P.J. Wu1, J. Taylor3, R. Natraj3, E. de Ritis4, P. Mama1, J.S. Reis-Filho2, A. Tutt1
1Breakthrough Breast Cancer Research Unit, King’s College London, London/UNITED KINGDOM, 2Breakthrough Breast Cancer Research Unit, King’s College London, London/UNITED KINGDOM, 3Breakthrough Breast Cancer Research Centre, The Institute Of Cancer Research, London/UNITED KINGDOM

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 overlaid 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 methylation status were identified. 87 genes showed a significant inverse correlation between their expression and DNA methylation status with ACVR1, EPH2A, ETS1, GSTP1, MET, PRRCD8P and VIM having higher expression and lower methylation in basal as compared to luminal BCCLs and vice versa. PRRCD8P and GSTP1 were both less methylated and showed a DNA copy number loss in the H1P5 genomic region containing these genes specifically within the basal-like BCCLs (MDA-MB157, MDA-MB436, SUM155 and SUM190). Genes reported to be preferentially expressed in luminal cancers (eg ESR1, TFF1, TGFβ3, 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.

11DQ INFLUENCE OF ESTROGEN RECEP'TORS GENES VARIANTS ON PROSTATE-SPECIFIC ANTIGEN EXPRESSION IN BREAST CANCER

D.M. Nita1, A. Anghel1, R.S. Iliru2, N.V. Cirea2
1Biochemistry, University of Medicine and Pharmacy, Timisoara/Romania, 2Clinic of Surgical Oncology, University of Medicine and Pharmacy, Timisoara/Romania

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 to 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) and the expression status 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 ERα (p=0.03). AR immunexpression was correlated with CAG repeats on AR gene, higher number of repeats being linked to a higher AR immunexpression (p=0.04). Performing logistic regression to investigate relationships with prognosis, we observed that PSA immunexpression (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.

Acknowledgments: Supported by CNCSIS grant IDEI 1197/2009

Disclosure: All authors have declared no conflicts of interest.

11DQ STROMA-DERIVED MARKERS PREDICTING BREAST CANCER PROGNOSIS AND TREATMENT RESPONSE

A. Oseman1, J. Paulsson1, T. Liu1, L. Ryder1, K. Amin2, S. Cullen3, S. Hinde4, M. Hair5, J. Bergh5
1Department of Oncology-Pathology, Karolinska Institutet, Stockholm/Sweden, 2Department of Surgery, Lund University, Lund/Sweden, 3Department of Pathology, Lund University, Lund/Sweden, 4Clinical and Translational Research, Karolinska Hospital, Stockholm/Sweden, 5University of Manchester and the Christie Medical Oncology Breast Unit, Manchester/UNITED KINGDOM

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 predictive markers. PDGF receptors are important regulators of tumor stroma through stimulatory effects on cancer-associated fibroblasts (CAF's) 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-predictive significance of stromal PDGF-β-receptor expression 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 trastuzumab-sensitivity of HER2+ breast cancer. In vitro studies indicate that activation of PDGFβ in fibroblasts leads to a paracrine signaling that reduces the inhibitory effects of trastuzumab on HER2+ breast cancer cells. Fibroblasts were

Disclosure: All authors have declared no conflicts of interest.
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.

111P | FOUR POLYMORPHISMS IN CYTOCHROME P450 1B1 (CYP1B1) GENE AND BREAST CANCER RISK: A META-ANALYSIS
K.P. Economopoulos, T.N. Sergentanis
School of Medicine, University of Athens, Athens/GREECE

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 procarcinogens 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, Arg849Cys, Ala1195Ser and Asn453Ser. This meta-analysis aims to examine whether the four abovementioned polymorphisms are associated with breast cancer risk. Eligible articles were identified by a search of MEDLINE bibliographic 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 Arg849Cys polymorphism (11,321 cases and 13,379 controls); eleven studies were eligible for Ala1195Ser (10,715 cases and 11,678 controls); and twelve studies were eligible regarding Asn453Ser (11,638 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 3.034 (0.930-1.050) 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 Arg849Cys, 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 Ala1195Ser, 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 (0.84-1.144) for homozygous Ser subjects. In conclusion, this meta-analysis suggests that CYP1B1 Arg849Cys, Ala1195Ser 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.

111P | TRYPTASE AND PROTEASE-ACTIVATED RECEPTOR-2 EXPRESSION PARALLELED WITH MICROVASCULAR DENSITY IN BREAST CANCER PATIENTS
M. Ammendoloso1, R. Patruno2, N. Zuzzo3, P. Valeria4, A. Minello5, V. Di Lecce6, K.P. Economopoulos, T.N. Sergentanis
1Clinical Pharmacology Unit, University Magna Graecia of Catanzaro Medical School, Catanzaro/ITALY, 2Surgery, Di Venere Hospital, Bari/ITALY, 3Chair of Pathological Anatomy, Università degli Studi di Bari, Bari/ITALY, 4Medical Experimental Oncology Unit, National Cancer Institute Giovanni Paolo II, Bari, Bari/ITALY, 5Department of Surgery, Surgery Unit, Di Venere Hospital Bari, Italy, bari/ITALY, 6Clinical Pharmacology Unit, University Magna Graecia of Catanzaro Medical School, Catanzaro, Catanzaro/ITALY, 7Department of Sperimental Oncology, National Cancer Institute, Bari/ITALY

Background: Tryptase, a serine protease stored and released from mast cells (MCs) granules has been identified as a new non-classical angiogenic 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 biopptic 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 (A14, Dako, Glostrup, Denmark), polyclonal anti-PAR-2 (N-19; sc-8206 Santa Cruz Biotecnology), and monoclonal anti-C3D4 (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 vesicles, 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 (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 parallel to each other suggesting a role 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 antiangiogenic drugs.

Acknowledgements: Work supported by grants from Alleanza Contro il Cancro – Istituto Superiore di Sanità, Ministero della Salute, Italy.

Disclosure: All authors have declared no conflicts of interest.

111P | THE ROLE OF OESTROGEN SIGNALLING IN BREAST CANCER
A. Muliukurupputation, B.A. Michael1, L.D. Miller2, Y. Tamada3, T.M. Allen1, R.C. Wool3, T. Wang1, E.J. Crampin1, A.N. Shelling1, C.G. Print1
1Obstetrics and Gynaecology, University of Auckland, Auckland/New Zealand, 2Biochemistry, University of Otago, Dunedin/New Zealand, 3School of Medicine, Wake Forest University, Winston-Salem/USA, 4Human Genome Centre, Institute of Medical Science, University of Tokyo, Tokyo/JAPAN, 5School of Biosciences, University of Nottingham, Loughborough/UNITED KINGDOM, 6Auckland Bioengineering Institute, University of Auckland,
Breast cancer is a leading cause of malignancy in women worldwide. Oestrogen receptor-positive (ER+), 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 aim is 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, GSE3790, 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 ER1, 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 ER1 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 these genes also being target genes of ER1. Analysis of this microarray data revealed that the knockdown of either ER1 or FOXA1 in MCF7 cells reduced the expression levels of many oestrogen target genes, including CCG1 and MYC, and reduced the activation state of many oestrogen target pathways. The level of ER1 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 ER1, 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.

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

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 alterations 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™ 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=76%-100%). 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.

ANALYSIS OF MRNA – MICROARRAY DATA OF BREAST CANCER FOR THE PREDICTION OF LYMPH NODE INVASION

A. Smeets1, O. Gevaert2, B. Claes3, A. Daemen4, D. Lombrecht5, H. Wildiers6, H. Vandeven7, P. Vandenberghe7, P. Neven3, M. Christiaens1


Objective: 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 Z=2.0016 (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=1.21-3.53). In ethnicity-subgroup analysis, we found that in the Caucasian group, the expression of P53 gene was stronger in the TNBC group (OR=2.26, 95%CI=1.21-4.23), 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.

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

F. Guo1, X. Ke1, Z. Liu1, H. Liu2

1Oncology, the General Hospital of Shenyang Military Region, Shenyang/CHINA, 2Biostatistics, Chinese Medical University, Shenyang/CHINA

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 Z=2.0016 (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=1.21-3.53). In ethnicity-subgroup analysis, we found that in the Caucasian group, the expression of P53 gene was stronger in the TNBC group (OR=2.26, 95%CI=1.21-4.23), 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.

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

V. Guo1, P.D. Middlo1, T. Brown2, B. Kumar1, S. Hari1

1Medical Oncology, Southern Health, East Bentleigh/AUSTRALIA, 2Biochemistry, Monash University, Clayton/AUSTRALIA, 3Anatomical Pathology, Southern Health, Clayton/AUSTRALIA, 4Surgical Oncology, Southern Health, East Bentleigh/AUSTRALIA

Human breast cancer is a complex disease with large inter-tumoural and intra-tumoural heterogeneity. A population of CD44+/CD24-/+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 subset so far immunophenotypically. 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 patients with breast cancer. We determined the expression of many oestrogen target genes, including CCND1 and MYC, 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 ER1, 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.
The goal of this analysis is to investigate the loss of miRNA binding to its targets in this paired microRNA-mRNA data set. Microarray (1752 genes) and microRNA (229 microRNAs of which 189 are known microRNAs) data are present from 83 ER positive, HER2 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. Spearmann 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 p value of 0.05. This resulted in 26 microRNAs. These miRNAs were significantly inversely correlated with their computationally predicted targets. These 26 microRNAs 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-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.

Identification of Novel Biomarkers for Breast Cancer, Including BRCA1-Associated Breast Cancer

M.A. Brown1, J.D. French1, S.L. Edwards1, K.M. Peters1, A. Wronski1, C.E. Smart1, B.L. Brewster1, J.E. Wee1, N. Waddell1, G.D. Francis1
1School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane/AUSTRALIA, 2Centre For Clinical Research, The University of Queensland, Brisbane/AUSTRALIA

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 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 sequences 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 exonic 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, BreastFR 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.

Computational Dissection of Tumor Expression Profiles

A.C. Eldu1, N. Juul1, Q. Li1, A.L. Richardson2, S. Salsali3
1Department of Systems Biology, Technical University of Denmark, Lyngby/ DENMARK, 2Department of Pathology, Brigham and Women’s Hospital, Boston/USA

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 histologicalcellularity 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 interpretation of tumor expression profiles that may improve accuracy in molecular characterization and drug response prediction.

Disclosure: All authors have declared no conflicts of interest.
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 positive correlation of TOP2A gene dosage with nodal status (p=0.042), tumour 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 quantification method with a use of amyloid precursor protein (APP) as a reference.

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 has limitations, and its use is cumbersome. The method that we used was real-time PCR assay and showed to be fast and easy to perform, which makes it possible for BMI 25-29 vs. BMI<25 = 1.7 [1.05-2.75] especially when premenopausal women with breast cancer, 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.

Disclosure: All authors have declared no conflicts of interest.
All authors have declared no conflicts of interest.

Disclosure:

In all patients, CGRP was significantly higher in grade II as compared to grade III was significantly higher, whereas ER significantly lower in IDC. CGRP (P=0.00008 and P=0.014, respectively). BD and SMM were significantly higher in patients with BD>25% compared to <25%BD patients. Overall positive correlation was found between BD and CGRP (r=0.577, P<0.05). CGRP and Ki-67 were significantly higher in grade II cancers had significantly higher expression level of MTDH mRNA in peripheral blood is closely related to poor prognostic factor and it maybe used as a predictive marker.

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 age=66 ±3.13 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 cm, mean age=58 ±5.13 years). Immunohistochemistry for CGRP, Ki-67 and ER status was performed in paraffin sections. BD and SMM were calculated using computer-assisted methods and were statistically correlated with CGRP expression. BD, SMM, Ki-67 and ER were statistically correlated between IDC and IDC+DCIS, while CGRP was significantly higher in patients with BD>25% and <25%, and was also compared to test with grade II and grade III in both groups.

Results:

Overall positive correlation was found between BD and CGRP (P=0.037, P<0.001). Positive correlation was established between SMM and CGRP only in IDC+DCIS (sSMM/IDC+DCIS: CGRP=0.643, P<0.05). CGRP and Ki-67 were significantly higher in patients with BD>25% compared to <25%BD patients (P=0.0008 and P=0.014, respectively). BD and SMM were significantly higher 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<0.05). 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 the IDC+DCIS is an entity more aggressive, ER-independent and possibly associated with a pathway linked to stromal involvement and GCRP activity. All authors have declared no conflicts of interest.

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

A. Klaunov, D. Kornov, S. Polkarpova, E. Roshin, E. Boguth Diagnostical Surgical Department, HROC RAMS, Moscow, RUSSIAN FEDERATION

Inflammatory breast cancer was defined as particular clinic-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 (T4N1-M0) and non-inflammatory locally advanced breast cancer featured by skin oedema (T4N1-M0). 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 T4N1-M0 group, 23/51 (45.1%) tumours were SR+ and PR+, 26/51 (50.9%) were Her2+, while in T4N1-M0 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 T4N1-M0 than in T4N1-3M0 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 T4N1-3M0 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 T4N1-M0 patients, the majority 28 (57.1%) had SR+/Her2+ tumour profile, 7 (14.3%) were SR+/Her2- and 14 (28.6%) patients had SR/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 (p=0.013) tumour profiles. Most of inflammatory breast cancers are SR negative and Her2 overexpressing non-tumour expression non-inflammatory oedematous locally-advanced breast cancers express SR positive and Her2 negative phenotype. All authors have declared no conflicts of interest.

AUTOCRINE GROWTH HORMONE EXPRESSION ENHANCES SDF-1/CXCR4-4 AXIS ACTIVITY TO INCREASE AGGRESSIVE PHENOTYPE OF BREAST CANCER CELLS

M. Mojarrad1, M. Momary2, R. Raaofifar2, M.H. Medaramees2, S.H. Ghaffarif2

1Medical Genetics, Mashhad University of Medical Sciences, Mashhad/RAN, 2Medical Genetics, Tehran University of Medical Sciences, Tehran/RAN, 3Hematology, Oncology and Bone Marrow Transplantation Research Center, Shariati Hospital, School of Medicine, Tehran University of Medical Sciences, Tehran/RAN

Autocrine growth hormone is one of the important factors in breast cancer tumourogenesis 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 SDF-1/CXCR4-4 interaction in breast cancer cells, 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 CXCR4/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.

**Disclosure:** M. Mojarrad: This experiment was funded by Tehran University of Medical Sciences. All other authors have declared no conflicts of interest.

**CHROMOSOMATIC ABERRATIONS IN BREAST CANCER PATIENTS IN SOUTH INDIAN REGION**

V. Balachandar1, R. Sangeetha1, S. Mohanadevi1, K. Sasikala2, S. Dharwatgar3

1Human Molecular Genetics Laboratory, Bharathiar University, Coimbatore/INDIA, 2Zoology, Bharathiar University, Coimbatore/INDIA, 3Department of Biotechnology, KLE medical University, Bangalore/INDIA

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 (experiments 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.

**Disclosure:** All authors have declared no conflicts of interest.