TGFB1–509TT genotypes were showed in the patient group in comparison to the controls ($p = 0.008$ and $p = 0.03$ respectively). We have observed an increased frequency in the VEGF-2578A/A genotype among women with positive regional lymph node metastases compared to patients with negative regional lymph node metastases ($p = 0.02$). A significant difference was found between the luminal B and luminal A subtype tumor of patients carrying the VEGF-2578C/C genotype ($p = 0.04$). In addition, patients with the FGFR2A/A genotype exhibited a non-statistically significant better response to neoadjuvant chemotherapy ($p = 0.06$). There was also trend for association between FGFR2G/G genotype and worse response to neoadjuvant chemotherapy in infiltrating ductal breast carcinoma patients ($p = 0.08$).

Conclusions: These findings indicate that genetic variants in VEGF-2578A/A, FGFR2G/G and IL10–592A/A are associated with infiltrating ductal breast carcinoma risk. Polymorphism in VEGF gene may serve as molecular marker related with regional metastasis and molecular subtype of tumor. The polymorphic variants of FGFR2 gene may be a potential prognostic factor for response to neoadjuvant chemotherapy in infiltrating ductal breast carcinoma patients.

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In patients with early breast cancer, populations of immature, cytokine producing plasmacytoid dendritic cells (PDC) decrease in tumour draining lymph nodes and express TLR9

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Background: Plasmacytoid dendritic cells (PDC) represent a distinct subset of dendritic cells (DC) capable of producing large amounts of type-1 interferons after stimulation of toll-like receptors (TLR)-7 and 9. In breast cancer draining LN, PDC secrete cytokines such as IL-10 and IL-12 with the potential to polarise local T cell responses. Despite being frequently identified within the tumour microenvironment, PDC remain poorly characterised in human cancer.

Materials and Methods: Plasmacytoid DC and myeloid DC (MDC) were identified using flow cytometry in cell suspensions of control LN and good and poor prognosis breast tumour draining axillary LN (TDLN) as defined by the Nottingham Prognostic Index (NPI). Immunohistochemistry and immunofluorescent microscopy was performed on frozen Control LN and TDLN sections to localise PDC and determine IL-10 and IL-12 expression. Immunofluorescence microscopy of magnetically sorted PDC populations was undertaken to determine TLR-9 expression.

Results: PDC constituted a prominent immature population in all LN studied. In both control and TDLN the maturation status of PDC and MDC subset populations was similar. When compared to poor prognosis LN, PDC proportions decreased in LN draining good prognosis breast cancer ($p < 0.05$). Immunohistochemistry identified CD123+ BDCA-2+ PDC within the cortex and sinus system of control and tumour draining LN. In LN containing metastatic breast cancer, CD123+ BDCA-2+ PDC were found within and at the cancer periphery. Immunofluorescence microscopy localised BDCA-2^+ PDC co-expressing IL-10 or IL-12 to the T cell areas of control and tumour draining LN. TLR-9 expression was identified on PDC sorted from control and tumour draining LN.

Conclusions: PDC were found in close proximity to malignant cells in metastatic LN as well as T cells in control LN and TDLN. The identification of PDC in the sinus system of control LN and TDLN suggests that they can gain access to tissue afferent lymphatics. The expression of IL-10 and IL-12 by PDC in the T cell areas of control LN and TDLN confirms that PDC are able to produce polarising cytokines. In patients with breast cancer, the migration and cytokine secretion of PDC populations may play a pivotal role in anti-tumour responses. The expression of TLR-9 by PDC also makes them a target for therapeutic intervention.

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Relation between methylation promoters gene and estrogen receptor (ERS1) and her2/neu status in breast cancer patients

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The CpG island metylator phenotype is associated with distinct clinicopathological caracteristics as Estrogen Receptor (ESR1) positive and amplification HER2 in breast cancer.

Objective: To investigate the relation between DNA promoter methylation and the prognostic clinico-pathological features of breast cancer, including diagnosis and treatment response and to evaluate epigenetics differences in tumor-related genes to ESR1 and HER2/neu status in primary breast cancer.

Material and Methods: We quantified methylation levels of promoter of 5 genes (ERS1, RAR-β, 14-3-3 sigma, APC, E-Cadherin) which are to confer growth advantage to cells in 107 women with breast cancer and 108 control subjects. Real Time QMS-PCR SYBR green (methylation-specific PCR) was used to analyze the hypermethylation. Tumours were classified as phenotype basal, luminal A, Luminal B and phenotype HER2+.

Results: Ours analyses revealed low or absent methylation *ESR1*and *14-3-3*σ in healthy controls and significant differences between breast cancer patients (pts) and healthy controls in relative serum levels of methylated gene promoters *ESR1* ($p = 0.0112$) and *14-3-3*s ($p = 0.0047$). Presence of methylated *ESR1* in serum of breast cancer patients was associated with ER-negative phenotype ($p = 0.0179$). Of the available cases, 60 pts (56%) were Luminal A, 10 pts (9.3%) Luminal B, 13 pts (12%) Basal like and 9 pts (8.4%) HER2+. We observed that methylated ERS1 was preferably associated with phenotype Basal Like and worse interval progression free and survival global though $p > 0.05$ and the amplification HER2+ was correlation with significant more frequent methylation gene ($p < 0.05$). Thet hypermethylation of normal ERS1 and 14-3-3σ combined differentiated between breast cancer patients and healthy controls ($p = 0.0001$) with a sensitivity of 81% (95% CI: 72−88%) and specificity of 88% (95% CI: 78−94%).

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. These showed that frequent methylation had a strong association with molecular phenotype of breast cancer and perhaps in the future can explain therapy resistance related to RE and HER2/neu status in breast cancer patients.

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Novel germline mutations in BRCA2 gene among breast and breast-ovarian cancer families from Poland

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Background: The aim of our study was an assessment of the spectrum of *BRCA2* gene mutations and their frequency in women and men with familial breast cancer and ovarian cancer, in whom no mutations were found in *BRCA1* gene.

Material and Methods: 105 probands were selected (97 women and 8 men) and treated in the Oncology Center in Warsaw and the Oncology Center − Branch in Cracow in the years 1998–2008 and remain in care of the Genetic Counselling Clinic, Oncology Center in Warsaw. The patients were aged 17−67 years; (median age 46 years). The presence of molecular changes was examined in DNA isolated from peripheral blood lymphocytes. Germline mutations in 27 exons of the *BRCA2* gene were screened by "touchdown" PCR amplification, DHPLC and sequencing. Missense mutations were classified by multiple-sequences alignments of orthologous BRCA2 protein sequences with T-Coffee software.

Results: Thirty-nine molecular variants were identified in the study group, including eight changes determined for the first time (five pathogenic

mutations and three variants of indefinite biological effect). Ten frame shift mutations were detected, the result of which was production of truncated protein. They included: 5467insT, 6174delT, 6192delAT, 6675delTA, 8141del5, 9152delT, 9326insA and 9631delC. The 8141del5 mutation was detected in 3 patients. The group of pathogenic mutations was completed with the nonsense change E394X and splice site mutation IVS23−2A>G. The presence of 10 missense type mutations was detected: N289H, N372H, T598A, G602R, N991D, D1420O, K1690N, T1915M, I2627F, N3124I. The frequency of N991D, D1420O and N3124I was compared between breast cancer patients and the control group of healthy subjects.

Conclusions: 1. A high diversity was found of the mutations detected in *BRCA2* gene; their frequency depended on the study population and family history of the patients subjected to genetic tests. 2. The determination of pathogenic status of molecular variants detected in BRCA2 gene, described in the BIC database as "unclassified variants" depends on many parameters. Most important is the assessment of the evolutionary conservation of their protein sequences and studying of the frequency of molecular variants detected in breast cancer and in healthy population.

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Phenotypic profile of triple negative and hormonal receptors positive breast cancer cells treated with growth factors for mammospheres formation − preliminary results

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Backgrounds: Breast cancer patients are stratified into 3 main groups: tumors expressing hormonal receptors (HR), HER2 positive tumors and triple negative tumors, the last one show a more aggressive clinical behavior.

In human breast cancer cell lines exposed to bFGF and EGF is possible to identify groups of spherical colonies in suspension with stem cell properties designated mammospheres. In solid tumors a subpopulation of tumorigenic cells is thought to express CD44+/CD24-, ESA+, Oct4, Musashi1 and CD133+, possibly representing stem cell markers.

The aim of this study is to analyze breast cancer cells exposed to bFGF and EGF considering HR expression, which is positive in MCF7 and triple negative in HCC1806 breast cancer cell lines, particularly CD24/CD44 expression.

Material and Methods: The adherent human breast cancer cell lines MCF7 and HCC1806 were cultured according to recomended procedures. To perform the mammospheres forming protocol, both cell lines were cultured in DMEM-F12 supplemented with bFGF and EGF. After, cell lines were analyzed by flow cytometry with anti-CD45, anti-CD44and anti-CD24 in a FACSCalibur. To access underlying cell death pathways, treated cells were also analyzed with Annexin-V (An).

Results: The cell lines studied showed different phenotypes in culture. MCF7 cells formed several spherical colonies in suspension and HCC1806 cells kept mainly the adherent phenotype with a few groups of spherical colonies in suspension.

The characterization of the adherent population of MCF7 identified 2 subpopulations, one representing 14−22%, with CD44 expression higher than the main population, which was CD44 negative. In contrast, suspended population presented a prevalent subpopulation (83−86%) expressing CD44. Regarding HCC1806 cells we found similar behavior, the adherent subpopulation expressed CD44 less frequently (15−25%) than suspended subpopulation (91−95%).

The An profile was positive in 11−17% of HCC1806 suspended subpopulation, with insignificant marks in the other cells studied.

Conclusions: The mammospheres forming protocol developed more suspended cell colonies in HR positive cells than in triple negative. The exposure of both cell lines to growth factors separated suspension population expressing CD44 in a higher degree than the adherent population, similar to cells in standard conditions. The former is thought to harbor stem cells properties, emphasized by CD44 positivity. Apoptosis marker (An) had an irrelevant expression.

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Early breast cancer detection: validation of a commercially available blood-based gene expression test

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Background: Despite screening programmes, breast cancer continues to be the second most common form of cancer death in women. Better diagnostic tools for the detection of cancer at early stages would contribute to increased survival. We have previously reported the development of a blood-based gene expression test for the detection of breast cancer. The test was developed from an initial whole genome array analysis for selection of informative assays. Further assay selection was performed that resulted in the present day, commercially available, 96-assay set. Gene expression is measured using reverse-transcriptase real-time PCR to determine mRNA levels. An algorithm was developed in a so-called calibration study to distinguish between BC and non-BC patients. The current study reports the calibration and validation results obtained with the gene expression test (BCtect®).

Materials and Methods: In a multicentre-study, blood samples were collected from women in 3 groups (1) Stage 0−III BC, (2) benign breast lesions, or (3) negative mammograms. Blood samples (2.5 mL per sample) were collected in PAXgene™ tubes and shipped on dry ice to a central laboratory for RNA extraction according to the manufacturer's instructions. Quality control of RNA was performed using the Agilent 2100 BioAnalyzer and Nanodrop ND-1000. Gene expression analysis was performed using real time RT-PCR (AB7900 HT) with a microfluidic card containing the BC-specific gene signature in a 96-gene assay format. Modelling was performed using Partial Least Square Regression providing an algorithm for application to gene expression data. Leave-one-out cross validation was used to obtain performance characterstics from the calibration study. The final algorithm was used with the test software to provide a test score for each subject in the independent validation cohort. A positive test score classified a subject as positive for BC, whilst a negative score classified a subject as negative for BC. Overall, 332 samples were included, 223 samples were used to develop the algorithm and 109 samples were used as an independent validation set to describe its performance.

Results: The model correctly predicted the class of 78 of the 109 validation samples and 162 of the 223 calibration samples (overall accuracy of 72%). Performance was similar for early and late stage cancer with a sensitivity of 74% for stage 0/1 breast cancer (stage 0 = in situ cancer, and stage 1 = T1N0M0; staging defined by AJCC 2002). The test performed equally well in pre- and post-menopausal women. Use of hormonal based contraceptives, hormone replacement therapy and common medications did not appear to affect the accuracy of the blood test.

Conclusions: The blood-based gene expression test showed efficacy for the detection of early breast cancer in both pre- and post-menopausal women. The test may be of clinical benefit as a complement to mammography for all women, and particularly for pre-menopausal women for whom mammography is known to have limitations.

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Knock-down of the estrogen receptor GPR30 in triple-negative breast cancer reduces cell proliferation

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Background: The G protein coupled receptors (GPCR) are a large family of membrane-bound receptors characterized by a 7-mer alpha helical structure. They mediate rapid response to a wide range of ligands including opiates, dopamine, chemokines and steroids. GPR30 is a G protein-coupled receptor that is activated not only by estrogen but also tamoxifen and other selective estrogen receptor modulating drugs. We have previously reported that GPR30 is frequently expressed in triple negative (ER−, PR, HER2 neu−) breast cancers, but its influence on breast cancer biology is unknown.

Hypothesis: GPR30 functions to stimulate cancer cell growth and increases resistance to tamoxifen (TAM).