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).