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**The occurrence and classification of the hereditary BRCA2 gene mutation in women and men with breast cancer**

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**Purpose:** Identification of mutations in the BRCA2 gene and estimation of their clinical consequences for women and men without pathogenic mutations in BRCA1 and with familial breast cancer treated in the Maria Sklodowska-Curie Memorial Cancer Center Warsaw, Poland in the years 1998–2006.

**Materials and Methods:** All the patients (23 women and 7 men) have a family history of at least 3 breast cancer or 2 breast cancer and ovarian cancer. 4 probands have bilateral breast cancer. The age at onset of breast cancer of mutation carriers in BRCA2 gene was $\leqslant$50 years (36−50). The presence of molecular changes were examined in DNA isolated from peripheral blood lymphocytes of patients. Germline mutations in 27 exons of the BRCA2 gene were screened by the PCR amplification "touchdown", denaturing high performance liquid chromatography (DHPLC) and sequencing. Missense mutation, detected during mutation screening of the BRCA2 gene were classified by multiple-sequences alignments of orthologous BRCA2 protein sequences with T-Coffee software.

**Results:** 25 molecular changes were identified in the BRCA2 gene in 30 of the investigated patients. In 4 patients the following pathogenic, frame shift type mutations, were identified: 5467insT (exon 10), 6174delT (exon 11), 9631delC (exon 25) and 10323delCins11 (exon 27). In 7 patients, the presence of 8 missense type mutations was detected, among them the following: Asn3124Ile (within DNA binding domain of BRCA2) and Asn372His (within a putative histone acetyltransferase P/CAF interacting region of BRCA2). All the identified missense mutations fall at the positions that are invariant in our alignments of mammalian BRCA2 sequences. The strongest evolutionary conservation (through pufferfish Tetraodon) was observed for the position of the missense mutation Asn3124Ile. In the 13 patients, 8 silent type mutations and 5 intron changes in the BRCA2 gene were identified.

**Conclusions:** The cosegregation of identified, missense variants, falling at the positions that are evolutionary conserved and/or in recognized domain of the BRCA2 gene with the disease is evaluated. The results of cosegregation analysis should improve our estimation of the risk of breast cancer, associated with the identified potentially pathogenic missense variants within the BRCA2 gene.

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**Promoter methylation profiles in breast cancer**

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**Background:** Aberrant DNA methylation of tumor suppressor genes has been accepted to be a common feature and early event in human cancer. The aim of this study is that analyze methylation profiles of 50 well established methylation-associated genes in breast cancer.

**Material and Methods:** Using HpaII-MspI-PCR, we determined methylation status of 50 genes in two breast cancer cell lines MCF7 and MDA-MB231 and 8 genes (APC, CALCA, CDH13, MTHFR, S100A2, H19, EDNRB, MUC2) were found to be methylated in at least 1 cell line. Methylation of all 8 genes was observed in tumor tissues with different methylation frequency.

**Results:** Methylation frequency of five genes is determined as follows: MTHFR (41.9%), APC (51.6%), EDNRB (77.4%), CALCA (80.6%), S100A2 (87.1%), CDH13 (93.5%), H19 (93.5%) and MUC2 (96.8%) respectively in breast cancer. The results represent that a panel of these 8 genes will be useful for detection of breast cancer.

**Conclusions:** Methylation of APC, MTHFR, CALCA, CDH13, H19, MUC2, EDNRB and S00A2 will be useful for detection of breast cancer. Detection of this abnormality may be useful in risk assessment and early detection of breast cancer.

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**Anti-apoptotic role of TNF-inducible zinc finger protein A20 through the interrupting ASK1-mediated JNK activation in breast cancer cells**

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**Background:** Nuclear factor kappa-B (NF-kB) is a strong anti-apoptotic factor, which is constitutively active in human breast cancer cells. Since the TNF-inducible zinc finger protein A20 has been known for a key player in the negative feedback mechanism responsible for terminating NF-kB activation during TNF-triggered signaling pathway, the down regulation of NF-kB pathway by A20 may responsible for inhibiting JNK-mediated apoptotic cell death pathway.

**Material and Methods:** The expression levels of A20 protein were compared by Western blotting in normal and cancerous tissues from 13 patients who underwent surgery for breast cancer. The transient transfection was performed using Lipofectamine plus reagent. Direct interaction between A20 and ASK1 was analyzed by co-immunoprecipitation assay. In vitro JNK activity was assessed by measuring the formation of phosphorylated GST-c-jun after immunoprecipitation with anti-JNK1 antibody. The transcriptional activity of NF-kB was assessed by p2xNF-kB-Luc luciferase reporter assay after each sample was normalized with $\beta$-galactosidase activity.

**Results:** In this study we found that high levels of A20 expression in human breast cancer tissues, and ectopic expression of A20 blunted TNF-induced apoptosis through repressing the apoptosis signal-regulating kinase 1 (ASK1) signaling pathway. In cotransfection experiments, A20 physically interacts with N-terminal half of ASK1, and the interaction was dependent on the cellular redox status. Furthermore, overexpression of A20 sufficiently blocked the sustained-JNK activation in response to TNF, but not the transient-JNK activation, ASK1 dependently.

**Conclusions:** Our study provides a novel mechanism for the A20-mediated suppression of JNK pathway to inhibit apoptotic cascade in the TNF receptor death signaling pathway.

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**The oestrogen receptor interacts with the correlation between HER-2 over-expression and age at diagnosis, tumour grade and lymph node involvement in operable breast cancers: a single centre experience**

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**Background:** Significant interactions between HER-2 and the oestrogen receptor (ER) that correlate with breast cancer pathology and age at diagnosis have been suggested (Journal of Clin Oncol 2007: 25: 4423−30). The HER-2 status was predicted by ER only above age 40 and by the lymph node status (LN) in ER− but not in ER+ tumours where HER-2 was predicted by the tumour grade. We analyse our database for an interaction between HER-2, ER-expression, tumour grade and lymph nodes status.

**Materials and Methods:** Our database contains 2552 consecutive women with a primary operated invasive breast cancer (January 2000 and December 2005). All women had a classical LN dissection, mostly level I-II. After June 2003, the sentinel lymph node procedure was performed in patients with a cT1 tumour. An LN dissection was only performed if the sentinel node was involved. Tumour grading was performed according to the Ellis and Elston grading system. LN were examined by H&E using 3 sections per node; sentinel lymph nodes and those from lobular breast cancers classified as negative on H&E were stained with epithelial markers. Expression of ER and HER-2 was demonstrated by IHC according to the Envision method using MoAb NLC-ER-6F11 for ER and CB11 for HER-2. Since 2005, highly sensitive rabbit MoAb (SP1) were used for the assessment of ER. IHC staining was performed according to standard procedures for clinical purposes. For ER, any nuclear staining of invasive tumour cells was considered as positive. For HER-2, either strong expression by IHC (score 3+) or HER-2 gene amplification by FISH was considered HER-2+.

**Results:** In our database 7.0% of patients were younger than 40 year of age, 41.0% and 38.4% were respectively grade 3 and LN positive; 15.4% of breast cancers in our institution were ER− and 11.6% were HER-2+. HER-2 over-expression correlated with tumour grade only in ER+ tumours ($p < 0.0001$, Mantel-Haenszel $\chi^2$). HER-2 was also related with LNI involvement (10.1% in LN− and 13.8% in LN+ cases; $p = 0.0038$, $\chi^2$). Although more women in the ER− '4 or more lymph node' group were HER-2+, this figure was not significant ($p = 0.2894$, $\chi^2$). However, in ER+ cases, HER-2 was related to the degree of LN involvement ($p = 0.0022$, $\chi^2$). Also, tumours with an ER+HER-2+ phenotype were more likely LN+ than tumours of any other ER-HER-2 status.

**Conclusion:** The risk of LN involvement among operable HER-2+ breast cancers is associated with the ER status.

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**High preoperative plasma TIMP-1 is prognostic for early relapse in primary breast carcinoma**

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TIMP-1 is a natural inhibitor of extracellular matrix degrading enzymes called matrix metalloproteinases (MMPs). In addition to its capacity to inhibit matrix degradation, TIMP-1 has been shown to promote cell growth and inhibit apoptosis. The expression of TIMP-1 in tumour tissue, as well as in circulating blood, has therefore been shown to associate with worsened survival in several malignancies.

In this study, a prospective series of 213 patients with primary breast carcinoma was assessed. Circulating pre- and postoperative TIMP-1 levels were assayed using ELISA analysis.

It was shown that high preoperative plasma TIMP-1 was a powerful predictor of systemic early relapse in breast carcinoma, with HR 8.1 (95% CI 1.8−37.6) ($P = 0.007$) as a log-transformed continuous variable in Cox regression univariate analysis. It was shown to be independent of, and superior to, nodal status as a prognostic variable in multivariate analysis, and not associated with any known prognostic clinicopathological parameters. Kaplan–Meier analysis showed that the patients belonging to the highest quartile of circulating TIMP-1 levels had a worsened recurrence-free survival of 79% compared to 94% RFS among patients in the lower quartiles ($P = 0.016$).

The postoperative levels of circulating plasma TIMP-1 were not found to be prognostic for relapse.

In conclusion, preoperative plasma TIMP-1 was found to be a powerful prognostic factor for early systemic relapse in primary breast carcinoma.

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**EGFR genetic mutations of exons 19 and 21 are rare in male breast carcinoma**

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**Background:** Breast carcinoma is a rare male neoplasm with poor outcome, despite its histological low grade features. We studied 12 cases of male breast carcinoma (MBC) to evaluate over-expression and genetic mutations of EGFR and Her 2/neu comparing clinical characteristics.

**Material and Methods:** All cases of MBC were collected in the period between 2000–2006. Histological features were reviewed; immunophenotyping analysis was performed for ER, PgR, Her 2/neu EGFR, and ki-67 with avidin-biotin method as previously described. Her 2/neu/CEP17 sonde from PathVysis was used for FISH assay. Genomic DNA was extracted and used to assay EGFR mutation analysis of exons 19 and 21 by PCR and confirmed by automated fragment analysis (PE Applied Byosystems, Foster City, CA) using fluorescent-labeled primers.

**Results:** Twelve cases were identified with a median follow-up of 17months (1−35mo). The mean age was 68yr (48−87yr). Ten patients (83%) showed advanced clinical stages; 5 patients (41.7%) presented lymph node involvement. The median size of the tumors was 4.5 cm (2−9 cm).Ten cases were ductal invasive carcinoma (83.3%), one case was papillary carcinoma (8.3%), and one case was mixed secretory and ductal carcinoma (8.3%). ER was expressed in 83.3%, PgR in 66.7%, Her 2/neu in 8.3%, EGFR in 25%, and ki-67 in 50% of the cases. None FISH amplification was found. Eleven cases amplified by PCR of EGFR; all amplified cases showed wild type sequences of exons 19 and 21 by fragment analysis.

The outcome showed: 5/12 patients (41.7%) were death with disease, 2/12 (17%) were alive with disease, and 5/12 patients (41.7%) were alive without disease. Large tumor size or advanced clinical stage were associated with poor outcome in 5/12 (41.7%) patients. Over-expression of EGFR was related with bad prognosis in 1/12 patient (8.3%), and ki-67 in 4/12 patients (25%).

**Conclusions:** Over-expression and genetic mutations of EGFR are two uncommon phenomena in MBC. The prognostic factors involved with poor outcome are related with clinical and histological aspects. In our study, the expression of ki-67 was associated with bad prognosis. More studies are necessary to establish different prognostic factors and new treatments.

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**EGFR analysis in metaplastic carcinoma of the breast**

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**Background:** Metaplastic carcinoma (MC) of the breast is considered a triple-negative receptor carcinoma, which over-expresses EGFR in 80%, and gene amplification is observed in 25% of the cases. EGFR is regulated by other proteins like ph-EGFR. We studied different molecular aspects of EGFR in a group of MC, in order to recognize alterations at transcriptional or regulatory protein levels.

**Material and Methods:** In a group of 22 MC, we assessed IHC studies for ER, PgR, Her 2/neu, EGFR and ph-EGFR with avidin-biotin method as previously described. Genomic DNA was extracted and used to assay EGFR mutation analysis of exons 19 and 21 by PCR and confirmed by automated fragment analysis (PE Applied Byosystems, Foster City, CA) using fluorescent-labeled primers. Sequencing was performed in suspicious mutant cases (PE Applied Byosystems, Foster City, CA).

**Results:** The assessment of immunophenotyping was negative for both hormonal receptors, and Her2/neu in all cases. The 100% of the cases were positive for EGFR with high intensity in 13/22 cases and moderate intensity in 9/22 cases. The expression of ph-EGFR was positive in 11/22 cases (50%), 9 cases were mixed tumors, and 2 cases were purely epithelial neoplams. Twenty of 22 cases amplified by PCR, and a 316bp band in the electrophoresis gel was observed. The fragment analysis of exon 19 showed 5/20 cases (25%) with two peaks at 192 and 207bp. Exon 21 was wild type in all cases. The sequencing of 1/5 cases (20%) presented a deletion in exon 19 of 15bp, and 4/5 cases (80%) presented wild type sequences. The case with genetic deletion was purely epithelial type, and negative for ph-EGFR; the 4/5 cases with wild type sequences were positive for ph-EGFR, 3 were mixed type and one case was purely epithelial tumor.

**Conclusions:** MC of the breast over-expresses EGFR and is a triple-negative receptor carcinoma. In our study, we observed the mutations of EGFR are rare (4.5%), but the high expression of ph-EGFR (50%) may explain the signalling of EGFR. EGFR presents different alterations at transcriptional levels, and regulatory proteins also are involved in over-expression in MC of the breast. New treatments may focus on different pathways of EGFR expression.

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**Semi-quantitative transcript analysis of Portuguese breast/ovarian cancer families with the BRCA1 founder mutation R71G of Galician origin**

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**Background:** We identified three unrelated Portuguese breast/ovarian cancer families with the c.211A>G (R71G) variant in the BRCA1 gene. In order to evaluate the functional effect of this variant localized at position −2 of the exon 5 donor splice site, we performed semi-quantitative transcript analysis. Furthermore, we have evaluated whether Portuguese and Galician families with this mutation share a common ancestry.

**Material and Methods:** RNA analysis of three carriers and control individuals was performed by RT-PCR. All amplification fragments were sequenced and semi-quantitative fragment analysis of the RT-PCR products was performed. Furthermore, segregation and loss of heterozigozity (LOH; in a patient with bilateral disease) analyses were performed. Histopathologic data from one proband with bilateral carcinomas and from an affected family member of a second family were obtained from medical records. BRCA1 haplotype analysis was performed using six microsatellite markers in the three Portuguese families and in one Galician family with the same R71G BRCA1 mutation.