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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 β-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+.