and TP53 coding regions in 90 unrelated probands with strong family history or early-onset breast cancer cases with or without family history, 50 sporadic breast cancer cases and 23 bilateral breast cancer patients without family history of breast and ovarian cancer.

As the observed distribution of mutations favour routine pre-screening using a simple and cost-effective test we used allele specific oligonucleotide PCR-based assays (ASO) followed by reamplification and sequencing on ABI377 automated sequencer.

Results: We found five different disease predisposing mutations in BRCA1 gene (185delAG, 300T/G, 4174delA, 5382insC, 5528del1+IV22-6), four mutation in BRCA2 gene (6174del, 6886del5, 9599A/T, 9631delC) and one mutation in TP53 (1095del8) which is the largest mutation ever detected in this gene. Five mutations detected in our study (9599A/T, 9631delC, 6886del5 in BRCA2; 5528del1+IV22-6 in BRCA1; 1095del8 in TP53) were not reported previously and may be specific to the southern Polish population while the others were recurrent. Mutation prevalence was substantially higher in cases with strong family history. Of the 90 women with family history 33 carried a germ-line mutation in BRCA1 (26 of these cases were 5382insC mutation caries) and 6 carried mutations in BRCA2. We have also identified a family with mutations in both genes BRCA1 and BRCA2 (185delAG BRCA1 and 6174delT BRCA2). Our results are in agreement with the idea that mutations in BRCA1 and BRCA2 significantly contribute to familial breast cancer risk and their contribution to sporadic breast cancer and bilateral breast cancer without family history of breast and ovarian cancer is much lower.

Conclusion: Further studies will be necessary to estimate more accurately the risk of the BRCA1, BRCA2 and TP53 mutations among breast and ovarian cancer patients in Polish population.

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Gene expression profiling of estrogen-responsive genes in human breast cancer: development of a DNA microarray system for monitoring hormone therapy

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Purpose: Estrogen plays an important role in carcinogenesis and development of human breast cancer. However, the essential downstream molecular targets of estrogen-signals in cancer have not been clarified hitherto. Moreover, along with the recent improvement of hormone therapy, accurate therapeutic prediction of breast cancer is desired earnestly.

Methods & Results: We first comprehensively analyzed the gene expression profiles for estrogen responsiveness among four estrogen receptor (ER)-positive cancer cell lines, MCF-7, MCF-7c9, T47-D and Ishikawa cells, using large-scale cDNA microarray technique. Approximately 4% of 9000 genes showed significant estrogen responsiveness, classified as the induction or repression types, among these cells. Many of the genes in the induction type were found to be growth-associated or tumor-associated genes, while tumor suppressor-related genes were found in the repression type. Based on the obtained information, a total of 138 genes, which showed high induction or repression in expression by estrogen stimulation, were selected and provided for custom microarrays. The results obtained from the custom microarray analysis of the cell lines were consistent with those from large-scale microarray analysis. The time course study of these 138 genes using the custom microarrays revealed that they were clearly categorized into the early- or late-response types. Furthermore, the custom microarray analysis of ER positive breast cancer tissues also showed similar but not identical profiles to those obtained with the cell lines.

Conclusion: Further analysis of these genes categorized into subgroup by custom microarray may provide new clues for elucidation of estrogen-dependent growth mechanisms of cancer. These observations indicate the usefulness of the custom microarrays for assessment of response by estrogen-agonists and antagonists in the human breast cancer. This custom microarray may be applicable for prediction of effectiveness of anti-hormone therapy for breast cancer.

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Shifts in phenotype of dendritic cells (DC) in healthy BRCA1 mutation carriers and in patients with early breast cancer

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Rationale: Investigations originating from our laboratory have reported deficiencies in antigen presentation and functionality of monocytes derived from patients with early breast cancer (EBC), but not from healthy women with germline mutations of BRCA1.We have now expanded our investigations to dendritic cells (DC), only insufficiently studied in patients with EBC in general and in BRCA1 mutant carriers in particular.

Objective: DC derived from patients with EBC, healthy women with BRCA1 germline mutations as well as EBC patients with BRCA1 mutations were analysed for the expression of CD1a, CD11c, CD83, CD80, CD86, CD54 and CD14. Antigen presentation by DC was evaluated by a T-cell proliferation assay.

Methods: Peripheral blood was obtained from 36 patients with EBC, 7 healthy women with germline mutations of BRCA1, 4 patients with EBC with BRCA1 mutations and 26 healthy age-matched control persons. PBMC were prepared for ex vivo DC generation using GM-CSF, IL4 and TNF-alpha according to standard procedures. DC phenotype was examined by FACS.T cell proliferation in response to TTX-pulsed DC was measured by (3H)thymidine assay.

Results: Phenotypically, DC derived from patients with EBC presented with a significantly reduced expression of CD1a, CD83, CD80, CD86, CD54, compared to DC from healthy control females. Moreover, DC derived from healthy women with BRCA1 germline mutations showed a significant increase in CD54 and CD80 antigens, as compared to DC derived from healthy control persons. Finally, DC derived from EBC patients with germline mutations of BRCA1 showed not only a higher expression of CD1a and CD83, but also of CD86 and CD54,compared to DC from patients with EBC without BRCA1 mutations.

Functionally, T cell-proliferation in response to TTX-pulsed autologous DC was significantly decreased in patients with EBC.

Conclusions: DC derived from patients with EBC had not only an immature phenotype, but also a functional impairment in antigen presentation. Our results showed, furthermore, that differences in DC phenotype preceded the clinical manifestation of breast cancer and shifts in antigen expression were also present in BRCA1 germline mutation carriers persisting throughout the development of EBC.These results suggested the presence of a characteristic phenotype in individuals with BRCA1 germline mutations.

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Comparative genomic hybridization in phyllodes tumors of the breast

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Purpose: Phyllodes tumors are a rare neoplasm of the breast and it is not easy to predict their clinical behavior, for instance, recurrence and metastasis. The aim of this study was to search for specific alterations of genes associated with various phyllodes tumor grades.

Methods: To analyse genetic alterations, we used the comparative genomic hybridization (CGH) method in 25 cases of archival paraffin embedded materials which were classified into 3 histological categories as benign, borderline and malignant.

Results: Chromosomal aberration was identified in 11 cases (44%) but it was difficult to identify a sequential DNA copy number change associated with histological type. However, the most interesting finding was the gain of the long arm in chromosome 1 that was shown in 9 cases. Gain of 1q was significantly associated with recurrences. Other changes were the loss of 4q, 6q, 9p and 13q and the gain of chromosomes 17,19, and 20.