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DNA methylation profiles in breast cancer – relation to hormone receptors and role in response to adjuvant endocrine treatment

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Recent advances suggest that it may be possible to more accurately predict clinical response from the molecular characteristics of a patient s tumor. Molecular profiling in breast cancer has thus far focused primarily on the use of gene expression (cDNA) microarrays. However, this technique is limited by the innate instability of RNA and is poorly compatible with procedures used in routine histopathology. Therefore, we have explored the use of DNA methylation markers as an alternative approach to molecular profiling. Hypermethylation of promoter CpG islands, frequently observed in breast cancer, is often associated with transcriptional silencing of the associated gene, thus providing a DNA-based surrogate marker for expression status. We have used a moderate-throughput, fluorescence-based, semi-automated quantitative technique called MethyLight, to screen a panel of 35 methylation markers in 148 cases of breast cancer. Interestingly, we found that among these 35 markers, the best predictor of estrogen receptor (ER) status, was methylation of the progesterone receptor gene (PGR). Conversely, the best predictor of progesterone receptor (PR) status was methylation of the estrogen receptor gene (ESR1). Interestingly, we found that ESR1 expression from Exon 1A was resistant to Exon 1A DNA methylation in PR positive tumors, but not in PR negative tumors, suggesting an interaction between PR and the ESR1 gene. We further showed that expression levels of the de novo DNA methyltransferase DNMT3B were statistically significantly lower in ER positive tumors. Selective estrogen-receptor modulators like tamoxifen, have been shown to dramatically reduce the risk of breast cancer and of breast cancer recurrence. Here we show that ESR1 methylation predicts survival only in tamoxifen treated patients, while ARH1 methylation predicts survival only in non-tamoxifen treated patients, while CYP1B1 methylation predicts survival differentially in tamoxifen-treated and non-treated patients. We propose that these differences in DNA methylation profiles reflect alternative pathways of tumorigenesis, possibly involving differential expression of DNA methyltransferases. It has to be determined whether DNA methylation based changes in breast tissue, serum or Nipple Aspirate Fluid - may also be used to predict responsiveness to chemo-preventive strategies. (Supported by grants from "Fonds zur F rderung der wissenschaftlichen Forschung", P15995-B05 and P16159-B05 to W.M.)

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Imunological phenotype of the blood and degree of paracortical hyperplasia in regional lymph nodes of young patients with breast cancer

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Introduction: Prognosis of young women with breast cancer is poor. The character of interrelationship between cellular immunity and local reactions in the regional lymph nodes (as one of the aspect influenced on survival of patients) is not reviled yet. The purpose of our study was to analyze the indices of cellular immunity dependent on degree of pathological reactions in regional lymph nodes in patients of different age with breast cancer.

Methods: The comparative analysis of the immunological phenotype of the mononuclear cells of peripheral blood was established in 60 young (20-35 years old) and 60 middle-aged (36-45 years old) breast cancer patients dependent on degree of hyperplasia of the paracortical zone in the regional lymph nodes.

Results: The maximal level of expression of CD3, CD4, HLA-DR, CD30, CD38 and RFB-1 antigens in population of peripheral blood cells of young patients proved to correlate with low degree of paracortical hyperplasia in the regional lymph nodes. The high degree of hyperplasia was not reveled in patients of young age. The maximal level of expression of a set of T-, B- cells markers, as well as activation's markers was observed in middle-age patients in the cases of absence of paracortical reactions in regional lymph nodes. Comparative analysis of two age groups revealed significant increase level of T-suppressors/killers, as well as CD3+, RFB-1 cells in young patients with low level of paracortical hyperplasia in the regional lymph nodes.

Conclusion: The different type of relationship between degree of reactions in regional lymph nodes and cellular immunity indices, especially activated lymphocytes, is discovered in young, compared with middle -aged patients, that may reflect an existence of basic differences in endocrine regulation of the immune system in breast cancer patients of different age groups.

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Determination of malignant or non-malignant phenotype of tumour cells

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In our opinion, tumour arises when transformed cells do not receive growth factors (GFs) required for their subsequent differentiation. As a result, the process of differentiation of mentioned cells is suspended at certain level. But these cells proliferate uninterruptedly and avoid apoptosis menace, because the oncoproteins of changed, permanently active oncogenes, present in these cells, generate unsanctioned signal which imitates GFs influence and thus activates the components of intracellular proliferative signal transduction cascade as well