P31

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.)

P32

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.

P33

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

as prevents from activity of proapoptotic cell components (Bax, Bad, AIF, cytochrome C, caspase 9 et al). The ability of tumour cells for certain motogens perception (as a result of this, cells begin to migrate and therefore form metastases) is absolutely determined by expression of genes of specific cellular receptors. Obviously, cells endeavour to migrate towards motogen source, i.e. to the side of motogen concentration increase. It follows from this that if motogen is produced by microenvironment cells and diffuses into tumour through intercellular space, the tumour is disposed mainly to infiltrating growth, but not to metastatic dissemination, because the latter has no sense in this situation. But, if the motogen is released by distant cells and reaches tumour from blood, the tumour cells will penetrate firstly into vascular system and subsequently into tissue which is motogen source; in these conditions mainly metastases must arise. According to the genes cluster conception, proposed by us, each discrete stage of every direction of cell differentiation is executed by separate specific gene group - genes cluster; activity of individual genes, determining certain features of cell phenotype, must depend completely on their presence in genes cluster that is active in this cell. Thus, as malignancy (and its grade) or non-malignancy of a tumour is determined a priori by expression or non-expression of motogens genes (and which exactly) in tumour cells, tumour phenotype features must be dependent on direction of differentiation of transformed cells and on stage, at which their specialization was suspended because of lack of necessary GFs. In our opinion, if the direction and grade of tumour cells differentiation are ascertained precisely, their characteristics can be foreseen.

P34

Melatonin with fraxiparine and hydrocortison as a new combination in prevention of the vascularization and metastasis in mice with Lewis lung carcinoma

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The blocking of angiogenesis is the strategy of a new preventive therapy. The evidence of abnormally high blood levels of vascular endothelial growth factor (VEGF) has been proven associated with poor prognosis in cancer patients. Melatonin-induced control of the neoplastic growth is associated with a decline in VEGF secretion. The combination of fraxiparine and hydrocortison influenced on the development of a new tumor blood vessels. The aim of the study was to investigate effect of melatonin in combination with fraxiparine and hydrocortison on the levels of the VEGF and on metastatic injury in C57Bl/6 mice bearing Lewis lung carcinoma (LLC). LLC transplantation was performed by injection i.m. of 0.02 ml of the tumor cell suspension consisting 4x105 cells. The combination of drugs were introduced in 4 groups of animals on day 14th after LLC transplantation: 1) intact animals - control, 0,2 ml of physiologic solution, b.w., s.c., ev.d. throughout the experiment; 2) melatonin (M), (Sigma), 5 mg/kg b.w., s.c., 6 p.m., ev.d. throughout the experiment; 3) fraxiparine (Fr.)+hydrocortison (Hk.) (Fraxiparine 2850 UI AXa/0,3 ml, (Sanofi, France), 1UI b.w in 0,1ml of physiologic solution, 9 in., s.c., ev.d.; Hydrocortison (Richter), 50 mk/kg b.w. in 0,2 ml of physiologic solution, 9 in., i.m.); 4) combination of Fr.+Hk.+M in the same doses. For monitoring of the primary tumor, the levels of tumor dissemination, the tumor volumes (VT, mm3), the number and volumes of the lung metastasis (VLM, mm3), and VEGF levels in serum were estimated. On days 14th, 24th, 34th, and 42 after primary tumor transplantation the levels of metastasis injury were evaluated and venous blood samples were collected. Blood serum levels of VEGF (ng/ml) were measured by an enzyme immunoassay. The influence of drugs on intensity of vascularization of the lung metastasis were evaluated by the calculation of the VLM: metastases in diameter up to 0.5 mm were estimated as metastases in avascular phase and metastases with size more than 0.5 mm - as vascular phase. All combination of drugs in nontoxic doses decreased serum levels of VEGF and VLM, especially in the group of mice with Fr.+Hk.+M treatment. This data was revealed that Fr.+Hk. increased the ability of melatonin to suppress neovascularization of the Lewis lung metastasis. We suggest that combination of drugs Fr.+Hk+M and Fr.+Hk. may be perspective as a new innovative antiangiogenic agents in prevention of tumor growth and metastasis.

P35

Lung tuberculosis and cancer

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Aims: The purpose of our research was studying frequency of erroneous statement of the diagnosis of tuberculoma and fibrocavernous tuberculosis in patients with a cancer of lungs.

Materials and methods: There were made by us the analysis of 137 case reports of patients of surgical department of a regional tubercular clinic of Donetsk for 2000-2002,which had operated concerning tuberculoma and fibrocavernous tuberculosis of lungs. Among them men were 96(70%), women 41 (30%). The greatest number of operated patients was at young able-bodied age: from 18 to 30 years-72(52.6%),from 31 to 40-38(27.7%),from 41 to 50-27(19.7%). All patients repeatedly were treated in ambulatory clinic and permanently and have admitted in connection with deterioration of a condition. Tuberculomas of lungs were in 26(19%) patients, fibrocavernous tuberculosis-11(81%).

Results: In the preoperative period in anybody from them cancer of lungs were not diagnosed, precise nodes it is not revealed and at macroscopic research. Only at histological research in 34(4.8%) patients it is found out squamous cancer, and in 11 (32.3) from them-high –differentiated, in 23 (67.7%)-low-differentiated. To look after any law of distribution of parts of malignancy in tissues of lungs at histological research it was not possible. Cancer of lungs among men was observed in 2 times more often 23 (16.8%),in women 11 (8%),and the majority of them 25 (73.5%) suffered fibrocavernous tuberculosis - 19 (55.9%) and 6 (17.6%) women, only in 9 (26.5%) were observed tuberculomas of lungs. The age of patients, basically, was till 30 years-29 (85.3%) and only 6 (14.7%) patients were more advanced age. All men and 8 women were chain smokers,