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Circulating Microparticles (MP) in breast cancer patients – comparison with established biomarkers

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Background: At present there is a growing interest to evaluate new markers for endothelial cell activation, coagulation and fibrinolysis, tumor angiogenesis and drug therapy monitoring in cancer patients. Recent investigations suggested a possible influence of circulating microparticles (MP) in various diseases.

The aim of the present prospective case-control study was to evaluate the putative relevance of circulating MP as a biomarker in breast cancer patients.

Materials and Methods: Endothelial cell- (EMP) and leukocyte-derived MP (LMP) were determined by flow cytometry in breast cancer patients (n = 41) and healthy controls (n = 25) and compared to carcinoembryonic antigen (CEA), cancer antigen (CA) 15-3 and von Willebrand factor antigen (vWF; marker of endothelial cell activation) levels by specificity-sensitivity profiles. Women with histologically proven breast cancer (n = 41) were included and classified according to tumor size, lymph nodes and metastatic disease: tumor size <2 cm (T1; n = 22) and tumor size 2−5 cm (T2; n = 13). Most patients had negative axillary lymph nodes (n = 26). Six patients had a metastatic disease. The control group was composed of 25 women without pathological findings in the mammogram. MP were examined by electron microscopy as well as flow cytometry using labels for annexin V, CD (EMP), CD62E (activated EMP) and CD45 (LMP).

Results: LMP, CEA and CA15-3 levels differed significantly between breast cancer patients and controls, whereas EMP and vWF did not. These specificity-sensitivity profiles of LMP and CA15-3 were similar. Increasing levels of circulating LMP (CD45+), CEA and CA15-3 correlated with increasing tumor size, thus reflecting disease stage.

Conclusion: LMP showed an equal specificity–sensitivity profile to the established marker CA15−3 and therefore might have the potential to become a new biomarker in breast cancer patients.

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Expression of epithelial-mesenchymal transition markers in metaplastic carcinoma of the breast – Immunohistochemical and immunoblotting study of E-cadherin and Snail

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Background: Metaplastic carcinoma (MC) is a rare breast neoplasm with poor outcome, which presents epithelial or mesenchymal components. Snail and E-cadherin are expressed in epithelial-mesenchymal transition (EMT), and are involved in epithelial tumor progression, invasion, and node-positive tumors. The aim of this study was to know the expression of these two markers to determine the EMT in both epithelial and mixed tumors of MC of the breast.

Material and Methods: Twenty-two cases with diagnosis of MC were selected in the period from 1995 to 2006. The cases were re-evaluated and reclassified by WHO classification. Immunohistochemical studies were performed for ER, PgR, Her 2/neu, EGFR, CK 5/6, E-cadherin, and Snail by avidin-biotin method as previously described. In a group of 5 mixed tumors, protein extraction was performed in different areas of normal, mesenchymal, and epithelial components. Purified proteins from each area were spotted; dot blot arrays were used to profile the expression of vimentin, EGFR, CK18, E-cadherin, and Snail by Western blotting protocols as previously described. The relation of intensity of antibody/sypro was used as standard of protein microarray.

Results: Ten of 22 cases (45.5%) were purely epithelial neoplasms, divided into 2 adenosquamous carcinomas, 7 high-grade squamous carcinomas and one spindle cell carcinoma. Twelve cases (54.5%) were mixed cases, divided into 10 chondroid matrix, one matrix-producing bone and one carcinosarcoma.

All cases were negative for hormonal receptors; Her 2/neu was negative in all cases but 2, which showed positive2 expression only in the intraductal component. EGFR was positive in all cases. E-cadherin was positive in 20/22 cases (90.1%); in 10/10 epithelial tumors (100%), and in 10/12 of mixed tumors (83.3%). Snail was positive in 16/22 cases (72.7%), 6/10 epithelial tumors (60%), and 10/12 mixed tumors (83.3%).

The relation of intensity of antibody/sypro was among 0−1.9 by protein microarray. In the epithelial areas, 3/5cases (60%) showed higher intensity of E-cadherin; but in the mesenchymal areas, the five cases (100%) showed higher intensity of Snail protein. We observed the same results in the pool of tissues.

Conclusions: The expression of EMT markers is frequent in MC. The down-regulation of E-cadherin, and the increasing of Snail in mesenchymal components, support the EMT in MC, and may explain the particular histology and poor outcome of this tumor.

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Influence of breast cancer cells on maturation and function of dendritic cells in lymph nodes in breast cancer patients

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Background: The interactions of tumor tissue, dendritic cells (DC) and T cells play a crucial role in human tumor immunopathogenesis. These have been previously studied mostly by using in vitro-generated DC which may not reflect the natural status of DC in vivo. Therefore we set out to investigate the effect of tumor presence on phenotype, maturation, and T cells activation ability of naturally occurring DC deriving from lymph nodes of breast cancer patients.

Material and Methods: To test whether the presence of a tumor affects maturation and the ability of DC to activate T cells, we established the isolation of genuine DC from the tumor-affected and tumor-unaffected lymph nodes (LN-DC) using a magnetic bead separation procedure. We assessed LN-DC phenotype by FACS and their activation capacity by mixed lymphocyte reaction before and after maturation by a cytokine cocktail.

Results: Freshly isolated LN-DC derived from tumor unaffected as well as tumor affected lymph nodes showed rather weak activation of T cells. However, stimulation of those LN-DC with a cytokine cocktail resulted in increased expression of CD83, CD80, and CD86 molecules as well as in increased T-cell stimulatory capacity. We furthermore observed in 2 patients that T-cell stimulatory capacity of activated DC, derived from unaffected LN, was stronger as compared to tumor affected LN-DC from the same patient. This was in spite of the fact that the increase in the expression of co-stimulatory molecules did not differ after maturation. In one case of totally unaffected lymph nodes, LN-DC derived from a Level 1 lymph node showed increased stimulation capacity after maturation as compared with Level 2 LN-DC.

Conclusions: Thus this data indicates that genuine DC are rather immature and additional maturation of the DC from affected as well as non affected lymph nodes is still required to render DCs capable of activating T cells. However, maturated DC from unaffected lymph node may have a better activation ability as compared to DC isolated from tumor affected lymph node from the same patient. This could indicate that the presence of tumor cells within lymph nodes may decrease immune responses in stage II and stage III breast cancer patients. Additional assays are necessary to identify the molecules responsible for this difference in activation capacity of LN-DC.

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The novel familial association between breast and gastric cancer in a Korean family

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Background: BRCA mutations cancer spectrum includes breast, ovary and other cancers including stomach and prostate cancers. In addition, despite the identification of a large number of sequence variants in BRCA1/2 mutation analyses, many genetic alterations have still not been characterized and it has been suggested that there are ethnic variations in BRCA mutations. Little has been reported on germline mutations in Asian population. We report a novel BRCA2 mutation in a Korean patient with bilateral breast cancers and extensive family history of cancers.

Materials: The proband was a 39 year old Korean female who was diagnosed to have bilateral breast cancer with bilateral mastectomy performed. She had a strong family history of cancer in the maternal line, affecting the grandmother (ovarian cancer); mother, maternal aunt and sister (signet-ring gastric cancer) and 2 materanl uncles (squamous cell carcinomas of tongue and larynx). Bilateral mastectomy was performed and endoscopy were undertaken to exclude an occult primary gastric cancer

due to the strong family history. Peripheral blood samples were collected from the breast cancer proband and was processed for DNA and RNA extraction. 16-exon CDH1 gene sequencing was performed to exclude the exon 13 mutation reported in a Korean kindred with coexisting ductal breast and gastric cancer. In addition, the entire coding regions and flanking introns of BRCA1/2 were screened for germline mutations using full gene sequencing and Multiplex Ligation-dependent Probe Amplification (MLPA).

Results: Sequencing of the 16-exon CDH1 gene was negative. An Exon 18 duplication causing a frameshift mutation and downstream premature termination codon (8047_8054dup GCAAAAAC, Leu2686GluX10) was revealed, this being a novel BRCA2 mutation not previously reported.

Conclusion: We identified a novel BRCA2 germline mutation in a Korean breast cancer patient with extensive history of non breast cancer. There is limited knowledge of the prevalence, spectrum of germline mutations and also the phenotypic presentations in Asian population. Research on the spectrum of mutations in such diversed ethnic groups has important implications on clinical management.

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Expression and functional analysis of PGRMC1 in breast cancer

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Background: In a proteomic screening approach we identified progesterone receptor membrane component 1 (PGRMC1) as a protein upregulated in frozen ER-negative breast cancer tissue samples making PGRMC1 a potential therapeutic target. PGRMC1 is a 28 kDa protein consisting of 194 amino acids and belongs to the "membrane-associated progesterone receptor" (MAPR) protein family.

Aim: The aim of this project is to characterize the potential biological function of PGRMC1 by structure analysis and generation of different mutant forms.

Material and Methods: Structure analysis was performed (http://scansite.mit.edu/motifscan_id.phtml) under high and medium stringency to identify protein motifs predicted for PGRMC1. Based on these predicted motifs PGRMC1-variants were generated by site-directed mutagenesis and MCF-7 breast cancer cells were stably transfected with the corresponding expression plasmids. Several PGRMC1-specific hybridoma were generated and tested by western blot analysis. Multiple immune fluorescence for PGRMC1, estrogen receptor alpha and the hypoxia marker glucose transporter 1 (Glut1) was applied to label tissue microarrays containing different breast cancer specimen. Additionally, posttranslational modification of PGRMC1 was investigated by phosphatase treatment of tissue lysates. Functional analysis was performed by stimulating transfected MCF-7 cells with a membrane-impermeable progesterone:BSA:fluoresceinisothiocyanate conjugate followed by analysis of cell proliferation by measuring the cellular ATP content.

Results: PGRMC1 contains a cytochrome b5 domain and several protein interaction domains making it a possible signalling adaptor protein. Further, PGRMC1 is posttranslationally modified. Stimulation of PGRMC1 expressing MCF-7 cells resulted in an increased proliferation compared to control cells grown in growth factor and hormone reduced medium. Western blot analysis of hybridoma produced a PGRMC1-specific signal at the predicted molecular weight of 28 kDa which is not detected after preincubation of the hybridoma with recombinant PGRMC1 protein. In immune fluorescence analysis of breast cancer tissue sections myoepithelial cells were highly positive for PGRMC1. Besides that PGRMC1 expression was upregulated in Glut1 positive, hypoxic areas in ductal carcinoma in situ of comedo type.

Conclusion: These data indicate that PGRMC1 is expressed in breast cancer and might functionally play a role in cell proliferation. Further determination of the so far poorly defined role of PGRMC1 in cancer biology could prove to be of great relevance to clinical cancer therapists.

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Peroxisome proliferator-activated receptor-gamma agonist, troglitazone has anticancer effect on breast cancer cells

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Background: It is reported that peroxisome proliferator-activated receptor-gamma (PPAR-γ) has become a potential target for the prevention and treatment of breast cancer. And also, recent studies suggest that PPAR-γ agonist could serve as negative regulators of breast cancer development and progression, but their mechanism is still unknown. The purpose of this study was to evaluate the mechanism that PPAR-γ agonist, troglitazone would induce antiproliferative effect on MCF-7 (ER-positive) and MDA-MB-231 (ER-negative) breast cancer cells.

Methods: Cytostatic/cytotoxic effects of troglitazone were measured with mitochondrial tetrazolium (MTT) assay. The cell cycle distribution and apoptosis induction were evaluated by using the flow cytometry. The expression of apoptosis-related proteins were measured with Western blotting. Detection of apoptosis was carried out using a DNA fragmentation assay based on TUNEL staining. For morphological examination of apoptotic changes, cells were stained with Hoechst 33342.

Results: Troglitazone showed antiproliferative effect on MCF-7 breast cancer cells with tamoxifen, respectively and synergically. Troglitazone and tamoxifen could induce cell cycle G1 arrest and apoptosis of MCF-7 cells, through upregulating or downregulating the expression of apoptosis-related genes. MDA-MB-231 cells exposed to troglitazone showed G1 arrest as well as induction of characteristic morphological changes of apoptosis. Accumulation of cells in G1 was accompanied by an attenuation of retinoblastoma (Rb) protein phosphorylation associated with decreased cyclin dependent kinase (CDK) 2 activity. Troglitazone increased the expression of CDK inhibitor, p21 and p27.

Conclusion: PPAR-γ agonist, troglitazone increase the sensitivity of anti-hormonal therapy in MCF-7 breast cancer cells and inhibits the proliferation of MDA-MB-231 cells. These results suggest that troglitazone has anticancer effect on both ER-positive and negative breast cancer cells.

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Tamoxifen as inhibitor of multidrug resistance mechanism in lung and breast cancer tumours

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Background: Tamoxifen (Tam) is an effective drug in standard therapy of breast cancer as an antiestrogen. At the same time there a lot of other important activities of Tam, one of them – increase chemotherapy efficacy by high doses of Tam. Analyzing the results we have taken notice that in all cases Tam was used in chemotherapy included anticancer drugs associated with multidrug resistance mechanism (MDR) that is determined by energy-dependent ABC-transporters, extruding MDR-drugs out of the cells. Besides it was shown that Tam increases cytotoxicity of MDR-drugs in cell cultures with expression of Pgp. That is why thinking over the reasons of increase chemotherapy efficacy under Tam action we supposed the one of the reasons for that may be Tam inhibition of ABC-transporter(s)' activity.

Material and Methods: Tam influence on intracellular accumulation of MDR-drug doxorubicin (Dox) in tumor cells of surgical biopsy specimens (breast cancer and non-small-cell lung cancer, totally – 22 specimens) was studied by a flowcytometry. MDR-phenotype (Pgp+ and/or MRP+) of the investigated tumors was determined as the change in Dox intracellular accumulation after preincubation of cell suspension with ABC-transporter(s)' inhibitors: verapamil (Ver) and genistein (Gen) – specific inhibitors of Pgp and MRP respectively.

Results:

1. Totally, stimulation of Dox intracellular accumulation after Ver and/or Gen action (Pgp+ and/or MRP+ phenotype) was shown in 70% of investigated tumor specimens.
2. Tam stimulated Dox intracellular accumulation in all tumor specimens with Pgp+ and/or MRP+ phenotype.
3. Significant increase in nuclear fraction and Dox binding to DNA was also revealed in stimulation of Dox intracellular accumulation under Tam action.

Conclusions:

1. Tam is an effective inhibitor of ABC-transporter(s)' functional activity namely Pgp and MRP.
2. Tam inhibition of ABC-transporter(s)' function resulting in increase in Dox intracellular accumulation and nuclear fraction of Dox could at least partly explain efficacy of MDR-drugs in combinations with high doses of Tam.

It means that Tam could be considered not only an effective antiestrogen drug but also an inhibitor of multidrug resistance mechanism increasing efficacy of MDR-drugs in tumors exhibited MDR-phenotype Pgp+ and/or MRP+. According to presented results it could be true at least for lung and breast cancer.

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