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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