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P13

Features of proteasome system functioning in the tumor and microenvironment cells of breast cancer

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Background: The tumor microenvironment plays an important role in the progression of cancer and may be regulated by proteolysis, including the proteasomes. The proteasomes modify the biologically important molecules involved in the pathogenesis and progression of a variety of malignancies, including breast cancer (BC). The aim of this study was to investigate the features of the subunit composition of proteasomes in the tumor and its microenvironment of breast cancer.

Material and Methods: The material for investigation was samples of tumor tissue of invasive ductal breast cancer. The study was conducted to estimate the distribution of the total pool of proteasome, proteasomes activator PA700, immune proteasome forms containing LMP7 and/or LMP2 subunit in tumor cells and stromal component using immunofluorescence. Furthermore, there was evaluated the distribution of the proteasome in the tumor. The expression of immune proteasomes and proteasomes activators in the cells was studied by immunofluorescence labeling of the cells by antibodies to immune proteasome subunits and cell markers. Fluorescence was analyzed by using a fluorescence microscope DM RXA2 ("Leica", Germany) and confocal microscope TCS SP ("Leica", Germany). The specificity of the primary antibodies was confirmed by check samples, at which the reaction was carried out only with the second antibody. No cross reaction between the first and second antibodies was tested by incubation of each primary antibodies with the opposing second antibodies. In addition, there was carried out labeling the cell nuclei by reagent Hoechst 33,342.

Results: It was found that the tumor cells comprise immune proteasomes, also PA700 and PA28αb activators. Activator PA28αb and immune proteasomes are localized in the cytoplasm of tumor cells, whereas α1, 2, 3, 5, 6, 7 subunits and PA700 activator detected in the cytoplasm and in the nuclei of tumor cells. Availability of α1, 2, 3, 5, 6, 7 subunits in the nuclei of tumor cells shows the expression of constitutive proteasome subunits. Stromal cells are characterized by a high ratio of the α1, 2, 3, 5, 6, 7 subunits to the immune LMP2 subunit as compared to cells of invasive ductal carcinoma. This means that the pool of proteasome in tumor cells of invasive ductal cancer is enriched by the immune proteasomes as compared to stromal cells. Thus, samples of invasive ductal breast cancer contain predominantly tumor cells enriched by immune proteasomes, activators PA700 and PA28αb. The presence of proteasomes in stromal component indicates that the tumor microenvironment also has active processes of proteolysis, with involving proteasome system. Probably the processes occurring in the stromal component contribute to the output of the proteasome into the extracellular space, which is confirmed by other researchers about the existence of circulating proteasome pools and their further participation in dissemination of cancer.

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T9

Metformin in breast cancer therapy

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Background: Metformin is a antidiabetic drug with anticancer properties. However, the mechanism action by which metformin affects various cancer cells still unknown. It is known that tumor growth is accompanied by changes in the metabolic cascade that includes overproduction of lactate and adenosine. The adenosine is released into the extracellular environment and regulates differentiation, proliferation, and angiogenesis of tumor mass. We found that lactate is activator of key enzyme of adenosine metabolism – adenosine deaminase (ADA).

Aim: The aim of our study was to investigate the catabolism of adenosine in the tumor while taking metformin.

Materials and methods: In this study we investigated the level of adenosine, inosine, hypoxanthine and ADA activity in 15 women aged 46–76 years, with breast cancer (BC) T2-4N1M0 (cancer tissues) during treatment with metformin, 1000 mg per day for 3 months. Control group – 15 women aged 46–76 years, with stage T2-4N1M0 breast cancer (cancer tissues) without metformin therapy.

Statistical analysis was performed using the license package StatSoft. Statistica 12.0.

Results: ADA activity during treatment with metformin was 2-fold increased: 12.1 ± 2.49 nmol/min*mg in comparison with 4.77 ± 0.943 nmol/min*mg. Concentration of catabolic products of adenosine degradation was increased before metformin therapy. Inosine level was 0.121 ± 0.041 micro mol/g tissue (BC tissues from women without metformin 0.042 ± 0.015 micro mol/g tissue). Hypoxanthine 2.45 ± 0.428 micro mol/g tissue (in comparison with 0.711 ± 0.269 micro mol/g tissue). Whereas, adenosine level in BC after metformin therapy was 0.226 ± 0.148 micro mol/g tissue (in comparison with 0.186 ± 0.056 micro mol/g tissue), that were not significantly different.

Conclusion: Thus, we have found that metformin significantly increases the rate of catabolism of adenosine, and this in turn reduces the inhibitory effect on the tumor microenvironment cytotoxic cells. Therefore, our data for the first time provide novel evidence for a mechanism that the anticancer activities of metformin are due to adenosine metabolism regulation.

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A73

Plant-produced substance antibodies against HER2/neu oncoprotein

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Background: Human epidermal growth factor receptor-2 (HER2/nue) is overexpressed in breast cancer. It has been shown that HER2-targeted therapies have radically changed the outcome of HER2-positive breast cancer patients. The aim of this study was to develop the technique for preparation of recombinant anti-HER2/neu antibody from plants and to provide with the basic characteristics of the HER2/neu oncoprotein.
Materials and Methods: We have designed and synthesized viral vectors to transform Agrobacterium tumifaciens affinity, ion exchange and gel filtration chromatography were used to purify the antibody. The quality of the substance was confirmed by SDS–PAGE and ELISA. The biological activity was tested by immunofluorescent analysis.
Results: Antibodies were purified by affinity and ion exchange chromatography from the Agrobacterium tumifaciens leaves extracts. Gel filtration chromatography was used for final purification of the protein. Immunocytochemical staining was performed to test the functional activity of the plant-made antibodies. Here we also show that plant-made antibodies bind to HER2/neu receptors on the surface of human SK-BR-3 breast cancer cells as effectively as the diagnostic antibody A0485 (DAKO, Denmark). Flow cytometry analysis was used for quantitative estimation of recombinant anti-HER2/neu antibodies: from 75.7% to 98.3% cells bound the plant-made antibodies. The same data were obtained with trastuzumab.
Conclusion: Based on the data obtained, we conclude that plant-made antibodies inhibit HER2/neu+ breast cancer cell proliferation. Additional experiments are required to prove that trastuzumab and plant-made antibodies share full identity in their biological activity.

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P82

Integrin receptors and their ligands as potential biomarkers in preoperative diagnosis of papillary thyroid carcinoma

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Papillary thyroid cancer (PTC) is the most common malignancy of the endocrine system. The most frequent genetic alteration in PTC is the BRAF V600E mutation, which affects the activation of several intracellular signaling pathways. As a result, changes in the expression levels of cell membrane integrin receptors and their ligands – extracellular matrix proteins – osteopontin (OPN) and thrombospondin -1 (TSP1) are observed. This process increases the metastatic potential of tumor cells. Thus, integrin receptors and their ligands are potential biomarkers of an aggressive PTC phenotype.

The aim of our study was to compare the gene expression profile of integrins ITGA2, ITGA3, ITGAV, ITGA6, ITGA9, ITGB1, ITGB3 and their ligands OPNa, OPNb, and TSP1 in PTC with different BRAF V600E mutation status.

Intraoperative thyroid tissue samples from 41 patients diagnosed with PTC ($n = 26$), diffuse nodular nontoxic goiter ($n = 10$) and follicular adenoma ($n = 5$) were analyzed to evaluate the expression levels of the investigated genes by real time RT-PCR. Fluorescent immunohistochemistry (IHC) was used to confirm the PCR results and to estimate the amount of protein products. For IHC, frozen and paraffin sections were used. The BRAF V600E mutation was determined using allele-specific amplification. Nonparametric criteria (Kruskal Wallis, Wilcoxon and Mann–Whitney tests) were used to evaluate group differences. P values of less than 0.05 were considered statistically significant.

The BRAF V600E mutation was observed in 12 PTC samples, which corresponds to 46% of PTC cases. An increase of gene expression level of ITGA3 (2.9-fold, $p = 0.014$), ITGAV (1.9-fold, $p = 0.038$), ITGB1 (1.7-fold, $p = 0.026$), OPNb (2.5-fold, $p = 0.0001$) and TSP1 (3.2-fold, $p = 0.017$) was identified in the PTC tissues, and a high gene expression level of OPNb (5.9-fold, $p = 0.003$) and TSP1 (12.1-fold, $p = 0.005$) was identified in the tissue samples of lymph node metastases compared to the conventionally normal tissue.

In the samples with advanced cancer (T3, T4, TNM) the expression levels of ITGA3, ITGA6 and ITGA9 were higher compared to the T1 samples. MRNA levels of ITGA3 and ITGAV were significantly higher in the PTC BRAF V600E positive samples than in the BRAF V600E negative samples.

Elevated levels of OPNa (11.4-fold, $p = 0.0112$), OPNb (10.2-fold, $p = 0.0216$) and TSP1 (33.5-fold, $p = 0.0005$) genes were observed in the follicular adenoma samples compared to the PTC tissues. For ITGA2 and ITGB3 there was a significant increase of expression in the PTC tissues compared to the benign thyroid tumors (8.9-fold, $p = 0.019$ and 38.4-fold, $p = 0.014$, respectively).

We also studied the distribution and localization of integrins ITGA2 and ITGB3 in the thyroid tissues by IHC. In normal thyroid tissues ITGA2 and ITGB3 were located mainly in the follicular membrane. In the PTC tissue samples another location of the integrins was registered: ITGA2 was located mainly in the papillary structures, whereas ITGB3 was seen both at the basal and apical surfaces of thyrocytes. Follicular adenoma was characterized by a uniform distribution of both ITGA2 and ITGB3.