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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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.