

The GPNMB/OA protein increased the invasiveness capability of human metastatic prostate cancer cells DU145 and PC3 through the activity of MMP-2 and MMP-9

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Introduction. By a microarray approach, using the experimental model of Nerve Growth Factor (NGF)-induced reduction of DU145 prostate cancer (PCa) cell line malignancy (1, 2), we identified the non-metastatic glycoprotein melanoma protein B (GPNMB) gene, encoding a type 1 transmembrane 572 amino acid protein (3). GPNMB, also known as osteoactivin (OA), is expressed in a wide array of normal and cancer tissues. In our model, untreated DU145 cells express the mRNA encoding for GPNMB/OA that is reduced after NGF exposure. It should be underlined that DU145 and PC3 cell lines derive from metastatic localization of human PCa. It could be thus hypothesized that GPNMB/OA could be involved in the progression of PCa. Indeed, dissolution of basement membrane components is a critical step in the multistep cascade that leads to metastasis and matrix-degrading enzymes, including matrix metalloproteinases, MMPs (4) play a main role in this phenomenon. Indeed, elevated levels of matrix metalloproteinases (MMPs) are associated with prostate cancer progression and metastasis. The aim of this study is to investigate the role of GPNMB/OA in PCa cell lines, namely DU145 and PC3.

Methods. DU145 and PC3 human PCa cell lines were cultured and treated with NGF as described (2-4). GPNMB/OA expression was analyzed by both Q-RT-PCR and indirect immunofluorescence, using specific antibodies. The role of GPNMB/OA in PCa progression has been identified by a siRNA approach, using a specific si-GPNMB/OA and scramble oligonucleotides (Thermo Scientific Dharmacon, USA). The cell proliferation rate was assessed by direct counting. A double staining with Acridine Orange and Ethidium Bromide (AO/EtBr) was performed to visualize and quantify the number of viable, apoptotic and necrotic cells, while the *in vitro* invasivity was evaluated by the Matrigel assay (BD Biosciences, USA). The expression and activity of MMPs has been investigated by both an western blot approach and by a zymography. Data analysis and graphics were obtained using GraphPad Prism 4 software (GraphPad Software, La Jolla, CA). The statistical analysis was made using the one-way ANOVA, with a post-hoc test (Bonferroni's test) for multiple comparisons, considering $p < 0.05$ as threshold for significant difference.

Results. In both cell lines, NGF exposure reduced the expression of GPNMB/OA expression, both at the mRNA and protein level. Transfection for 5 days with the si-GPNMB/OA (50 nM for DU145 cells and 10 nM for PC3 cells, respectively) significantly reduced GPNMB/OA and MMPs mRNA and protein levels, measured by Q-RT-PCR and western blot. The GPNMB/OA and MMPs extracellular fragments, that accumulated in the cell culture media, were as well strongly reduced. The GPNMB/OA knock down slightly reduced the cell proliferation rate of both cell lines. In particular, the reduction of the cell proliferation rate was significant in si-GPNMB/OA-treated DU145 cells and the double staining with AO/EtBr indicate that cells died prevalently by apoptosis. The migration capability was strongly inhibited in si-GPNMB/OA-treated DU145 and PC3 cells.

Discussion. GPNMB/OA is emerging as an important protein that plays a role in the progression of human PCa cell lines. In particular, GPNMB/OA-expressing cells showed the tendency to invade adjacent tissue and to promote metastasis, through MMP-2 and MMP-9 gelatinase activity.

References.

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