ABSTRACT

Author: Veronika Barešová
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Charles University, Faculty of Pharmacy in Hradec Králové
Department of Biological and Medical Sciences

Ovarian carcinomas are the deadliest group of gynecological malignancies. This is due to a high rate of metastasis and acquired drug resistance. Metastasis is characterized by a phenotypic change of cells from epithelial to mesenchymal, resulting in the loss of cell-cell and cell-extracellular matrix contacts and subsequent cell movement. This process is called epithelial-mesenchymal transition and has been shown to also play a role in cell resistance to cisplatin. The movement of individual cells is mediated by the Rho GTPase family and can be divided into mesenchymal and amoeboid types. The amoeboid type is characterized by the Rho/ROCK signaling pathway, while mesenchymal movement is mediated by the Rac/Cdc42 pathways. In my thesis, we investigated the differences in expression of selected proteins in both signaling pathways in ovarian carcinoma cells.

Initially, we focused on comparing the A2780 and A2780cis cell lines, which are resistant to cisplatin. We monitored markers of amoeboid movement, RhoA and phosphorylated myosin, and markers of mesenchymal movement, Wave-2, PAK, and phosphorylated PAK. In the second phase, we studied the effect of EGF and the inhibitor Y27632 on the change in cell movement of both lines.

To achieve our goals, we cultured cells in a 3D environment of collagen gel, which simulates the natural environment of the tumor stroma in the organism. After culturing and influencing with the selective ROCK inhibitor Y27632 and epidermal growth factor, we immunohistochemically stained selected proteins and observed them under a microscope. We also isolated active forms of Rho GTPases, which we subsequently subjected to western blot detection.

We found that the resistant A2780cis line expressed a greater amount of RhoA kinase and phosphorylated myosin than the sensitive A2780 line. The A2780cis line also exhibited a greater amount of active forms of RhoA, indicating that A2780cis cells mainly use amoeboid movement, while A2780 cells mainly use mesenchymal movement. Furthermore, the experiments showed that EGF reduces the expression of RhoA and myosin phosphorylation and, conversely, increases PAK phosphorylation, suggesting that it potentiates the shift from amoeboid to mesenchymal movement. The inhibitor Y27632 had similar effects, reducing myosin phosphorylation, accompanied by a decrease in RhoA activity, and increasing PAK phosphorylation.

Keywords: ovarian carcinomas, amoeboid movement, mesenchymal movement.