“EGFR and c-Met inhibitors as factors limiting the invasiveness of human melanoma cells”

Melanoma is a type of cancer characterized by low incidence rate, while its mortality rate reaches ca. 80% among all of the skin malignancies. More and more new cases are diagnosed every year, however there is no sufficient cure for the melanoma patients who developed metastases to the lymph nodes or distant organs. The high invasiveness of this particular cancer is a result of molecular mechanisms connected to signaling pathways activated by growth factors as well as to proteins regulating cell cytoskeleton organization, including actin-rich invasive structures – invadopodia. Among the growth factor receptors, which mutations and genes overexpression were identified in patients suffering from melanoma, is EGFR (*Epidermal Growth Factor Receptor*) and c-Met (*Hepatocyte Growth Factor Receptor*). In normal cells they control physiological processes like growth and migration, however in cancer cells these receptors may contribute to tumor progression and metastasis, which renders them potential therapeutic targets for small-molecule inhibitors or monoclonal antibodies.

The main aim of this work was to determine the effect of EGFR and c-Met inhibitors on viability and invasive abilities of human melanoma cells able to form invadopodia, adhesive protrusions with proteolytic abilities. Therefore, the purpose of this project was to verify the thesis that growth factor receptors may be a potential target for therapy aimed at suppressing metastasis of melanoma cells by blocking the activity of their invasive structures.

In the first stage of the project growth factor receptors’ expression in human melanoma cell lines derived from primary tumor (A375, WM1341D) and metastases to the lymph nodes (Hs294T, WM9, WM239) was evaluated and sensitivity of these cells to treatment with inhibitors of EGFR (erlotinib, gefitinib, lapatinib) and c-Met (crizotinib, foretinib) was determined. Based on collected preliminary data three cell lines (A375, Hs294T and WM9) and two pairs of inhibitors (foretinib with lapatinib or gefitinib), which exhibited synergistic cytotoxic effect on melanoma cells, were selected for further studies. Next, the influence of chosen drugs’ combinations on invasive abilities of cancer cells was verified. Spontaneous migration tests, scratch assays and experiments involving Boyden chambers showed the advantage of treatment with duets of inhibitors compared to drugs used individually. Moreover, the parameters associated with cell invasion through the extracellular matrix - the number of invadopodia and proteolytic activity of melanoma cells, were reduces upon inhibitors’ administration.

Furthermore, the direct involvement of EGFR and c-Met on melanoma cells' invasiveness was also studied. To achieve this goal cell lines exhibiting overexpression or silencing of growth factor receptors genes were generated. It was demonstrated that elevated EGFR level contributes to cell invasiveness, while reduced expression of EGFR or c-Met correlates with diminished motility and proteolytic activity of melanoma cells.

In the final stage of the study melanoma cell lines resistant to vemurafenib, a mutant BRAF kinase inhibitor, commonly used among patients suffering from metastatic melanoma, were generated. Obtained cell lines exhibited elevated invasive abilities compared to the parental lines as well as the presence of some stem cells traits. The combination therapy (foretinib with lapatinib) efficiently blocked the proliferation of resistant cells, while its impact on generated cells motility was less pronounced.

Obtained results seem to confirm proposed thesis and indicate that administration of EGFR and c-Met inhibitors, especially used as drug duets, efficiently reduced melanoma cell proliferation as well as lowers their migratory and invasive abilities by blocking the formation of active invadopodia.