Title: Regulation of tumor microenvironment by calcitriol and its analogs in mouse mammary gland cancer metastasis

The main goal of this study was to determine the role of calcitriol and its analogues PRI-2191 and PRI-2205 in the regulation of tumor microenvironment in the process of metastasis of 4T1 mammary gland carcinoma.

In vivo studies showed that calcitriol and its analogues potentiated metastasis of 4T1 mammary gland carcinoma but did not affect the growth of the primary tumor in this model. It has been proven that increased metastasis was accompanied by elevated OPN level in tumor and lung tissue as well as increased TGF-β level in plasma and lung tissue. These changes were correlated with an increase of 17β-estradiol plasma concentration in the initial stage of tumor growth and a decrease of vitamin D receptor expression in the tumor tissue in the final phase of experiment. Moreover, in tumors of mice treated with tested compounds elevated level of CCL2, IL-10, Arg1 as well as decreased iNOS concentration was also noted, which indicates the induction of immunosuppression of tumor microenvironment and stimulation of the protumoral phenotype of cells present in it, such as CAFs and TAMs. Additionally, it has also been demonstrated that normalized tumor blood vessels in concert with activated endothelium stimulated by OPN, TGF-β and 17β-estradiol significantly contributed to increased metastasis of tumor cells in the groups receiving tested agents.

In vitro studies showed that 4T1 cells, in contrary to 67NR, were not sensitive to the antiproliferative effects of calcitriol and its analogues, despite the higher expression of VDR receptor in 4T1 than in 67NR cells. Moreover, in 67NR cells tested compounds increased the level of CYP24A1 and CYP27B1 enzymes, retinoid x receptor alpha (RXRα), N-cadherin, estrogen receptor alfa (ERα) and β-catenin. It was also proved that calcitriol and its analogues elevated OPN secretion into the supernatant only in 67NR cultures. Additionally, increased production of OPN and CCL2 in, respectively, mouse BALB/3T3 fibroblasts and mouse RAW 264.7 macrophages cultures was also been demonstrated.

In conclusion, calcitriol and its analogues increased prometastatic potential of 4T1 cells by modifying the tumor microenvironment, therefore the validity of vitamin D_3 supplementation in the advanced stage of invasive breast cancer should be considered.