

According to the World Health Organization databases, breast cancer is the most common malignancy in women – more than 1.5 million new cases and 0.5 million deaths have been reported in 2012 (GLOBOCAN 2012). Despite the increasing availability of screening tests many cases are detected in advanced stage, characterized by an inauspicious prognosis. Most of the fatalities are related to the late diagnosis associated with the presence of metastases in distant organs.

Metastasis is multistage and complex process that is the result of numerous interaction between cancer and normal cells present in tumor microenvironment, i.e. stromal cells. Tumor associated macrophages (TAMs) are dominant population of tumor infiltrating cells. The plasticity of their phenotype determines their antitumor or protumoral activity. At the advanced stages of many malignant cancers, including breast cancer, the polarization to M2 macrophages is often observed and is associated with poor prognosis. M2 tumor-associated macrophages on account of the profile of secreted cytokines and chemokines promote tumor growth, neovascularization, invasion and metastasis.

It is well-known that proper vitamin D level is essential to maintaining homeostasis of the organism. Numerous studies indicate that its deficiency may be a prognostic factor and its supplementation is recommended in many therapeutic regimens to alleviate bone symptoms. However, there are no studies about the safety of its use in advanced stages of invasive breast cancer, especially with regard to its immunomodulating properties.

The main goal of our research is to clarify whether vitamin D can affect invasiveness and metastasis of breast cancer cells *via* modification of macrophages properties, determined by their phenotype polarization. The proposed project is based on *in vitro* co-culture and 3D culture experiments. For this purpose, we will use a model of murine macrophages that will be stimulated by different cytokines to activate their phenotype to M1 or M2. First of all, the effect of vitamin D on the activation status of macrophages (M1 or M2) will be evaluated by analysis of genes and proteins expression characteristic for different class of macrophages. In the next step, the impact of different subpopulations of macrophages on the proliferation and ability of normal and breast cancer cells to adhesion, invasion, migration and spheroids forming will be examined. Moreover, the influence of factors secreted by M1 or M2 macrophages on the normal and breast cancer cells properties will be investigated by analysis of markers related to EMT and genes and proteins associated with invasion and metastasis. To determine the effect of vitamin D on these processes, tumor cells and macrophages used in co-cultures will be preincubated in the presence or absence of biologically active form of vitamin D, i.e. calcitriol.

The results obtained in this project will contribute to a better understanding of molecular and cellular development and progression of breast cancer, what seems to be necessary to create new, more effective therapeutic strategies. Moreover, they will also allow us to consider the safety of vitamin D supplementation in the context of its direct effect on the macrophage present in tumor environment.