Worldwide, breast cancer is the most common invasive cancer in women. About 70% of breast cancers rely on supplies of estrogen to grow. They are known as estrogen-sensitive cases. Estrogens are the main female sex hormones responsible for the physical characteristics and reproduction of women. Estrogen-sensitive breast cancer cells possess estrogen receptors (the main one is ERα) and are classified as ER-positive. When estrogen interacts with its receptor, it binds to DNA, regulates transcription (i.e. gene activity), and ultimately stimulates cell growth and migration (and thus cancer expansion). Therefore, therapies targeting either estrogen production or the receptor itself, so-called hormone therapy, are widely used in patients with ER-positive breast cancer. Tamoxifen is the best-known anti-estrogen drug, which is the standard for ER + systemic treatment of breast cancers. Hormonal treatment allows for objective clinical responses in approximately 50% of patients. Acquired endocrine resistance associated with the activation of non-estrogen receptor signaling pathways is responsible for the failure of hormone therapy in the second group of patients with ER + breast cancer. Thus, seeking new therapeutic targets in the treatment of ER + breast cancer is important to overcome tumor resistance and improve treatment outcomes.

The main goal of the project is to establish whether Heat Shock Factor 1 (HSF1) could be a potent therapeutic target that limits metastases formation by ER+ breast cancer cells and improves the efficiency of hormonal therapy. HSF1 is a well-known protein that regulates the response of cells to disturbances associated with damage in the protein structure. In addition, HSF1 regulates other biological processes related to the cell cycle, protein synthesis, and glucose metabolism. In our *in vitro* studies, we showed that HSF1 is activated in ER+ breast cancer cells grown in media supplemented with estrogen. Moreover, the reduction of HSF1 expression inhibits estrogen-stimulated motility of ER+ breast cancer cells. The ability of cells to migrate to some extent associated with the invasion of cancer cells is responsible for the formation of metastases.

In this project, we will use human ER + breast cancer cells (MCF7), which will be administered to athymic (athymic) mice. The athymic mice do not produce T cells of the immune system, which are responsible for rejecting transplants of another species. Human breast cancer cells will be labeled by introducing the luciferase gene and green fluorescent protein, so that cell growth and metastasis can be observed by bioluminescence imaging. We will also construct human cell lines with reduced HSF1 expression. The human ER+ breast cancer MCF7 cells will be injected into the milk duct system of immunocompromised mice and tumor growth and metastases formation will be monitored. In the next step, we will study whether HSF1 down-regulation will increase the efficiency of hormonal therapy. We will inject cells into the milk duct of athymic mice to study the effect of HSF1 down-regulation on the efficiency of hormonal treatment at an early stage, or inject cells into the tail vein to model the treatment of the advanced metastatic setting. For these experiments, we will use either unmodified cells or cells knockout for HSF1. We will also use an HSF1 inhibitor to down-regulate its activity. Mice with established tumor growth will be treated with an HSF1 inhibitor in the combination with (i) tamoxifen, (ii) fulvestrant, or (iii) Palbociclib. The tumor growth will be monitored by *in vivo* bioluminescence. At the end of the experiments, mice will be sacrificed, the tumors will be collected and used for analysis by staining for cell proliferation index, blood vessel formation, cell death, and others.

We expect that these experiments will show whether inhibition of HSF1 function will reduce estrogen-dependent ER + growth of breast cancer cells and their metastasis. Thus, we will obtain knowledge of whether HSF1 may be an appropriate therapeutic target in the treatment of hormone-dependent breast cancers.