Estrogens are the primary female sex hormones which play an essential role in sexual and reproductive development. They are produced by organism almost for the whole life but could be also delivered as external compounds: they are used as part of some oral contraceptives or in estrogen replacement therapy for postmenopausal women. Additionally, many chemicals present in the environment (i.e. bisphenol A) possess estrogenic features (so called xenoestrogens), which may interfere with the functioning of the body and hormonal balance.

Both the deficiency and excessive exposure to estrogens are not healthy. A variety of clinical studies implicate them (especially the most potent physiological estrogen, 17β-estradiol, E2) as risk factors for developing breast and endometrial tumors, which account for almost 40% of cancer incidence among women. Beside of epidemiological studies, it was experimentally proved that 17β-estradiol, as well as some xenoestrogens are able to induce neoplastic transformation of human mammary epithelial cells. Consequently, the National Institute of Environmental Health Sciences (NIEHS) in USA added estrogen to its list of known cancer-causing agents.

Worldwide, breast cancer is the most common invasive cancer in women. It comprises more than 20% of all cancers, with more than one million cases diagnoses per year. Furthermore, about 80% of breast cancers, once established, rely on supplies of the estrogen to grow: they are known as estrogen-sensitive. It means that cancer cells possess an estrogen receptor (they are ER-positive). The estrogen binds to its receptor which then acts as transcription factor: interacts with DNA and regulates gene expression what stimulates cell divisions.

The number of cases worldwide has significantly increased since the 1970s, a phenomenon partly attributed to the modern lifestyles. However, the exact mechanism of mutagenesis induced by E2 is still not exactly known. The two major hypotheses have been proposed to explain the mechanisms of action of estrogens in breast tumorigenesis: 1) estrogen activity via the estrogen receptors; this leads to enhanced proliferation of target mammary (breast) cells and increases the possibility of genomic mutations during DNA synthesis; 2) the ability of estrogen metabolites to bind to DNA and create genotoxic DNA adducts (complexes between the metabolites and the DNA); this process will also ultimately lead to accumulation of mutations. Our preliminary studies have revealed that 17β-estradiol treatment leads to unusual, rapid elevation of HSF1 (Heat Shock transcription Factor 1) level with subsequent increased HSPs (Heat Shock Proteins) expression in the human mammary epithelial cells and also in breast cancer cells. HSF1 is primarily known as master regulator of the heat shock response. In model systems it has been shown that HSF1 facilitates malignant transformation, cancer cell survival, and proliferation. Moreover, its high level is associated with increased mortality of ER-positive breast cancer patients and endometrial cancer patients. Thus, we hypothesize that 17β-estradiol and HSF1 could cooperate in promoting of neoplastic transformation and in the tumor growth. Such hypothesis is completely new, thus we suppose to find a new face of the estrogen mediated carcinogenesis.

Although both HSF1 and estrogen, are known for their role in cancerogenesis, little is known about the interactions between the signaling pathways induced by them. The important question is whether and how they interact with each other in normal and cancer cells. The aim of this project is therefore to find out HSF1-dependent mechanisms associated with malignant transformation induced by estrogen as well as mechanisms promoting the growth of cancer cells.

The main part of the project is focused on explaining which signaling pathways activated by estrogen in breast epithelial cells depend on HSF1. For this, we will compare the changes induced by estrogen in cells with a normal level of HSF1 (HSF1+) and in the cells without HSF1 (HSF1-). Analysis will be done in both: normal and cancer cells. It is known that estrogen treatment of the normal (non-cancerous) breast epithelial cells leads to the development of the cancer phenotype. In vitro it manifests by an anchorage independent growth, the loss of an ability to form ductules in three-dimensional culture, increased motility, etc. Such cells after neoplastic transformation are able to growth and create a cancer when introduced into the body (in opposition to normal cells). Studies on non-cancerous cells with different levels of HSF1, after short-term or long-term exposure on estrogen, should explain what mechanisms are triggered in the early and late stages of malignant transformation induced by estrogen and what is the impact of HSF1 on this process.

The growth of the breast cancer cells also strongly depends on estrogen. Assessment of changes which occur in the estrogen treated cancer cells with different levels of HSF1 should enable us to assess what is the meaning of HSF1 in maintaining the growth of cancer cells.

Evaluation of the changes induced in the cells by the estrogen will be possible through the use of modern genomic methods such as RNA-Seq, i.e. the entire transcriptome sequencing. This method allows the assessment of global changes in gene expression, both quantity and quality of the transcripts. In addition, we are planning to identify all the estrogen receptor DNA binding sites (using the ChIP-Seq method). We will get information if HSF1 affects binding of the estrogen receptor to DNA, and which changes in gene expression are directly dependent on the binding of the receptor.

An important element of the project is to examine the response of cells to the promising HSF1 inhibitor (KRIBB11). We will investigate whether the impact of KRIBB11 on transcriptome is the same/similar to the effect induced by HSF1 depletion. We will also check the combination of KRIBB11 and tamoxifen (which is currently used for the treatment of both early and advanced estrogen receptor positive breast cancer in pre- and post-menopausal women). We belief that a better understanding of a role of HSF1 in breast cancer pathogenesis may help to resolve a clinical and scientific problems relating to (breast) cancer prevention, diagnosis, and treatment.