Identification and functional evaluation of candidate mutations related to genome destabilization in uninvolved, non-tumor mammary glandular tissue samples from breast cancer patients.

Breast cancer is the primary reason of malignant carcinoma incidence, as well as the leading cause of cancer related mortality. The key factors that determine therapeutic outcome are prophylactics and diagnostics which facilitate early detection of disease. Despite significant improvement of prophylactics in the recent years, the limiting factor is diagnostics, because the prevailing majority of methods applies when clinically detectable tumors have been formed. The molecular diagnostics DNA based methods are unique in this regard, as they enable detection of damaging alterations in sequence of the human genome, which are referred to as mutations. Mutations may have very limited scope (point mutations), or they may encompass considerable part of the genome (structural rearrangements) leading to genome destabilization. The molecular methods enable evaluation of genetic predisposition, i.e. the risk of getting condition, which is essentially predetermined by inherited factors/mutations. The molecular methods also permit detection of early genetic alterations, so called somatic mutations in the "source" tissue for the specific cancer, before any clinical symptoms become apparent. In the case of breast cancer, the "source" tissue is referred to as the mammary epithelial glandular tissue, which under normal conditions is responsible for lactation. That is why, in the studies of breast cancer, the mammary epithelial glandular tissue may constitute research model for detecting early genetic alterations which are related to carcinogenesis. This model is supported by numerous indications implying that carcinogenesis begins long before any clinical manifestations occur. Furthermore, studying early levels of carcinogenesis may aid in elucidating functional significance and interplay between the effects of different mutations, which is not a trivial task. Currently, majority of studies uses full-blown tumors, or isolated cancer cells *in vitro* that underwent the complete transformation. This type of material is characterized by multitude of secondary effects that are related to late stages of carcinogenesis. These secondary effects impede interpretation of effects of inherited or early somatic mutations, which are *de facto* the primary cause of carcinogenesis.

Hence, the main objective of the proposed project is identification and functional characterization of mutations in uninvolved (non-tumor) mammary glandular tissue from breast cancer patients. As the assessment of all possible functional effects exceeds the scope of a single study, we will focus on the relationship between point mutations and structural rearrangements that ultimately lead to genome destabilization.

The choice of genome destabilization as the effect under study is justified by high frequency of this molecular disturbance in the course of breast cancer, as well as by its causal role for the cascade of secondary events and deregulation of basic cellular functions. Several research groups, including ours, showed that the genome destabilization may occur during early stages of carcinogenesis, i.e. in seemingly normal mammary glandular tissue. At the same time, because of the vast genomic scope, the destabilization is relatively easy to discover. Hence, we will first preselect the uninvolved glandular tissue samples that show genomic destabilization. Next, we will seek the underlying genetic cause, i.e. alterations of nucleotide sequence in the genes that are responsible for maintenance of genome integrity. To get better grasp of existing interactions in clinical, patient derived samples we will carry out analysis of different functional layers, i.e. in addition to DNA sequence and structural rearrangements, we will perform analysis of gene expression on transcript level (RNA), as well as we will study one aspect of so called epigenetic modifications by means of DNA methylation. Both, gene expression and DNA methylation changes are inherently bound to mutations and genome destabilization. We will also conduct *in vitro* experiments based on induction of stress response during hormonal stimulation of mammary epithelial glandular cells of the patients. These conditions are considered to facilitate early genome destabilization under physiological conditions, i.e. during puberty or pregnancy. By recreating these conditions *in vitro* we anticipate to achieve more pronounced effect of mutations than that of clinical samples. This should ultimately facilitate observations and interpretation of results.

We will apply state-of-the-art high throughput genomics along with advanced data analysis throughout the project tasks. These methods will provide basis for broad, multilateral evaluation of the observed effects and their molecular context.

Evaluation of functional links between mutations and genome destabilization in the uninvolved mammary glandular tissue may impact on diagnostic approaches, prognosis and therapeutic options in the future.