Description for the general public

Genes mutation can induce alteration and malignant cellular transformation. Significant influence on the organism has mutations occurring within the oncogenes. The development of many cancer is associated with mutations within the *BRAF* (*B-raf proto-oncogene*) gene. The protein encoded by this gene is component of the MAPK/ERK (*mitogen-activated protein kinases/extracellular signal-regulated kinases*) signaling pathway, transmitting information from outside to the cell nucleus. The main function of MAPK/ERK pathway is regulation of cell growth, migration and proliferation. The most common mutation in the *BRAF* gene structure is V600E. This variation causes continuous activation and signal transduction, regardless of the external stimulus. As a consequence, proliferation and cell invasion are enhanced. V600E mutations have been found in patients with melanoma, colon cancer, myeloid leukemia and other.

Sequencing of genetic material isolated from patients with liver cancer identified new mutations in the *BRAF* gene. So far, alterations in this gene have not been associated with liver cancer development. Therefore, we intend to determine the influence of new *BRAF* mutations on hepatocytes pathogenesis. Furthermore, we assume that the mutation locus affects the differentiated the mechanism of signal transduction within the MAPK/ERK path. Others studies have shown that mutations in the *BRAF* gene enhance or even reduce BRAF kinase activity. These results revealed different gene functions depending on mutation locus. It is necessary to develop personalized anti-cancer therapy, counteracting the effects of specific mutations.

We plan to perform the functional analysis of newly discovered mutations in two systems: *in vitro* and *in vivo* (mouse model). Mutations will be generated using CRISPR/Cas9 method. Next, the ability to proliferate, migrate and invade mutated hepatocytes will be determined. In the second part of our study, mutations will be introduced into a fertilized oocyte by microinjection of ctRNP (Cas9, crRNA, tracrRNA) complex. Next, mutated embryos will be transfer into the pseudo-pregnant female mice. Further investigations will be based on the analysis of material collected from animals suffering from cancer. Protein expressions and protein-protein interactions will be determined. Moreover, we plan to perform histopathological and immunohistological analysis of liver and other organs involved in the tumor process.

Our results allow to investigate the effects of new, rare BRAF gene mutations in liver cancer development. In addition, the results of our study can be used to develop personalized therapeutic strategy. This is especially crucial because, the number of patients with liver cancer has been increasing recently. Except that an inappropriate therapy could lead to the opposite effect and promote tumor growth.