The *HNF4A* gene is one of an array of genes involved in organogenesis and in modulation of the cancer development process in liver. In particular, it is known in mice, which removal of this gene from the genome, leads to tumour development in liver. Hence, it is postulated that any loss-of-function variation to the gene structure/composition (mutation) can trigger liver cancer development and its progression. As far, a throughout functional analysis of the HNF4A mutations in human liver cancer have not been performed. This study will allow us to investigate for the first time the importance of the HNF4 gene mutations in liver cancer. We have already found that the HNF4 mutations are accumulated at the evolutionally sustained regions of the gene, which is partly the reason of our interest in those mutations. Since the HNF4A gene modulates liver tissue differentiation and liver cancer cell fate, the loss of its function is associated with a poor prognosis. Our study aims to demonstrate that pathogenic mutations of HNF4A cause dysregulation of several gene expressions and induce hepatic tumorigenesis. Therefore, the immediate gain in knowledge expected from this project is evaluation of the role of the HNF4A mutations in liver cancer onset. However, given the increasing information on single DNA units' variants and alterations of their copy number stemming from the many cancer sequencing projects going on world-wide, there is strong need of functional validation to identify potential drivers – a pre-requisite to translate this knowledge into new diagnostic or therapeutic leads. For this reason, we perform functional study of HNF4A mutations as a model case.

Our preliminary results show that the mutations of the HNF4A gene, referred to as G79C, F83C, and M125I, result in impaired gene functionality. We have also identified three mutations located within another region of the gene (referred to as 164X, 191X, and 247X) which all resulted in a reduction of the gene activity. Although we found that HNF4A mutations G79C, F83C, and M125I are possible pathogenic mutations, it is unclear how the HNF4A mutations impact the pathogenesis of liver cancer. To answer this question, we will introduce mutations using CRISPR-Cas9 system in mouse and human normal liver cell lines and subsequently perform functional assays.

Gene mutations can lead to altered gene function and malignant cellular transformation. To improve the early detection of mutations, it is indispensable to have a complete map of cancer driver genes and their hot spots. Our study aims to support the ongoing efforts at improving the early detection of cancers, thereby contributing to the field of preventive medicine. Although the modern molecular tools have let us determine multiple mutations linked to several cancers, the data comes only from limited number of countries**.** Our study may help to identify pathogenic mutations without performing tedious analyses, but by using an already existing dataset.