TP53 mutations drive alternative *FGFR1* splicing - the study of consequences for cancer progression and therapeutic opportunities

Despite ongoing advancements in diagnostic techniques and therapeutic strategies, cancer remains a leading cause of mortality globally, according to the World Health Organization (WHO). The most common types of cancer include lung, colon, pancreatic and head and neck cancers. Therefore, it is essential to expand our knowledge of the molecular pathways involved in tumor development and to develop new therapeutic strategies. **This project aims to investigate how mutations in the *TP53* gene affect alternative splicing, which is an important process in cancer development, and to explore the possibility of targeting this process for therapeutic purposes**. *TP53* mutations are among the most common in various types of cancer. They can cause changes to the p53 protein, resulting in the loss of its tumor-suppressing functions and the acquisition of new properties that accelerate cancer development. The p53 protein with such properties is called mutant p53 gain-of-function (GOF). One of the processes affected by mutant p53 GOF is alternative mRNA splicing, which allows different protein isoforms to be generated from the same gene. These splicing isoforms may display different functions. For example, one may be pro-oncogenic, while the other may be anti-oncogenic. It has already been demonstrated that mutant p53 GOF can promote the production of oncogenic protein isoforms that favor blood vessel formation in tumors or alter cellular signalling pathways. **The goal of this project is to explain how mutant p53 protein may alter the splicing of a specific gene called *Fibroblast Growth Factor Receptor 1 (FGFR1)*, which plays a key role in various cellular processes.**

Alternative splicing of *FGFR1* is common in human cancers and enables different versions of the FGFR1 protein to be produced. For example, the splicing of exon 3 produces two isoforms: FGFR1 alpha and FGFR1 beta. Depending on whether exon 8 or exon 9 is included, two other variants can be produced: FGFR1 IIIb and FGFR1 IIIc. Production of these different versions of FGFR1 can affect how strongly the protein binds to its signalling molecules, thereby influencing the phenotype of cancer cells. Notably, these variations can also impact how cancers respond to certain treatments, thereby complicating therapy. Overall, alternative splicing of *FGFR1* plays a significant role in both the development of cancer and its resistance to treatment.

Initial results from RNA sequencing (RNA seq), that we obtained during the SONATA project at the Laboratory of Human Disease Multiomics (MMRI, PAS), where I am a PhD student, reveal the influence of mutant p53 on factors from Serine/Arginine Rich Splicing Factors (SRSFs) family and on Serine-arginine Protein Kinase 1 (SRPK1). These factors regulate the splicing process, with SRPK1 being responsible for the activity of SRSFs. I have experimentally demonstrated the interactions of mutant p53 with the SRSFs and with SRPK1. In addition, RNA-seq data revealed that mutant p53 influences the splicing of exons 3, 4 and 11 of the *FGFR1* gene. These results were confirmed through experimentation.

The project aims to investigate the impact of *TP53* mutations on *FGFR1* splicing and the influence of different versions of the FGFR1 protein on the behaviour of cancer cells. This knowledge will be used to develop new therapeutic protocol. I will investigate the interactions between the mentioned splicing factors and FGFR1 isoforms, as well as the influence of FGFR1 isoforms on cancer cell characteristics such as proliferation, migration, and invasion. In addition, drugs targeting mutant p53 and FGFR1 and the splicing machinery, will be tested on cancer cell lines and 3D organoid cultures. The synthetic lethality strategy will be employed, whereby targeting two proteins or processes at the same time will kill cancer cells more effectively. Additionally, the study will examine the impact of different FGFR1 isoforms on resistance to therapy. The ultimate goal is to use these molecular insights to find more effective ways to treat cancer.