Abstract

Inherited mutations in predisposing genes significantly contribute to the development of various cancers. Current genetic testing via panel sequencing efficiently identifies pathogenic germline mutations in the *TP53* gene, which can cause hereditary Li-Fraumeni syndrome (LFS) associated with greatly increased risk of tumor development. However, variants of uncertain significance (VUS) can complicate clinical interpretation. This thesis aims to evaluate the functional significance of selected variants in the *TP53* gene, identified by genetic screening particularly in the Czech population. *TP53* is crucial for cell cycle regulation, induction of apoptosis, DNA repair and other functions and its dysregulation is associated with cancer.

Using CRISPR/Cas9 technology, we generated a cellular model with inactivated *TP53* gene. Further work included preparing expression plasmids carrying coding sequences for fluorescent markers, cDNA of selected *TP53* variants, and selection cassettes. The function of the mutated versus the wild-type gene forms was compared after transfection and generation of stable cell lines. Eventually, the expression of *CDKN1A* and *MDM2* genes among the cell lines after nutlin-3a treatment was assessed. Additionally, the role of selected *TP53* variants in the p53 tetramer formation and colony formation assay was analysed.

This work contributes to a deeper understanding of the cellular functions of several *TP53* mutations. The findings provide insights into their roles in the cell cycle regulation, which will assist in evaluating cancer risk for mutation carriers in collaboration with clinical genetics. Overall, out of 35 analysed mutations we defined 14 as loss-of-function, 20 as benign and one of them (R267W) as functionally intermediate. Finally, we found that the E339del3 variant recently identified in a Czech cancer patient is a loss-of-function mutation and can promote LFS.

Keywords: *TP53*, variants of uncertain significance, cancer, *CDKN1A*, *MDM2*