Abstract

Inactivation of tumor supressor gene *BRCA1* causes a life-long risk of breast carcinoma development. Genetic screenings of indicated individuals from high-risk families help to identify large number of sequence variants in known predisposing genes. Majority of discovered variants doesn't have clinical significance yet which causes a big problem for diagnostics. Some of these variants are found within regulatory non-coding regions of gene. A part of the clinical classification of variants is their functional characterization. The goal of this thesis was to create a model system for functional characterization of variants in non-coding regions and to verify its function. Model system was based on targeted gene manipulation by co-transfecting CRISPR-Cas9 construct and donor construct that contained a portion of *BRCA1* gene sequence with analyzed modifications, into U2 OS cells. The cells have stably integrated DR-GFP system which allows the activity of homologous recombination (HR) to be determined. Monoallelic modifications were induced into U2 OS cells. These modifications were in a Kozak sequence region of *BRCA1* gene. Expression level of *BRCA1* mRNA was determined by qRT-PCR, which showed the same levels of mRNA in all cells with analyzed alterations. Next, expression level of BRCA1 protein was determined by ELISA method. It showed that some of the analyzed alterations had significantly reduced level of BRCA1 protein. Next the activity of HR was determined by DR-GFP assay. BRCA1 participates in HR, and it's assumed that the reduced activity of this mechanism might cause a malignant transformation. It was found that the activity of HR positively correlates with the concentration of BRCA1 protein. Variants with negative effect on protein expression had significantly reduced HR. This way the functionality of model system was verified. It was also confirmed that reduced levels of BRCA1 cause a reduced activity of HR and that alterations in non-coding regulatory sequences of *BRCA1* gene can negatively affect biological activity of the gene expression product which might contribute to the risk of tumorigenesis in mammary gland.