ABSTRACT

Breast cancer is the most common tumor disease diagnosed in women worldwide. The hereditary character of this disease is observed in 5-10 % of all cases, and it is usually caused by a pathogenic mutation in one of the predisposition genes. Although a variety of pathogenic mutations in the coding sequences of these genes was described, the cause of the disease is still unknown in many familial cases (> 50%). A great number of identified pathogenic mutations were localized in the consensus splicing sites, which results in the formation of aberrant mRNA splicing variants and their damaged protein isoforms. However, little is known about mutations affecting regulatory splicing sites, which can result in the translation of similarly affected mRNAs.

In this work, we proposed a method for indirect detection of mutations affecting the natural splicing pattern of any gene of our interest based on multiplex PCR and NGS with high sensitivity. Verification of this method on the BRCA1 model gene revealed the presence of the total of 94 splicing variants in peripheral leucocytes and healthy breast and adjacent fat tissues. This is the most detailed catalogue of physically occurring BRCA1 mRNA variants thus far.

The most commonly occurring variants, maintaining open reading frame, were quantified by RT-qPCR which resulted in the characterization of 6 ubiquitously expressed alternative splicing variants with a relative expression > 1 % of total BRCA1 (Δ5; Δ9_10; Δ9_10,11q; ▼13 and IRIS). Furthermore, we detected tissue specific levels in the expression of Δ9_10, ▼13 and IRIS variants. The majority of ubiquitous variants probably result in protein isoforms which maintain the BRCA1 wild-type character or an unknown (probably regulatory) function.

This study fully reveals the qualitative and quantitative splicing pattern of BRCA1 mRNA variants in relevant healthy human tissues. Based on this, we can instantly detect aberrantly spliced BRCA1 mRNA variants e.g. in tumor tissue and reveal the presence of mutation affecting the regulatory splicing site in cases of negative mutation analysis of *BRCA1*.

Key words: Alternative splicing, BRCA1, breast cancer, gene expression analysis