Abstract:

Pancreatic cancer (PC) is extremely severe malignant disease with a five-year survival of less than 5%. Currently there is no reliable tool for the diagnosis of PC in its early stages. At the time of clinical symptoms most patients are in an advanced stage of the disease and the treatment does not usually have a significant effect. For these reasons emphasis is gradually shifting to the search for the suitable molecular markers for improvement of the diagnosis and assessment of the survival prognosis with respect to a possibility of surgical treatment. MiRNA represent one of the most promising markers, although, their examination in pancreatic tissue is a complicated process. One of the reasons is the very small amount of the source material coming from a fine needle biopsy. A second cause of problems is the subtle character of the pancreatic tissue resulting in significantly lower yields of molecular genetic analysis when compared to other epithelial tissues. An additional negative factor is heterogeneity of the tissue resulting in disproportionate representation of tumor cells within the sample. A suitable choice of procedures for isolation of nucleic acids (NA) and subsequent analysis including quantification of tumor cells is critical for accurate evaluation of the miRNA levels.

This work is focused on optimization of the entire process from the initial acquisition of the sample from pancreatic tissue biopsy and isolation of NA, tumor cells quantification based on mutation detection in KRAS gene, cDNA synthesis and, finally, measurement of levels of selected miRNAs by qPCR. The partial steps were evaluated based on the quantities of nucleic acids and the yield and quality of the PCR products amplified from DNA and cDNA templates. The efficiency of the complete procedure was verified by a successful analysis of the KRAS gene, two miRNAs (miR-21 and miR-10b) and a reference gene RNU6b on a set of 110 biopsy samples with a success rate of 100% (KRAS and miR-21), 99% (RNU6b) and 93% (miR-10b). This confirmed a successful development and validation of an efficient procedure for reliable quantification of miRNAs from biopsy tissue samples of the pancreas applicable in routine clinical practice.

Keywords:

Pancreatic cancer, EUS-FNB, fine needle biopsy, miRNA, optimization, chronic pancreatitis, RNA isolation, DNA isolation, qRT-PCR, heteroduplex analysis, *KRAS*, diagnostics, prognosis.