ABSTRACT

Ductal adenocarcinoma (PDAC) is the most common primary pancreatic neoplasm. It's frequently diagnosed in late inoperable stage and has high resistance to chemotherapy; this situation contributes to its unfavourable prognosis. Therefore, there is a need for biomarkers enabling early detection of PDACs and by this way to improve patient prognosis. MicroRNAs (miRNA), short non-coding RNA molecules involved in post-transcriptional regulation of gene expression, belong to such markers.

Contrary to other RNA molecules, miRNAs are stable in biological samples. Their expression is measured by several analytical methods, including real-time quantitative PCR (RT-qPCR). Normalization of methods determining miRNA levels requires adequate endogenous controls. However, variable expression of endogenous controls in tumors may cause bias in determining miRNA levels. The aim of the first study was to investigate the expression of six miRNAs isolated from formalin fixed paraffin embedded (FFPE) samples of PDACs. Four controls were chosen for RT-qPCR result normalization: artificial spike miR-39 from C. elegans, U6 snRNA, miR-16 and snoRNA U91. Expression values of all studied miRNAs in tumors were significantly different depending on selected endogenous controls. Additionally, stability of the controls varied significantly in individual tumors, U91 was determined to be the most stable according to the NormFinder algorithm. Thus, U91 can be recommended as endogenous control for miRNA expression in PDAC tissues.

The possibility to utilize miRNAs as diagnostic and prognostic biomarkers in malignancies has been the subject of multiple publications. The aim of the second study was to investigate the expression of seven selected miRNAs isolated from FFPE samples of 54 PDAC patients. The relationship of miRNA expression levels with tumor histology, clinico-pathological characteristics, patient overall survival (OS) and progression-free survival (PFS) was subsequently evaluated. Overexpression of miR-21, miR-155 and miR-210 was observed in PDACs, in comparison with non-neoplastic pancreatic tissue. On the contrary, miR-96 and miR-217 were significantly downregulated in PDACs. Positive correlation of miR-210 levels was observed with patient age (ρ=0.35). Expression levels of all selected miRNAs failed to demonstrate significant correlation with tumor parameters – grade, degree of local progression, lymph node involvement, perineural invasion, vascular invasion and length of patient survival. Additionally, elevated levels of miR-148a and miR-217 have shown positive correlation with tubular arrangement of tumors; decreased miR-148a expression was associated with

dissociative tumor growth. Elevated miR-155 levels were linked to high mitotic activity in cancer cells.

This work demonstrated significance of the choice of endogenous controls for normalization of RT-qPCR results during miRNA expression analysis. The results have confirmed abnormal miRNAs expression in PDACs in comparison with adjacent non-neoplastic tissue. Significant association was detected between histological structure, mitotic activity and miRNA expression in tumors. Finally, no correlation of miRNA levels with tumor progression and length of patient survival could be demonstrated.