Abstract

Laryngeal squamous cell carcinomas (LSCC) belong to the heterogeneous group of head and neck cancers (HNSCC). Despite the development of medicine and science, there is still a lack of effective methods of personalized molecular treatment of LSCC tumors and the commonly used partial or total laryngectomy significantly reduces the patient's quality of life. In addition, patients are diagnosed at an advanced stage of the disease which has a direct influence on low 5-year survival rate approximately 50-60%. Therefore, there is still a need to better understand the genetic as well as epigenetic changes characteristic of LSCC. Deregulation of proto-oncogene and tumor suppression gene expression resulting from aberrant expression of microRNAs is one of the epigenetic mechanisms involved in tumorigenesis.

The main aim of this doctoral dissertation consisted of a series of three publications, was to extend the knowledge about the microRNA role in pathogenesis of LSCC by identification of potentially oncogenic microRNAs.

The first step was to compile the current state of knowledge about deregulated microRNAs in diverse types of HNSCC, including LSCC The summary of the collected data clearly showed that HNSCC tumors are characterized by an altered level of microRNA expression in relation to non-cancerous controls. In addition, it was emphasized that each of the HNSCC subtypes has its own unique microRNA expression profile, which translates into the need to conduct research on tumors from specific locations. It has also been pointed out that microRNA molecules are important regulators of key processes for the survival of a cancer cell, and therefore have a chance to become targets of new anti-cancer therapies.

Then, taking into account the conclusions of the review that each HNSCC subtype is characterized by specific changes in the level of microRNA expression, profiles for the expression of the LSCC microRNA and non-cancerous controls were created. This allowed the selection of potential oncogenic microRNAs previously not described in the context of laryngeal cancer, namely hsa-miR-1290, hsa-miR-1246 and hsa-miR-4317. The verification of the obtained results on an independent group of tumors and non-cancer controls confirmed the overexpression of hsa-miR-1290 in LSCC. In order to establish the biological role of hsa-miR-1290, potential target genes with the binding site of this microRNA in their 3'UTR sequence and at the same time characterized by reduced expression in LSCC cell lines were selected. The *MAF* and *ITPR2* genes were chosen for further research due to their repeated

down-regulation in a group of 20 LSCC tumors as well as the described role as regulators of apoptosis. Then, functional analysis showed a significant increase in MAF protein expression after blocking hsa-miR-1290 activity in LSCC cell lines using a microRNA inhibitor.

The next step was to further elucidate the relationship between hsa-miR-1290 and MAF and their importance in the LSCC. The conducted analyzes allowed to confirm the direct interaction of hsa-miR-1290 with the 3'UTR sequence of the second variant of the *MAF* gene NM_001031804. Therefore, it was assumed that overexpression of the studied microRNA observed in LSCC directly silences the expression of *MAF*. Moreover, based on the results of the immunohistochemical study, it was shown that 51% of LSCC samples had MAF protein only in the cytoplasm, and 6% had no MAF protein. Because MAF protein is a transcription factor, its presence in non-neoplastic squamous epithelial cells is observed both in the nucleus and in the cytoplasm. This allows the conclusion that even if the MAF protein is present in LSCC cells, its activity is disturbed. In order to demonstrate the function of *MAF* downregulation in LSCC, genes containing *MAF* binding motif sites in their promoter region sequence and characterized by reduced expression in LSCC cell lines were searched. As a result, the *PRODH* gene was selected, which, according to the results of GO analysis and literature data, participates in the regulation of apoptosis. Furthermore, it was shown that enhancing hsa-miR-1290 overexpression in LSCC cells with an expression vector resulted in a reduction of *MAF* expression, which in turn translated into a decrease in *PRODH* expression.

In summary, this doctoral dissertation is part of the research on the role of microRNAs in LSCC. The expression profiles obtained on a homogeneous group of laryngeal tumors led to the indications of hsa-miR-1290, previously not described in the LSCC. Moreover, the conducted research allowed to describe the connections between hsa-miR-1290, *MAF* and *PRODH*, which may play a role in the regulation of apoptosis in laryngeal squamous cell carcinoma.