## ABSTRACT

Laryngeal squamous cell carcinoma (LSCC) is the most common cancer among HNSCCs. The 5-year survival rate for LSCC remains low (approx. 50%), which is associated with high rate of lymph node metastases, second primary tumors and recurrences. The research performed in this field of tumor genetics is focused on the discovery of molecular markers for early LSCC diagnosis, prognosis and targeted therapies development. It was also found that despite of the dominant influence of environmental and lifestyle-related LSCC factors, various genetic predispositions to this type of cancer are also observed.

Because of the significance of the process of metastasis formation in this tumor the aim of the dissertation was to determine the importance of the *DIAPH2*, *DIAPH3* and *PCDH17* genes in LSCC pathogenesis and lymph node metastases. The research conducted in this study included the determination of the mechanisms of these genes inactivation in laryngeal cancer.

To perform the research, a number of molecular techniques (DNA and expression microarrays, pyrosequencing, targeted next generation sequencing - NGS and Sanger sequencing) and the immunohistochemical method were applied. *In vitro* studies were performed to inhibit the DNA methylation in LSCC cell lines using decitabine. Depending on conducted experiments, following materials were used: 18 cell lines derived from LSCC and their lymph node metastases; DNA from 108 tumors; 125 tumors embedded in paraffin blocks. Results of the studies were compared with appropriate controls such as DNA obtained from peripheral blood or oral swabs from healthy donors; demethylated and methylated DNA; WGA and other controls described in the main part of the dissertation.

Based on the DNA and expression microarray results as well as literature search, the *DIAPH2*, *DIAPH3* and *PCDH17* genes were selected for further studies. This choice was made because of the recurrent deletions or significant transcriptional downregulation of the selected genes as compared to non-cancer controls. Then, the estimation of the frequency of the alterations of these genes as well as the mechanisms of their inactivation in LSCC was performed.

In the first step, DNA hypermethylation of the *PCDH17* promoter region in all LSCC cell lines ($p < 0.0001$) and increased methylation in most LSCC tumors ($p < 0.0001$) were identified. This suggests that these changes play a significant role in the *PCDH17* silencing in laryngeal cancer. Such results were not observed for the *DIAPH2* gene, therefore further studies were focused mainly on the genetic changes in this gene.

Afterwards, a DNA sequence analysis in the selected genes in LSCC cell lines using Sanger sequencing was performed. The most interesting changes include the deletion of exon 23 of the *DIAPH2* gene with the simultaneous insertion of an intron 23 derived sequence (NM_006729:c.3116_3240delinsGTACTGTTGAGCCATGTTCCTTAACAAAAAGCTAC) in the UT-SCC-17 line. In case of the *DIAPH3* gene, two genetic variants were identified (rs36084898 and rs200345616), which are likely to alter the protein structure and impair its function.

Because of the interesting Sanger sequencing results, further analysis of genetic changes in the *DIAPH2* and *DIAPH3* genes were performed using the NGS method in 95 tumor samples from patients. This analysis led to the identification of four hemizygous changes in the *DIAPH2* gene, mainly in cases with diagnosed lymph node metastases - N+ (3/42). Statistical analysis for the studied group of patients combined with data from the cBioPortal database showed a higher frequency of the *DIAPH2* alterations in metastatic tumors as compared to non-metastatic tumors ($p = 0.036$; $\chi 2$ test).

In the *DIAPH3* gene, the majority of alterations (5/6 variants) are located in gene regions encoding respective protein domains and two of them (rs111260336 and rs150023947) are described as cancer-associated (FATHMM). Moreover, higher frequency of the A|A genotype of the rs111260336 variant was found in the Polish population as compared to the European Caucasian population.

Next, an *in vitro* study on LSCC cell lines using decitabine was performed which confirmed that inhibition of *de novo* DNA methylation restores *PCDH17* transcription. This indicates that the DNA methylation of the promoter region is an important mechanism of silencing of this gene in LSCC.

Moreover, miRNA expression microarray analysis allowed to demonstrate a significant upregulation of a number of miRNAs which potentially regulate the *DIAPH2* and *PCDH17* expression. Additionally, a strong negative correlation was demonstrated between the expression of miRNAs related to the *PCDH17* gene (hsa-miR-15b-5p, hsa-miR-16-2-3p and hsa-miR-30a-3p) and *PCDH17* expression in LSCC.

In conclusion, the results obtained during the performed analysis for this doctoral dissertation allowed for a better understanding of the LSCC molecular background, including the molecular background of metastasis formation, both on the genetic and epigenetic level. Moreover, these results can potentially contribute to the improvement of LSCC diagnosis by providing new biomarkers of carcinogenesis and metastasis and to the development of innovative targeted therapies for this type of cancer.