

## SUMMARY

Head and Neck Squamous Cell Carcinoma (HNSCC) is an important medical and social problem. Despite development in medicine, including better diagnostic and therapy methods, the morbidity as well as mortality rates associated with this type of cancer remain still high. Application of molecular techniques for a better understanding of cancer genetics, identification of potential markers of carcinogenesis or targets for personalized therapy is a promising approach for diagnosis and treatment. So far, numerous studies have been conducted to expand the knowledge of cancer development and progression. Numerous genes were indicated as oncogenes (like *PIK3CA*, *EGFR*) or tumor suppressor genes (such as *TP53*, *RBI*) involved in head and neck carcinogenesis.

Head and neck tumors are very heterogeneous group of cancers. Although they arise in the same site of the body, their biology and genetics is different. Thus, in the current thesis, the studies were focused on tumors developed only in larynx. Identification of alterations specific for carcinogenesis of this site is crucial for diagnosis, therapy and prognosis of patients.

In the current thesis, a group of genes was analyzed in terms of their involvement in laryngeal squamous cell carcinoma (LSCC) pathogenesis and regarding their oncogenic or suppressive potential. Genes were selected based on significant changes in their expression level observed in laryngeal squamous cell carcinoma cell lines as compared to non-cancer control samples. Six genes with increased expression level, namely: *LAPTM4B*, *ATAD2*, *CDK1*, *SERPINH1*, *SNAI2*, *NETO2* and four genes with decreased expression level: *CLCA4*, *FUT3*, *CEACAM6* and *SFRP2* were selected. The correlation of gene expression with clinicopathological data of patients, whose tumors were used to establish the cell lines, allowed to analyze the involvement of genes: *ATAD2*, *CDK1*, *CEACAM6*, *CLCA4*, *FUT3* and *LAPTM4B* in different stages of laryngeal cancer pathogenesis.

To determine the molecular mechanisms leading to the observed gene deregulation several techniques were applied. Changes in gene promoter DNA methylation level were analyzed by bisulfite pyrosequencing for each of the genes and *CEACAM6* was found to be hypermethylated in LSCC cell lines. Next, using DNA demethylating agent (DAC) it was shown that demethylation of *CEACAM6* in LSCC cell lines results in gene expression restoration. However, the results obtained in the current study were insufficient to confirm the potential suppressor function of *CEACAM6* in LSCC.

*In silico* data mining based on cBioPortal database indicated putative copy number alteration of *ATAD2* and *LAPTM4B* genes. However, analysis of data from microarray-based DNA copy number analysis (array-CGH) obtained during the presented study do not support this observation. Also, for the remaining genes analyzed in presented study, no changes of DNA copy number were shown. *In silico* analysis of data collected in the cBioPortal and COSMIC databases made it possible to exclude DNA sequence mutations of analyzed genes as the causative factor of changes in their expression. Therefore, the molecular mechanism causing the observed gene deregulation remained undetermined.

Based on the significantly increased expression of the *CDK1* gene in laryngeal cancer, as well as its function in the cell, *CDK1* was selected as a potential oncogene. To understand its significance in laryngeal carcinoma the consequences of *CDK1* silencing by siRNA in LSCC cell lines were analyzed. Despite the involvement of CDK1 protein in cell cycle control neither the decrease in cell viability nor in proliferation was observed. Thus, the oncogenic potential of *CDK1* was not confirmed in LSCC cell line model. As a reason for the lack of expected effects of *CDK1* silencing, a compensation of CDK1 deficiency by the activity of other genes was proposed. Among the genes most likely involved: *CDK6*, *CALD1*, *FYN* were indicated, as their expression level was highly increased in cell lines following *CDK1* knockdown.

In conclusion, in the current thesis the group of genes with altered gene expression level was analyzed and the potential mechanisms leading to observed gene deregulation were indicated. Presented studies were performed mostly with the use of LSCC cell lines. Therefore, to extend the understanding of the role of the selected genes in the process of carcinogenesis, the analysis of the tumors samples (clinical material) appears to be the next and obligatory step. Moreover, to confirm the oncogenic and suppressor potential of respective genes, functional studies should be extended to include analysis using a non-cancer epithelial cell line or animal model.