IN SEARCH FOR POTENTIAL BIOMARKERS: DEREGULATION OF SELECTED MICRORNAS IN SQUAMOUS CELL SINONASAL CARCINOMA

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Introduction
Malignant tumors arising from nasal cavity and paranasal sinuses make up 3% of all cancers of head and neck area with squamous cell carcinoma (SCC) being the most common subtype. Sinonasal carcinomas (SNC) are characterized by unfavorable outcome due to difficult diagnosis, treatment and prognosis of the disease corresponding with the anatomic complexity of the region. Risk factors for developing SNC include cigarette smoking professional exposure to various cancerogenous substances (wood-dust, leather dust) and HPV infection [1]. MicroRNAs (miRNAs) are short (18 – 25 nt) non-coding RNA molecules that are part of gene expression and their primary role is negative regulation of translation as part of the RNA-induced silencing complex (RISC) [2]. The aim of this study was to investigate relative expression levels of selected miRNAs in squamous cell sinonasal carcinoma samples and to compare the results with recorded clinicopathological data.

Methods
A total of 63 formalin fixed, paraffin embedded samples of squamous cell sinonasal carcinoma and normal sinonasal tissue were analyzed (46 sinonasal cancer samples and 17 samples of control tissue). Relative expression of miR-21, miR-9, miR-145, miR-99a, miR-137, miR-484 and let-7d were measured by real-time PCR with specific TaqMan® Advanced miRNA Assays (Applied Biosystems) on Rotor-Gene Q and calculated using the \(2^{-\Delta\Delta Ct}\) method [3]. One-way analysis of variance and regression analysis were used to analyze the correlation between relative expression levels of miRNA and recorded clinicopathological characteristics such as gender, age at the time of diagnosis, smoking history, occupation, tumor localization, TNM classification, tumor subtype, invasion, recurrence, metastasis or HPV infection status. The Kaplan-Maier method and Logrank test were used to determine overall survival rate and corresponding statistical significance.

Results
Real-time PCR data show statistically significant upregulation of three miRNA miR-21 (\(p < 0.001\)), let-7d (\(p < 0.001\)), and miR-9 (\(p < 0.001\)) in SCC samples in comparison to control tissue. On the other hand miR-145 was significantly downregulated (\(p < 0.001\)). Kaplan-Maier survival analysis showed, that survival of patients with high upregulation of miR-21 (\(p = 0.063\)) and high expression of miR-137 (0.0278) was significantly impaired. Patients with higher upregulation of miR-9 (0.026) and high expression of miR-99a (0.068) survived longer. After comparing relative expression levels of the miRNAs with clinicopathological data, we identified correlation between expression of miR-21 and tumor localization (nasal cavity × maxillary sinus, \(p = 0.026\)). Higher expression of miR-145 was in patients with angioinvasion (\(p = 0.037\)), lower expression of miR-99a had tumors with
perineural spread (p = 0.0055). Expression of miR-137 and let-7d correlated with local recurrence (p = 0.045, p = 0.025), miR-9 expression with regional recurrence (p = 0.045) and miR-145 and miR-484 expression with HPV infection status (p = 0.019 and p = 0.016).

Discussion
Although miRNA research is currently on the rise and many authors investigated expression of miRNAs in many cancerous tissues including head and neck cancer subtypes, there are not many information about miRNA expression and regulation in sinonasal cancer. We observed statistically significant upregulation of miR-9, let-7d and miR-21, which corresponded with other head and neck cancer studies [4] and downregulation of miR-145 also observed by Karatas et al. [5] in head and neck cancer. Kaplan Maier survival analysis showed significant differences between groups of patients with different miRNA expression levels. Higher expression of miR-21 and miR-137 resulted in impaired survival. On the other hand high upregulation of miR-9 and miR-99a corresponded with longer survival time of the patients. This confirms that selected miRNAs might be used as prognostic biomarkers of the disease [6]. Correlation analysis with clinicopathological data shows that levels of expression of some miRNAs correlate with localization of the tumor, angioinvasion, perineural spread, local recurrence and HPV infection status.

Conclusion
Our data show that our selected miRNAs may represent important regulatory molecules involved in development and progression of the disease and survival time of sinonasal carcinoma patients. On top of that, they could be potentially used as valuable prognostic biomarkers of the disease.

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Literature