The role of etiological factors in carcinogenesis in young head and neck cancer patients

PhD thesis

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1. Introduction

The term, head and neck squamous cell carcinoma (HNSCC) in the strict sense includes tumors of the lip, oral cavity, oro-hypopharynx and larynx. HNSCC was estimated to be the sixth most common cancer with approximately 800,000 new cases annually worldwide based on the Global Cancer Observatory (GCO) database. It usually occurs in patients over 50 years, however, in the past half century the incidence also increased in young adults. The most accepted definition for young HNSCC patient is currently an age of ≤39 years defined by the National Comprehensive Cancer Network (NCCN) Adolescent and Young Adult (AYA) Oncology's guideline. The cause of early-onset HNSCC is yet unclear. Of note, when considering etiological factors, it is necessary to highlight that extrinsic (traditional risk factors: tobacco and alcohol consumption) and intrinsic risks (genetic susceptibility) might mutually enhance each others impact and this network of interacting risks is largely yet to be explored. Several further mechanisms may influence

carcinogenesis, of which epigenetic mechanisms play a role in the carcinogenesis.

The enhancer of zeste homolog 2 (EZH2) is a member of the family of polycomb group genes, which are important epigenetic regulators by repressing transcription. EZH2 has a well-defined oncogenic role and is frequently upregulated in different cancer types (prostate, breast, bladder, esophagus, gastric and non-small cell lung carcinoma and lymphomas). EZH2 exerts its impact on tumor viability through the regulation of cell proliferation, apoptosis and tumor progression. It modulates the effect of the cisplatin treatment and it is associated with poor prognosis also in head and neck cancer. Due to its outstanding role in modulating both tumor cell survival and tumor immunity EZH2 seemed to make a suitable target to further characterize the tumors of young patients.

2. Objectives

It is still a matter of debate whether young HNSCC patients might form a distinct disease entity or sub-category. Our aim was to clarify the relation of this group with the general patient population. The study design consisted of two sequential parts.

1. We aimed to characterize this group epidemiologically. Three main aspects seemed to have outstanding relevance. Firstly, we hypothesized that an etiologically distinct group has to have its own age distribution. In contrast, the distortion of the age distribution towards the older age groups is against a group-specific causative factor. Secondly, we aimed to compare the distribution of the primary tumor localizations and assess the prevalence of classical risks (namely smoking and alcohol consumption) in both study groups. We also intended to compare the tumor variables (grade, stage, TNM status) and the prognosis (including 5-year survival rates) in the young and the general HNSCC patient population.

2. We aimed to characterize the molecular phenotype of the tumors of young patients in comparison with the

general HNSCC patient population. This might be indicative of whether tumor characteristics (i.e. aggressivity) or permissive patient factors might rather characterize the increased risk in this patient subpopulation. Having a well-defined oncogenic role in cancer initiation, progression, metastasis, metabolism, and drug resistance, and in the modulation of antitumor immunity, EZH2 has been defined as an effective marker of the tumor aggressivity and tumorigenic potential. Thus, we determined the EZH2 expression in combination with P53 of our examined groups' tumors.

3. Methods

3.1. Study design

In the first part of the study we collected and analyzed the medical records of 85 young patients with histologically verified lip, oral cavity, meso-hypopharynx or laryngeal cancer between 2000 and 2018. Young patients were defined as individuals age under 40 years (≤39) at the time of the diagnosis according to the NCCN AYA Oncology's

guideline. Patient data (age, gender, drug and clinical history, tobacco and alcohol consumption, tumor site, stage, grade, and nodal status) were compared with a control group of 140 consecutive general HNSCC patients from year 2014.

In the second part of the study we involved formalin fixed, paraffin embedded tissue blocks of 68 random young HNSCC patients (≤39 years, between 2000-2018) in comparison with the samples of 58 gender and tumor localization matched general HNSCC subjects (all diagnosed in the year 2014).

3.2. Immunohistochemical staining

Paraffin-embedded tissue sections were used for the confirmation of the diagnosis and the immunohistochemical staining for $p16^{INK4a}$ antigen with Roche CINtec® p16 ($E6H4^{TM}$) antibody (Roche, Basle, Switzerland).

EZH2 expressions of the tumors were also detected by immunohistochemical staining (mouse monoclonal anti-EZH2 antibody, clone 11, BD Biosciences, Franklin

Lakes, NJ, USA). The proportion of the positive tumor cells (%) and the intensity of the nuclear staining (0, 1+, 2+, 3+) were recorded for each slide. The P53 protein levels were also examined by immunohistochemistry (mouse monoclonal anti- P53 antibody, clone DO7, DAKO, Glostrup, Denmark). For P53 staining intensity, the positive control was a monoclonal, P53-expressing, high-grade serosus ovarian cancer cell line. Negative nuclear staining corresponded to a score of 0, focal or heterogenous staining patterns corresponded to a score of 1, and diffuse intensive nuclear staining in ≥80% of the tumor cells was classified as a score of 2.

3.3. Statistical analysis

Statistical analyses were conducted using KyPlot 5.0 (KyensLab Inc., Tokyo, Japan) and Statistica 14.0.1.25 (TIBCO Software Inc., Palo Alto, CA) softwares. Kolmogorov–Smirnov test was used to assess the distributions of numeric variables. T test or Mann–Whitney U test (MWU) were used to compare independent variables. Kruskal–Wallis test was used for non- parametric multiple comparisons and Dunn's test for

post hoc analysis. Chi-square test (CS) and Fisher's exact test (FE) were used to compare the compositions of groups. Survival data were processed using the Kaplan–Meier method, while the survival of subgroups was compared using the Log-Rank (Cox–Mantel–Haenszel) test. Univariate and multivariate analyses of prognostic factors were performed using the Cox's regression model. A $p \leq 0.05$ was considered significant.

4. Results

4.1. First part

In both groups male predominance (young group: 78.9%, male/female: 67/18; general HNSCC group: 74.3%, male/female: 104/36) was found. Two-third of the young age group were 35-39 years old. The prevalence of smoking and alcohol consumption (65.8% and 48.1% respectively) in the young group exceeded the regional population average (30.6%; 32.3%) but was below the

institutional (87.9% and 56.4% respectively) control HNSCC patient population.

Primary tumor sites in the young group were as follows: oral cavity (56.4%), oropharynx (17.6%), hypopharynx (11.7%), and larynx (14.1%). Cumulative five-year overall survival (OS) was 44.2% in the young group. Significantly better OS was observed in patients with early T (T1-2 vs. T3-4: 52.6% vs. 26.7%; $p = 0.0058$) and N0 status (N0 vs. N+: 65.2% vs. 32.3%; $p = 0.0013$).

Univariate Cox regression model for the entire study population revealed that young age, alcohol abstinence, earlier stage and laryngeal tumor site might predict a better prognosis.

4.2. Second part

Lower EZH2 expression was found to be characteristic of the tumors of young vs general HNSCC patients (median EZH2 staining intensity: 1 vs. 1.5 respectively, $p<0.001$; median fraction of EZH2 positive tumor cells: 40% vs. 60% respectively, $p=0.003$, MWU). In the subgroup

analysis a lower EZH2 expression both regarding the proportion of expressing cells and the intensity of staining was characteristic for young patients in all tumor localizations, although the difference only reached statistical signficance for the oral cavity and hypopharynx when regarding proportions. Univariate Cox analysis identified a more advanced T status (T3-4 vs. T1-2), a positive nodal status, and alcohol consumption above the WHO medium risk category as significant predictors of mortality. Neither a higher proportion of EZH2-expressing tumor cells (defined as exceeding the group median of 40%) nor intratumoral P53 expression were identified as significant predictors of an unfavorable outcome among young HNSCC patients.

5. Conclusions

In conclusion our results reveal that early- onset head and neck carcinomas occur mostly between 35-39 years, thus their distribution is heavily distorted toward the older age group. It might suggests that the majority of young

patients are unlikely to carry independent etiologies such as typical tumor predisposition syndromes, as these rather manifest at a younger age and are expect to carry their own age distribution. In the young group the traditional risk factors (tobacco and alcohol consumption) might play a significant role in the development of cancer, however the exposition time of the harmful agents are necessarily shorter. Based on these findings we concluded that young patients with HNSCC might represent an extreme value within the spectrum of the general HNSCC patient population, and we suggest that this group does not form a distinct entity. Histological examination indicated that young patients suffered from significantly more differentiated tumors in comparison with general HNSCC patients group, however there were no other histological markers that differed between the two groups.

We hypothesized increased vulnerability in the background of the early carcinogenesis, which might be either tumor or host (patient factors) induced. For assessing the biological aggressivity of the young patients' tumors our choice fell on EZH2, which has an outstanding

role in modulating tumor cell survival, proliferation and tumor immunity. We found lower EZH2 expression in the young group, which discourages the assumptions that we are dealing with biologically more aggressive cancer phenotypes in young patients. Of note, the P53 immunohistochemical staining intensitiy was similar in the two study groups. Further studies should probably put more focus on patient characteristics, including a potentially altered tumor immunity and also the cellular capacity to restore DNA damages. Understanding the EZH2 mechanisms of action and interplay with other regulator proteins can guide the development of targeted therapies. Pharmacological trials might need to be supplemented with appropriate subgroup studies to investigate potential differences in the efficacy of EZH2 inhibitor therapies in different age groups.

The study was conducted under the ethical permission of the Scientific and Research Ethics Committee of the Medical Research Council (approval number: BMEÜ/3719- 1 2022/EKU) in accordance with the Helsinki Declaration of 1975, as revised in 2008.

6. Bibliography of the candidate's publications

6.1. Publications related to the thesis

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