Markers of progression and prognosis in head and neck cancers

Ph.D. Theses

Kornél Pál Dános M.D.

Semmelweis University
Doctoral School of Pathological Sciences

Supervisor: László Tamás M.D. Ph.D. associate professor

Official reviewers: Péter Móricz M.D. Ph.D., head of hospital dept.
Zsuzsanna Orosz M.D. Ph.D., assistant lecturer

Chairman of Final Examination Committee:
Henriette Farkas M.D. Ph.D., university professor
Members of the Final Examination Committee:
Tibor Glasz M.D. Ph.D., associate professor
Csilla Katona M.D. Ph.D., head physician

Budapest
2015

Introduction

General considerations

Head and neck cancers (HNC) are defined as malignant lesions arising from the mucosal regions of the head and neck. The vast majority of these cancers (90%) are squamous cell carcinomas. According to the GLOBOCAN data published in 2015, about 600.000 new head and neck cancers are diagnosed annually worldwide. In Hungary, this rates about 5000 new cases per year. Regarding its mortality, this entity shows a great diversity: patients with early stage cancers (T1-2N0M0) have an average of 5-year overall survival of 80-90%. The 5-year overall survival of patients suffering from locoregionally advanced (T3-4N0M0 or T1-4N1-3M0) malignancies is between 40-50%, while the median overall survival of patients having recurrent/metastatic cancer is about 7-9 months.

The main factors affecting prognosis are TNM-stage, localization of the primary disease, co-morbidities, the depth of invasion, and in case of lymph node metastasis, the presence of extracapsular spread. Another important prognostic factor is the human papillomavirus (HPV-) status of the tumor, it is known that HPV-positive cancers show a more favorable outcome compared with HPV-negative, alcohol-, and tobacco-related cancers.

The biomarkers involved into the study

p16

$p16^{INK4}$ is a cyclin-dependent kinase (CDK) inhibitor preventing the formation of active cyclin D – CDK 4/6 complex. As a consequence,

the retinoblastoma protein is not phosphorilated, and the cell cycle stops at G1 phase. Several articles have been published for the past decade stating that about 80% of cancers showing $p16^{INK4}$ overexpression are HPV-positive, thus it can be used as a surrogate marker for HPV-positivity.

Ki67

The physiological function of Ki67 has not been fully elucidated yet. However, it is known that this protein is only present in G1, S, G2 and M phase, and is absent in cells being in G0 (resting) phase. Accordingly, the detection of Ki67 can be used as a marker of proliferation.

p53

The main function of p53 (also known as the guardian of the genome) is to prevent getting unrepaired mutations into newly formed cells. Cancers associated with tobacco smoking and/or alcohol consumption often harbor the loss of p53 function caused by mutations. Some studies suggest overexpression of p53 is associated with poorer prognosis.

EGFR

EGFR is a transmembrane protein (receptor) physiologically activated by EGF (Epidermal Growth Factor) or TGF (Transforming Growth Factor). It plays a role in proliferation, differentiation, migration.

Connexin 43

Connexins are structural proteins forming gap juctions. The role of these gap junctions is intercellular communication by transmission of small molecules and ions between adjacent cells. Currently, 21 different connexin isotypes are identified differing from each other in charge selectivity and size. It is known that Cx43 expression is

decreased in breast cancer and renal cell cancers, and that in certain tumors, reduction of connexin expression is associated with poor outcome.

Phosphatidyl-inositol 3-kinase (PI3K)

The PI3K/Akt/mTOR cascade is one of the most commonly disrupted pathways in human cancers. Mutations of the first member of this cascade is frequent in breast and colorectal cancers while its copy number gain is often found in cervical and lung cancers.

c-MET

c-MET is a receptor having tyrosine kinase activity. Its physiological ligand is HGF (Hepatocyte Growth Factor). The protein plays a role in epithelial-mesenchymal transition (EMT), proliferation, migration and invasion. Its overexpression is frequent in squamous cell carcinomas and is often associated with unfavorable prognosis.

Aims

Both scientific literature and our own clinical experience suggest that cancers of the same stage and localization often show notable differences in prognosis and therapeutic responses. The aim of our studies was to identify prognostic markers that can help to predict the tumor aggressiveness and could help in selecting the most appropriate therapeutic option, in particular:

1. To verify if head and neck cancers can be regarded as one entity based on their biomarker expression profile;
2. To investigate the correlation between Ki67 expression and prognosis, disease progression;
3. To investigate the correlation between cytogenetic alterations of phosphatidyl-inositol 3-kinase and c-MET and disease stage;
4. To explore the role of connexin 43 (Cx43) expression alterations in head and neck cancers.

Methods

Patients

We used tissue samples of patients having head and neck squamous cell cancer being treated between 2000 and 2008 at the Department t. of Oto-Rhino-Laryngology of Jahn Ferenc Hospital. Two cohorts were included into our study: one of them consisted of 226 patients treated with irradiation or surgical therapy. The other group included 90 patients treated with radiotherapy.

Histological processing

Tissue Microarray (TMA) block were prepared from the patients' tumor samples. Immunohistochemical staining was applied to detect expression of biomarkers detailed above. In order to detect cytogenetic alterations of PI3K and c-MET, fluorescent in situ hybridization (FISH) was used.

Subsequently, the slides were digitalized.

Histological evaluation, statistical analysis

The evaluation of slides were performed using computers with the Panoramic Viewer software (3DHISTECH). The scoring was carried out by 3 independent assessors. Based on the percentage of positively stained tumor cells, a four-tier scoring system was applied: I: 0-5%; II: 6-20%; III: 21-60%; IV: 60-100%. After the evaluation, the scores were dichotomized. In case of FISH reactions, a modified Cappuzzo system was used: nuclei containing 2, 3 and 4 or above gene copies were registered along with gene/centromere ratios.

Based on the results disomy, trisomy, polisomy and amplification were distinguished.

For statistical analysis, Pearson's chi-square tests or Fischer's exact tests were applied. For survival evaluation, Kaplan-Meier analysis with log-rank tests and Cox-regression were used. The significance level was defined as 0.05.

Results

1. Verifying if head and neck cancers can be regarded as one entity based on their biomarker expression profile

Among the studied biomarkers, Ki67 and EGFR showed significant correlation with anatomical localization.

92.9% of hypopharyngeal cancers, 85.4% of supraglottic tumors, 76.5% of transglottic cancers, 75.4% of oropharyngeal tumors, 58.3% of oral cavity cancers proved to be Ki67-positive, while only 38.5% of glottic cancers were Ki67-positive ($P<0.001$).

Regarding EGFR, the results were as follows: oral cavity – 100%, supraglottic larynx – 95.1%, hypopharynx – 88.1%, oropharynx – 78.3%, transglottic tumors – 76.5%, while in cancers of glottic larynx – 71.1% ($P=0.023$).

2. Investigating the correlation between Ki67 expression and prognosis, disease progression

Significant correlation was revealed between Ki67 expression and survival of patients: patients with Ki67-positive primary disease had significantly worse survival rates compared to those having Ki67-negative disease (median survival: 20 vs. 27 months; HR: 1.619; 95%CI: 1.050-2.498; $P=0.029$). Ki67-positivity was significantly more common in advanced T ($P<0.001$) and advanced N ($P=0.007$) stages.

3. Investigating the correlation between cytogenetic alterations of phosphatidyl-inositol 3-kinase and c-MET and disease stage

When assessing the cytogenetic alterations of these genes, we found that elevated copy number of PIK3CA (the catalytic subunit-alpha of PI3K gene) (i.e. amplification or polysomy) was associated with significantly higher T-stages (P=0.008). No significant correlation between c-MET copy number gain and tumor stage was found.

4. Exploring the role of connexin 43 (Cx43) expression alterations in head and neck cancers

A strong positive correlation was found between disease specific survival of patients and the expression of the protein: Cx43-positive cancers showed a significantly better prognosis (HR: 0.509; 95% CI: 0.315 – 0.822; P=0.006). The median survival time in the Cx43-positive group was 15 months, while only 5 months in the Cx43-negative group (log-rank P=0.004). Significant correlation was found between Cx43 and p53 expression: p53 overexpression was associated with high (normal) Cx43 levels. (P=0.036).

Conclusion

1. While studying the biomarker expression profiles of head and neck squamous cell cancers, we found that, nevertheless these cancers possess similar histological appearance, they cannot be regarded as a same entity based on their protein expression profiles. Overexpression of the proliferation marker Ki67 was significantly more common among hypopharyngeal cancers, whereas proved to be the least frequent among glottic tumors. Similarly, EGFR overexpression was detected all in the oral cancers (100%), whereas it was the least frequent in glottic tumors.

2. Significant correlation was unveiled between Ki67 expression and disease-specific survival of the patients. Ki67 positivity was associated with worse prognosis. Additionally, Ki67-positivity was associated with higher T and N stage. Knowing the facts that hypopharyngeal cancers show the worst survival rates, in contrast glottic cancers having the best ones among head and neck cancers, and glottic cancers harbor Ki67 positivity least frequently while it is the most frequent among hypopharyngeal cancers, we can conclude that the higher proliferation rate (thus the more aggressive behavior) of hypopharyngeal cancers might be accountable for the difference in prognosis (and vice-versa).

3. Amplification and polysomy phosphatidyl-inostiol 3-kinase (more precisely, its catalytic subunit alpha) were more frequent in advanced tumor (T) stage. No correlation between c-MET copy number gain and tumor stage was found.

4. The loss of or decrease in connexin 43 expression was associated with significantly poorer prognosis. Interestingly, the Cx43-positive (i.e. normal Cx43 level) cancers harbored p53 overexpression more commonly. This observation could lead to the conclusion that the disruption of Cx43 expression might be dominant in cancers with normal p53 expression.

Author's own publications:

Publications used in the Ph.D. thesis

Danos K, Brauswetter D, Birtalan E, Pato A, Bencsik G, Krenacs T , Petak I, Tamas L
The Potential Prognostic Value of Connexin 43 Expression in Head and Neck Squamous Cell Carcinomas
APPLIED IMMUNOHISTOCHEMISTRY & MOLECULAR MORPHOLOGY In press: p. In press. (2015)
DOI: 10.1097/PAI.0000000000000212

Szentkuti G, **Danos K**, Brauswetter D, Kiszner G, Krenacs T, Csako L, Repassy G, Tamas L
Correlations Between Prognosis and Regional Biomarker Profiles in Head and Neck Squamous Cell Carcinomas.
PATHOLOGY AND ONCOLOGY RESEARCH 21:(3) pp. 643-650. (2015)
DOI: 10.1007/s12253-014-9869-4

Publications related to, but not used in the Ph.D. thesis

Szentkuti G , Brauswetter D , **Danos K** , Birtalan E , Tamas L
A fej-nyaki daganatok magas incidenciájának és mortalitásának okai hazánkban.
OTORHINOLARYNGOLOGIA HUNGARICA 60:(3) pp. 77-80. (2014)

Tamas L , Szentkuti G , Eros M , **Danos K** , Brauswetter D , Szende B , Zsakovics I , Krenacs T
Differential Biomarker Expression in Head and Neck Cancer Correlates with Anatomical Localization
PATHOLOGY AND ONCOLOGY RESEARCH 17:(3) pp. 721-727. (2011)
DOI: 10.1007/s12253-011-9376-9