HPV18 Associated with E-cadherin Expression in Head and Neck Squamous Cell Carcinoma

SIMINA BOIA[1], EUGEN RADU BOIA[2], RALUCA AMALIA CEAUSU[3]*, CONSTANTIN NICOLAE BALICA[2], OVIDIU ALEXANDRU MEDERLE[3]

[1]Victor Babes University of Medicine and Pharmacy, Periodontology Department, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania,

[2]Victor Babes University of Medicine and Pharmacy, ENT Department, 2 Eftimie Murgu Sq, 300041, Timisoara, Romania

[3]Victor Babes University of Medicine and Pharmacy, Department of Microscopic Morphology/Histology, Angiogenesis Research Center, 2 Eftimie Murgu Sq, 300041, Timisoara, Romania

HPV is an important oropharyngeal cancer cause, but it may have a role in other head and neck cancers? HPVpositive head and neck squamous cell carcinoma (HNSCC) epithelial-mesenchymal transition role is unclear. We included 38 cases: 20 laryngeal, 3 corresponding lymph nodes; 5 oropharyngeal, 5 hypopharyngeal, 2 rhynopahryngeal, 2 pharyngolaryngeal and 1 naso-sinusal case. Immunoreactivity was positive in nuclear expression cells, accordingly: score 1 (10-30%), 2 (30-50%) and 3 (>50%). HPV18 immunoexpression appeared in 18 cases (47.36%), (11 laryngeal, 4 oropharyngeal, 1 hypopharyngeal, 1 pharyngolaryngeal and 1 naso-sinusal). The score was 1 in larynx well differentiated type. The score was between 1 and 3 in larynx moderately differentiated types, and a significant correlation HPV18/E-cadherin was found (p=0.031). HPV18+/E-cadherin low values were noticed in larynx, oropharynx, pharyngo-larynx and naso-sinusal well and moderately differentiated types. HPV18-/E-cadherin low values were present in larynx, hypo and rhyno-pharynx moderately and poorly differentiated and larynx well differentiated types. Larynx presented HPV18/E-cadherin and moderately differentiated type significant correlation. Rhyno, hypo-pharyngeal and laryngeal presented HPV18–/E-cadherin low values association for moderately, poorly and undifferentiated types. The oropharyngeal location was associated with E-cadherin maximum values, independently of HPV18 status.

Keywords: HPV18, head and neck squamous cell carcinoma, mesenchymal epithelial transition

Characterized by phenotypic, biological, aetiological, and clinical heterogeneity, HNSCCs represented the sixth most common cancer worldwide, affecting 600.000 new patients each year [1].

Human papillomaviruses (HPVs) are a heterogeneous group of small non-enveloped epitheliotropic DNA viruses belongs to the Papillomaviridae family and targeting the basal cells of stratified epithelia at either mucosal or cutaneous sites. The HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 were classified by the IARC (International Agency for Research on Cancer) Working Group as carcinogenic and 68 type as probably carcinogenic to humans [2].

HPV was found in 25% of all HNSCC [3, 4]. HPV represented the major cause of oropharyngeal (tonsillar and tongue base) cancer in developed countries, detected in 45–90% of cases, in a smaller subset of laryngeal (24%) and oral cavity cancers (23%) [5-8]. The demonstrated role of HPV18, until now, is minor compare to HPV16. Using HPV16 and 18 type-specific PCR, Quintero et al. showed that 20% of primary HNSCCs analysed cases were HPV positive. Among these 82% were HPV16 and 18% were HPV18 positive cases [9].

While HPV is an important cause of oropharyngeal cancer, it is unclear whether HPV may have a role in head and neck cancers with other location.

E-cadherin, a calcium-dependent cell-surface protein, characterized by long cytoplasmic and extracellular domains, is the main protein of adherents junctions that anchor oral epithelial cells to each other. Regarding the involvement of E-cadherin in HNSCCs there is some major direction debate in the literature. Such as, it was showed that aberrant E-cadherin expression was associated with a poor prognosis in patients with HNSCC [10]. Loss or sequestration of E-cadherin in the nucleus releases β-catenin, which translocates to the nucleus to induce transcription of EMT genes [11]. It was found that HPVpositive oropharyngeal squamous cell carcinoma tissues were significantly associated with EMT-induction, progression of lymph node metastasis and better prognosis than HPV negative cases [12]. Some studies demonstrated the lowest E-cadherin levels in poorly differentiated type of HNSCC, the hypermethylation of the E-cadherin gene and a similar expression between primary tumors and metastases, perhaps because of MET in metastatic tumors [13, 14]. It was shown the involvement of E-cadherin reduced production in the inhibition of protective immune responses in HNSCC as a result of the activation of migration and function of Langerhans and dendritic cells [15].

The aim of this work was to describe the possible correlations between HPV18 and E-cadherin expression for different location of head and neck cancer.

Experimental part

Material and method

We included in the present study 38 cases of HNSCC with different localisation: larynx (20 cases) and 3 corresponding lymph nodes, oropharynx (5 cases), hypo-pharynx (5cases), rhynopahrynx (2cases), pharyngo-larynx (2 cases) and naso-sinusal (1 case). Immuno-histo-chemical techniques included heat-induced epitope retrieval with Novocastra Bond Epitope Retrieval Solution 2, a ready-to-use, *p*H 9.0 solution (Leica Biosystems,

* email: ra.ceausu@umft.ro

All authors contributed equally to this work.

Newcastle Ltd, Newcastle UponTyne NE 12 8EW, U.K.) for 20 minutes. Endogenous peroxidase blocking was realised with 3% hydrogen peroxide for 5 minutes. E-cadherin (monoclonal, clone 36B5, ready to use, Leica Biosystems, Newcastle UponTyne, U.K., 30 minutes incubation time) and HPV 18 (clone BF7, dilution 1:50, Novus Biologicals, Cambridge, U.K. CB4 0FQ, 30 minutes incubation) were used as primary antibodies. The Bond Polymer Refine Detection System was used for visualisation. As chromogen 3, 3 diamino-benzidine dyhidrochloride was applied for 10 minutes and hemotoxylin for 5 minutes, as counterstain. The entire immunohistochemical procedure was performed with Leica Bond- Max (Leica Biosystems, Newcastle uponTyne, U.K.) autostainer.

Immunoreactivity was estimated as positive in the cells with nuclear expression, according to the following score: score 1 (10-30%), score 2 (30-50%) and score 3 (>50%) positive cells. Microscopic evaluation and image acquisition was performed with Axiocam 506 color, Zeiss, Jena, Germany.

Results and discussions

Histopathological evaluation indicated the presence of well (6 cases), moderately (27 cases), poorly (4 cases) and undifferentiated (1 case) types.

The immunoexpression of HPV 18 was noticed in 18 cases, with the following distribution: larynx (11 cases), oropharynx (4cases), hypopharynx (1 case), pharyngo-larynx (1 case) and naso-sinusal (1 case). From the 3 lymph node corresponding to larynx cases, 2 were positive and one negative for HPV18.

In the category of laryngeal HNSCC well differentiated cases, 3 cases were HPV18 positive and one case was negative. We noticed a score value of 1 (10-30% positive tumor cells) in all of these cases. As a particular aspect, the stromal cells with cytoplasmic expression was present in one irradiated case (fig. 1a).

The corresponding lymph node was HPV18 positive. The case score value was 2, with heterogeneous distribution in the tumor area, the highest nuclear intensity cells disposed in tumor area periphery, in lymphoid tissue vicinity (fig. 1b).

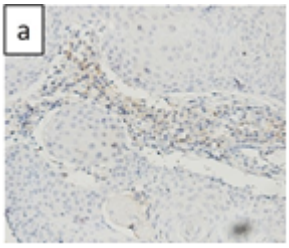

Fig. 1a. Stromal cells HPV18 immunoexpression, magnification X200.

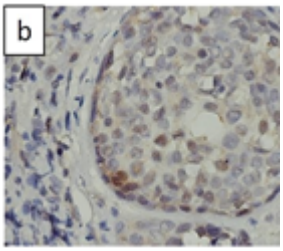

Fig. 1b. Larynx SCC lymph node metastasis, the most intensely HPV18 positive cells in tumor area periphery, magnification X400.

Moderately differentiated carcinomas were evaluated in 13 cases, 6 cases being HPV18 positive. The score values were 1 (3cases), 2 (2 cases) and 3 in 1 case. The distribution pattern was heterogeneous, with tumor area center and periphery highest nuclear expression cells (fig. 1c).

The HPV18 positive stromal cells were found. One of the corresponding lymph nodes was positive and one negative. The positive one presented a score value of 1 and tumor area isolated positive cells, without a predominant disposition to the tumor area periphery.

One of the two poorly differentiated cases with laryngeal origin was HPV18 positive. The score value was 1, with the most intense reaction in the cells nucleus, from the tumor area center. In the quasinormal larynx, the HPV18 nuclear immunoexpression was noticed in basal, intermediate and superficial layers cells (fig. 1d).

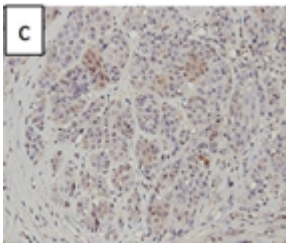

Fig. 1c. Larynx moderately differentiated SCC, heterogeneous distribution pattern, magnificationX100.

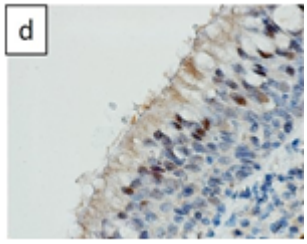

Fig. 1d. Basal, intermediate and superficial layers quasinormal larynx cells HPV18 nuclear immunoexpression, magnification X400.

At the pseudostratified and stratified epithelium junction, HPV18 expression remained in the basal cell nuclei and in suprabasal layer (fig.1e).

In the 5 cases of oropharyngeal HNSCC the tumor grading was G2. In 2 out of 5 cases we encountered the highest score value of 3 and the immunoexpression extended in the surface epithelium full height and the most intense reaction was found in tumor area both center and periphery (fig. 1f).

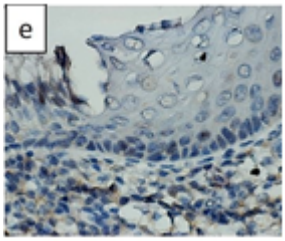

Fig. 1e. The border between pseudostratified and stratified epithelium, larynx poorly differentiated SCC – cell's nuclei HPV 18 expression in the basal and suprabasal layers, magnification X 1000.

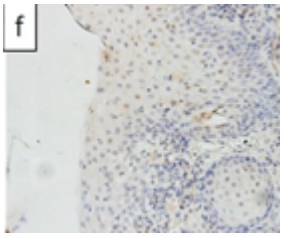

Fig. 1f. Surface epithelium and tumor area HPV18 immunoexpression (oropharynx moderately differentiated SCC), magnification X200.

The value score of 2 (1 case) and 1(1 case) were noticed, as well.

Besides oropharynx, the score value of 3 was present in naso-sinusal region (G3 type). The distribution pattern was heterogeneous, with tumor area periphery highest intensity.

The hypo-pharynx was characterized by the score value of 2. The positive cells were found in the tumor area, with highest intensity in the periphery.

The maximum E-cadherin immunoexpression score value was 3.

HPV18 positive cases, presented this value as follow: larynx, well differentiated type (2 cases), moderately differentiated type (5 cases), poorly differentiated type (1 case), oropharynx (2 cases) and hypo-pharynx (1 case).

The E-cadherin score value of 3 was found in a few HPV18 negative laryngeal cases, well differentiated type (1case), moderately differentiated type (1 case), 1 oropharyngeal case, 1 hypopharyngeal case, 1 pharyngo-laryngeal case and 1 rhyno-pharyngeal case.

The E-cadherin score value of 2 was noticed in the HPV18 positive cases, according to the histological grade: larynx well differentiated (1 case) and moderately differentiated type (6 cases), oropharynx (1 case),

pharyngo-larynx (1 case) and 1 poorly differentiated naso-sinusal HNSCC.

The HPV18 negative cases were not associated to E-cadherin immunoexpression score value decrease well differentiated type, only in moderately, poorly and undifferentiated types of HNSCC. Six laryngeal HNSCC cases diagnosed as G2 and 3 hypo-pharyngeal HNSCC cases (G2, G3) were characterized by the HPV18 absence and of and E-cadherin score value of 2.

Two laryngeal and hypo-pharyngeal HNSCC poorly differentiated cases and one rhyno-pharyngeal un-differentiated case presented HPV18 negative and E-cadherin score value of 2.

In cases of laryngeal lymph node metastases the HPV18 positive and negative had the same E-cadherin score value of 3. A significant correlation between the HPV18 and E-cadherin immunoexpression was noticed in moderately differentiated laryngeal HNSCC cases ($p=0.031$). The E-cadherin immunoexpression was noticed in the tumoral islands (fig. 1g) and covering epithelium, in suprabasal and intermediary layers (fig. 1h).

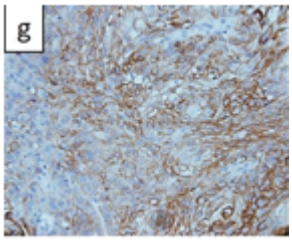

Fig. 1g. Tumor islands Ecaderin immunoexpression, magnification X200

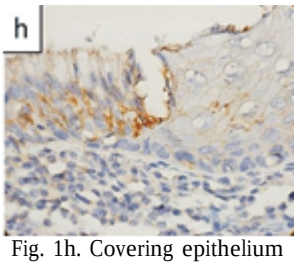

Fig. 1h. Covering epithelium Ecadherin immunoexpression, larynx poorly differentiated SCC, magnification X1000

The HPV16 and HPV18 are among the high risk classified types, of both, genital and non-genital tract. Together with alcohol and tobacco consumption, they represent the main risk factors for the development of HNSCCs, especially with tonsils and tongue base localization.

Some data [3, 16] showed the role of HPV in different head and neck cancer such as larynx and oral cavity, but in smaller proportion, compare with oropharyngeal cancer. It was noticed that the distribution of HPV in HNSCCs may vary with site. The rare presence of HPV18 in the oropharynx was confirmed, heaving a special tropism for glandular tissue [3]. Other study showed a 17% of HPV18 positive and a 69.2% for HPV16 in the laryngeal carcinoma cases [17]. In our study, 47.36% of cases were HPV18 positive, with the following distribution: larynx (28.94%), oropharynx (10.52%), hypo-pharynx (2.63%), pharyngo-larynx (2.63%), naso-sinusal (2.63%) and rhyno-pharynx (0%).

Literature data report a favourable prognosis in patients with HPV-positive HNSCCs [18-20]. On the contrary, some studies showed no association between HPV positivity and patient prognosis [21-23]. The others underlined that the HPV-positive subgroup correlates with a higher risk of recurrence or developing a second primary tumor [24, 25]. In our study we noticed in a case, which received treatment, tumor and stromal cells HPV18 presence.

The HPV18 positive stromal cells were also found in our study in the moderately differentiated type of larynx HNSCC, and a HPV18/E-cadherin immunoexpression significant correlation was identified.

Kim et al. [26], demonstrated in their study that the E-cadherin negative group has more moderate and poor differentiation type than the higher E-cadherin expressing group. In our study, we noticed that the HPV18 negative cases were associated with the lower score values of E-cadherin immunoexpression in the larynx, hypo-pharynx, rhyno-pharynx moderately, poorly and undifferentiated type.

In a study which used the p-16 positive CERV196 and p-16 negative HNSCC22B SCC cell lines, Umbreit et al.[27], found a strong expression of beta-catenin and E-cadherin in both SCC lines, independently of HPV status, but the last one, positive influenced by treatment time with EGF and EGF/TGF beta 1. We found a maximum value of E-cadherin score, to the positive and negative HPV18 cases localized only in oropharynx.

Conclusions

A significant correlation between HPV18, E-cadherin immunoexpression and moderately differential grade was found in the laryngeal cancer. The rhyno, hypo-pharyngeal and laryngeal HPV18 negative cases presented a decrease of E-cadherin values for the moderately, poorly and undifferentiated types. The maximum value for E- cadherin, independently of HPV18 status was associated with the oropharyngeal sub-sites.

Acknowledgements:This work was supported by internal funds from Victor Babes University of Medicine and Pharmacy, Timisoara, Romania: Doctoral Grant No.: 13901/19.11.2014; Research Grant No. PII-C5-TC-2017-07; Research Grant No. 15250/19.12.2012

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Manuscript received: 15.02.2018