Send Orders for Reprints to reprints@benthamscience.ae

Current Topics in Medicinal Chemistry, 2017, *17*, 1-5

REVIEW ARTICLE

Searching for New Targets and Treatments in the Battle Against Squamous Cell Carcinoma of the Head and Neck, with Specific Focus on Tumours of the Tongue

Nicola Sgaramella[1,2,3,*], Xiaolian Gu[1], Linda Boldrup[1], Philip J Coates[4], Robin Fåhraeus[1,4,5], Luigi Califano[3], Gianpaolo Tartaro[2], Giuseppe Colella[2], Lena Norberg Spaak[6], Adrian Ström[6], Torben Wilms[6], Lorenzo Lo Muzio[7], Giovanni Dell'Aversana Orabona[3], Mario Santagata[2], Lotta Loljung[1], Riccardo Rossiello[8], Karin Danielsson[9], Klas Strindlund[1], Sandra Lillqvist[1] and Karin Nylander[1,*]

[1]*Department of Medical Biosciences, Umeå University, Umeå, Sweden;* [2]*Second University of Naples, Multidisciplinary Department of Medical, Surgical and Dental Specialties, Naples, Italy;* [3]*Department of Neuroscience Reproductive and Dentistry Sciences, University of Naples Federico II, Naples, Italy;* [4]*RECAMO, Masaryk Memorial Cancer Institute, Zluty kopec 7, 656 53 Brno, Czech Republic;* [5]*University Paris Diderot, INSERM UMRS1162, 27 rue Juliette Dodu, Paris, 75010, France;* [6]*Department of Clinical Sciences/ENT, Umeå University, Umeå, Sweden;* [7]*Department of Clinical and Experimental Medicine, University of Foggia, 71122 Foggia, Italy;* [8]*Dipartimento Universitario di Anatomia Patologica, Seconda Universita' Degli Studi di Napoli, Piazza Miraglia, Naples, Italy;* [9]*Department of Odontology, Umeå University, Umeå, Sweden*

Abstract: Squamous cell carcinoma of the head and neck, SCCHN, is a heterogeneous group of tumours not only concerning the site of origin but also regarding aetiology. The 5-year survival for the whole group of SCCHN tumours has not significantly improved over the last 20-25 years. Apart from tumour spread to lymph nodes, N status, gains and losses of specific chromosomes are the only factors shown to be independent prognostic markers for these tumours. Worldwide, an increasing number of people ≤ 40 years are seen being affected by tongue SCC, the most common tumour within the SCCHN group. Even without any clinical signs of metastasis, up to 30% of all tongue SCC have histologically detectable spread to lymph nodes.

In this mini review, field cancerization, tumour microenvironment, the so called EMT (epithelial mesenchymal transition) process and the role of viruses in development of SCCHN are discussed as well as potential new therapeutic targets.

For the group of tongue SCC, with the increasing incidence seen in young patients and particularly women, new data with impact on prognosis and treatment are urgently needed. But as long as data from the analyses of several sub sites are presented as valid for the whole group of tumours, this vital point is missed.

ARTICLE HISTORY

Received: August 31, 2017
Revised: November 29,2017
Accepted: December 11, 2017

DOI:
10.2174/1568026618666180116121624

Keywords: Squamous cell carcinoma, tongue, prognosis, therapy, miRNA, HPV, EBV, p63.

1. SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK, SCCHN, AND SUB-SITES

The group squamous cell carcinoma of the head and neck, SCCHN, encompasses tumours in different anatomical locations, such as oral cavity, pharynx, larynx, nasal cavity, paranasal sinuses and salivary glands. Of the SCCHN tumours located in the oral cavity, the majority are tumours in the tongue, which is a complicated organ with varying histology. The tongue is covered by squamous epithelium which on the upper surface has developed into specific papillary structures, some of which harbour taste buds. Most SCC of the tongue develop on the lateral border, where the two types of epithelia meet. Interestingly, it is clear that tongue SCC differ from SCC in other intra-oral sites regarding, for example, expression of proteins and miRNAs [1, 2]. Also in clinically normal tissue adjacent to tumour, a difference can be seen based on the sub-site, showing the need to take sub-site into consideration in studies of the group of SCCHN, also when concentrating on intraoral SCC only [1, 2].

All over the world, there is a worrying trend with an increasing number of young people (≤ 40 years) being affected by tongue SCC [3]. In the whole group of SCCHN tumours,

*Address correspondence to these authors at the Department of Medical Biosciences, Bldg 6M, 2nd floor, Umeå University, SE-901 85; Umeå, Sweden; Tel/Fax: +46 705580523; E-mail: karin.nylander@umu.se (K. Nylander) Second University of Naples, Multidisciplinary Department of Medical, Surgical and Dental Specialties, Naples, Italy; Tel/Fax: +39 3393313912; E-mail: sgaramellanicola12@gmail.com (N. Sgaramella)

1568-0266/17 $58.00+.00

there is no difference in the prognosis between young and "normal aged" patients [4], whereas among tongue SCC, a significantly higher frequency of recurrence has been reported in young compared to old patients [5].

The 5-year survival for the whole group of SCCHN tumours has not significantly improved over the last 20-25 years. One factor of major prognostic importance is the presence of lymph node metastases (N-status). Even without any clinical signs of metastasis, up to 30% of all tongue SCC have histologically detectable spread to lymph nodes [6, 7]. This is possible due to a well developed net of lymph vessels in the tongue, which also crosses the midline, easing spread to the contra lateral side [6].

Even if there are several hundreds of published reports concerning prognostic factors only a few such as gains of chromosome 3q21-29, 11q13 and loss of 8p21-22, have shown to be independent prognostic markers, with even higher significance than clinical node status [8].

2. FIELD CANCERIZATION AND TUMOUR MICROENVIRONMENT

For tongue SCC and other intra-orally located SCC, the so called "field effect" or "field cancerization" is a well known phenomenon. Since it was first described 60 years ago, the definition has slightly changed and now, based on molecular knowledge, is "one or more areas consisting of epithelial cells that have genetic alterations" [9]. Thus, not only the tumour itself but also the surrounding tissue shows genetic changes related to the neoplastic process. For intra-oral SCC, such genetically changed fields can be detected within 7 cm from the tumour [9], meaning that practically the whole oral cavity is affected. These changes can not be seen in routine histology, but could be of great value for early detection of a relapse or a newly arising tumour. One example of a field cancerization change is deregulation of microRNA 424, miR-424. miRNAs which comprise around 3% of the known genes in the human genome can either have tumour suppressor function or act as oncogenes (oncomiRs) [10], and regulate up to 30% of all human genes [11]. For miR-424, levels in tongue tissue adjacent to the tumour were significantly lower compared to both tumour and normal tongue tissue from healthy volunteers indicating that, apart from being a marker of field cancerization, miR-424 could also be important for tumour development [12]. However, comparing miRNA profiles from different studies of tongue SCC does not give a uniform pattern of up- and down-regulated miRNAs [7], and results also vary in cell lines derived from tongue SCC. The thought of using miRNAs as both biomarkers and potential therapeutic targets remains tempting and investigations are ongoing [13]. A complicating factor is the potential ability of each individual miRNA to target hundreds or even thousands of different mRNAs [10], a fact that must be taken into consideration when planning new therapeutic strategies.

At this stage the role of miRNAs as biomarkers seems clear, and mapping alterations in expression of them in tumour and the surrounding field in individual patients provides information about abnormalities and also shows how the tumour influences its' stroma, the so called tumour microenvironment.

In connection to most tumours a host response in the form of inflammation can be seen. Tongue SCC patients with a dense inflammatory infiltrate as judged by the grading introduced by Brandwein-Gensler and coworkers [14,15] have better prognosis compared to patients with tumours with limited or no inflammatory response [5]. Regulation of inflammation in correlation to tumours is a complicated process. One important factor is OAS2 (oligoadenylate synthetase 2), which plays a role in down-regulation of the specific T-cell response [16]. When comparing tongue SCC to tonsillar SCC, tongue tumours show considerably higher expression of OAS2 [56], indicative of a less active T-cell response in this disease, which also has a worse prognosis.

Another factor involved in the inflammatory response is PD-L1, programmed death ligand 1, which by binding to the co-receptor PD-1 on T-cells can inhibit T-cell proliferation and thus a proper inflammatory response [17]. As levels of PD-L1 are upregulated in many types of tumours and its expression has been correlated with poor prognosis, blocking of the receptor with antibodies is a potential way of enhancing immune reactivity against tumours. So far, pre-clinical studies from blocking of the PD-L1:PD-1 pathway in SCCHN show promising results with increased anti-tumour immune responses. To have effect, there is need for an activated immune system in the microenvironment of the tumour [18].

3. TUMOUR SPREAD

In the process of tumour spread, Epithelial Mesenchymal Transition, EMT, is important, at least *in vitro*, whereas its existence *in vivo* is not convincingly proven so far [19]. During EMT, cells of epithelial origin achieve mesenchymal characteristics, measured as loss and gain of epithelial- and mesenchymal-specific markers, respectively. This more mesenchymal appearance eases spread through a mesenchymal environment like the connective tissue. One epithelial-specific factor down-regulated during EMT is E-cadherin, an epithelial calcium dependent adhesion molecule [20] important in cell adhesion through binding to β-catenin [21,22]. When E-cadherin is down regulated, β-catenin is no longer attached to the cell membrane and is instead transported into the nucleus where it can act as an oncogenic transcription factor [23]. A connection between levels of E-cadherin and prognosis has been seen in SCCHN, where tumours with low expression show poor prognosis in contrast to tumours with high expression showing good prognosis [24].

When evaluating the effect of anti PDL-1 therapy in melanomas, it was found that β-catenin within the tumours caused exclusion of T-cells and thus also resistance to this therapy [25]. If results are valid also in other tumours, targeting of β-catenin could be used to gain an immunostimulatory effect in SCCHN.

A protein known to enhance cell motility and tumour invasion in for example SCC is the transmembrane protein podoplanin, PDPN, [26] which apart from being expressed by certain tumour cells, is also expressed in endothelial cells in lymph vessels. Young patients with tongue SCC ($\leq$ 40 years) show significantly higher levels of this protein compared to old patients, indicative of a specific role for podoplanin in young patients [27]. The reason for this is not

known, but as a PDPN-antibody and lectin have shown the ability to inhibit PDPN expression and thus also motility and invasion [28], PDPN is a potential therapeutic target in the group of young patients with tongue SCC and a known worse prognosis [27].

4. VIRUS

4.1. Human Papilloma Virus, HPV

Based on the increase seen in oropharyngeal cancers caused by HPV [29, 30], the importance of viruses in development of SCCHN has been a focus for discussion. Both oropharyngeal and oral SCC infected with HPV have shown better outcome compared to non-infected tumours [31, 32]. In the clinical situation, detection of the p16 protein has been used as a surrogate marker for high risk HPV in many pathology laboratories, especially types 16, 18, 31, 33, 34, 35, 39, 51, 52, 56, 58, 59, 66, 68 and 70, but p16 can also be expressed independently of HPV [33]. In tonsillar SCC, expression of p16 shows high concordance with HPV infection [34], whereas HPV16 is not detected in tongue SCC by *in situ* hybridization, but p16 is expressed, and thus there is no correlation between HPV16 and p16 expression in this tumour type [35]. Whether this completely excludes involvement of high risk HPV in tongue SCC is hard to say, as it must be kept in mind that smaller amounts of virus may need more sensitive methods for detection.

The reason for less HPV-infected tongue SCC compared with tonsillar SCC is not easily explained, especially not as the possibility for the virus to enter the tissue seems comparable between the two, judged by presence of the HPV-receptor syndecan-1 [36] which is equally expressed in tongue and tonsil [35].

Looking at p16 expression irrespective of HPV-status, an improved prognosis is seen for p16-positive tongue SCC. This could be due to many causes, for example the ability of p16 to induce a senescent phenotype resulting in slower tumour growth, or the role of p16 in repair of DNA double strand breaks [33, 37, 38, 39]. The use of p16 as a clinical indicator of worse prognosis particularly in patients $\leq$ 40 years with tongue SCC could thus be of value [35] advocating more aggressive treatment.

With the ongoing vaccination programme against high risk HPV types (primarily introduced to prevent cervical cancer) it will be interesting to see if, in the long run, this affects the increasing frequency of tongue SCC seen among young people, and primarily women. Such a decrease would thus indicate a role for HPV also in development of tongue SCC, but in doses too low to be detected with the common methods used today. If this is seen, a wider programme for vaccination could be implicated.

4.2. Epstein Barr Virus, EBV

Epstein Barr virus, EBV, or human herpesvirus 4, is one of the most common human viruses found all over the world. During their life time, the majority of people at some point become infected. In general the primary infection occurs in the first few years of life and is asymptomatic, but infection at later stages, typically in adolescents or young adults, manifests as infectious mononucleosis in around half of the cases [40]. A strong association between EBV infection and nasopharyngeal cancer has been known for many years.

EBV-infected cells, where B-cells are the primary target, express so called EBV-encoded RNAs, EBERs, which are small non coding RNAs of two types, EBER-1 and EBER-2 [41]. Detection of EBERs by *in situ* hybridization is therefore used as a sign of EBV infection in tissue. As EBERs can induce transcription of growth factors in different cell types, they are important contributors to carcinogenesis in tissue of lymphoid as well as epithelial origin [42].

In clinically healthy normal oral mucosa, EBV infection is rarely detected, whereas in the whole group of oral SCC up to 40% of tumours are infected [43]. Looking at tongue SCC specifically, 67% were positive, all showing signals from epithelial cells as well as lymphocytes using *in situ* hybridization. In only two cases, signals from lymphocytes only were seen, showing the high propensity of epithelial cells to be EBV infected [44]. In contrast, our own recent data showed no evidence of EBV infection in SCC of the mobile tongue [57].

More recently the importance of interaction between different types of viruses in tumour development has been discussed and for tongue SCC a case of a young woman with both HPV18 and EBV infection has been reported [45]. The value of this co-infection is difficult to evaluate based on individual cases, but could indicate the importance of mapping not only HPV but also EBV status in these tumours. This is further supported by results from a recent study of nasopharyngeal carcinoma (NPC) indicating that a vaccine inducing antibodies against the EBV glycoprotein, gp350, as well as B-cell neutralizing antibodies, could reduce the risk of developing NPC and thus hopefully also other EBV-related cancers [46]. Also other EBV-encoded proteins like EBNA1 and ZEBRA are promising therapeutic targets for treatment of EBV-infected cancers [47].

4.3. p63

The individual members of the p63 family play important roles at different stages in formation of the oral mucosa [48, 49, 50]. When studying their role in tumours, results from the whole group of SCCHN vary, with some showing p63 expression to be correlated to poor outcome and aggressive behaviour [51, 52]. Looking at tongue SCC specifically, high expression of p63 correlates to poorer survival [53].

When treating p63 expressing tumours with cisplatin, the drug specifically degrades p63 and thereby withdraws an important signal for survival [54]. When exposing cells *in vitro* to cisplatin, p63 upregulates several miRNAs affecting DNA methylation. By inhibiting these miRNAs as well as the epigenetic enzymes they regulate, cisplatin resistant cells became more sensitive to cisplatin exposure [55]. As resistance to cisplatin is a clinical problem, this finding could be of potential clinical relevance.

PERSPECTIVES

As pointed out previously, SCCHN is a heterogeneous group of tumours not only concerning site of origin but also regarding aetiology. Whereas smoking and alcohol are well known risk factors for tumours in many of these sites, UV-

irradiation is the major risk factor for developing lip cancer, a tumour type often included in studies of SCCHN tumours. This pinpoints the very important fact that it is reasonable to include the whole group of SCCHN in analysis, but when it comes to interpretation of results dividing samples based on sub-site is a prerequisite for aiding new knowledge of potential value to the field of these tumours. Another important aspect is the need for inclusion of full clinical data ensuring complete control over how samples have been treated and handled.

When it comes to evaluating data, many different systems are used, making it complicated or sometimes even impossible to compare findings between different research groups. One typical example being choice of scoring system in evaluation of immunohistochemical stainings. But before reaching that level of discrepancies between studies, it is important to state clearly how analyses were performed. In the case with immunohistochemical stainings, some use an automated staining machine whereas others perform manual staining. Conditions can be considered stable when using an automated staining machine, whereas in the case of manual staining many factors like ambient temperature, incubation time etc can influcence results.

For the group of tongue SCC, with the increasing incidence seen in young patients and particularly women, new data with impact on prognosis and treatment are urgently needed. But as long as data from analyses of several sub sites are presented as valid for the whole group of tumours, this vital point is missed.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The studies were supported by grants from Lion's Cancer Research Foundation, Umeå University, The Swedish Cancer Society contract number 17 0663, Umeå University and project MEYS-NPSI-LO1413 in the Czech Republic.

REFERENCES

[1] Boldrup, L.; Coates, P.J.; Laurell, G.; Nylander, K. Differences in p63 expression in SCCHN tumours of different sub-sites within the oral cavity. *Oral Oncol.,* **2011***, 47*, 861-865.

[2] Boldrup, L.; Coates, P.J.; Wahlgren, M.; Laurell, G.; Nylander, K. Sub-site based alterations in miR-21, miR-125b and miR-203 in squamous cell carcinoma of the oral cavity and correlation to important target proteins. *J. Carcinog.,* **2012,** *11*:18.

[3] Annertz, K.; Anderson, H.; Björklund, A.; Möller, T.; Kantola, S.; Mork, J.; Olsen, J.H.; Wennerberg, J. Incidence and survival of squamous cell carcinoma of the tongue in Scandinavia, with special reference to young adults. *Int. J. Cancer,* **2002,** *101*, 95-99.

[4] Goldstein, D.P.; Irish, J.C. Head and neck squamous cell carcinoma in the young patient. *Curr. Opin. Otolaryngol. Head Neck Surg*., **2005,** *13(4),* 207-211.

[5] Lundqvist, L,; Stenlund, H.; Laurell, G.; Nylander, K. The importance of stromal inflammation in squamous cell carcinoma of the tongue. *J. Oral Pathol. Med*., **2012,** *41(5),* 379-383.

[6] Sano, D.; Myers, J.N. Metastasis of squamous cell carcinoma of the oral tongue. *Cancer Met. Rev.*, **2007,** *26*, 645-662.

[7] Yu, X.; Li, Z. MicroRNA expression and its implications for diagnosis and therapy of tongue squamous cell carcinoma. *J. Cell Mol. Med*., **2016**, *20(1),* 10-16.

[8] Bockmühl, U.; Schlüns, K.; Küchler, I.; Petersen, S.; Petersen, I. Genetic imbalances with impact on survival in head and neck patients. *Am. J. Pathol.,* **2000,** *157*, 369-375.

[9] Braakhuis, B.J.; Tabor, M.P.; Kummer, J.A.; Leemans, C.R.; Brakenhoff, R.H. A genetic explanation of Slaughter's concept of field cancerization: evidence and clinical implications. *Cancer Res.,* **2003,** *63(8),* 1727-1730.

[10] Lin, S.; Gregory, R.I. MicroRNA biogenesis pathways in cancer. *Nat. Rev. Cancer,* **2015,** *15(6):* 321-333.

[11] Cummins, J.M.; Velculescu, V.E. Implications of micro-RNA profiling in cancer diagnosis. *Oncogene,* **2006,** *25*: 6220-6227.

[12] Boldrup, L.; Coates, P.J.; Laurell, G.; Wilms, T.; Fahraeus, R.; Nylander, K. Down-regulation of miRNA-424 - a sign of field cancerization in clinically normal tongue adjacent to squamous cell carcinoma. *Br. J. Cancer*, **2015,** *112(11),* 1760-1765.

[13] Gelato, K.A.; Shaikhibrahim, Z.; Ocker, M.; Haendler, B. Targeting epigenetic regulators for cancer therapy: modulation of bromodomain proteins, methyltransferases, demethylases, and microRNAs. *Expert Opin. Ther. Targets,* **2016**, *22*: 1-17.

[14] Brandwein-Gensler, M.; Teixeira, M.S.; Lewis, C.M.; Lee, B.; Rolnitzky, L.; Hille, J.J.; Genden, E.; Urken, M.L.; Wang, B.Y. Oral squamous cell carcinoma: histologic risk assessment, but not margin status, is strongly predictive of local disease-free and overall survival. *Am. J. Surg. Pathol.,* **2005**; *29 (2),* 167-178.

[15] Brandwein-Gensler, M.; Smith, R.V.; Wang, B.; Penner, C.; Theilken, A.; Broughel, D.; Schiff, B.; Owen, R.P.; Smith, J.; Sarta, C.; Hebert, T.; Nason, R.; Ramer, M.; DeLacure, M.; Hirsch, D.; Myssiorek, D.; Heller, K.; Prystowsky, M.; Schlecht, N.F., Negass,a A. Validation of the histologic risk model in a new cohort of patients with head and neck squamous cell carcinoma. *Am. J. Surg .Pathol.,* **2010**; *34(5)*: 676-688.

[16] Dar, A.A.; Pradhan, T.N.;, Kulkarni, D.P.; Shah, S.U.; Rao, K.V.; Chaukar, D.A.; D'Cruz, A.K.; Chiplunkar, S.V. Extracellular 2'5'-oligoadenylate synthetase 2 mediates T-cell receptor CD3-ζ chain down-regulation via caspase-3 activation in oral cancer. *Immunology,* **2016,** *147(2),* 251-264.

[17] Chen, J.; Jiang, C.C.; Jin, L.; Zhang, X.D. Regulation of PD-L1: a novel role of pro-survival signalling in cancer. *Ann. of Oncol.,* **2016,** *27(3) :*409-416.

[18] Zandberg, D.P.;Strome, S.E. The role of the PD-L1:PD-1 pathway in squamous cell carcinoma of the head and neck. *Oral Oncol.,* **2014,** *50***:** 627-632.

[19] Savagner, P. The epithelial-mesenchymal transition (EMT) phenomenon. *Ann. of Oncol.,* **2010,** *21 (Supplement 7),* vii89-vii92.

[20] Wu, H.; Lotan, R.; Menter, D.; Lippman, S.M.; Xu, X.C. Expression of E-cadherin is associated with squamous differentiation in squamous cell carcinomas. *Anticancer Res.,* **2000,** *20,* 1385-1390.

[21] Palacios, F.; Tushir, J.S.; Fujita Y.; D´Souza-Schorey, C. Lysosomal targeting of E-cadherin: a unique mechanism for the donwregulation of cell-cell adhesion during epithelial to mesenchymal transitions. *Mol. Cell. Biol.* **2005***, 25,* 389-402.

[22] Bremnes, R.M.; Veve, R.; Hirsch, F.R.; Franklin, W.A. The E-cadherin cell–cell adhesion complex and lung cancer invasion, metastasis, and prognosis. *Lung Cancer,* **2002,** 36, 115–124.

[23] González-Moles, M.A.; Ruiz-Ávila, I.; Gil-Montoya, J.A.; Plaza-Campillo, J.; Scully, C. β-catenin in oral cancer: an update on current knowledge. *Oral Oncol*., **2014**, **50(9),** 818-824.

[24] Foschini, M.P.; Leonardi, E.; Eusebi, L.H.; Farnedi, A.; Poli, T.; Tarsitano, A.; Cocchi, R.; Marchetti, C.; Gentile, L.; Sesenna, E.; Marucci, G.; Montebugnoli, L. Podoplanin and E-cadherin expression in preoperative incisional biopsies of oral squamous cell carcinoma is related to lymph node metastases. *Int. J. Surg. Pathol*., **2013,** *21(2),* 133-141.

[25] Spranger, S.; Bao, R.; Gajewski, T.F. Melanoma-intrinsic β-catenin signaling prevents anti-tumour immunity. *Nature,* **2015,** *523,* 231-235.

[26] Martín-Villar, E.; Scholl, F.G.; Gamallo, C.; Yurrita, M.M.; Muñoz-Guerra, M.; Cruces, J.; Quintanilla, M. Characterization of human PA2.26 antigen (T1alpha-2, podoplanin), a small membrane mucin induced in oral squamous cell carcinomas. *Int. J. Cancer,* **2005,** *113(6)*, 899-910.

[27] Sgaramella, N.; Lindell Jonsson, E.; Boldrup, L.; Califano, L.; Coates, P.J.; Tartaro, G.; Lo Muzio, L.; Fahraeus, R.; Colella, G.; Dell'Aversana Orabona, G.; Loljung, L.; Santagata, M.; Rossiello, R.; Wilms, T.; Danielsson, K.; Laurell, G.; Nylander, K. High expression of podoplanin in squamous cell carcinoma of the tongue occurs predominantly in patients ≤ 40 years but does not correlate with tumour spread. *J. Path. Clin. Res.* **2016,** *3,* 3-8.

[28] Ochoa-Alvarez, J.A.; Krishnan, H.; Pastorino, J.G.; Nevel, E.; Kephart, D.; Lee, J.J.; Retzbach, E.P.; Shen, Y.; Fatahzadeh, M.; Baredes, S.; Kalyoussef, E.; Honma, M.; Adelson, M.E.; Kaneko, M.K.; Kato, Y.; Young, M.A.; Deluca-Rapone, L.; Shienbaum, A.J.: Yin, K.; Jensen, L.D.; Goldberg, G.S. Antibody and lectin target podoplanin to inhibit oral squamous carcinoma cell migration and viability by distinct mechanisms. *Oncotarget,* **2015,** *6(11),* 9045-9060.

[29] Marur, S.; D´Souza, G.; Westra, W.H.; Forastiere, A.A. HPV-associated head and neck cancer: a virus-related cancer epidemic. *Lancet Oncol.,* **2010,** *11***,** 781-789

[30] Chaturvedi, A.K.; Engels, E.A.; Pfeiffer, R.M.; Hernandez, B.Y.; Xiao, W.; Kim, E.; Jiang, B.; Goodman, M.T.; Sibug-Saber, M.; Cozen, W.; Liu, L.; Lynch, C.F.; Wentzensen, N.; Jordan, R.C.; Altekruse, S.; Anderson, W.F.; Rosenberg, P.S.; Gillison, M. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *J. Clin. Oncol.,* **2011,** *29(32),* 4294-4301.

[31] Pannone, G.; Santoro, A.; Carinci, F.; Bufo, P.; Papagerakis, S.M.; Rubini, C.; Campisi, G.; Giovanelli, L.; Contaldo, M.; Serpico, R.; Mazzotta, M.; Lo Muzio, L. Double demonstration of oncogenic high risk human papilloma virus DNA and HPV-E7 protein in oral cancers. *Int. J. Immunpath. Pharm.,* **2011,** ***24 (2S),* 95-101.**

[32] Shah, N.G.;Trivedi, T.I.; Tankshali, R.A.; Goswami, J.V.; Jetly, D.H.; Shukla, S.N.; Shah, P.M.; Verma, R.J. Prognostic significance of molecular markers in oral squamous cell carcinoma: a multivariate analysis. *Head Neck,* **2009***, 31*, 1544-1556

[33] Witkiewicz, A.K.; Knudsen, K.E.; Picker, A.P.; Knudsen, E.S. The meaning of $p16^{ink4a}$ expression in tumor. Functional significance, clinical associations and future developments. *Cell Cycle,* **2011,** *10 (15),* 2497-2503

[34] Loizou, C.; Laurell, G.; Lindquist, D.; Öfverman, C.; Stefansson, K.; Nylander, K.; Olofsson, K. Incidence of tonsillar cancer in northern Sweden Impact of human papilloma virus. *Oncol. Letters*, **2015,** *10(6),* 3565-3572.

[35] Sgaramella, N.; Coates, P.J.; Strindlund, K.; Loljung, L.; Colella, G.; Laurell, G.; Rossiello, R.; Lo Muzio, L.; Loizou, C.; Tartaro, G.; Olofsson, K.; Danielsson, K.; Fåhraeus, R.; Nylander, K. Expression of p16 in squamous cell carcinoma of the mobile tongue is independent of HPV infection despite presence of the HPV-receptor syndecan-1. *Br. J. Cancer,* **2015,** *113***,** 321-326.

[36] Shafti-Keramat, S.; Handisurya, A.; Kriehuber, E.; Meneguzzi, G.; Slupetzky, K.; Kirnbauer, R. Different heparin sulfate proteoglycans serve as cellular receptors for human papillomaviruses. *J. Virol.,* **2003,** *77(24),* 13125-13135.

[37] Rieckmann, T.; Tribius, S.; Grob, T.J.; Meyer, F.; Busch, C.J.; Petersen, C.; Dikomey, E.; Kriegs, M. HNSCC cell lines positive for HPV and p16 possess higher cellular radiosensitivity due to an impaired DSB repair capacity. *Radiother. Oncol.,* **2013,** *107,* 242-246.

[38] Dok, R.; Kalev, P.; van Limbergen, E.J.; Asbagh, L.A.; Vázquez, I.; Hauben, E.; Sablina, A.; Nuyts, S. $p16^{INK4a}$ impairs homologous recombination-mediated DNA repair in human papillomavirus-positive head and neck tumors. *Cancer Res.,* **2014,** *74,* 1739-1751.

[39] Romagosa, C.; Simonetti, S.; Lòpez-Vicente, L.; Mazo, A.; Lleonart, M.E.; Castellvi, J.; Cajal, S.R. $p16^{INK4a}$ overexpression in cancer: a tumor suppressor gene associated with senescence and high-grade tumors. *Oncogene,* **2011,** *30***,** 2087-2097.

[40] Iwakiri, D.; Zhou, L.; Samanta, M.; Matsumoto, M.; Ebihara, T.; Seya, T.; Imai, S.; Fujieda, M.; Kawa, K.; Takada, K. Epstein-Barr virus (EBV)-encoded small RNA is released from EBV-infected cells and activates signaling from Toll-like receptor 3. *J. Exp. Med*., **2009**, *206(10)*, 2091-2099.

[41] Ahmed, W.; Khan, G. The labyrinth of interactions of Epstein-Barr virus-encoded small RNAs. *Rev. Med. Virol.,* **2014,** *24***,** 3-14.

[42] Iwakiri, D.;Takada, K. Role of EBERs in the pathogenesis of EBV infection. *Adv. Cancer Res.,* **2010,** *107,* 119-136.

[43] Sand, L.P.; Jalouli, J.; Larsson, P.A.; Hirsch, J.M. Prevalence of Epstein-Barr virus in oral squamous cell carcinoma, oral lichen planus, and normal oral mucosa. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endo.,* **2002,** *93***,** 586-592**.**

[44] Shimakage, M.; Horii, K.; Tempaku, A.; Kakudo, K.; Shirasaka, T.; Sasagawa, T. Association of Epstein-Barr virus with oral cancers. *Hum. Pathol.,* **2002,** *33(6),* 608-614.

[45] Hermann, R.M.; Füzesi, L.; Pradier, O.; Christiansen, H.; Schmidberger, H. Presence of human papillomavirus-18 and Epstein–Barr virus in a squamous cell carcinoma of the tongue in a 20-year-old patient. Case report and review of the current literature. *Cancer/Radiothérapie,* **2004,** *8 (4),* 262-265.

[46] Coghill, A.E.; Bu, W.; Nguyen, H.; Hsu, W.L.; Yu, K.J.; Lou, P.J.; Wang, C.P.; Chen, C.J.; Hildesheim, A.; Cohen, J.I. High levels of antibody that neutralize B-cell infection of Epstein-Barr virus and that bind EBV gp350 are associated with a lower risk of nasopharyngeal carcinoma. *Clin. Cancer Res.,* **2016**, Feb 26. pii: clincanres.2299.2015. [Epub ahead of print]

[47] Daskalogianni, C.; Pyndiah, S.; Apcher, S.; Mazars, A.; Manoury, B.; Ammari, N.; Nylander, K.; Voisset, C.; Blondel, M.; Fåhraeus, R. Epstein-Barr virus-encoded EBNA1 and ZEBRA: targets for therapeutic strategies against EBV-carrying cancers. *J. Pathol*., **2015, 235(2)**, 334-341.

[48] Yang, A.; Kaghad, M.; Wang, Y.; Gillett, E.; Fleming, M.D.; Dötsch, V.; Andrews, N.C.; Caput, D.; McKeon, F. p63, a p53 homolog at 3q27-29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities. *Mol. Cell.,* **1998**, **2**, 305-316.

[49] Koster, M.I.; Roop, DdR. The role of p63 in development and differentiation of the epidermis. *J. Derm. Sci.,* **2004**, *34(1),* 3-9.

[50] Thurfjell, N.; Coates, P.J.; Uusitalo, T.; Mahani, D.; Dabelsteen. E.; Dahlqvist, A.; Sjöström, B.; Roos, G.; Nylander, K. Complex p63 mRNA isoform expression patterns in squamous cell carcinoma of the head and neck. *Int. J. Oncol.,* **2004,** *25(1),* 27-35.

[51] Lo Muzio, L.; Santarelli, A.; Caltabiano, R.; Rubini, C.; Pieramici, T.; Trevisiol, L.; Carinci, F.; Leonardi, R.; De Lillo, A.; Lanzafame, S.; Bufo, P.; Piattelli, A. p63 overexpression associates with poor prognosis in head and neck squamous cell carcinoma. *Hum. Pathol.,* 2005, *36(2),* 187-194.

[52] Moergel, M.; Abt, E.; Stockinger, M.; Kunkel, M. Overexpression of p63 is associated with radiation resistance and prognosis in oral squamous cell carcinoma. *Oral Oncol.,* **2010**, *46*, 667-671.

[53] Loljung, L.; Coates, P.J.; Nekulova, M.; Laurell, G.; Wahlgren, M.; Wilms, T.; Widlöf, M.; Hansel, A.; Nylander, K. High expression of p63 is correlated to poor prognosis in squamous cell carcinoma of the tongue. *J. Oral. Path. Med.*, 2014, *43(1),* 14-19.

[54] Leong, C.O.; Vidnovic, N.; DeYoung, M.P.; Sgroi, D.; Ellisen, L.W. The p63/p73 network mediates chemosensitivity to cisplatin in a biologically defined subset of primary breast cancers. *J. Clin. Invest.,* 2007, *117(5),* 1370-1380.

[55] Ratovitski, E.A. Phospho-ΔNp63α/microRNA network modulates epigenetic regulatory enzymes in squamous cell carcinomas. *Cell Cycle,* **2014**, *13(5),* 749-761.

[56] Gu, X.; Boldrup, L.; Coates, PJ.; Fahraeus, R.; Nylander, E.; Loizou, C.; Olofsson, K.; Norberg-Spaak, L.; Gärskog, O.; Nylander, K. Epigenetic regulation of OAS2 shows disease-specific DNA methylation profiles at individual CpG sites. *Sci Rep*., **2016**, *6,* 32579.

[57] Wilms, T.; Khan, G.; Coates, P.J.; Sgaramella, N.; Fåhraeus, R.; Hassani, A.; Philip, P.S.; Norberg Spaak, L.; Califano, L.; Colella, G.; Olofsson, K.; Loizou, C.; Franco, R.; Nylander, K. No evidence for the presence of Epstein-Barr virus in squamous cell carcinoma of the mobile tongue. *PLoS ONE*, **2017**, *12*(9), e0184201.

PMID: 29345578