Histol Histopathol (2015) 30: 833-839

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Markers of squamocolumnar junction cells in normal tonsils and oropharyngeal cancer with and without HPV infection

Patrizia Morbini[1,2], Gianluca Capello[2], Paola Alberizzi[2], Marco Benazzo[3], Chiara Paglino[4], Patrizia Comoli[5] and Paolo Pedrazzoli[4]

[1]Department of Molecular Medicine, Unit of Pathology, University of Pavia, [2]Department of Pathology IRCCS Policlinico S. Matteo Foundation, [3]Department of Otolaryngology, University of Pavia and IRCCS Policlinico S. Matteo Foundation, [4]Department of Oncology, IRCCS Policlinico S. Matteo Foundation and [5]Pediatric Hematology/Oncology, IRCCS Policlinico S. Matteo Foundation, Pavia, Italy

Summary. HPV infection has been identified recently as the causative agent of a subset of squamous cell carcinomas arising in oropharyngeal tonsils. Factors influencing the susceptibility of tonsillar epithelium to HPV-induced oncogenesis are far from being elucidated. A 5-protein signature including cytokeratin (CK)7, anterior gradient (AGR)2, cluster differentiation (CD)63, matrix metalloproteinase (MMP)7, and guanine deaminase (GDA) has recently been found to identify a residual embryonic cell population in the squamo-columnar (SC) junction of the cervix, susceptible to HPV infection, and cancers originating from these cells. The expression of SC junction markers was investigated with immunohistochemistry in normal tonsils and in oropharyngeal carcinomas (OPC) fully characterised for HPV.

All markers were constantly expressed in the reticulated epithelial cells of the tonsillar crypts, with variable diffusion and intensity; in OPC, positivity was observed in 36.5%, 29.2%, 39%, 17%, and 25% of cases with respectively AGR2, CK7, GDA, CD63, and MMP7 antibodies. No OPC was positive for all markers; 6 were completely negative. AGR2 and CK7 showed significant association with tumor- and HPV-related parameters. AGR2 expression was associated with tumor origin in the tongue base (p=0.013); CK7 was associated with non-keratinising morphology (p=0.013). p16 tumor cell expression was associated with AGR2 (p=0.021); transcriptionally active HPV infection was associated with AGR2 and CK7 (p=0.024 and 0.043).

Expression of SC junction markers in tonsillar crypt cells might be related to the embryological development of tonsillar structures; their partial association with HPV oncogenic infection could help to identify HPV-susceptible cells and related OPC.

Key words: HPV-associated oropharyngeal carcinoma, Squamocolumnar junction, Tonsillar crypt, Anterior gradient 2, Cytokeratin 7

Offprint requets to: Patrizia Morbini, MD, PhD, Department of Molecular Medicine, Unit of Pathology, Via Forlanini 16, 27100 Pavia, Italy. e-mail: patrizia.morbini@unipv.it

DOI: 10.14670/HH-11-590

Introduction

HPV infection has been identified recently as the causative agent of a subset of squamous cell carcinomas (SCC) arising in the oropharyngeal region with a specific tropism for tonsillar epithelium (Jemal et al., 2013). The incidence of HPV-associated oropharyngeal carcinomas (OPC) has increased significantly in most western countries, particularly among men younger than 60 years, and now represents over two-thirds of OPC (Marur et al., 2010; Joseph and D'Souza, 2012) occurring in this population. This epidemiological trend is paralleled by reduced tobacco and alcohol exposure in younger cohorts. Oncogenic HPV infection in other areas of the head and neck compartment on the other hand has been documented sporadically, with conflicting

results in different series (Marur et al., 2010). Factors influencing the selective susceptibility of tonsillar epithelium to HPV-induced oncogenesis are far from being elucidated (Kim et al., 2007). Being the uterine cervix by far the preferential target of HPV infection and related neoplastic transformation, translating the data already collected on the mechanisms underlying infection in the cervical mucosa to the tonsillar region could help to identify mechanisms of HPV susceptibility and possibly to devise preventive strategies.

Herfs et al. (2012) have recently identified a population of cuboidal cells located at the squamo-columnar (SC) junction of the cervix that show a unique gene expression profile, different from adjacent ectocervical and endocervical transitional zone epithelium. With gene expression analysis the Authors identified a 5 protein signature that uniquely recognized SC cells, whose expression was further documented in early stages of cervical embryonic development, in adult SC junction, in HPV-infected cell lines and in all analyzed HPV-associated cervical dysplasias and cancers. The proteins that specifically recognized SC cells were cytokeratin (CK)7, anterior gradient (AGR)2, cluster differentiation (CD)63, matrix metalloproteinase (MMP)7, and guanine deaminase (GDA). The Authors concluded that the profile of expression of these 5 markers allowed the recognition of a residual embryonic cell population in the cervix, susceptible to HPV infection, and of cancers originating from these cells. The mechanisms by which these proteins could be implicated in virus-host cell interactions leading to malignancy in the SC junction population were however not addressed. Considering the lack of information on factors regulating the susceptibility of tonsillar epithelium to HPV infection and neoplastic transformation, the present study was designed to analyze the expression profile of the described SC junction markers in normal tonsils and in oropharyngeal carcinomas, and to correlate this profile with tumor HPV status and with the presence of a transcriptionally active (i.e. oncogenic) infection.

Materials and methods

Tissue samples

Fourty-one consecutive OPC collected between January 2010 and June 2013 were fully characterized for HPV status by means of HPV DNA in situ hybridization (ISH), HPV DNA amplification and genotyping with SPF LiPA, HPV16 E6 gene amplification; viral transcriptional activity was assessed indirectly with p16 immunostain, and directly with HR HPV E7 mRNA ISH (Morbini et al., 2015). The protocol has been reviewed and approved by the Institutional Ethical Review Board and is in compliance with the Helsinki Declaration. Each subject enrolled in the project signed a detailed informed consent form. The mean patient age was 63.68 years, 34 were males. Twenty OPC showed viral mRNA and p16 expression and were classified as HPV-associated, while 21 were negative, although 5 of them hosted transcriptionally inactive HR HPV DNA.

Twenty-four normal palatine tonsils (NT) obtained at tonsillectomy performed for benign conditions were selected in order to cover all age decades between 1st and 8th (range 3-82 years, all males). All subjects or their legal representatives had signed an informed consent form for tissue storage and possible re-usage for research purposes.

Immunohistochemistry

Unstained slides obtained from both NT and OPC were immunostained with rabbit anti-GDA (Sigma-Aldrich, Saint Louis, MO), mouse anti-AGR2 clone EPR3278 (GeneTex Inc, Irvine, CA), mouse anti-CD63 cloneNK1/C3 (Abcam, Cambridge, UK), mouse anti-CK7 clone OV-TL 12/30 (Dako, Glostrup, DE), and rabbit anti-MMP-7 (Neomarkers, Fremont, CA) antibodies. p16 immunostain (MTM Laboratories AG, Heidelberg, Germany) was also performed on NT samples. All reactions were performed on the Benchmark XT automated immunostainer (Ventana Medical Systems Inc, Tucson, AZ), using the Ultraview DAB v3 revelation system.

Semiquantitative analysis was used to evaluate the results of the immunohistochemical reactions: in NT, expression in epithelial cells lining tonsillar crypts was distinguished from surface squamous epithelium; absence of staining was graded 0, the presence of scattered isolated positive cells as grade 1, clustered positive cells as grade 2, and diffuse immunostain of the crypt epithelium as grade 3. OPC samples were scored according to the H scoring system (Jordan et al., 2012). Briefly, the highest staining intensity present in tumor cells for each marker was given a score of 0 to 3. The percentage of tumor cells staining at the highest intensity in the sample was also estimated within 5% increments with respect to the total tumor area. The H score was derived from the cross-product of the intensity score (0 to 3) and of the percentage of tumor staining at the highest intensity (0% to 100%). An H score of >60 was defined as positive.

Statistical analysis

The relationship between patient age and marker expression in normal tonsils was investigated with correlation coefficient analysis. The Fisher exact test was used to assess the association between OPC marker expression and tumor and HPV-related variables (tumor site and morphology, p16 expression, presence of HPV DNA and mRNA). P values <0.05 were considered to be statistically significant; all tests were two-sided. Data analysis was performed with MedCalc statistical package (release 9.0.1.1, MedCalc Software bvba,

Ostend, BE).

Results

Normal tonsils

In NT, AGR2 and CK7 stained the deep reticular cells of crypt epithelium at the interface with lymphoid tissue, and isolated keratinocytes in the surface squamous epithelium. GDA was expressed in reticular cells and in the squamous superficial lining of the crypts, while the external surface of the tonsil was negative. MMP7 was expressed in reticular and squamous epithelia, both in the crypts and on the surface. CD63 was diffusely expressed in stromal plasma cells, and, to a lesser extent, in deep reticular cells of the tonsillar crypts. Typical immunostaining for each marker and for p16 is depicted in Fig. 1.

AGR2 grade 1, 2 and 3 expression was found respectively in 8 (33.3%), 12 (50%), and 4 (16.6%) NT; CK7 grade 1, 2 and 3 expression was found in 4 (16.6%), 11 (45.8%), and 9 (37.5%); GDA grade 2 and 3 expression was found in 4 (16.6%) and 20 (83.4%); grade 1 expression was not observed. CD63 grade 1 and 2 expression was found in 17 (70.8%) and 7 (29.2%) NT samples; grade 3 expression was not observed. MMP7 grade 1 and 2 expression was found in 14 (58.3%) and 10 (41.7%) NT; grade 3 expression was not observed. p16 was strongly expressed in crypt cells in all NT samples. Increasing patient age was non-significantly associated with reduced AGR2 expression (p=0.052), no association was observed for the other markers.

Oropharyngeal cancers

H-score higher than 60 was observed in 15 (36,5%) OPC with AGR2, 12 (29,2%) with CK7, 16 (39%) with GDA, 7 (17%) with CD63, and 6 (25%) with MMP7. No OPC was positive for all markers; 6 were completely negative. Typical positive immunostain for each marker is depicted in Fig. 2. AGR2 and CK7 showed significant association with tumor- and HPV-related parameters, while no association was observed for GDA, CD63 and MMP7 (Table 1). AGR2 expression was associated with tumor origin in the base of tongue as compared with origin in the palatine tonsils or soft palate (p=0.013), while CK7 showed a trend in the same direction (p=0.057). Non-keratinising morphology was associated with CK7 expression (p=0.004). Considering the association of tonsillar origin, either palatine or lingual., and lack of keratinisation as indicators of possible crypt phenotype as opposed to superficial epithelial phenotype, CK7 was also significantly associated with crypt phenotype (p=0.018); a non-significant association for AGR2 was observed (p=0.051).

Analyzing the association with HPV-related parameters, p16 tumor cell expression was associated with AGR2 (p=0.021). None of the markers reached a

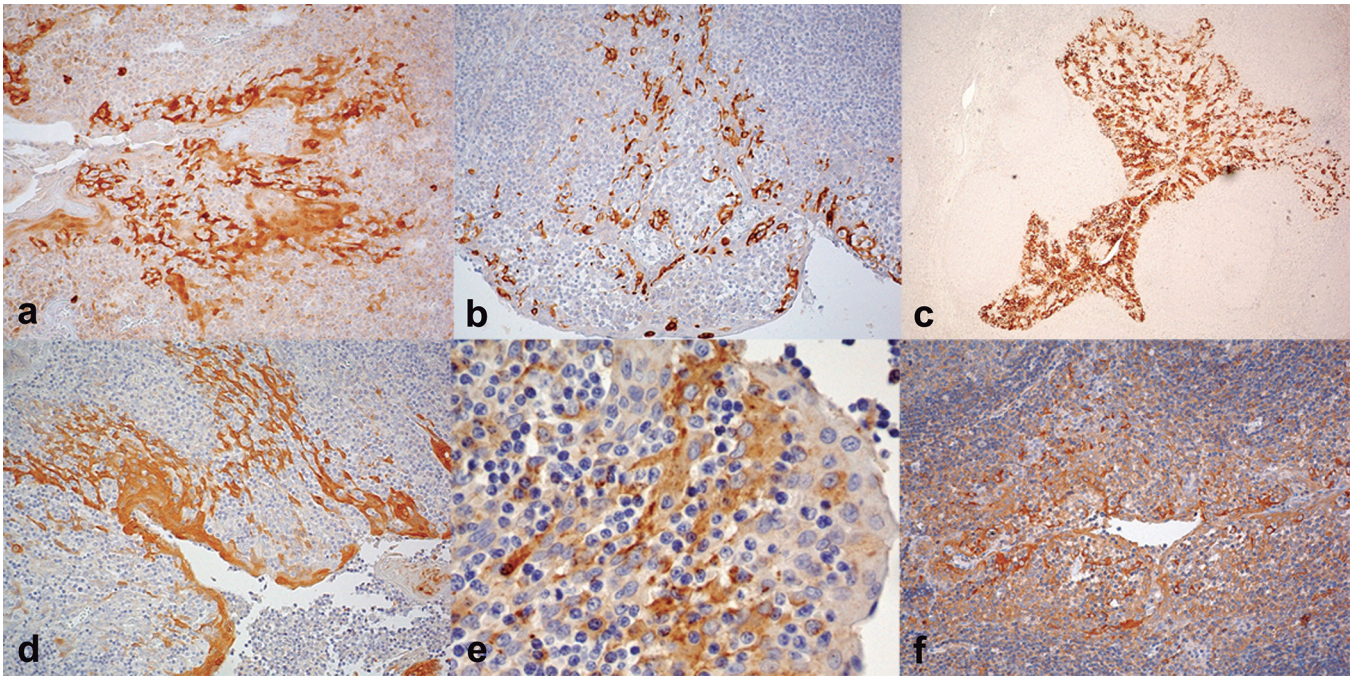

Fig. 1. Micrographs showing representative examples of p16 **(a),** AGR2 **(b),** CK7 **(c),** and GDA (**d)** grade 3 expression, and CD63 **(e)** and MMP7 **(f)** grade 2 expression in tonsillar crypts. p16 **(a)** AGR2 **(b)** and CK7 **(c)** are expressed only in the deepest layer of crypt epithelium; GDA **(d)** and MMP7 **(f)** stain superficial squamous and deep reticular cells, and CD63 **(e)** stains both reticular epithelial and plasma cells. a, b, d, f, x 20; c, x 1; e, x 40

significant association with the presence of HR HPV DNA, as assessed by amplification and/or DNA ISH, while AGR2 and CK7 were associated with the presence of HR HPV mRNA in tumor cells (p=0.024 and 0.043). Combining possible crypt vs superficial phenotype as defined above and HPV transcriptional status, we found that CK7 expression rate was similar in HPV-positive tumors, independently of their origin, but among HPV-negative tumors it was significantly higher in those of possible crypt origin (p=0.015).

Discussion

A 5 protein signature that has been shown to characterise an embryonic cell population of the uterine cervix susceptible to HPV infection (Herfs et al., 2012) was found to be expressed, albeit with variable diffusion and intensity, in the reticulated epithelial cells of the tonsillar crypts that lay at the interface with lymphoid tissue. This is the first study documenting a protein expression profile associated with HPV susceptibility in tonsillar epithelia. Despite the increasing burden of HPV-associated OPC observed in western countries (Marur et al., 2010; Joseph and D'Souza, 2012), the pathways and natural history of HPV infection and neoplastic transformation in the head and neck are poorly characterized. Several studies have highlighted differences and similarities between tonsillar, cervical., and other HPV-associated SCC, including morphology, chromosomal alterations, gene expression profiles, and miRNA expression (Pyeon et al., 2007; Wilting et al., 2009; Lajer et al., 2012); the peculiar close relationship between epithelial and immune cells in tonsillar crypts, absent in other HPV-targeted epithelia, prompted the hypothesis that immuno-mediated mechanisms may play a key role in tonsillar HPV infection (Andersen et al., 2014).

The SC junction cell population identified by Herfs et al. in the cervix bears several differences from crypt reticulated cells, both on morphological and developmental grounds: SC junction cells are described as a single layer of cuboidal cells, while the tonsillar crypts are lined by polygonal cells that form a reticular tridimensional meshwork. Strong evidence supports the theory that the initial steps of HPV infection in the cervical mucosa consist of viral binding to basement membrane glycosaminoglycanes and laminin 5, prior to cell surface binding (Kines et al., 2009; Sapp and Bienkowska-Haba, 2009; Horvath et al., 2010), while this aspect has never been investigated in the tonsils.

Table 1.

	AGR2	CK7	GDA	CD63	MMP7
Topography					
Palatine tonsil	6/21 (28.5)	8/21 (38)	8/21 (38)	5/21 (23.8)	8/21 (38)
Lingual tonsil	7/10 (70)	4/10 (40)	4/10 (40)	2/10 (20)	5/10 (50)
Soft palate	1/10 (10)	0/10 (0)	4/10 (40)	0/10 (0)	8/10 (80)
p:	0.013	0.057	ns	ns	ns
Morphology					
Keratinising	3/16 (18.7)	1/16 (6)	7/16 (43.7)	1/16 (6.2)	10/16 (62.5)
Non-keratinising	12/25 (48)	11/25 (44)	9/25 (36)	6/25 (24)	11/25 (44)
p:	ns	0.013	ns	ns	ns
Combined morphology and topography					
Tonsillar keratinising or non-tonsillar (superficial epithelium)	3/18 (16.6)	1/18 (5.5)	8/18 (44.4)	1/18 (5.5)	12/18 (66.6)
Tonsillar non-keratinising (crypt)	11/23 (47.8)	11/23 (47.8)	8/23 (34.7)	6/23 (26)	9/23 (39)
p:	0.051	0.004	ns	ns	ns
HPV status					
p16 +ve	12/22 (54.5)	9/22 (40.9)	8/22 (36.3)	5/22 (22.7)	11/22 (50)
p16 -ve	3/19 (15.7)	3/19 (15.7)	8/19 (42.1)	2/19 (10.5)	10/19 (52.6)
p:	0.021	ns	ns	ns	ns
HR HPV DNA +ve*	12/25 (48)	10/25 (40)	10/25 (40)	6/25 (24)	11/25 (44)
HR HPV DNA -ve*	3/16 (18.7)	2/16 (12.5)	6/16 (37.5)	1/16 (6.2)	10/16 (62.5)
p:	ns	0.083	ns	ns	ns
Transcriptionally active HR HPV	11/20 (55)	9/20 (45)	8/20 (40)	5/20 (25)	9/20 (45)
Transcriptionally inactive HR HPV	4/21 (19)	3/21 (14.2)	8/21 (38)	2/21 (9.5)	12/21 (57.1)
p:	0.024	0.043	ns	ns	ns
Combined origin and HPV status (transcriptionally active infection)					
Crypt, HPV +ve	10/17 (58.8)	8/17 (47)			
Crypt, HPV -ve	2/6 (33.3)	3/6 (50)°			
Superficial epithelium, HPV -ve	2/15 (13.3)	0/15 (0)°			
Superficial epithelium, HPV +ve	1/3 (33.3)	1/3 (33.3)			

CK: cytokeratin; AGR: anterior gradient; CD: cluster differentiation; MMP: matrix metalloproteinase; GDA: guanine deaminase; ns: non-significant; +ve: positive; -ve: negative; HPV: human papillomavirus; HR: high risk. *any positive with DNA ISH, and/or HPV16 amplification, and/or SPF LiPA (HR genotypes). ° p=0.0150

Although basement membrane is discontinuous and porous in adult tonsillar crypts (Choi et al., 1996), its abundant laminin component, including alpha-5 chain (Määttä et al., 2004) could possibly facilitate HPV cell infection associated with discontinuation of the superficial epithelial layers (Kines et al., 2009; Horvath et al., 2010) analogously to what happens in the cervix.

There is no available evidence at present to answer the question if the proteins identified by Herfs et al. in the cervix play a direct biological role in the susceptibility to HPV infection, in mechanisms of viral internalization, survival or replication, or if they rather merely define a cell population targeted by HPV for other, unrelated reasons. Interestingly, the Authors reported the identification of a limited positive cell population in the anorectal junction, but not in the epithelium of the vulva, vagina and penis where no SC junction is anatomically present. Reticulated cells of the tonsillar crypt are thus a third non-squamous cell population expressing the susceptibility markers in an epithelium prone to HPV infection and neoplastic transformation. The hypothesis that these markers characterize embryonic cell populations that are conserved in the adult mucosa (Hosoya et al., 2010; Lepreux et al., 2011) was supported in the cervical SC junction by their presence in samples of embryonic as well as of adult SC junction cells. Similarly, since tonsillar reticulated cells differentiate during the 4th fetal month (von Gaudecker, 1988) and persist thereafter until advanced age, their marker expression profile could be related to their embryonic origin.

In Herfs's study (2012), the SC junction signature was expressed in all HPV-associated in situ and invasive squamous neoplasms of the uterine cervix. Our research showed that only AGR2 and CK7 were associated with HPV status in OPC. Both markers were more frequently expressed in tumors originating from the lingual or palatine tonsils, characterized by non-keratinizing morphology, which were considered of possible origin from the tonsillar crypts. AGR and CK7 were also associated with the presence of a transcriptionally active HR HPV infection, but not of viral DNA. It is generally agreed that the presence of HR HPV DNA in SCC is not sufficient to document an oncogenic infection (Jung et al., 2010), and that latent HPV infection is common in head and neck SCC and patients' oral mucosa (Morbini et al., 2013). According to most recent studies, the gold standard for the identification of HPV-associated tumors is the demonstration of viral transcriptional activity in tumor cells (Rautava and Syrjänen, 2012). The strong association of AGR2 and CK7 expression with HPV transcriptional activity but not with virus DNA in tumor supports their correlation with HPV-related oncogenesis.

While the presence of AGR2 and of CK7 in tumors

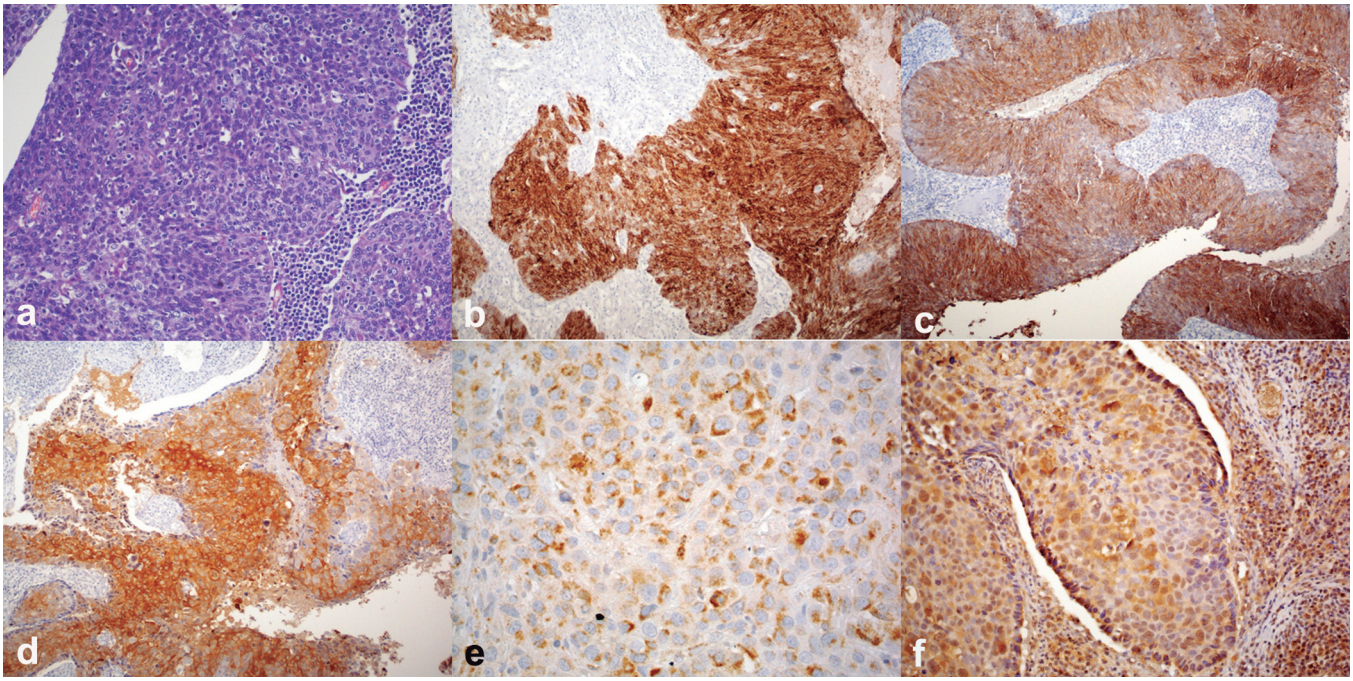

Fig. 2. Typical non-keratinising, HPV-associated squamous cell carcinoma originating from the tonsillar crypt. H&E **(a)** highlights the lack of keratinisation and the so-called "basaloid" morphology of neoplastic tissue. AGR2 **(b)**, CK7 **(c)**, and GDA **(d)** positivity was associated with strong and diffuse stain of the majority of neoplastic cells. CD63 **(e)** expression was characterized by strong cytoplasmic stain limited to the perinuclear area; MMP7 **(f)** reactivity in epithelial cells was partially blurred by diffuse immunoreactivity in associated inflammatory cells. Magnification: a-d, f, x 20; e, x 40

with crypt phenotype can be related with their expression in normal crypt cells before neoplastic transformation, the nature of their association with transcriptionally active HPV infection is less clear. AGR2 is a recently described protein expressed in normal epithelia, where it is involved in epithelial barrier function, and in several tumor histotypes (Brychtova et al., 2011); it has also been proposed as a marker of metastatic oral SCC (Sweeny et al., 2012; Chen et al., 2013). Preliminary data showed AGR2 to be involved in p53 regulation and in cell survival control (Pohler et al., 2004), thus hinting at a possible relationship between AGR2 overexpression in HPV-associated tumors and HPV-mediated disruption of p53 pathways (Wilting at al., 2009). CK7 was almost absent in tumors deriving from the surface epithelium, and showed a strong association with non-keratinizing OPC with crypt phenotype even among HPV negative tumors. Low molecular weight cytokeratins such as CK7 are reliable markers of glandular differentiation in neoplastic cells and are only expressed in subsets of SCC (Yamada et al., 2008; Clarke et al., 2013). CK7 expression in non-keratinising OPC has not been reported previously, but could be usefully employed to classify tonsillar tumors on the basis of their anatomical origin, shedding additional light on the relationship between tonsillar cell populations, HPV infection and OPC development.

The mechanisms underlying HPV targeting of tonsillar crypt epithelial cells are far from being clarified, although that knowledge would be highly relevant for the prevention and early diagnosis of HPV-related OPC, whose incidence is increasing, especially in younger cohorts. We have documented a group of markers that selectively identify crypt cells, part of which also appear to be associated with HPV oncogenic infection. Further studies are needed to assess the utility of these markers in identifying susceptible cells and HPV-related OPC.

Aknowledgements. The study was partially supported by grants RC08017800/12 and RC08053903/12 from the Italian Health Ministry to the IRCCS Policlinico San Matteo Foundation, Pavia, G11961 from the Associazione Italiana per la Ricerca sul Cancro (AIRC), and Ricerca Finalizzata Ministero della Salute project code RF-2011-02351315.

References

Andersen A.S., Koldjaer Sølling A.S., Ovesen T. and Rusan M. (2014). The interplay between HPV and host immunity in head and neck squamous cell carcinoma. Int. J. Cancer 134, 2755-2763.

Brychtova V., Vojtesek B. and Hrstka R. (2011). Anterior gradient 2: a novel player in tumor cell biology. Cancer Lett. 304, 1-7.

Chen Y.T., Ho C.L., Chen P.K., Chen Y.L. and Chang C.F. (2013). Anterior gradient 2: a novel sensitive tumor marker for metastatic oral cancer. Cancer Lett. 339, 270-278

Choi G., Suh Y.L., Lee H.M., Jung K.Y. and Hwang S.J. (1996). Prenatal and postnatal changes of the human tonsillar crypt epithelium. Acta Otolaryngol. Suppl. 523, 8-33.

Clarke L.E., Conway A.B., Warner N.M., Barnwell P.N., Sceppa J. and Helm K.F. (2013). Expression of CK7, Cam 5.2 and Ber-Ep4 in cutaneous squamous cell carcinoma. J. Cutan. Pathol. 40, 646-650.

Herfs M., Yamamoto Y., Laury A., Wang X., Nucci M.R., McLaughlin-Drubin M.E., Münger K., Feldman S., McKeon F.D., Xian W. and Crum C.P. (2012). A discrete population of squamocolumnar junction cells implicated in the pathogenesis of cervical cancer. Proc. Natl. Acad. Sci. USA 109, 10516-10521.

Horvath C.A., Boulet G.A., Renoux V.M., Delvenne P.O. and Bogers J.P. (2010). Mechanisms of cell entry by human papillomaviruses: an overview. Virol. J. 7, 11.

Hosoya A., Kwak S., Kim E.J., Lunny D.P., Lane E.B., Cho S.W. and Jung H.S. (2010). Immunohistochemical localization of cytokeratins in the junctional region of ectoderm and endoderm. Anat. Rec. 293, 1864-1872.

Jemal A., Simard E.P., Dorell C., Noone A.M., Markowitz L.E., Kohler B., Eheman C., Saraiya M., Bandi P., Saslow D., Cronin K.A., Watson M., Schiffman M., Henley S.J., Schymura M.J., Anderson R.N., Yankey D. and Edwards B.K. (2013). Annual Report to the Nation on the Status of Cancer, 1975-2009, featuring the burden and trends in human papillomavirus (HPV)-associated cancers and HPV vaccination coverage levels. J. Natl. Cancer Inst. 105, 175-201.

Jordan R.C., Lingen M.W., Perez-Ordonez B., He X., Pickard R., Koluder M., Jiang B., Wakely P., Xiao W. and Gillison M.L. (2012). Validation of methods for oropharyngeal cancer HPV status determination in US cooperative group trials. Am. J. Surg. Pathol. 36, 945-954.

Joseph A.W. and D'Souza G. (2012). Epidemiology of human papillomavirus-related head and neck cancer. Otolaryngol. Clin. N. Am. 45, 139-164.

Jung A.C., Briolat J., Millon R., de Reyniès A., Rickman D., Thomas E., Abecassis J., Clavel C. and Wasylyk B. (2010). Biological and clinical relevance of transcriptionally active human papillomavirus (HPV) infection in oropharynx squamous cell carcinoma. Int. J. Cancer 126, 1882-1894.

Kim S.H., Koo B.S., Kang S., Park K., Kim H., Lee K.R., Lee M.J., Kim J.M., Choi E.C. and Cho N.H. (2007). HPV integration begins in the tonsillar crypt and leads to the alteration of p16, EGFR and c-myc during tumor formation. Int. J. Cancer 120, 1418-1425.

Kines R.C., Thompson C.D., Lowy D.R., Schiller J.T. and Day P.M. (2009). The initial steps leading to papillomavirus infection occur on the basement membrane prior to cell surface binding. Proc. Natl. Acad. Sci. USA 106, 20458-20463.

Lajer C.B., Garnaes E., Friis-Hansen L., Norrild B., Therkildsen M.H., Glud M., Rossing M., Lajer H., Svane D., Skotte L., Specht L., Buchwald C. and Nielsen F.C. (2012). The role of miRNAs in human papilloma virus (HPV)-associated cancers: bridging between HPV-related head and neck cancer and cervical cancer. Br. J. Cancer 106, 526-534.

Lepreux S., Bioulac-Sage P. and Chevet E. (2011). Differential expression of the anterior gradient protein-2 is a conserved feature during morphogenesis and carcinogenesis of the biliary tree. Liver Inter. 31, 322-328.

Määttä M., Liakka A., Salo S., Tasanen K., Bruckner-Tuderman L. and Autio-Harmainen H. (2004). Differential expression of basement membrane components in lymphatic tissues. J. Histochem. Cytochem. 52, 1073-1081.

Marur S., D'Souza G., Westra W.H. and Forastiere A (2010). HPV-

associated head and neck cancer: a virus-related cancer epidemic. Lancet Oncol. 11, 781-789.

Morbini P., Dal Bello B., Alberizzi P., Mannarini L., Tinelli C., Mevio N., Garotta M., Mura F., Bertino G and Benazzo M. (2013). Oral HPV infection and persistence in patients with head and neck cancer. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. 116, 474-484.

Morbini P., Alberizzi P., Tinelli C., Paglino C., Bertino G, Comoli P., Pedrazzoli P and Benazzo M. (2015). Identification of transcriptionally active HPV infection in formalin-fixed, paraffin-embedded biopsies of oropharyngeal carcinoma. Hum. Pathol. (in press).

Pohler E., Craig A.L., Cotton J., Lawrie L., Dillon J.F., Ross P., Kernohan N. and Hupp T.R. (2004). The Barrett's antigen anterior gradient-2 silences the p53 transcriptional response to DNA damage. Mol. Cell. Proteomics 3, 534-547.

Pyeon D., Newton M.A., Lambert P.F., den Boon J.A., Sengupta S., Marsit C.J., Woodworth C.D., Connor J.P., Haugen T.H., Smith E.M., Kelsey K.T., Turek L.P. and Ahlquist P. (2007). Fundamental differences in cell cycle deregulation in human papillomavirus-positive and human papillomavirus-negative head/neck and cervical cancers. Cancer Res. 67, 4605-4619.

Rautava J. and Syrjänen S. (2012). Biology of human papillomavirus infections in head and neck carcinogenesis. Head Neck Pathol. 6, S3-15.

Sapp M. and Bienkowska-Haba M. (2009). Viral entry mechanisms: human. papillomavirus and a long journey from extracellular matrix to the nucleus. FEBS J. 276, 7206-7216.

Sweeny L., Liu Z., Bush B.D., Hartman Y., Zhou T. and Rosenthal E.L. (2012). CD147 and AGR2 expression promote cellular proliferation and metastasis of head and neck squamous cell carcinoma. Exp. Cell Res. 318, 1788-1798.

von Gaudecker B. (1988). Development and functional anatomy of the human tonsilla palatina. Acta Otolaryngol. Suppl. 454, 28-32.

Wilting S.M., Smeets S.J., Snijders P.J., van Wieringen W.N., van de Wiel M.A., Meijer G.A., Ylstra B., Leemans C.R., Meijer C.J., Brakenhoff R.H., Braakhuis B.J. and Steenbergen R.D. (2009). Genomic profiling identifies common HPV-associated chromosomal alterations in squamous cell carcinomas of cervix and head and neck. BMC Medical Genomics 2, 32.

Yamada A., Sasaki H., Aoyagi K., Sano M., Fujii S., Daiko H., Nishimura M., Yoshida T., Chiba T. and Ochiai A. (2008). Expression of cytokeratin 7 predicts survival in stage I/IIA/IIB squamous cell carcinoma of the esophagus. Oncol. Rep. 20, 1021-1027.

Accepted February 3, 2015