



Letter to the editor

Beta human papillomaviruses in middle ear squamous cell carcinoma



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Middle ear squamous cell carcinoma (MESCC) is a rare tumor but is the most commonly found cancer in middle ear [1]. Frequency of MESCC is very low, about one case per 8000–10,000 patients admitted to hospital with otologic disorders [1]. Due to its rare incidence, studies trying to explain the development and mechanism of carcinogenesis in MESCC are lacking. Only few case reports showed presence of alpha HPV and/or EBV in these tumors [2,3]. In addition, the presence of papillary growth and koilocytosis, which are characteristic of papillomavirus-induced lesions, suggests that HPV may play a role in the development of this neoplasm. Indeed, some types of HPV are implicated in the development of human squamous cell carcinoma in a variety of body sites including the oropharynx where the percentage of cancer related to high risk HPV infection is approximately 40% of cases [4].

The present study was conducted on patients undergoing surgery at our Department of Otolaryngology Head and Neck Surgery from 2004 to 2015 for middle ear tumors according to the classification by Arriaga et al. [5]. The study was performed in accordance with the Declaration of Helsinki and patients signed a consent form. Regina Elena Ethical Committee approved the study (# RS852010). Presence of HPV and EBV DNA was ascertained by nested PCR with consensus primers followed by direct sequencing according to previously reported standard procedures [4]. P16INK4a detection was performed with a commercial kit (CINtec® p16INK4a, Roche Diagnostics, Basel, Switzerland) and was defined as a strong and diffuse nuclear and cytoplasmic staining in $\geq 70\%$ of the tumor cells. The non-parametric Chi-Square test was applied for association between HPV and p16INK4a.

The patients enrolled in the study were 25 males and 8 females with a mean age of 77 (44–94 yrs.). CT/MRI or PET-TC scans of our series did not detect any lesions in the sinonasal, nasopharynx or pharyngeal cavity that may leave opened the auditory tube. None of the patients had a past history of papillomas of the external ear canal or oropharynx. Ectopic migration of ectodermal tissue to the middle ear, viruses, chronic inflammation, allergies and carcinogenic exposure have all been considered as possible etiological factors for this neoplasm. Among these factors, viral infections have long been considered involved in squamous cell carcinoma of skin and mucosa. A possible mechanism of infection could be postulated, as follows: middle ear is incidentally involved via the Eustachian tube or via the external acoustic meatus. This last mechanism could be related to the chronic

external and middle ear infection that are frequently related to ear cancer; the manoeuvres that usually the patients do to alleviate the pruritus in external eczematous otitis could be a cause of the migration of the viruses in this particular anatomic region [1].

Analysis by nested PCR of a large number of HPVs [4] showed their presence in the 66% of our MESCC. In particular, high risk mucosal alpha HPV accounted for 32% of total detected viruses whereas beta HPV accounted for the remaining 78% (Table 1). Same tumors were also analysed for the expression of p16INK4a protein that is a surrogate marker of transformation by high risk mucosal HPV. As in the table, a number of MESCC scored positive stain for p16INK4a, but there was no statistically significant association with HPV infection confirming that p16INK4a is not always linked to viral transformation. There was no association between HPV and any histological grade or Arriaga's stage. Analysis for the presence of EBV by PCR failed to demonstrate the presence of this virus in our samples in contrast with previous reports. However, these literature data are from small case series [3]. Direct sequence analyses of amplified products revealed the presence of two high risk mucosal (HPVs 16 and 18), and several beta papillomaviruses; multiple infection was revealed only in one patient (HPVs 16 and 20) (Table 1). Presence of high risk HPV was already reported in this tumours and could be associated to tumor development [1,2]. Indeed, it is well known that the E6-E7 oncogenes of high risk HPV are essential for cell transformation and maintenance of transformed status in cervical cancer [6,7]. On the contrary, presence of beta1 and beta2 HPV was never reported opening the question about its significance in this localization. A meta-analysis showed that some beta HPVs are significantly associated with non-melanoma skin cancer [8]. Beta HPV types play a role at an early stage of carcinogenesis, facilitating the accumulation of UV-induced mutations in the host genome, which in turn can lead to cellular transformation [6]. This hypothesis is less plausible in MESCC as the anatomical site is not exposed to UV irradiation; this model of carcinogenesis could be postulated only in some patients treated with previous radiotherapy. However, the large presence of these beta HPVs in our samples could reflect their involvement together with other unknown carcinogenic factors like 4-nitroquinoline 1-oxide (4NQO), a molecule that mimics exposure to tobacco products. Indeed, beta HPV49 E6/E7-transgenic mice are highly susceptible to carcinogenesis upon initiation with 4NQO [6]. In addition, a more

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Table 1
Histology, clinical data, and HPV/EBV presence in MESSC.

ID	Age at surgery	Sex	Histology grade	Arriaga's stage	EBV	HPV type	p16INK4a score
3751	68	f	1	3	neg	98*	neg
3752	56	m	2	2	neg	16	3+
3753	70	m	2	4	neg	16	neg
3754	87	m	3	4	neg	16/20*	neg
3755	85	m	3	3	neg	99*	neg
3756	75	f	3	3	neg	159†	neg
3757	91	m	2	2	neg	150†	3+
3758	69	m	3	4	neg	18	neg
3759	79	m	3	3	neg	18	neg
3760	76	f	2	4	neg	18	3+
3761	86	m	3	3	neg	18	neg
3781	86	m	2	2	neg	neg	neg
3782	64	m	3	4	neg	18	3+
3783	74	m	3	3	neg	23*	neg
3784	72	m	3	4	neg	neg	1+
3785	75	m	2	4	neg	neg	3+
3786	80	m	2	4	neg	neg	1+
3787	85	m	2	4	neg	8*	3+
3788	94	f	2	4	neg	neg	3+
3789	84	m	3	3	neg	100*	neg
3790	77	f	2	4	neg	120†	neg
3791	44	m	2	3	neg	25*	neg
3792	80	f	2	2	neg	113†	neg
3793	79	m	2	3	neg	98*	neg
3882	79	m	1	2	neg	neg	neg
3883	82	m	2	3	neg	neg	3+
3884	81	m	3	4	neg	neg	neg
3885	85	m	3	4	neg	14*	neg
3886	75	m	3	4	neg	129†	neg
3887	87	f	3	3	neg	neg	3+
3888	87	m	3	3	neg	20*	3+
3889	59	f	3	4	neg	neg	3+
3890	84	m	3	4	neg	neg	neg

* Beta Papillomavirus.

direct involvement was also suggested for some other beta HPV, i.e. for HPV 8 that was detected in one of our patient [9]. The HPV presence as mere passenger is also another hypothesis that is less realistic as middle ear is not easily accessible by the virus, a situation completely different from that of skin where HPV detection is very common even in healthy population. Unfortunately, RNA in paraffin samples was degraded probably for their long storage and clear data on a possible biological activity of these HPV at least as mRNA could not be ascertained. In conclusion, beta HPVs were detected for the first time in MESSC and our data are in favour of a possible association of alpha and beta HPV with these tumors, possibly with different mechanisms of transformation. The clinical significance of this association as well as the HPV involvement as carcinogens or co-carcinogens requires further studies, like a perspective patient follow-up and/or viral RNA analyses in fresh samples.

Conflict of interest

The authors declare no conflict of interest.

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