Modern Pathology (2011) 24, 1306–1312

Undifferentiated carcinoma of the oropharynx: a human papillomavirus-associated tumor with a favorable prognosis

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Undifferentiated carcinoma (undifferentiated carcinoma, nasopharyngeal type, or lymphoepithelial carcinoma) is an uncommon and histologically distinct tumor in the oropharynx, which in Western countries, has been clearly shown not to harbor Epstein Barr virus (EBV). We sought to analyze these tumors for human papillomavirus (HPV) and to examine their clinical outcomes. All cases of oropharyngeal carcinoma diagnosed as 'undifferentiated' or 'lymphoepithelial' were retrieved from the department files at Barnes-Jewish Hospital. After consensus review by all three study pathologists, 16 were found to have diagnostic histological features and to lack distinguishing characteristics of other oropharyngeal cancers. Immunohistochemistry for p16 and p53 and *in-situ* hybridization for HPV and EBV encoded small RNA were performed. p16-positive but HPV *in situ* hybridization-negative cases were analyzed by polymerase chain reaction for high-risk HPV types. The results were correlated with pathological findings and clinical follow up. There were 16 patients. The average age was 59.2 years, 14 patients (88%) were smokers, and 13 (81%) had nodal metastases. In all, 14 cases (88%) were p16 positive and 15 (94%) were HPV positive by *in situ* hybridization and/or polymerase chain reaction. All cases were negative for EBV, and p53 was overexpressed in five (33%), four of which were HPV positive. Disease recurred in only three patients and two of these died with disease at 38 and 136 months, respectively. Three year overall, disease-free, and disease-specific survival rates were 54, 78, and 100%, respectively. In summary, in our patient population, the majority of oropharyngeal undifferentiated carcinomas harbor transcriptionally active HPV but not EBV. Almost all overexpress p16, and few have p53 overexpression. Disease-specific survival is comparable to published rates for other HPV-related oropharyngeal squamous cell carcinoma variants and is better than that of HPV-negative carcinomas.
Modern Pathology (2011) **24,** 1306–1312; doi:10.1038/modpathol.2011.87; published online 13 May 2011

Keywords: human papillomavirus; lymphoepithelial; lymphoepithelioma; p16; undifferentiated

In the head and neck, undifferentiated carcinoma (also known as lymphoepithelial carcinoma or lymphoepithelioma) has historically been most frequently found in the nasopharynx. Nasopharyngeal undifferentiated carcinoma has a strong etiologic relationship with Epstein Barr virus (EBV),[1] the tumor is distinctly radiosensitive,[2,3] and, despite the undifferentiated appearance of the tumor cells histologically, the prognosis is relatively favorable.[3] Tumors with these identical morphological features can also occur outside of the nasopharynx. The most common anatomic subsites are the oropharynx[4] and major salivary glands,[5,6] but they can also occur in other sites.[7–9] The oropharynx anatomically extends from the plane of the hard palate superiorly to the plane of the hyoid bone infereriorly. It is separated from the oral cavity by the junction of the soft and hard palate superiorly, the line of the circumvallate papillae inferiorly, and the anterior pillars of the fauces laterally. It includes the base of tongue ('lingual tonsil') and bilateral palatine tonsils. It is unique from the oral cavity in both its normal tissues and in the tumor types that develop there. Undifferentiated carcinomas, although rare in the oropharynx, are exceedingly rare in the oral cavity proper. Non-nasopharyngeal undifferentiated carcinoma is usually associated with EBV in

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Received 21 December 2010; revised 8 February 2011; accepted 16 February 2011; published online 13 May 2011

endemic areas such as Southeast Asia,[6,10] but in Western countries and most other areas of the world, almost all of the cases are EBV negative.[11,12,7]

Oropharyngeal squamous cell carcinoma is frequently associated with transcriptionally active high-risk human papillomavirus (HPV). HPV-related tumors typically have a distinctive non-keratinizing morphology,[13–15] show strong p16 protein expression,[16] and have lower rates of p53 mutation[17] than most other head and neck squamous cell carcinoma. The prognosis for p16-positive oropharyngeal squamous cell carcinoma is very good and much better than for tumors which are p16 negative.[16,18,19] Squamous cell carcinomas with biologically and clinically relevant HPV are largely limited to the oropharynx among head and neck sites; however, occasional examples outside of the oropharynx have been demonstrated. Tumors of the oral cavity are clearly different, the vast majority being lacking transcriptionally active HPV.

Just like in the uterine cervix,[20–22] a number of histological variants of squamous cell carcinoma of the oropharynx, such as basaloid squamous cell carcinoma,[23] adenosquamous carcinoma,[24] and papillary squamous cell carcinoma[25] are HPV-related. Indeed, in the oropharynx, transcriptionally active HPV has been identified in the majority of papillary and basaloid squamous cell carcinoma.[25,26] In a recent study, HPV has also been demonstrated in oropharyngeal lymphoepithelial carcinomas.[4] Regardless of their morphology, HPV-related squamous cell carcinoma variants appear to have a good prognosis, essentially the same as for typical oropharyngeal HPV-related non-keratinizing squamous cell carcinoma.[23]

The purpose of this study was to determine the prevalence of HPV in undifferentiated carcinoma of the oropharynx, to characterize the immunohistochemical profiles of these tumors, and to determine patient outcomes.

Materials and methods

Case Identification

The surgical pathology department files of Barnes-Jewish Hospital/Washington University were electronically searched from for the terms 'undifferentiated' and 'lymphoepithelial' and the anatomic subsite 'oropharynx.' Cases were reviewed by all three study pathologists (DHC, SEM, and JSL) using the WHO definition/features for undifferentiated-type nasopharyngeal carcinoma (Figure 1) including solid sheets, irregular islands, dyscohesive sheets,

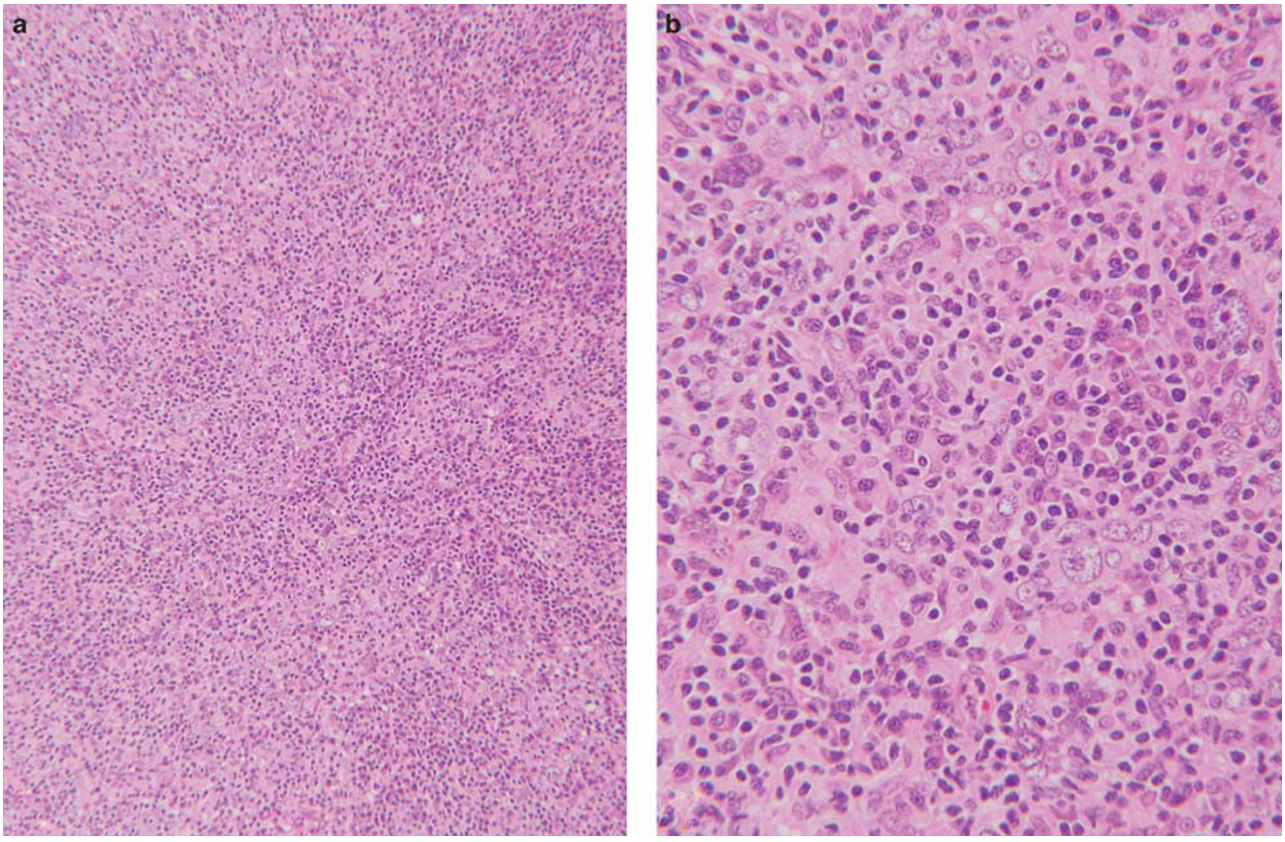

Figure 1 Morphology of undifferentiated (lymphoepithelial) carcinoma of the oropharynx. (**a**) Low-power view showing sheets of neoplastic cells with ill-defined borders and set in a background of mixed lymphoid tissue (hematoxylin and eosin; 100× magnification). (**b**) High-power view showing cells with markedly atypical tumor cells with nuclei with vesicular chromatin and prominent nucleoli (hematoxylin and eosin; 400× magnification).

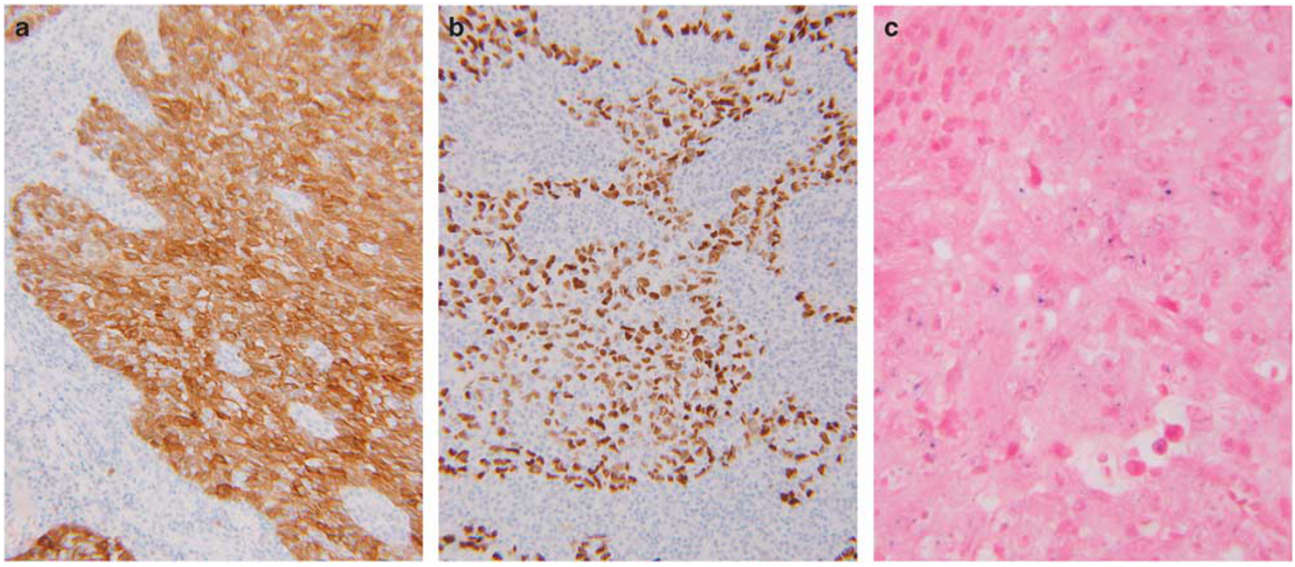

Figure 2 Special staining results in oropharyngeal undifferentiated carcinoma. (**a**) Immunohistochemistry for p16 showing strong, diffuse cytoplasmic, and nuclear staining (200 × magnification). (**b**) Immunohistochemistry for p53 showing strong expression in over 90% of the tumor cell nuclei (200 × magnification). (**c**) *In-situ* hybridization for high-risk HPV positivity with punctate, blue nuclear staining (600 × magnification). HPV, human papillomavirus.

and trabeculae of carcinoma intimately intermingled with variable numbers of lymphocytes and plasma cells. Further, the tumors had to have syncytial-appearing large tumor cells with indistinct cell borders, round to oval vesicular nuclei, and large central nucleoli; however, sometimes the nuclei could have chromatin-rich rather than vesicular nuclei.[2] Cases were included only after consensus review and agreement by all three pathologists that the features were diagnostic. Cases were excluded if they had distinguishing characteristics of other tumor types. This included cases that were more discretely nested and less syncytial, which we considered to be (according to our previously characterized histological typing system of oropharyngeal SCC[13]) non-keratinizing SCC or non-keratinizing SCC with maturation.

Immunohistochemistry

Immunohistochemistry was performed for p16 and p53 on representative 4-μm sections cut from formalin-fixed, paraffin-embedded tissue on a Ventana Benchmark XT automated immunostainer (Ventana Medical Systems, Tucson, AZ, USA) according to standard protocols and with appropriate positive controls. Antigen retrieval, standard on the machine, used the Ventana CC1, EDTA-Tris, pH 8.0 solution. We used monoclonal antibodies to p16 (MTM Laboratories; 1:1 dilution) and p53 (Ventana Medical Systems; prediluted). The staining pattern for p16 was nuclear and cytoplasmic in all cases and was considered positive if more than 50% of the tumor cells were reactive. p53 immunostaining was nuclear in all cases and was classified according to the percent positive cells as either negative (<25% nuclear staining) or positive (25% or greater). This scoring system is based on a concept that cases with greater than 25% of cells with nuclear staining are more likely to harbor p53 mutations[27] (Figure 2).

In Situ Hybridization

In situ hybridization was performed on formalin-fixed, paraffin-embedded 4-μm tissue sections using for high-risk HPV and for EBV-encoded small RNA (EBER) using an I View Blue Plus Detection Kit (Ventana Medical System). The assays used the Ventana INFORM EBER probe and the Ventana HPV III family 16, probe B, a cocktail recognizing the high-risk HPV (HR HPV) types 16, 18, 31, 33, 35, 45, 51, 52, 56, 58, 59, 68, and 70. Staining was in a totally enclosed system and was performed according to the manufacturer's instructions. Ventana Red Counterstain II (Ventana Medical System) was used. Positive staining was identified as blue nuclear dots. Any definitive nuclear staining in the tumor cells was considered positive. Cases were classified in a binary manner as either positive or negative.

HPV PCR

Molecular detection of HPV DNA by polymerase chain reaction (PCR) was carried out using 2 mm cores of formalin-fixed paraffin-embedded tumor identified from the corresponding H&E slide. These cores were deparaffinized using a series of xylene and ethanol washes. Next, they were subjected to a rigorous proteinase K digestion with an incubation

time tailored for recovery of DNA. The methodology for DNA purification included RNase treatment, protein precipitation, and DNA precipitation. PCR was then performed using the INNO-LiPA HPV Genotyping Extra kits. Part of the L1 region of the HPV genome is amplified using SPF10 primers. For high-risk HPV, a 65-bp fragment is amplified. An additional primer pair for the amplification of the human *HLA-DPB1* gene is added to monitor sample quality and extraction. For specimens where the HPV amplification product was present on initial reaction, they were hybridized to type-specific probes immobilized as parallel lines on membrane strips pre-made by the manufacturer. After hybridization and stringent washing, streptavidin-conjugated alkaline phosphatase was added. Incubation with BCIP/NBT chromogen yielded a purple precipitate. The results were visually read and compared with the provided interpretation chart to type the HPV.

Results

A total of 16 cases were identified in our files that met the criteria for undifferentiated carcinoma as described above. These cases were from 1992 to 2009. Clinical and pathological characteristics of the cases are shown in Tables 1 and 2. Patients were predominantly male, the majority smokers (either current or lifetime), and had an average age of 59 years (range 36–81; median 60). In all, 13 of the tumors were from the palatine tonsil (81%). The other three were either base of tongue or soft palate. There were only four biopsy cases and 12 surgical resections. Two patients were treated by primary surgery alone, 10 by primary surgery followed by postoperative radiation therapy, and four by primary radiation. Six patients received chemotherapy.

Table 3 shows viral status, immunohistochemical profile, and patient outcome in each case. Tables 4 and 5 summarize the results. Of the 16 cases, 14 (88%) were p16 positive with staining in at least 50% of the tumor cells. One of the remaining two cases had focal, weak staining and the other was completely negative. All 14 of the p16-positive tumors had high-risk HPV either by DNA *in situ* hybridization or by PCR. Only five tumors were p53 positive (>25% staining and thus presumed to have gene mutation).

The average follow-up was 44.0 months or 3.6 years (range 1.9–136.0 months). Of the 16 patients, three (19%) had tumor recurrence, two had regional recurrence only, and an additional patient had local and regional recurrence, as well as distant metastases. Only two of these patients (13% of the total) died with evidence of disease (which we thus assume as dying of disease). These two patients, age 52 and 62, respectively, died 11.2 and 3.2 years after diagnosis. At last follow-up, seven of 16 (44%) patients were still alive. For those patients with adequate follow-up, 2-year overall survival was 79% (11 of 14 patients alive) and disease-specific survival was 100% (all 14 patients alive or died without evidence of disease). For 11 patients, 3-year overall survival was 55% (six of 11 patients alive) and disease-specific survival was 100% (all 11 patients alive or dead without evidence of disease).

Table 1 Clinical characteristics of the cases

Patient characteristics (n)	*% of cases*
Gender	
Male (14)	88
Female (2)	12
Tobacco use	
Smoker, current or ever (14)	88
Non-smoker (1)	6
Unknown (1)	6
Average age	59.2
Treatment type	
Surgery alone (2)	13
Surgery+postoperative IMRT (10)	63
Primary IMRT (4)	25

Discussion

Non-nasopharyngeal undifferentiated (lymphoepithelial) carcinomas of the head and neck region are uncommon tumors that occur predominantly in the oropharynx and major salivary glands. There is a very strong association between nasopharyngeal undifferentiated carcinoma and EBV.[2] However, the literature on the non-oropharyngeal cases shows that in so-called 'endemic' areas such as Southeast Asia and in Eskimo populations they are still

Table 2 Pathological characteristics of the cases

Tumor characteristics (n)	*% of cases*
Tumor site	
Tonsil (13)	81
Base of tongue (2)	13
Soft palate (1)	6
TNM stage	
I (1)	6
II (2)	13
III (0)	0
IV (13)	81
Tumor stage	
T1 (5)	31
T2 (6)	38
T3 (4)	25
T4 (1)	6
Lymph-node metastases	
Not present–No. (3)	19
Present (13)	81
N2 (11)	69
N3 (2)	13

Table 3 Test results and clinical outcomes for individual cases

Case	*HPV*	*p16*	*p53*	*EBV (EBER)*	*Recurrence*	*Time, in months*	*Outcome*
1	+	+	−	−	R	33	Alive
2	+	+	−	−	None	127	Dead
3	+	+	−	−	None	22	Alive
4	+	+	−	−	None	48	Dead
5	+	+	−	−	None	9	Dead
6	+	+	−	−	None	68	Alive
7	+	+	−	−	None	2	Alive
8	+	+	−	−	None	15	Dead
9	+	+	−	−	L/R/D	136	Dead
10	−	+	No tumor	−	None	27	Dead
11	+	+	−	No tumor	R	38	Dead
12	+	+	+	−	None	29	Alive
13	+	−	+	−	None	33	Alive
14	+	+	+	−	None	26	Dead
15	+	+	+	−	None	79	Alive
16	−	−	+	−	None	13	Dead

Abbreviations: L, local recurrence; R, regional recurrence; D, distant metastasis; HPV, human papillomavirus; EBV, Epstein Barr virus; EBER, Epstein Barr virus-encoded small RNA.

Table 4 Summary of testing results of the cases

p16	*HPV status*	*p53*	*EBV status (EBER)*
Positive: 14 (88%)	Positive: 14 (88%) ISH+, PCR n/a: 8 ISH−, PCR+: 6	Positive: 5 (33%) No tumor present: 1	Positive: 0 (0%) No tumor remaining: 1
Negative: 2 (12%)	Negative: 2 (12%)	Negative: 10 (67%)	Negative: 15 (100%)

Abbreviations: HPV, human papillomavirus; ISH, *in-situ* hybridization; PCR, polymerase chain reaction; EBV, Epstein-Barr virus; EBER, Epstein Barr virus-encoded small RNA.

usually EBV-related, whereas in Western countries, they are typically EBV-negative.[7] In the current study on oropharyngeal undifferentiated carcinomas, all were EBV-negative. Conversely, almost all cases harbored high-risk HPV as demonstrated by *in situ* hybridization and/or PCR. These tumors also expressed a molecular profile (ie, high p16 and low p53) characteristic of HPV-related carcinomas. p16 overexpression is considered a surrogate marker of transcriptionally active HPV. It is a tumor suppressor protein that is aberrantly overexpressed when high-risk HPV protein E7 is produced. E7 degrades retinoblastoma protein, which normally serves to suppress p16 transcription.[18,28]

We found that these tumors have essentially the same demographics as other HPV-related/p16 positive oropharyngeal squamous cell carcinoma, being predominantly found in men, an average age of ~60 years, and most patients being smokers. This latter rate of 88% may be higher than that for oropharyngeal squamous cell carcinoma in general, which has a significant minority of lifetime non-smokers, however, our sample size is too small to draw any meaningful conclusion.[16,29,30] Pathological features were similar as well, with most (~70%) patients presenting with T1 or T2 tumors[16,29] and most (~80%) presenting with cervical lymph-node metastases.[31] Despite this very high rate of nodal metastases, the prognosis was quite favorable. Only three patients developed recurrent disease and only one developed distant metastatic disease. Interestingly, the distant metastasis was 9 years after the initial diagnosis. All of the clinical, pathological, and molecular findings are consistent with an HPV etiology.

Table 5 Clinical outcomes of the patients

Outcome		*% of cases*
Recurrence (3)		19
Local (1)		6
Regional (3)		19
Distant (1)		6
Survival rates[a]	*2 year (%)*	*3 year (%)*
Overall survival	79	55
Disease-specific survival	100	100

[a]For patients dying before 2 (or 3) years and surviving patients with at least 2 (or 3) years of clinical follow-up, respectively.

All of our cases were negative for EBV, which is consistent with the literature on EBV in oropharyngeal undifferentiated carcinoma in Western patient populations.[7] A recent study also evaluated

oropharyngeal undifferentiated (lymphoepithelial) carcinomas for HPV.[4] It was demonstrated in 19 of their 22 cases (86%) by DNA *in situ* hybridization and all 22 of their cases (100%) were also p16 positive. All 22 of their cases were also EBV negative by *in situ* hybridization for EBER.

It is of interest that our observations are consistent with recent findings that HPV-related oropharyngeal squamous cell carcinoma may present a variety of morphological patterns, yet, essentially always shows favorable clinical outcomes. This has been demonstrated in non-keratinizing,[13,14,16] basaloid,[26,23] and papillary squamous cell carcinoma[25] of the oropharynx, and we have also found it in adenosquamous carcinoma (unpublished data).

In summary, undifferentiated (lymphoepithelial) carcinoma of the oropharynx is almost always associated with biologically active high-risk HPV. Unlike nasopharyngeal undifferentiated carcinoma, when this tumor type occurs in the oropharynx in Western countries, it is not EBV-related, and, despite the alarming histology, these tumors seem to be responsive to therapy and have a favorable prognosis.

Acknowledgements

We would like to thank Bridgette Sims RN for her help in obtaining clinical information on these patients, and also Walter Clermont for his help in preparing the figures for the manuscript.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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