HPV-16 Infected Oropharyngeal Tumors as Oncovirus Reservoirs in the Context of HIV/AIDS

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Abstract

HPV-associated cancers are dramatically increased in HIV/AIDS. Therefore, it is imperative to know whether patients with HPV-associated oropharyngeal carcinoma have chronic active infection that is transmissible to their HIV+ partners. Importantly, unlike cervical carcinoma, oropharyngeal tumors often contain HPV 16 DNA in an episomal, not integrated, form that might be poised for re-activation under certain circumstances. Further, no investigator has directly assessed the oral cavity of patients with HPV-associated oropharynx cancer for active virus nor correlated the results of this assessment with cancer treatment. We have created a tissue repository of approximately 70 samples of HPV-associated oropharynx tumors, as well as control oropharynx tissue samples. Our laboratory work is focused on developing a low-cost, streamlined technique for detection of episomal HPV DNA in oropharynx tumor samples in order to determine the prevalence of this condition. Two HPV16-positive HNSCC cell lines have been carefully analyzed for the expression of the viral genes E1, E2, E4, E5, E6, E7, L1 and L2 and the expression of the HPV16 E2 viral protein. A clinical study is now underway analyzing saliva samples from patients with HPV-associated oropharynx cancer for encapsidated HPV 16 before and after definitive cancer treatment.

Background

- HPV is a small DNA virus that is capable of infecting human keratinocytes of the skin and mucous membranes. Although there are more than 100 subtypes of HPV, HPV 16 accounts for 90% of all HPV-related head and neck cancers (1).
- There is an epidemic of human papillomavirus (HPV)-associated oropharynx cancer in the US and Europe, with a rise in incidence of at least 225% since the late 1990s, while oropharynx cancers associated with tobacco and alcohol have declined during the same time period (2). The reason for this increase has been attributed to changing sexual practices including oral-genital sexual practices combined with a lengthy latency period from oropharyngeal exposure to oncogenic HPV types, mainly HPV 16.
- HIV-positive patients are at increased risk for HPV-induced head and neck squamous cell carcinomas (HNSCC), including those of the oropharynx. The lower CD4+ T cell count is significantly associated with increased HPV-related cancer risk, indicating that immunosuppression could be a contributing risk factor for HPV-positive HNSCC (3).
- Papillomavirus E2 is a multifunctional viral protein that regulates many aspects of the viral life cycle including viral episome maintenance. Constitutive expression of oncogene transcripts are required in HPV-related oropharyngeal squamous cell carcinomas.

Methods

- A tissue repository of approximately 70 samples of HPV-associated oropharynx tumors and control oropharynx tissue samples was assembled. We are actively seeking samples from AIDS and Cancer Specimen Resource (ACSR), from an Argentine HIV Consortium, and saliva samples from HIV patients diagnosed with HPV-related non-oropharyngeal tumors.
- An immortalized cell line from one of our tumor samples (Tip1) was created, and two HNSCC (UM-SCC-47 and UM-SCC-104) cell lines were acquired .
- In cell culture, HPV16 E2 viral protein was studied by immunofluorescent staining and HPV16 viral gene expression was determined by real-time PCR.

Results

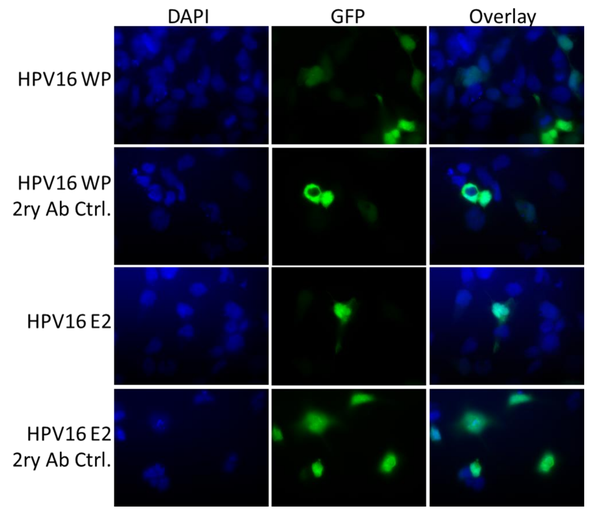

Figure 1. Immunofluorescence staining for detection of HPV16 E2 protein in AD293 cells transfected with the entire HPV16 or the HPV16 E2 plasmid. Transfection was done in the presence of GFP to track efficiency.

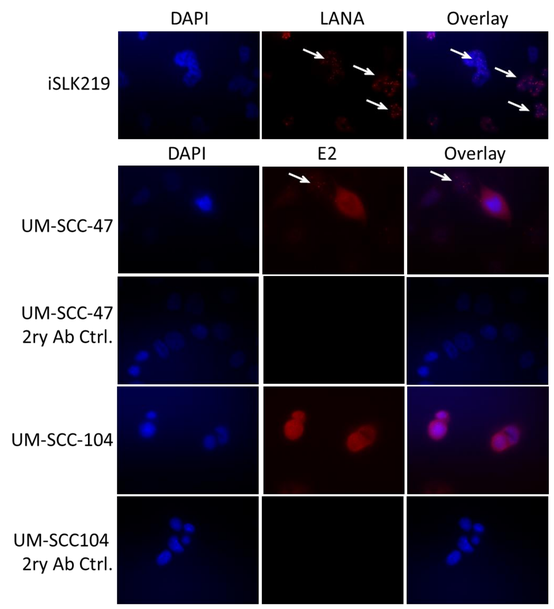

Figure 2. Immunofluorescence staining for detection of HPV16 E2 protein in UM-SCC-47 and UM-SCC-104 cells. LANA, a viral latency protein detected in the majority of KS lesions, was used as a positive control for nuclear episomal staining in iSLK219 cells.

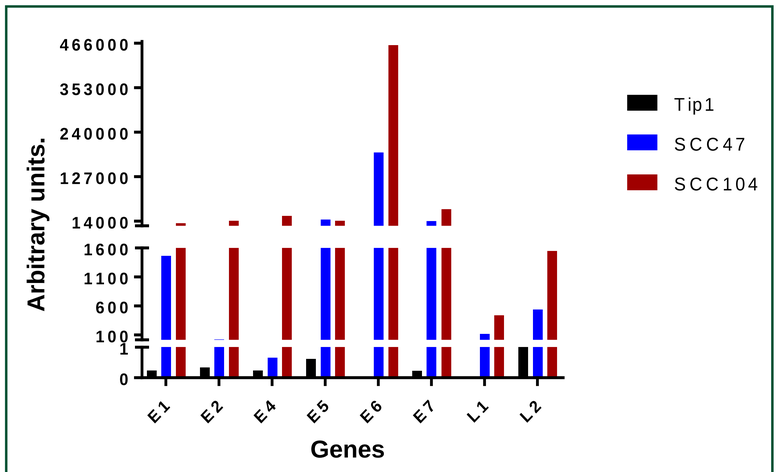

Figure 3. mRNA expression of the HPV16 genes E1, E2, E4, E5, E6, E7, L1 and L2 in the UM-SCC-47, UM-SCC-104 and a patient tumor cell line.

Table 1. Patient Repository of HPV-associated oropharynx samples

Lab Sample #	TBCF #	Tissue	Sample	Block	Sex
1	3577	Tonsil; p16+	1	A1	NA
2	3650	Tonsil; p16+	2	2	M
3	3727	Parapharyngeal; p16+	3	R1	M
4	3751	Tonsil; p16+	4	A1	M
5	3765	Tonsil; p16+	5	1	M
6	3771	Tonsil; p16+	6	A	M
7	3774	Tonsil; p16+	7	F1	NA
8	3848	Tonsil; p16+	8	1	M
9	3911	Tongue; p16+	9	H1	M
10	3920	Tonsil; p16+	10	1	M
11	4095	Tonsil; p16+	11	2	F
12	4171	Tonsil; p16+	12	1	M
13	4316	Tonsil; p16+	13	1	M
14	4345	Tonsil; p16+	14	1	M
15	4430	Tongue	15	A	F
16	4756	Tonsil; p16+	16	1	F
17	4776	Tongue	17	A3	M
18	4780	Tongue	18	M5	M
19	5063	Pharynx	19	1	M
20	5069	Tonsil; p16+	20	1	M
21	5172	Tonsil; p16+	21	1	NA
22	3699	Tonsil; normal	22	2	NA
23	3728	Tonsil; normal	23	2	NA
24	3812	Normal tonsil;	24	2	NA
25	3729	Blood	25	2	NA
26	3753	Blood & FFPE	26	2	NA
27	3884	Blood	27	2	NA
28	3887	Blood	28	2	NA

Conclusions

- A punctate staining pattern of the HPV16 E2 protein is observed in the squamous cell carcinoma UM-SCC-47 cell line.
- Similar HPV16 gene transcript levels of E5, E7 and L1 is observed in the squamous cell carcinoma UM-SCC-47 and UM-SCC-104. Different HPV16 gene transcript levels of E1, E2, E4, E6 and L2 is observed in the squamous cell carcinoma UM-SCC-47 and UM-SCC-104.
- We have an established repository of HPV-associated oropharynx samples and created a structure for ongoing collection.
- A clinical study is now underway analyzing saliva samples from patients with HPV-associated oropharynx cancer for encapsidated HPV 16 before and after definitive cancer treatment.

References

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