Maelle SALIBA[1]
Muhammad SHAHEEN[1]
Rana EL HAJJ[1]
Fatmeh Ibrahim ABBAS[1]
Shaarif BASHIR[2]
Umer Nisar Nisar SHEIKH[2]
Rami MAHFOUZ[1]
Asif LOYA[2]
Ibrahim KHALIFEH[1]

[1] Department of Pathology and Laboratory Medicine, American University of Beirut Medical Center, Beirut, Lebanon
[2] Department of Pathology, Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore, Pakistan

**Reprints:** Ibrahim Khalifeh
<ik08@aub.edu.lb>

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# Sebaceous neoplasms: prevalence of HPV infection and relation to immunohistochemical surrogate markers

*Background*: Sebaceous neoplasms (SNs) and carcinomas (SCs) represent rare skin adnexal tumours. *Objectives*: To establish the prevalence of HPV in SNs, assess the relationship between HPV, p16 and p53 expression, and further elucidate the carcinogenetic course of SCs. *Materials & Methods*: A total of 113 resected SNs (five sebaceous adenomas, 10 sebaceomas and 98 SCs) from the Near-East were reviewed. Clinical information (age, gender, size and anatomical location), microscopic variables, and expression of several immunohistochemical markers (EMA, CK5/6, p63, p40, AR, p16 and p53) were documented. Cases were evaluated by fluorescently labelled PCR for HPV detection, followed by DNA microarray hybridization for subtype detection. *Results*: HPV infection was detected in 9.4% of SNs: 28.6% sebaceous adenomas (HPV-16 and HPV-66), 9.1% sebaceomas (HPV-18) and 8.1% SCs. High-risk HPV types (HPV-16, -18, -52 and -66) constituted 90.9% of HPV infections. Histologically, HPV-positive SCs showed significantly milder cytologic atypia and patchy cellular necrosis. p16 was expressed in SNs irrespective of HPV status (20.0%, 33.3% and 65.5% of HPV-negative sebaceous adenomas, sebaceomas, and SCs, respectively), and p53 was abnormally expressed in 95.5% of HPV-negative SCs and all HPV-positive SCs. *Conclusion:* HPV infection is significantly present in benign and malignant SNs. HPV-positive SCs exhibit less cytologic atypia and necrosis than HPV-negative cases. p16 is not a surrogate marker of HPV infection in the SN setting. Further elucidation of various carcinogenic mechanisms in SCs will allow clinicians to single out the various populations at risk, optimize possible preventive strategies and develop targeted therapies

**Key words:** human papilloma virus, p16, polymerase chain reaction, sebaceous neoplasms

Human papillomaviruses (HPVs) infect cutaneous and mucosal epithelia of various human tissues [1]. High-risk HPV (HR-HPV) causes all cervical cancers and some head and neck squamous cell carcinomas forms (HNSCCs) [2]. The presence of E6 and E7 oncoproteins was reported in malignant and premalignant HPV-related lesions [3]. The oncogenic role of HPV in the development of sebaceous carcinomas (SCs) is debatable. While some studies report a null incidence of HR-HPV in SC, others report an incidence reaching 57% [4-6]. The literature, however, has not addressed the role of HPV in benign sebaceous neoplasms (SNs), namely sebaceous adenomas and sebaceomas. An understanding of the tumorigenic role of HPV will provide further insights into the link between SN entities. Furthermore, laying out the oncogenic pathways that promote SC development will unveil potential candidates for targeted therapy. Herein, 117 SN cases were evaluated and tested for HPV by the polymerase chain reaction (PCR). Our research objectives were three-fold: to establish the prevalence of HPV in SNs, assess the relationship between the presence of viral genomes and patterns of immunohistochemical markers, p16 and p5, and understand the carcinogenetic course of SCs.

## Methods

### Patients and clinical data

A cohort of 117 resected SN (seven sebaceous adenomas, 11 sebaceomas and 99 SCs), dating from 2000 to 2018, was retrospectively reviewed. Cases were retrieved from archives of two regional referral centres (American University of Beirut Medical Center and Shaukat Khanum Memorial Cancer Hospital and Research Centre). Diagnoses were reviewed and verified according to definitions in the literature [7]. Upon diagnostic confirmation,

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patients' demographic (age, gender) and clinical information (lesion size, anatomical location) were collected. Lesions were considered as periocular when involving the upper/lower eyelid, caruncle and periorbital/perioricular areas, and extraocular when involving all other areas.

### Histopathological examination

Microscopic examination using haematoxylin and eosin-stained slides revealed variable tumoral features (architectural invasion pattern, intraepithelial component, cytological features, percentage of sebaceous differentiation, mitotic count, necrosis, and apoptosis).
The invasion pattern was characterized as:

– Nodular: expansile tumour nests with rounded borders and a centripetal arrangement.
– Infiltrative: irregular/trabeculated to angulated tumoral nests.
– Comedocarcinoma: nodular-like pattern with central comedonecrosis in at least 50% of tumoral nests.
– Reticulated: trabecular, ribbon-like/corded cell arrangement with no specific direction.
– Papillary: variable tumour cell layers growing on fibrovascular cores.
– Mixed.

### Immunohistochemical profiling

Expression of markers was evaluated by immunohistochemistry using anti-p53 (clone DO-7; Leica) and anti-p16 (clone E6H4; Roche). Expression was assessed qualitatively and semi-quantitatively by consensus based on at least two observers (IK, MSa and MSh). Qualitative assessment documented tumour staining pattern and distribution. Semiquantitative p16 immunoreactivity was recorded with a three-tier system; 1 =negative (< 5% of positive tumour cells), 2 =focal to patchy (5-70% of positive tumour cells), and 3 =diffuse (>70% of positive tumour cells). For p53, 1 = null expression (<1% of tumour cells with adequate controls), 2= low expression (1-80% of tumour cells) and 3= high expression (>80% of tumour cells). Low p53 expression represented wild-type *TP53*, while null and high expression represented aberrant *TP53*.

### HPV testing

Cases were screened for 30 human high-risk and low-risk papilloma viruses (HPV 6, 11, 16, 18, 26, 31, 33, 35, 39,40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 66, 68, 70, 72, 73, 81, 82, 89). Testing was based on the amplification of defined gene sections from the listed HPV subtypes, and subsequent detection via a hybridization reaction with immobilized DNA probes using a microarray system (Euroarray test, Germany). DNA was extracted from paraffin-embedded tissues. Firstly, regions of E6 and E7 viral oncogenes from each sample were amplified and fluorescently labelled by PCR using a multiplex primer system. Secondly, products were detected using an oligonucleotide microarray. Specific hybridization was detected using a Microarray Scanner (Euroimmun, Germany).

### Ethical considerations

Both data and tissues were collected pursuant to an institutional review board-approved protocol at the primary investigator's institution. Data were anonymized and patient consent was waived.

### Statistical analysis

Quantitative variables were analysed by t-test or ANOVA as appropriate. Categorical variables were analysed using Chi-square test. Simple correlation between variables was performed using Pearson's correlation. A two-tailed $p < 0.05$ was required for statistical significance. Analyses were performed using SPSS version 22 (IBM Inc., Somers, NY, USA).

## Results

### Clinical data

The cohort included 117 patients consisting of 63 women and 54 men (F = 53.8%, M = 46.2%). Age at diagnosis ranged from 19 to 90 years (mean= 54.6; SD= 14.5). Lesions ranged from 0.2 to 7 cm (mean= 1.8; SD= 1.1).
Periocular lesions constituted 71.6% of cases. Extraocular lesions constituted the rest and mostly involved the nose (6.9%), forehead (5.2%), scalp (5.2%), back (4.3%) and cheeks (3.4%). Less frequent locations included the lips, parotid gland, ears and chin (0.9% each).

### Histopathological and immunohistochemical findings

#### SC architectural features

Major SCs showed a nodular invasion pattern (56.6%). An infiltrative pattern was seen in 26.3%. A comedocarcinoma-like pattern was seen in 14.1%. Rare cases displayed a reticulated pattern (2.0%). One case involved only the intraepithelium, with no invasive component.

#### Intraepithelial spread of SCs

SC malignant intraepithelial spread was seen in a total of 83.5% (flat *in situ* component in 58.8%, nested in 18.8%, pagetoid in 4.7% and mixed in 1.2%).

#### Sebaceous differentiation

Sebaceous differentiation significantly differed between SCs and sebaceomas and sebaceous adenomas (means = 7.2%,18.8%, 80.0%; SD 7.7, 9.7, 11.5, respectively).

### Cytological features

Most SCs showed severe cytologic atypia (67.7%). A minority displayed moderate (26.3%) and mild atypia (6.1%). Sebaceomas displayed moderate (72.7%) or mild atypia (27.3%), while adenomas displayed mild (85.7%) or no atypia (14.3%).

**Table 1.** Immunohistochemical staining profiles of sebaceous neoplasms grouped by diagnosis and anatomical location.

| | | Diagnosis | | | | | |
|---|---|---|---|---|---|---|---|
| | | Sebaceous adenoma | | Sebaceoma | | Sebaceous carcinoma | |
| | | Periocular | Extraocular | Periocular | Extraocular | Periocular | Extraocular |
| P16 | Negative | 100.0% | 25.0% | 25.0% | 50.0% | 10.8%[a] | 10.0% [a] |
| | Patchy | 0 | 50.0% | 0 | 33.3% | 14.9% [a] | 45.0% [a] |
| | Diffuse | 0 | 25.0% | 75.0% | 16.7% | 74.3% [a] | 45.0% [a] |
| P53 | Wild-type | 100.0% | 100.0% | 50.0% | 83.3% | 2.7% | 10.0% |
| | Aberrant | 0 | 0 | 50.0% | 16.7% | 97.3% | 90.0% |

[a] $p<0.05$

**Mitotic activity, cellular apoptosis and necrosis**

Mitotic activity significantly differed between SNs, with a mean mitotic count of 1.9/mm$^2$ for adenomas (SD: 1.7), 12.7/mm$^2$ (SD: 11.0) for sebaceomas and 30.1 /mm$^2$ (SD: 9.7) for SCs. Brisk cellular apoptosis was only seen in 78.8% of SCs and 27.3% of sebaceomas. Patchy cellular necrosis was seen in 17.2% of SCs. Sebaceous adenomas did not exhibit necrosis. Comedo-type necrosis was seen in 18.2% of sebaceomas and 57.6% of SCs.

**Immunohistochemical staining**

Overall, 85.5% of cases exhibited p16 staining (62.7% diffuse and 22.7% patchy). Aberrant p53 was seen in 87.2% (null expression in 29.4% and overexpression in 57.8%). Periocular SCs significantly expressed diffuse p16. Diffuse p16 staining differed significantly between periocular (74.3%) with extraocular SCs of the head and neck (47.1%). p16 and p53 profiles were grouped according to anatomical location and diagnostic subcategory (*table 1*).

## HPV status

**HPV and diagnostic subcategory**

HPV infection was detected in 9.4% of all SNs, 28.6% of sebaceous adenomas (HPV-16-positive, n = 1; and HPV-66-positive, *n* = 1), 9.1% of sebaceomas (HPV-18-positive, *n* = 1) and 8.1% of SCs (HPV-16-positive, *n* = 4; HPV-43-positive, *n* = 1; HPV-52-positive, *n* = 1; and HPV-66-positive, *n* = 1). High-risk HPV types (HPV-16, -18, -52 and -66) constituted 90.9% of HPV infections.

**HPV and clinical data**

HPV infection showed no significant association with gender (13% of males vs 6.3% of females), and there was no difference between the mean age of HPV-positive (mean = 57.7) and HPV-negative patients (mean= 54.24).

**HPV and anatomical location**

HPV infection showed no significant association with the anatomical location of neoplasms and was detected in 9.1% and 4.8% of periocular and extraocular SCs, respectively. HPV-positive cases arose in the periocular region in 72.7%, on the back in 18.2% and scalp in 9.1%.

**HPV and histopathological features**

A significant association was noted between HPV status and each tumoral cytologic atypia with regards to degree and necrosis type. HPV-positive cases showed milder cytologic atypia compared to HPV-negative cases, with 45.5%, 18.2% and 36.4% of positive cases showing mild, moderate, and severe cytologic atypia, respectively. HPV-positive cases exhibited significantly more patchy cellular necrosis (36.4% versus 12.3%) with less comedonecrosis (18.2% versus 53.8%).

There was no significant association between HPV status and architectural invasion pattern, intraepithelial spread, percentage of sebaceous differentiation, mitotic activity, or cellular apoptosis. HPV-positive cases showed nodular and infiltrative invasion patterns in 50.0% of cases, respectively. An intraepithelial component was present in 85.7% of cases consisting of a flat *in situ* component. The percentage of sebaceous differentiation averaged between 20.2% for HPV-positive cases and 11.9% for HPV-negative cases. The mean mitotic activity of the two groups was 20.7 versus 27.4/mm$^2$, respectively. Brisk apoptotic activity was seen in 70.8% of HPV-negative cases versus 54.5% of HPV-positive cases.

**HPV and immunohistochemical staining**

p16 and p53 immunohistochemical staining profiles were grouped according to HPV status and diagnostic subcategory (*table 2*).

# Discussion

HPVs are small double-stranded DNA viruses that infect skin keratinocytes and the mucosa [1, 8, 9]. HR-HPV subtypes are associated with human carcinogenesis, particularly anogenital carcinomas and a subset of head and neck cancers [1, 9]. These specific subtypes induce the development of malignancy with no additional risk factor [1]. The products of E6 and E7 viral genes are major oncoproteins that dysregulate tumour suppressor genes, TP53 and retinoblastoma (Rb), respectively [1]. Persistent HR-HPV infection is essential for malignant proliferation, as constant expression of viral oncogenes is required for the maintenance of the phenotype and transformation.

**Table 2.** Immunohistochemical staining profiles of sebaceous neoplasms grouped by diagnosis and HPV status.

| | | Diagnosis | | | | | |
|---|---|---|---|---|---|---|---|
| | | Sebaceous adenoma | | Sebaceoma | | Sebaceous carcinoma | |
| | | HPV negative | HPV positive | HPV negative | HPV positive | HPV negative | HPV positive |
| **P16** | **Negative** | 40.0% | 0 | 44.4% | 0 | 11.5% | 0.0% |
| | **Patchy** | 40.0% | 0 | 22.2% | 0 | 23.0% | 12.5% |
| | **Diffuse** | 20.0% | 100.0% | 33.3% | 100.0% | 65.5% | 87.5% |
| **P53** | **Wild-type** | 100.0% | 100.0% | 66.7% | 100.0% | 4.5% | 0.0% |
| | **Aberrant** | 0.0% | 0 | 33.3% | 0.0% | 95.5% | 100.0% |

Sebaceous glands are composed of sebaceous ducts, lined by stratified squamous epithelium, and sebocyte-rich lobules [10]. The transformation zone where the ductal stratified squamous epithelium progresses into the specialized appendegeal epithelium of sebaceous lobules harbours self-renewing junctional stem cells [11, 12]. Thus, these sites are similar to the cervical, anal and even tonsillar crypt transformation zones. It is generally accepted that HR-HPV-associated neoplasia develops primarily at these vulnerable sites [13].

The oncogenic role of HPV in SC development has been previously discussed. Reported HPV incidence in periocular SCs varies, from nil to 57% [4-6]. The role of HPV has been highlighted more clearly in Eastern than Western studies [5, 10]. A relatively recent study revealed HR-HPV in 13.8% of periocular SCs [11]. We detected HPV genome in 9.4% of all SNs, with 9.6% for periocular SCs and 8.1% for SCs for all anatomical sites. HR-HPV subtypes constituted 90.9% of these infections, increasing the potential tumorigenic role of E6 and E7. Unexpectedly, our data did not reveal a correlation between patient age and HPV status. Interestingly, although SCs slightly predominate in female patients, we noted HPV mostly in men. As previously reported, HPV-positive cases showed significantly less cytologic atypia [11].

p16 can reliably be used as a surrogate marker for HR-HPV infection in certain anatomical sites, such as for non-keratinizing squamous cell carcinoma of the oropharynx, where p16 positivity precludes viral studies [6, 11]. Consistent with prior studies, our series shows that p16 overexpression does not predict HPV infection in SNs, as up to 65.5% of HPV-negative SCs were diffusely positive for HPV (*figure 1*) [6]. This is likely because the majority of HPV-negative SCs harbour inactivating *RB1* mutations, correlating with elevated p16 levels, regardless of HPV status [11]. In addition to malignant SNs, we also detected p16 in HPV-positive and negative benign SNs (*figure 2*). Of note, p16 was overexpressed in 58% of cutaneous carcinomas (60% of squamous cell carcinomas and 50% of basal cell carcinomas) and is associated with sun-exposed skin, suggesting that p16 may be over-expressed in response to UV radiation [12]. We thus suggest p16 as one of the diagnostic immunomarkers for SNs, rather than a surrogate marker for HPV infection.

Tetzlaff *et al*. have shown that the most common carcinogenic route for SC development is characterized by a high frequency of somatic mutations in *TP53* and/or *RB1* [11]. These tumours arising in older patients, relative to than HPV-associated SCs, are histologically higher-grade lesions, exhibiting a local aggressive behaviour. Though non-specific for *Tp53* mutations, aberrant p53 expression correlates with dysregulation of the Tp53 signalling pathway in nearly all SCs [13]. This was noted for HPV-positive and negative cases, periocular and extraocular. Thus, as stated by Tetzlaff *et al*., aberrant p53 expression is associated with SC development that closely relies on TP53 dysregulation, whether mutational or viral protein-mediated [11].

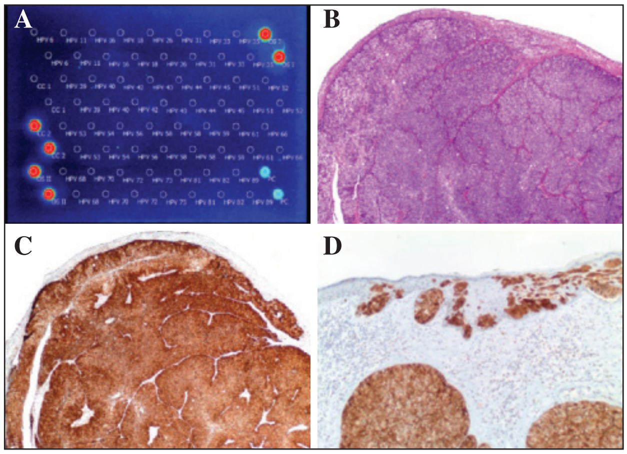

**Figure 1.** p16 expression in an HPV-negative sebaceous carcinoma. **A**) Negative micro-array for low and high-risk HPV subtypes with adequate internal controls (Euroimmun test, Germany). **B**) Poorly differentiated sebaceous carcinoma with minimal sebocytic differentiation (haematoxlyin and eosin; 4x). **C**) Diffuse and strong block-like p16 positivity in the tumour (4x). **D**) p16 highlights the malignant nested and flat intraepithelial spread (10x).

There are conflicting opinions on the role of p53 mutations in SC invasiveness [14]. In our cohort, two of three cases of pure SC *in situ* displayed aberrant p53 expression. As such, there was no correlation between *Tp53* status and the invasive/non-invasive nature of malignant SNs. Our data also show that unlike sebaceous adenomas, a subset of sebaceomas show aberrant p53 expression,

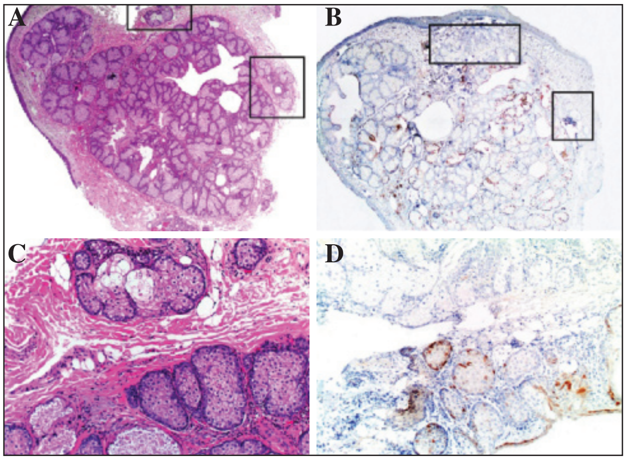


**Figure 2.** p16 expression in benign sebaceous neoplasms. **A**) Low-power view of a sebaceous adenoma with normal pilosebaceous units in the upper part and right side of the image (haematoxylin and eosin; 2x). **B**) p16 expression in the proliferating adenoma compared to no expression in the background pilosebaceous units (2x). **C**) High-power view of the tumour cells with overlying normal sebaceous glands (haematoxylin and eosin; 10x). **D**) p16 expression by the outermost tumoral cells, compared to no expression in the overlying normal sebaceous glands (10x).

comparable to that of SCs. This is consistent with Shalin *et al*. but contradictory to the findings of Cabral *et al*. [13, 15]. who demonstrated similar staining between sebaceomas and adenomas. We thus agree with the explanation of Shalin *et al*. and propose that dysregulation of the Tp53 pathway, itself, does not necessarily translate to malignancy.

Limitations to this study include its retrospective nature and the use of a surrogate p53 immunohistochemical marker for the evaluation of *Tp53* status and p53 pathway integrity. Also, it is worth mentioning that it is unlikely that HPV genome detection could have resulted from contamination. Moreover, though our study identifies HPV genomes in sebaceous neoplasia, it does not demonstrate viral transcriptional activity. We have also shown that, in contrast to data for the head and neck, p16 positivity is not a surrogate marker for active HPV infection.

## Conclusion

Our series reaffirms that different mutational processes in SNs are histopathologically related but clinically distinct [16]. A viral HPV factor, constituted predominantly of HR-HPV, potentially plays a tumorigenic role in SN development, possibly by dysregulating the p53 pathway. We therefore support the addition of E6 and E7 oncogenes to the proposed model of convergent, though non-overlapping, SC pathway carcinogenesis. Histologically, HPV-positive SNs have less cytologic atypia and more necrosis. Although HPV-positive cases are more likely to be p16 positive, the latter is not a surrogate marker for HPV infection in this setting [17-20]. The additional model of HPV-driven tumorigenesis presents an opportunity for elaborating novel preventive strategies to decrease the incidence of SC. Applying prophylactic HPV vaccination in cervical cancer patients with sebaceous neoplasia could help to protect vulnerable populations, such as organ transplant recipients. More studies are needed to investigate the clinical, preventive, and therapeutic significance of underlying viral infections in sebaceous neoplasms. ■

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