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ORIGINAL ARTICLE

Retrospective Cohort Study

Expression rates of p16, p53 in head and neck cutaneous squamous cell carcinoma based on human-papillomavirus positivity

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Abstract

BACKGROUND

The high prevalence of human papillomavirus (HPV) infection in oropharyngeal squamous cell carcinoma (SCC) is well established, and p16 expression is a strong predictor. HPV-related tumors exhibit unique mechanisms that target p16 and p53 proteins. However, research on HPV prevalence and the combined predictive value of p16 and p53 expression in head and neck cutaneous SCC (HNCSCC), particularly in Asian populations, remains limited. This retrospective study surveyed 62 patients with HNSCC (2011-2020), excluding those with facial warts or other skin cancer.

AIM

To explore the prevalence of HPV and the predictive value of p16 and p53 expression in HNCSCC in Asian populations.

METHODS

All patients underwent wide excision and biopsy. Immunohistochemical staining for HPV, p16, and p53 yielded positive and negative results. The relevance of each marker was investigated by categorizing the tumor locations into high-risk and middle-risk zones based on recurrence frequency.

RESULTS
Of the 62 patients, 20 (32.26%) were male, with an average age of 82.27 years (range 26-103 years). High-risk included 19 cases (30.65%), with the eyelid and lip being the most common sites (five cases, 8.06%). Middle-risk included 43 cases (69.35%), with the cheek being the most common (29 cases, 46.77%). The p16 expression was detected in 24 patients (38.71%), p53 expression in 42 patients (72.58%), and HPV in five patients (8.06%). No significant association was found between p16 expression and the presence of HPV ($P > 0.99$), with a positive predictive value of 8.33%.

CONCLUSION
This study revealed that p16, a surrogate HPV marker in oropharyngeal SCC, is not reliable in HNCSCC, providing valuable insights for further research in Asian populations.

Key Words: Squamous cell carcinoma; Oropharyngeal; Non-oropharyngeal; Human papillomavirus; The p16; The p53

Core Tip: Human papillomavirus (HPV) plays an important role in certain head and neck cancers; however, its role in these cancers remains unclear. We examined 62 patients to determine the prevalence of HPV and its relationship with p16 and p53 expression levels. These results indicate that p16 is not a reliable HPV marker in these skin cancers, unlike in other head and neck cancers. This study provides insights into the interactions between HPV, p16, and p53 in head and neck skin cancers, particularly in Asian populations, potentially improving our understanding and treatment of these cancers.

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INTRODUCTION

After basal cell carcinoma, squamous cell carcinoma (SCC) is the second most common skin cancer, followed by basal cell carcinoma[1]. SCC typically arise from precancerous lesions, often in sun-exposed regions, notably in the head and neck. Risk factors for the development of SCC include ultraviolet (UV) radiation, chemical carcinogens, genetic predisposition, immunosuppression, drugs, and viral infection[1]. Viral infections, particularly those caused by human papillomavirus (HPV), are potential carcinogens that indirectly impair DNA repair mechanisms or apoptotic responses to UV-induced damage[1]. The carcinogenic potential of HPV infection in SCC is relatively well known, particularly in cases of epidermodysplasia verruciformis (EV). EV, an autosomal recessive hereditary dermatosis, presents a heightened risk of SCC development and exhibits high susceptibility to infection with the beta genera HPV-5 and HPV-8[2]. In EV, SCC develops in chronically HPV-infected skin, particularly in sun-exposed areas[2]. Numerous studies have investigated the occurrence of HPV infections in oropharyngeal SCC (OPSCC)[3]. However, studies on the relationship between HPV and non-oropharyngeal cutaneous SCC, such as head and neck cutaneous SCC (HNCSCC), are limited. In addition, most studies have been conducted in Caucasian individuals, and few have been conducted in Asian individuals.

P53 is an important driver mutation in SCC with a tumor suppressor function against UV-induced damage[4]. However, to the best of our knowledge, no studies have been conducted on the rate of p53 mutations in HNCSCC and their association with HPV, which interferes with the repair of UV-induced damage. P16 is known as a surrogate marker for HPV with a high positive predictive value in OPSCC and is associated with a favorable prognosis[5]. However, its association with HNCSCC has yet to be studied. Numerous studies have highlighted that HPV-positive status is associated with a favorable prognosis in cervical carcinoma and oropharyngeal cancers[5,6]. This is due to HPV's role in promoting p16 expression, which is often used as a surrogate marker for HPV infection and has been linked to better treatment outcomes in these cancers[7]. However, the prognostic value of HPV infection in non-OPSCC, particularly HNCSCC, remains unclear. Similarly, p53 mutations are commonly associated with poor prognosis across various cancers, including SCC, as p53 inactivation allows for uncontrolled cell division and evasion of apoptosis. Despite this knowledge, the roles of p16 and p53 expression in predicting recurrence and metastasis in HNCSCC are yet to be fully understood. This study therefore aimed to examine not only the prevalence of HPV infection in HNCSCC among an Asian population but also to investigate whether p16 and p53 expression could serve as prognostic markers, specifically in relation to recurrence and metastasis. Our findings contribute to the growing understanding of HPV-related carcinogenesis in cutaneous SCC and the potential utility of these markers in clinical prognostication.

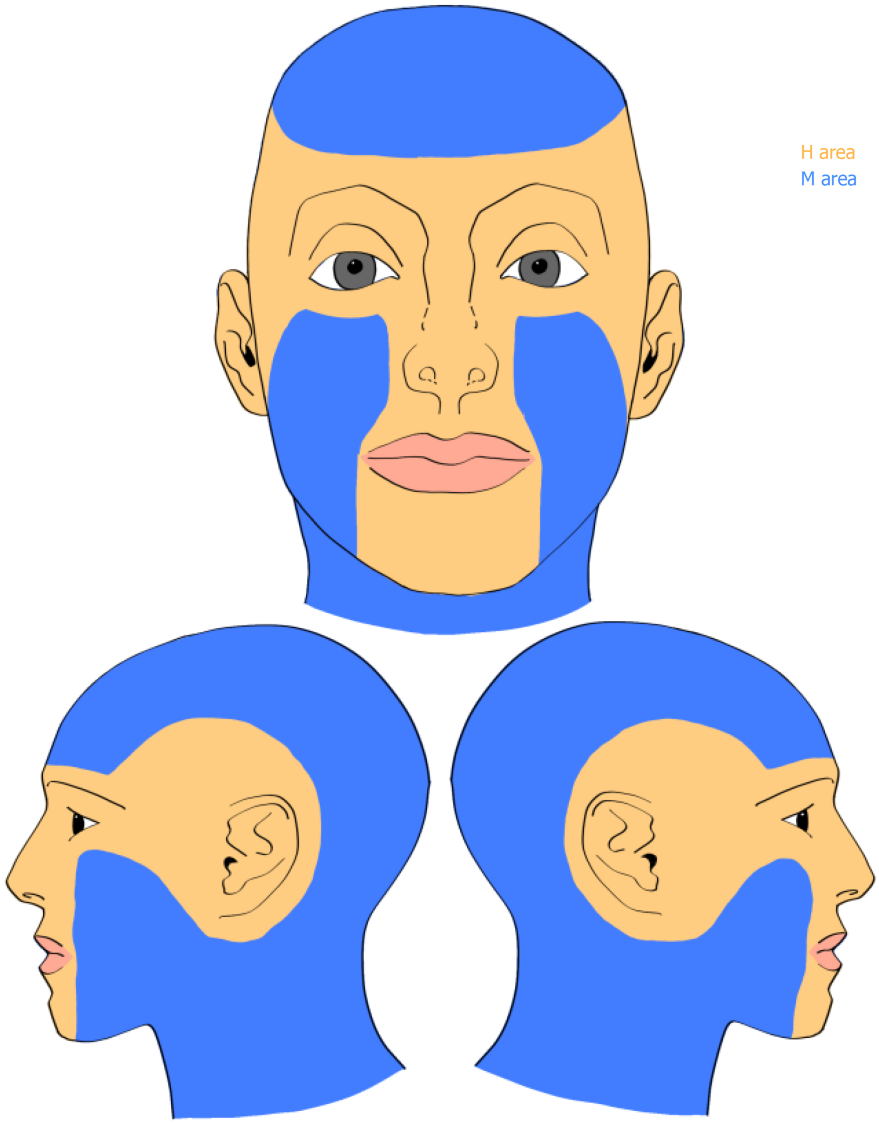

Figure 1 Classification of tumor location into high-risk and mid-risk zones. Area H (yellow area) encompasses central facial regions, including eyelids, eyebrows, periorbital area, nose, lips, chin, mandible, preauricular, and postauricular areas; area M (blue area) includes the cheeks, forehead, and scalp.

MATERIALS AND METHODS

Inclusion and exclusion criteria

We identified 62 patients diagnosed with HNCSCC who underwent excision and biopsy at a single institution between 2011 and 2020. All participants provided informed consent prior to participating in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Institutional Review Board of Soon-Chunhyang University Hospital, approval No. 2024-05-014. All patients were histopathologically diagnosed with SCC. Following registration, a computer-generated randomization process was conducted, resulting in the enrollment of 62 of the 269 patients. The process was balanced by incorporating clinical data, including age, sex, and tumor location. Patients diagnosed with verruca or other cutaneous head or neck malignancy were excluded. Additionally, individuals of races other than Asian were excluded from the study to maintain homogeneity within the sample population. Patients with a history of treatment for head and neck cancer or incomplete medical records were also excluded to ensure data reliability.

Clinical information

Clinical information, including sex, age, and medical history, was also collected. Additionally, data on tumor size, depth, lymphovascular invasion, and perineural invasion were recorded. Tumor location was categorized as a high-risk zone (area H) comprising the central facial regions (eyelids, eyebrows, periorbital, nose, lips, chin, mandible, preauricular, and postauricular areas) and a middle-risk zone (area M) consisting of the cheek, forehead, and scalp (Figure 1). Recurrence and metastasis data were also collected for each patient, based on follow-up examinations and imaging studies to assess the long-term prognosis of HNCSCC in relation to p16, p53, and HPV status.

Construction of tissue microarray

Hematoxylin and eosin-stained slides were prepared from formalin-fixed, paraffin-embedded tissue blocks. Slides were examined under a light microscope to identify the most representative cancerous regions. The corresponding areas of each paraffin block were then sectioned twice using a 2 mm-diameter cylinder and transferred to a recipient paraffin block using a tissue microarrayer (Unitma, Seoul, Korea).

Immunohistochemistry and interpretation

Immunohistochemical (IHC) staining of individual 4-μm thick slide sections derived from tissue microarray blocks was performed using the Ventana Bench Mark XT automated staining system (Ventana Medical Systems, Tucson, AZ, United States), according to the manufacturer's protocol. The following primary antibodies were used: Anti-p16 (dilution 1:2; clone E6H4; Roche, Tucson, AZ, United States), anti-HPV (dilution 1:100; clone K1H8; Dako, Glostrup, Denmark) and anti-p53 (dilution 1:900; clone DO-7; Dako). HPV and p16 expression in tumor cells were classified as negative or positive (> 1%) by cytoplasmic and nuclear staining, respectively (Figure 2A, B, C and D). The p53 expression in tumor cells was evaluated as negative or positive by nuclear staining (Figure 2E and F).

Statistical analysis

All statistical analyses were performed using the IBM SPSS Statistics for Windows (version 26.0; IBM Corp., Armonk, NY, United States). Categorical variables were expressed as counts (percentages), while continuous variables were presented as mean ± SD. The χ^2 test or the Fisher exact test for categorical variables and independent sample *t*-test for continuous variables were used to compare the two groups. The Mann-Whitney *U* test was used to analyze continuous variables that were not normally distributed. Analyses of recurrence and metastasis in relation to p16, p53, and HPV status were conducted using χ^2 tests and logistic regression to assess potential associations. Statistical significance was set at $P > 0.05$.

RESULTS

Patient demographics and tumor characteristics

Patient demographics and tumor characteristics are listed in Table 1. Among the 62 patients with HNSCC, 20 (32.26%) were male. The average age of the patients was 82.27 years, and 93.55% were aged > 65 years. Nineteen (30.65%) and 43 (69.35%) patients had tumors in areas H and M, respectively. The average tumor size and depth were 2.73 ± 4.60 cm^2 and 4.06 ± 3.62 mm, respectively. The incidence of lymphovascular and perineural invasion was 4.84% (3/62). Diabetes mellitus and hypertension were present in 14.52% (9/62) and 43.55% (27/62) of patients, respectively (Table 1).

Location of tumors

The tumor locations are presented in Table 2. Area H accounted for 30.65% (19/62) of the cases, of which the eyelids and lips were the most common (8.06%), followed by the ears (6.46%), nose (4.84%), and chins (3.23%). Area M comprised 69.35% (43/62), and the cheek (46.77%) was the most common, followed by the forehead (17.74%) and scalp (4.84%) (Table 2).

IHC study

HPV, p16, and p53 statuses are presented in Table 3. P16 was positive in 24 (38.71%), p53 in 42 (72.58%), and HPV in 5 (8.06%) patients with HNSCC. HPV expression varied in intensity and was primarily observed in well-developed tumor cells (Figure 2B). Diffuse p53 staining often revealed strong nuclear expression (Figure 2D). P16 positive staining showed strong nuclear and cytoplasmic expression in continuous tumor cells, which was defined as block-positivity (Figure 2F). The positivity rates for HPV, p53, and p16 were not significantly different according to sex or age ($P = 0.165$, 0.125, and 0.331, respectively). There was no significant difference in the presence of HPV according to the area H/M; however, the area M ratio in the HPV-positive group was 80%, which was higher than that in the HPV-negative group (68.42%) ($P > 0.99$). No significant association was observed between p53 expression and HPV infection ($P > 0.99$). The H ratio in the p53-positive group was 35.56%, which was higher than that in the p53-negative group (17.65%), but the difference was not statistically significant ($P = 0.172$). There was no significant association between p16 expression and the presence of HPV, and the positive predictive rate of p16 for HPV was 8.33% ($P > 0.99$). According to the tumor location, the area H ratio in the p16-positive group was 25%, which was lower than that in the p16-negative group (34.21%; $P = 0.444$, Table 3).

Recurrence and metastasis in relation to p16, p53, and HPV status

The rates of recurrence and metastasis based on p16, p53, and HPV expression are summarized in Table 3. Patients with positive p16 expression showed a higher recurrence rate (50%) compared to those with negative p16 expression (15.79%), with statistical significance ($P = 0.007$). Similarly, the recurrence rate in p53-positive patients was significantly higher (35.56%) than in p53-negative patients (11.76%; $P = 0.022$). HPV-positive patients also exhibited a higher recurrence rate (60%) compared to HPV-negative patients (26.32%; $P = 0.045$). In terms of metastasis, p16-positive patients had a metastasis rate of 33.33%, which was significantly higher than the 7.89% observed in p16-negative patients ($P = 0.015$). P53-positive patients showed a metastasis rate of 20%, while only 5.88% of p53-negative patients experienced metastasis ($P = 0.041$). Additionally, HPV-positive patients had a metastasis rate of 40%, significantly higher than the 14.04% in HPV-negative patients ($P = 0.037$). These findings suggest that p16 and p53 expression, along with HPV positivity, may

Table 1 Patient demographics and tumor characteristics, *n* (%)

Patient characteristics	*N* = 62
Sex	
Male	20 (32.26)
Female	42 (67.74)
Age group (years)	
≤ 50	2 (3.22)
51-65	2 (3.22)
> 65	58 (93.55)
Location	
Area H	19 (30.65)
Area M	43 (69.35)
Size of tumor (cm^2), mean ± SD	2.73 ± 4.60
Invasion depth (mm), mean ± SD	4.06 ± 3.62
Lymphovascular invasion	
+	3
-	59
Perineural invasion	
+	3
-	59
DM	
+	9
-	53
HTN	
+	27
-	35

Area H: High-risk zone; Area M: Middle-risk zone; DM: Diabetes mellitus; HTN: Hypertension.

be associated with a higher likelihood of recurrence and metastasis in HNCSCC patients.

DISCUSSION

SCC is the second most common type of non-melanoma skin cancer, accounting for approximately 40.2% of all malignancies of the head and neck region[8]. Various risk factors for HNSCC development have been identified, including tobacco smoking, alcohol consumption, dietary factors, and HPV infection[9]. The role of HPV in oral and oropharyngeal carcinogenesis was first described by Syrjänen *et al*[10] in 1983. Since then, numerous studies have investigated the role of HPV in HNSCC, particularly OPSCC. The HPV positivity rate in OPSCC varies widely, typically ranging from 40% to 80%, depending on the sensitivity and specificity of the detection method[3]. Notably, HPV-positive OPSCC exhibits distinct clinical and demographic characteristics compared to HPV-negative tumors[11]. For instance, HPV-positive OPSCC is more frequently observed in certain regions, such as the United States, than in Asia or Europe[12]. This geographical variation in HPV prevalence may be attributed to differences in sexual behavior, cultural practices, and HPV vaccination rates. Moreover, HPV-positive OPSCC tends to occur more frequently in younger patients and individuals with a history of multiple sexual partners and higher oral sex exposure[13]. These behavioral risk factors contribute to the transmission of HPV, particularly area H HPV genotypes such as HPV-16 and HPV-18, which are associated with oncogenic transformation and the development of OPSCC. Importantly, HPV-positive OPSCC is associated with a more favorable prognosis than HPV-negative tumors[14-16]. This improved prognosis is thought to be related to the distinct molecular and biological characteristics of HPV-positive tumors, including the overexpression of the p16 protein and inactivation of the p53 tumor suppressor gene. These molecular alterations contribute to enhanced sensitivity to radiotherapy and chemotherapy, resulting in better treatment responses and overall survival rates in HPV-

Table 2 Location of tumors, *n* (%)

Characteristics	*N* = 62
Area H	19 (30.65)
Eyelid	5 (8.06)
Nose	3 (4.84)
Lip	5 (8.06)
Chin	2 (3.23)
Ear	4 (6.46)
Area M	43 (69.35)
Forehead	11 (17.74)
Scalp	3 (4.84)
Cheek	29 (46.77)

Area H: High-risk zone; Area M: Middle-risk zone.

Table 3 Human papillomavirus, p16, and p53 status with human papillomavirus genotyping, *n* (%)

Characteristics	HPV (-)	HPV (+)	High-risk HPV	*P* value	The p53 (-)	The p53 (+)	*P* value	The p16 (-)	The p16 (+)	*P* value
n	57	5	3	-	17	45	-	38	24	-
Sex										
Male	20 (35.09)	0 (0.00)	0 (0.00)	0.165	8 (47.06)	12 (26.67)	0.125	14 (36.84)	6 (25.00)	0.331
Female	37 (64.91)	5 (100.00)	3 (100.00)	-	9 (52.94)	33 (73.33)	-	24 (63.16)	18 (75.00)	-
Age group										
≤ 50 years	2 (3.51)	0 (0.00)	0 (0.00)	> 0.99	1 (5.88)	1 (2.22)	0.733	1 (2.63)	1 (4.17)	0.768
51-65 years	2 (3.51)	0 (0.00)	0 (0.00)	-	0 (0.00)	2 (4.44)	-	2 (5.26)	0 (0.00)	-
> 65 years	53 (92.98)	5 (100.00)	3 (100.00)	-	16 (94.12)	42 (93.33)	-	35 (92.11)	23 (95.83)	-
HPV										
Negative	-	-	-	-	16 (94.12)	41 (91.11)	> 0.99	35 (92.11)	22 (91.67)	> 0.99
Positive	-	-	3 (60.00)	-	1 (5.88)	4 (8.89)	-	3 (7.89)	2 (8.33)	-
Location	-	-	-	-	-	-	-	-	-	-
Area H	18 (31.58)	1 (20.00)	0 (0.00)	> 0.99	3 (17.65)	16 (35.56)	0.172	13 (34.21)	6 (25.00)	0.444
Area M	39 (68.42)	4 (80.00)	3 (100.00)	-	14 (82.35)	29 (64.44)	-	25 (65.79)	18 (75.00)	-
Recurrence										
Yes	15 (26.32)	3 (60.00)	2 (66.67)	0.045	2 (11.76)	16 (35.56)	0.022	6 (15.79)	12 (50.00)	0.007
No	42 (73.68)	2 (40.00)	1 (33.33)	-	15 (88.24)	29 (64.44)	-	32 (84.21)	12 (50.00)	-
Metastasis										
Yes	8 (14.04)	2 (40.00)	1 (33.33)	0.037	1 (5.88)	9 (20.00)	0.041	3 (7.89)	8 (33.33)	0.015
No	49 (85.96)	3 (60.00)	2 (66.67)	-	16 (94.12)	36 (80.00)	-	35 (92.11)	16 (66.67)	-

HPV: Human papillomavirus; Area H: High-risk zone; Area M: Middle-risk zone.

positive OPSCC patients[17-19]. Similar findings have been reported in other cancer types, such as urothelial carcinoma of the bladder, where HPV DNA has shown prognostic value in predicting outcomes[7].

Clinically, HPV-positive OPSCC typically presents as a single localized tumor in the oropharynx, most commonly in the tonsillar region or base of the tongue[20]. These tumors often have a smaller primary tumor size and a lower rate of lymph node involvement than HPV-negative tumors[21,22]. Unlike HPV-negative tumors, HPV-positive OPSCC is less

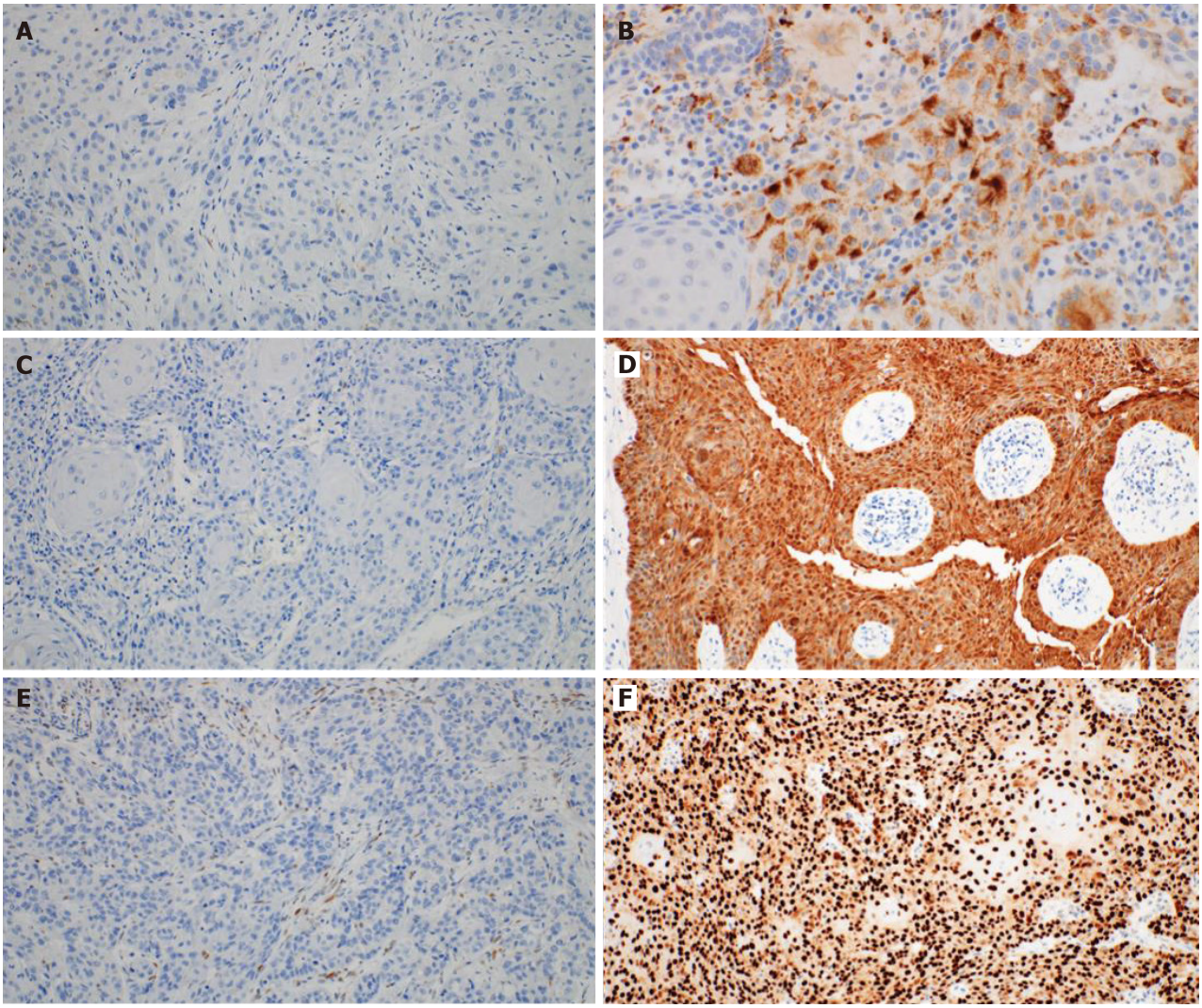

Figure 2 Representative images of immunohistochemical study are shown. A: Images of human papillomavirus staining, negative (× 200); B: Images of human papillomavirus staining, positive (× 400); C: Images of p16 staining, negative (× 200); D: Images of p16 staining, positive (× 200); E: Images of p53 staining, negative (× 200); F: Images of p53 staining, positive (× 200).

frequently associated with tobacco and alcohol use, and patients may present with fewer comorbidities and better performance status at the time of diagnosis[23]. Unlike OPSCC, the pathogenesis of HNCSCC remains unclear. However, the HPV infection rate is higher in SCC, including those occurring in the head and neck regions, than in normal skin[24]. UV exposure, a known trigger for SCC, can induce immunosuppression, potentially increasing the susceptibility to HPV infection. Conversely, evidence suggests that HPV infection may exacerbate UV-induced damage and potentially contribute to SCC development. However, their role in SCC maintenance remains unclear[25]. Previous studies have reported HPV positivity rates in HNCSCC ranging between 5% and 20%; however, it is unclear whether HPV is directly involved in the pathogenesis of HNCSCC[15,26]. Furthermore, most studies have been conducted in Caucasian populations, with few examining the role of HPV in HNCSCC in Asian populations[3].

In this study, the positive detection rate of HPV using IHC for HNCSCC was 8.06% (5/62). This was lower than that reported in a previous study using the polymerase chain reaction (PCR) method in North American individuals, in which the HPV positivity rate was 21.8%[27]. The difference in test methods is considered one of the reasons for this result. PCR is the most sensitive method for detecting HPV and may show a higher positivity rate than the IHC method used in this study[28]. In addition, as mentioned above, HPV-positive OPSCC occurs more frequently in the United States than in Asia[12]. Due to similar racial and cultural differences, HPV-positive HNCSCC is also thought to be lower than that reported in previous studies targeting North American populations. Our study further analyzed the effects of HPV, p16, and p53 expression on recurrence and metastasis in HNCSCC. Results indicated that HPV-positive HNCSCC cases exhibited higher recurrence and metastasis rates compared to HPV-negative cases (60% and 40%, respectively; $P = 0.045$ and $P = 0.037$), which contrasts with findings in OPSCC where HPV positivity is typically associated with a favorable prognosis. These findings align with other studies, such as those in urothelial carcinoma of the bladder, where HPV DNA presence has also been correlated with poorer outcomes[7]. Additionally, p16 and p53 expression were both associated with increased recurrence and metastasis rates. P16-positive patients showed a recurrence rate of 50% and a metastasis rate of 33.33%, compared to 15.79% and 7.89% in p16-negative patients ($P = 0.007$ and $P = 0.015$, respectively). Similarly, p53-positive patients had significantly higher recurrence (35.56%) and metastasis (20%) rates compared to p53-negative

patients (11.76% and 5.88%; $P = 0.022$ and $P = 0.041$, respectively). These findings suggest that p16 and p53 expression, along with HPV positivity, may be poor prognostic indicators in HNCSCC, indicating higher risks of recurrence and metastasis.

P53 is the most commonly mutated gene in SCC, occurring in up to 90% of SCC cases[29]. When a mutation occurs, tumor cells resist apoptosis and cell cycle arrest, leading to clonal expansion. Mutations in p53 caused by UV damage are involved in SCC development[4]. In the past, studies have been conducted on the effect of p53 overexpression on the treatment response or prognosis in HNCSCC, but a consensus has yet to be reached[30]. Additionally, a study has been conducted on the association between the presence of HPV and p53 in SCC. HPV E6 and E7 suppress p53-mediated transcription. This may interfere with the UV-activated cell cycle checkpoint, exacerbating UV-induced DNA damage and, ultimately, the oncogenic potential of HPV[31-33]. The contrasting roles of p16 and p53 in OPSCC *vs* HNCSCC underscore the complex interplay between HPV infection, UV radiation exposure, and molecular alterations unique to HNCSCC. Whereas p16 positivity is a favorable indicator in OPSCC, in HNCSCC, p16 overexpression may indicate a higher likelihood of recurrence and metastasis, potentially due to UV-related damage overriding p16's tumor-suppressive functions. This study highlights the need for further investigation into the prognostic role of these markers in HNCSCC.

In this study, p53 was overexpressed in 72.58% (45/62) of patients with HNSCC. This is similar to the 79% reported in previous studies on Korean patients with SCC[34]. The incidence of UV-induced SCC is inversely proportional to latitude; the closer it is to the equator, the higher the incidence[35]. Moreover, the incidence was higher in the UV-sensitive fair skin type than in the dark skin type. This study was conducted in South Korea, and the frequency of p53 mutations caused by UV damage was estimated to be lower than that reported in previous studies in Caucasian individuals[34]; however, the difference was not significant. In addition, no significant association was observed between HPV and p53 expression in HNCSCC ($P > 0.99$). P16 has emerged as a cost-effective surrogate marker for HPV in OPSCC, owing to its high sensitivity and specificity for detecting HPV-related tumors. IHC staining for p16 can reliably identify tumors with transcriptionally active HPV, making it a valuable tool for diagnostic and prognostic purposes. Additionally, p16 expression in OPSCC has been associated with favorable prognosis, as HPV-positive tumors generally exhibit a better response to treatment and improved survival outcomes than HPV-negative tumors. However, the role of p16 in HNCSCC remains to be elucidated. A study including HNCSCC patients with HNSCC reported that p16 expression was not related to area H HPV or prognosis[36]. The positivity rate for p16 in this study was 38.71% (24/62), which is similar to the 31.9% reported in a previous study on HNCSCC. The positive predictive value of p16 for HPV was 8.33%, which was very low compared with 92.9% for OPSCC in a previous study, and there was no significant relationship between HPV and p16 ($P > 0.99$)[36]. This finding suggests that the relationship between p16 expression and HPV status may differ between OPSCC and HNCSCC, highlighting the need for further research to elucidate the role of p16 in HNCSCC pathogenesis and its potential utility as a prognostic biomarker.

One possible explanation for the low positivity rate of p16 in HNCSCC is the influence of UV radiation exposure, which is a known risk factor for cutaneous SCC. UV exposure induces DNA damage and promotes genomic instability, leading to the accumulation of mutations in key regulatory genes such as p53, which may disrupt normal cell cycle regulation and override the compensatory mechanism of p16 overexpression in HPV-related OPSCC[25]. Furthermore, other environmental and genetic factors specific to cutaneous SCC may modulate p16 expression independently of HPV status, further complicating its utility as a prognostic biomarker for HNCSCC. Overall, the contrasting positivity rates of p16 in OPSCC and HNCSCC highlight the complex interplay between HPV infection, UV radiation exposure, and other etiological factors involved in the pathogenesis of head and neck cancer. Further research is required to elucidate the mechanisms underlying p16 expression in HNCSCC and its potential prognostic significance in these tumors.

The location of HNCSCC was classified as either area H or M to confirm the relationship between p16 expression and HNCSCC prognosis indirectly. According to the National Comprehensive Cancer Network guidelines, SCC located in area H has a high probability of recurrence and metastasis and requires Mohs microsurgery[37]. In this study, the relationship between p16 and tumor location was not statistically significant ($P = 0.444$), indicating no correlation between p16 and favorable prognosis in HNCSCC. This study had certain limitations. First, it was conducted at a single institution and focused on a specific racial demographic, which may have limited the generalizability of the findings to other populations. Additionally, the sample size was relatively small, comprising only 62 cases with a limited number of HPV-positive samples. Furthermore, HPV, p16, and p53 statuses were determined using IHC staining methods rather than more sensitive techniques such as PCR or HPV genotyping, limiting the ability to assess specific area H HPV types potentially associated with prognosis. While IHC staining is commonly used for clinical diagnosis, its sensitivity and specificity for detecting HPV and other molecular markers may vary, potentially leading to the misclassification of cases [38]. Furthermore, recurrence and metastasis data were collected retrospectively, which may limit accuracy in tracking long-term outcomes and survival. These limitations suggest the need for larger, multi-center studies with extended follow-up to better understand the implications of HPV, p16, and p53 expression on HNCSCC prognosis.

CONCLUSION

This study found that the HPV infection rate in HNCSCC was lower than that reported in studies conducted in North America. Furthermore, the positive rate of p53 did not demonstrate a significant difference compared with the findings in Caucasian populations. Notably, p16, which is often considered a surrogate marker for HPV in OPSCC, was not significantly associated with HPV in HNCSCC, and its overexpression was linked to increased recurrence and metastasis risks, indicating its limited utility as a prognostic marker in HNCSCC. Additionally, p16 expression was not associated with a favorable prognosis in HNCSCC. This study contributes to our understanding of the relationship between HPV,

p53, and p16 in HNCSCC, which has received less attention than OPSCC in previous studies. Moreover, the focus of this study on Asian populations provides valuable insights, as most previous studies have been conducted on Caucasian populations. Our findings suggest that p16 and p53 expression, along with HPV positivity, may be associated with poorer prognostic outcomes in HNCSCC, particularly in terms of recurrence and metastasis risks. Overall, this study may aid in further elucidating the complex interplay between HPV infection and molecular markers of HNCSCC in Asian populations, thus providing future research directions and clinical management strategies.

FOOTNOTES

Author contributions: Nam HJ and Lee JH contributed to manuscript writing; Ryu H contributed to conceptualization and methodology; Lee DW and Byeon JY contributed to project administration; Lim S contributed to data collection; Kim JH and Choi HJ contributed to manuscript review, editing, visualization, analysis, and supervision; and all authors have read and approved the final manuscript.

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REFERENCES

1 **Aldabagh B**, Angeles JG, Cardones AR, Arron ST. Cutaneous squamous cell carcinoma and human papillomavirus: is there an association? *Dermatol Surg* 2013; **39**: 1-23 [PMID: 22928516 DOI: 10.1111/j.1524-4725.2012.02558.x]

2 **Harwood CA**, McGregor JM, Proby CM, Breuer J. Human papillomavirus and the development of non-melanoma skin cancer. *J Clin Pathol* 1999; **52**: 249-253 [PMID: 10474513 DOI: 10.1136/jcp.52.4.249]

3 **Wookey VB**, Appiah AK, Kallam A, Ernani V, Smith LM, Ganti AK. HPV Status and Survival in Non-Oropharyngeal Squamous Cell Carcinoma of the Head and Neck. *Anticancer Res* 2019; **39**: 1907-1914 [PMID: 30952732 DOI: 10.21873/anticanres.13299]

4 **Que SKT**, Zwald FO, Schmults CD. Cutaneous squamous cell carcinoma: Incidence, risk factors, diagnosis, and staging. *J Am Acad Dermatol* 2018; **78**: 237-247 [PMID: 29332704 DOI: 10.1016/j.jaad.2017.08.059]

5 **Jiromaru R**, Yamamoto H, Yasumatsu R, Hongo T, Nozaki Y, Nakano T, Hashimoto K, Nakagawa T, Oda Y. p16 overexpression and Rb loss correlate with high-risk HPV infection in oropharyngeal squamous cell carcinoma. *Histopathology* 2021; **79**: 358-369 [PMID: 33450095 DOI: 10.1111/his.14337]

6 **Lei J**, Ploner A, Lagheden C, Eklund C, Nordqvist Kleppe S, Andrae B, Elfström KM, Dillner J, Sparén P, Sundström K. High-risk human papillomavirus status and prognosis in invasive cervical cancer: A nationwide cohort study. *PLoS Med* 2018; **15**: e1002666 [PMID: 30273338 DOI: 10.1371/journal.pmed.1002666]

7 **Sarier M**, Usta SS, Turgut H, Öztürk SA, Soylu A, Emek M, Kukul E, Bozcuk H, Sepin N. Prognostic value of HPV DNA in Urothelial Carcinoma of the Bladder: A Preliminary Report of 2-Year Follow-up Results. *Urol J* 2021; **19**: 45-49 [PMID: 33931844 DOI: 10.22037/uj.v18i.6429]

8 **Ciążyńska M**, Kamińska-Winciorek G, Lange D, Lewandowski B, Reich A, Sławińska M, Pabianek M, Szczepaniak K, Hankiewicz A, Ułańska M, Morawiec J, Błasińska-Morawiec M, Morawiec Z, Piekarski J, Nejc D, Brodowski R, Zaryczańska A, Sobjanek M, Nowicki RJ, Owczarek W, Słowińska M, Wróbel K, Bieniek A, Woźniacka A, Skibińska M, Narbutt J, Niemczyk W, Ciążyński K, Lesiak A. The incidence and clinical analysis of non-melanoma skin cancer. *Sci Rep* 2021; **11**: 4337 [PMID: 33619293 DOI: 10.1038/s41598-021-83502-8]

9 **zur Hausen H**. Papillomaviruses in the causation of human cancers - a brief historical account. *Virology* 2009; **384**: 260-265 [PMID: 19135222 DOI: 10.1016/j.virol.2008.11.046]

10 **Syrjänen K**, Syrjänen S, Lamberg M, Pyrhönen S, Nuutinen J. Morphological and immunohistochemical evidence suggesting human papillomavirus (HPV) involvement in oral squamous cell carcinogenesis. *Int J Oral Surg* 1983; **12**: 418-424 [PMID: 6325356 DOI: 10.1016/s0300-9785(83)80033-7]

11 **Gordis TM**, Cagle JL, Nguyen SA, Newman JG. Human Papillomavirus-Associated Oropharyngeal Squamous Cell Carcinoma: A Systematic Review and Meta-Analysis of Clinical Trial Demographics. *Cancers (Basel)* 2022; **14**: 4061 [PMID: 36011055 DOI: 10.3390/cancers14164061]

12 **Kreimer AR**, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiol Biomarkers Prev* 2005; **14**: 467-475 [PMID: 15734974 DOI: 10.1158/1055-9965.EPI-04-0551]

13 **Lewis JS Jr**, Thorstad WL, Chernock RD, Haughey BH, Yip JH, Zhang Q, El-Mofty SK. p16 positive oropharyngeal squamous cell carcinoma:an entity with a favorable prognosis regardless of tumor HPV status. *Am J Surg Pathol* 2010; **34**: 1088-1096 [PMID: 20588174 DOI: 10.1097/PAS.0b013e3181e84652]

14 **Chaturvedi AK**, Engels EA, Pfeiffer RM, Hernandez BY, Xiao W, Kim E, Jiang B, Goodman MT, Sibug-Saber M, Cozen W, Liu L, Lynch CF, Wentzensen N, Jordan RC, Altekruse S, Anderson WF, Rosenberg PS, Gillison ML. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *J Clin Oncol* 2011; **29**: 4294-4301 [PMID: 21969503 DOI: 10.1200/JCO.2011.36.4596]

15 **D'Souza G**, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, Westra WH, Gillison ML. Case-control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med* 2007; **356**: 1944-1956 [PMID: 17494927 DOI: 10.1056/NEJMoa065497]

16 **Fakhry C**, Westra WH, Li S, Cmelak A, Ridge JA, Pinto H, Forastiere A, Gillison ML. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst* 2008; **100**: 261-269 [PMID: 18270337 DOI: 10.1093/jnci/djn011]

17 **Westra WH**. The changing face of head and neck cancer in the 21st century: the impact of HPV on the epidemiology and pathology of oral cancer. *Head Neck Pathol* 2009; **3**: 78-81 [PMID: 20596995 DOI: 10.1007/s12105-009-0100-y]

18 **Ang KK**, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF, Westra WH, Chung CH, Jordan RC, Lu C, Kim H, Axelrod R, Silverman CC, Redmond KP, Gillison ML. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med* 2010; **363**: 24-35 [PMID: 20530316 DOI: 10.1056/NEJMoa0912217]

19 **Lassen P**, Eriksen JG, Hamilton-Dutoit S, Tramm T, Alsner J, Overgaard J; Danish Head and Neck Cancer Group (DAHANCA). HPV-associated p16-expression and response to hypoxic modification of radiotherapy in head and neck cancer. *Radiother Oncol* 2010; **94**: 30-35 [PMID: 19910068 DOI: 10.1016/j.radonc.2009.10.008]

20 **D'Souza G**, Dempsey A. The role of HPV in head and neck cancer and review of the HPV vaccine. *Prev Med* 2011; **53** Suppl 1: S5-S11 [PMID: 21962471 DOI: 10.1016/j.ypmed.2011.08.001]

21 **O'Rorke MA**, Ellison MV, Murray LJ, Moran M, James J, Anderson LA. Human papillomavirus related head and neck cancer survival: a systematic review and meta-analysis. *Oral Oncol* 2012; **48**: 1191-1201 [PMID: 22841677 DOI: 10.1016/j.oraloncology.2012.06.019]

22 **Dayyani F**, Etzel CJ, Liu M, Ho CH, Lippman SM, Tsao AS. Meta-analysis of the impact of human papillomavirus (HPV) on cancer risk and overall survival in head and neck squamous cell carcinomas (HNSCC). *Head Neck Oncol* 2010; **2**: 15 [PMID: 20587061 DOI: 10.1186/1758-3284-2-15]

23 **Gillison ML**, Broutian T, Pickard RK, Tong ZY, Xiao W, Kahle L, Graubard BI, Chaturvedi AK. Prevalence of oral HPV infection in the United States, 2009-2010. *JAMA* 2012; **307**: 693-703 [PMID: 22282321 DOI: 10.1001/jama.2012.101]

24 **Gillison ML**, Chaturvedi AK, Anderson WF, Fakhry C. Epidemiology of Human Papillomavirus-Positive Head and Neck Squamous Cell Carcinoma. *J Clin Oncol* 2015; **33**: 3235-3242 [PMID: 26351338 DOI: 10.1200/JCO.2015.61.6995]

25 **Hasche D**, Stephan S, Braspenning-Wesch I, Mikulec J, Niebler M, Gröne HJ, Flechtenmacher C, Akgül B, Rösl F, Vinzón SE. The interplay of UV and cutaneous papillomavirus infection in skin cancer development. *PLoS Pathog* 2017; **13**: e1006723 [PMID: 29190285 DOI: 10.1371/journal.ppat.1006723]

26 **Gillison ML**, Koch WM, Capone RB, Spafford M, Westra WH, Wu L, Zahurak ML, Daniel RW, Viglione M, Symer DE, Shah KV, Sidransky D. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst* 2000; **92**: 709-720 [PMID: 10793107 DOI: 10.1093/jnci/92.9.709]

27 **Mehanna H**, Beech T, Nicholson T, El-Hariry I, McConkey C, Paleri V, Roberts S. Prevalence of human papillomavirus in oropharyngeal and nonoropharyngeal head and neck cancer--systematic review and meta-analysis of trends by time and region. *Head Neck* 2013; **35**: 747-755 [PMID: 22267298 DOI: 10.1002/hed.22015]

28 **Awan MS**, Irfan B, Zahid I, Mirza Y, Ali SA. Comparison of Polymerase Chain Reaction and Immunohistochemistry Assays for Analysing Human Papillomavirus Infection in Oral Squamous Cell Carcinoma. *J Clin Diagn Res* 2017; **11**: XC10-XC13 [PMID: 28764278 DOI: 10.7860/JCDR/2017/24742.10119]

29 **Soussi T**, Wiman KG. Shaping genetic alterations in human cancer: the p53 mutation paradigm. *Cancer Cell* 2007; **12**: 303-312 [PMID: 17936556 DOI: 10.1016/j.ccr.2007.10.001]

30 **Tandon S**, Tudur-Smith C, Riley RD, Boyd MT, Jones TM. A systematic review of p53 as a prognostic factor of survival in squamous cell carcinoma of the four main anatomical subsites of the head and neck. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 574-587 [PMID: 20142252 DOI: 10.1158/1055-9965.EPI-09-0981]

31 **Dong W**, Arpin C, Accardi R, Gissmann L, Sylla BS, Marvel J, Tommasino M. Loss of p53 or p73 in human papillomavirus type 38 E6 and E7 transgenic mice partially restores the UV-activated cell cycle checkpoints. *Oncogene* 2008; **27**: 2923-2928 [PMID: 18026133 DOI: 10.1038/sj.onc.1210944]

32 **Münger K**, Baldwin A, Edwards KM, Hayakawa H, Nguyen CL, Owens M, Grace M, Huh K. Mechanisms of human papillomavirus-induced oncogenesis. *J Virol* 2004; **78**: 11451-11460 [PMID: 15479788 DOI: 10.1128/JVI.78.21.11451-11460.2004]

33 **Thomas M**, Pim D, Banks L. The role of the E6-p53 interaction in the molecular pathogenesis of HPV. *Oncogene* 1999; **18**: 7690-7700 [PMID: 10618709 DOI: 10.1038/sj.onc.1202953]

34 **Lee SY**, Lee M, Yu DS, Lee YB. Identification of genetic mutations of cutaneous squamous cell carcinoma using whole exome sequencing in non-Caucasian population. *J Dermatol Sci* 2022; **106**: 70-77 [PMID: 35365379 DOI: 10.1016/j.jdermsci.2022.03.007]

35 **Lomas A**, Leonardi-Bee J, Bath-Hextall F. A systematic review of worldwide incidence of nonmelanoma skin cancer. *Br J Dermatol* 2012; **166**: 1069-1080 [PMID: 22251204 DOI: 10.1111/j.1365-2133.2012.10830.x]

36 **Satgunaseelan L**, Chia N, Suh H, Virk S, Ashford B, Lum T, Ranson M, Clark J, Gupta R. p16 expression in cutaneous squamous cell

carcinoma of the head and neck is not associated with integration of high risk HPV DNA or prognosis. *Pathology* 2017; **49**: 494-498 [PMID: 28663082 DOI: 10.1016/j.pathol.2017.04.002]

37 **Schmults CD**, Blitzblau R, Aasi SZ, Alam M, Andersen JS, Baumann BC, Bordeaux J, Chen PL, Chin R, Contreras CM, DiMaio D, Donigan JM, Farma JM, Ghosh K, Grekin RC, Harms K, Ho AL, Holder A, Lukens JN, Medina T, Nehal KS, Nghiem P, Park S, Patel T, Puzanov I, Scott J, Sekulic A, Shaha AR, Srivastava D, Stebbins W, Thomas V, Xu YG, McCullough B, Dwyer MA, Nguyen MQ. NCCN Guidelines® Insights: Squamous Cell Skin Cancer, Version 1.2022. *J Natl Compr Canc Netw* 2021; **19**: 1382-1394 [PMID: 34902824 DOI: 10.6004/jnccn.2021.0059]

38 **Wentzensen N**, Schwartz L, Zuna RE, Smith K, Mathews C, Gold MA, Allen RA, Zhang R, Dunn ST, Walker JL, Schiffman M. Performance of p16/Ki-67 immunostaining to detect cervical cancer precursors in a colposcopy referral population. *Clin Cancer Res* 2012; **18**: 4154-4162 [PMID: 22675168 DOI: 10.1158/1078-0432.CCR-12-0270]

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