Cytokeratin 17 and Ki-67: Immunohistochemical markers for the differential diagnosis of keratoacanthoma and squamous cell carcinoma

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Abstract. The clinical and histopathological distinction between keratoacanthoma (KA) and squamous cell carcinoma (SCC) is essential, but frequently difficult to make. The utility of CK17 and Ki-67 expression in distinguishing between KA and SCC was investigated. Immunohistochemical staining patterns for CK17 and Ki-67 were evaluated in 24 KA and 27 SCC cases. The pattern of staining was evaluated as central, peripheral or diffuse, according to the basal/peripheral and suprabasal/central cell staining of tumor lobules. The sensitivity and specificity of the central CK17 staining pattern in the identification of KA were 92 and 70%, respectively. Additionally, the sensitivity and specificity of the diffuse Ki-67 staining pattern in the identification of SCC were 81 and 100%, respectively. The results of the present study suggest that a diffuse Ki-67 staining pattern may be used to diagnose SCC, while a central CK17 staining pattern indicates KA. However, the KA-like SCC cases exhibited mixed patterns, which limits the effectiveness of these markers.

Introduction

Cutaneous squamoproliferative lesions with crateriform architecture are frequently encountered (1,2). KAs are keratin-plugged, crater-shaped nodules that develop predominantly on the surfaces of the body exposed to the sun and typically grow rapidly, prior to regression (2). Although the majority of KAs are solitary and sporadic, multiple lesions may develop in association with certain syndromes (3). The etiology of KAs remains unclear; however, it is currently considered that they develop from the pilosebaceous unit (3,4).

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Histopathological differentiation between keratoacanthoma (KA) and squamous cell carcinoma (SCC) is difficult. It is occasionally impossible to distinguish between these lesions through clinical and histopathological analysis, particularly in small biopsy specimens (1). Therefore, multiple immunohistochemical markers have been investigated to overcome this limitation (5). Proliferation marker protein Ki-67 (Ki-67) is a cell cycle-regulating protein frequently studied in squamoproliferative lesions and the most useful marker among immunohistochemical markers (6,7). Cytokeratin (CK) 17 is a basal/myoepithelial cell-associated keratin protein that is expressed in the outer root sheath of wild-type hair follicles (8). It is not expressed in the normal epidermis but induced in activated keratinocytes (9), and its expression is associated with disease progression in SCCs of the anus, oral cavity and uterine cervix (10-12). To the best of our knowledge, CK17 expression has not been investigated in KA. The aim of the present study was to investigate the utility of CK17 and Ki-67 as adjunctive markers for the differential diagnosis of KA and SCC.

Materials and methods

Case selection. Tissue samples of patients diagnosed with KA or SCC of the skin between 1st January 2011 and 1st January 2015, were obtained from the Pathology Laboratory of the Istanbul Research and Educational Hospital (Istanbul, Turkey). Patient number, age and gender are presented in Tables I and II. Hematoxylin and eosin-stained slides of 43 cases of SCC and 24 cases of KA were re-examined. All lesions had been completely removed by surgery. Cases of moderately and poorly differentiated SCC were excluded. Due to the considerable overlap with KA, only grade 1 cases of SCC exhibiting a predominantly endophytic growth pattern were included in the present study. KA-like and crateriform cases of SCC were determined using the original characterization criteria of Misago *et al* (2). KA-like SCC lesions exhibit the characteristic histopathological architectural pattern of KA, and the characteristic neoplastic lobules of KA in certain regions. This includes the regular distribution of two types of cells: Large, pale pink cells with a glassy appearance thinly surrounded by a few layers of basophilic cells, which

are also asymmetrical and/or deeply invasive lesions, and the majority exhibit nuclear atypia and frequent mitotic figures. Crateriform SCC lesions arise from actinic keratosis, have crateriform architecture and lack KA-resembling cytological features (2).

Immunohistochemistry. Immunohistochemical reactions were performed on paraffin tissue sections using an automated immunohistochemical stainer (Ventana BenchMark ULTRA, Ventana Medical Systems, Inc., Tucson, AZ, USA), according to the manufacturer's protocol. Detection was performed using the Ventana ultraVIEW DAB Detection kit (Ventana Medical Systems, Inc.). The 4-μm-tissue sections were deparaffinized using the EZ Prep solution (Ventana Medical Systems, Inc.; cat. no. 05279771001). Heat-induced antigen retrieval was performed using the Cell Conditioning 1 solution (Ventana Medical Systems, Inc.; cat. no. 05424569001) at 98°C for 60 min. Endogenous peroxidase activity was blocked by treatment with the ultraVIEW inhibitor (Ventana Medical Systems, Inc.; cat no. 05269806001) in 3% H_2O_2 for 4 min at 37°C. Slides were incubated with the following primary antibodies for 60 min at 37°C: Ready-to-use CONFIRM anti-Ki-67 rabbit monoclonal antibody (cat. no. 30-9; Ventana Medical Systems); and ready-to-use anti-CK17 rabbit monoclonal antibody (cat. no. SP95; Ventana Medical Systems, Inc.). The ultraView Universal DAB Detection kit incorporates multimer technology, whereby the ultraVIEW streptavidin-horseradish peroxidase (HRP) enzyme is directly conjugated to the secondary antibody. Slides were incubated with a secondary antibody of ultraVIEW HRP Multimer (Ventana Medical Systems, Inc.; cat. no. 05269806001) at 37°C for 8 min and a diaminobenzidine + H_2O_2 substrate for 8 min, which was followed by counterstaining with hematoxylin and bluing reagent at 37°C, for 16 and 4 min, respectively. Slides were washed with Tris buffer (pH 7.6) and mounted using a xylene-based mounting media.

The pattern of immunohistochemical expression of CK17 was determined for all lesions. As reported in previous studies (8,10,13-15), the interpretation of the CK17 staining pattern was modeled on the physiological immunolocalization of keratin expression in the basal and suprabasal layers of human epithelia, specifically in the hair follicle. The staining patterns were evaluated as central and diffuse, according to the basal/peripheral and suprabasal/central cell staining of the tumor lobules. The following conditions were considered to indicate a central pattern (CP) of staining: i) CK17 expression was limited to the center (suprabasal or inner two-thirds of the tumor cell lobules) without peripheral staining; ii) the peripheral/basal cells of the tumor lobules stained weaker than the central/suprabasal cells; iii) mixed staining patterns were observed as described in i) and ii); and iv) strong expression was present in <10% of peripheral/basal cells, while the rest of the lesion exhibited the staining pattern described in i) and/or ii). A diffuse pattern (DP) of staining was diagnosed if the strong staining of peripheral/basal cells and that of central/suprabasal cells comprised >10% of the whole lesion.

A peripheral pattern (PP) of staining was diagnosed if the basal or peripheral cells of the tumor lobules were stained. Suprabasal or central staining of the lobules with basal/peripheral staining was interpreted as a DP.

Statistical analysis. Statistical analysis was performed using SPSS statistical software (version 13.0; SPSS, Inc., Chicago, IL, USA). The sensitivity, specificity and positive/negative predictive values of the CK17 CP staining for the detection of KA and of the Ki-67 DP staining for the detection of SCC were calculated as follows: Sensitivity=[true positive/(true positive + false negative)]; specificity=[true negative/(false positive + true negative)]; positive predictive value=[true positive/(true positive + false positive)]; and negative predictive value=[true negative/(true negative + false negative)]. CK17 and Ki-67 expression patterns were compared between KA and SCC cases using the two-tailed Fisher's exact test. The Kruskal-Wallis and Mann-Whitney U tests were used to examine other quantitative data. P<0.05 was considered to indicate a statistically significant difference.

Results

A total of 24 KA and 27 SCC samples were obtained. Prominent crateriform architecture was present in 10/27 SCC samples, Of these, 6 were defined as KA-like SCC and 4 as crateriform SCC. The clinicopathological and immunohistochemical features of each sample are summarized in Tables I and II.

The KA samples were obtained from 10 male and 14 female patients with a mean age of 63.7 years (range, 42-86 years). The tumors were located predominantly in the head and neck region (67%). The median tumor size was 0.9 cm (range, 0.4-3.5 cm), and the median duration of the lesions was 2.5 months. Duration of the lesions was obtained from the patient history files. The SCC samples were obtained from 22 male and 5 female patients with a mean age of 74.6 years (range, 56-90 years). The tumors were located predominantly in the head and neck region (85%). The median tumor size was 1.7 cm (range, 0.4-3.5 cm), and the median duration of the lesions was 5 months. The mean age of the patients with SCC was significantly higher compared with that of patients with KA (P=0.0358). The number of male patients with SCC was significantly higher compared with that of female patients (P=0.0261). The duration of the KA lesions (mean, 3.8 months) was lower compared with that of the SCC lesions (mean, 13.3 months).

A total of 51/51 (100%) cases stained positive for CK17 following immunohistochemical analysis. A total of 22/24 (92%) KA cases exhibited a CK17 CP (Figs. 1-3), while 2/24 cases (8%) exhibited a CK17 DP. A total of 8/27 (30%) SCC cases exhibited a CK17 CP, while 19/27 cases (70%) exhibited a CK17 DP (Table III). The incidence of a CK17 CP was increased in the KA cases compared with that in the SCC cases. The sensitivity and specificity of a CK17 CP in the identification of KA was 92 and 70%, respectively (Table IV). All KA cases (24/24) exhibited a Ki-67 PP. A total of 22/27 (81%) SCC cases exhibited a Ki-67 DP (Fig. 4) and the remaining 5 SCC cases exhibited a Ki-67 PP (Table V). The incidence of a Ki-67 DP was increased in the SCC cases compared with that in the KA cases. The sensitivity and specificity of a Ki-67 DP in the identification of SCC was 81 and 100%, respectively (Table VI).

The majority of KA cases exhibited a Ki-67 PP and CK17 CP; therefore this staining pattern was named the

Table I. Clinicopathological and immunohistochemical features of keratoacanthoma samples.

Patient no.	Age (years)	Gender	Location	Duration of the lesions (months)	Size (cm)	CK17 staining pattern	Ki-67 staining pattern
1	58	M	Face	2.0	0.5	C	P
2	76	M	Upper extremity	3.0	0.7	C[a]	P
3	42	M	Neck	3.0	1.0	C	P
4	71	F	Face	4.5	1.0	C[a]	P
5	69	F	Face	1.0	0.8	C[a]	P
6	60	F	Face	1.0	2.0	C[a]	P
7	53	F	Upper extremity	2.0	Unknown	C[a]	P
8	59	M	Head	Unknown	0.8	C[a]	P
9	79	M	Face	1.5	1.7	C[a]	P
10	47	M	Lower extremity	Unknown	1.1	C[a]	P
11	84	M	Face	Unknown	Unknown	D	P
12	54	F	Face	Unknown	0.6	C[a]	P
13	48	F	Neck	Unknown	Unknown	C	P
14	57	F	Trunk	12	0.6	D	P
15	60	F	Neck	6.0	0.4	C	P
16	82	M	Upper extremity	3.0	0.9	C	P
17	82	F	Face	2.0	3.5	C[a]	P
18	45	F	Trunk	1.0	1.0	C	P
19	72	M	Face	2.0	1.2	C	P
20	67	F	Head	5.0	0.8	C[a]	P
21	56	F	Trunk	11.0	0.6	C	P
22	66	M	Upper extremity	1.0	1.0	C[a]	P
23	56	F	Neck	6.0	0.4	C	P
24	86	F	Face	Unknown	1.7	C	P

[a]These cases exhibited weak peripheral/basal cell staining. CK17, cytokeratin 17; Ki-67, proliferation marker protein Ki-67; M, male; F, female; C, central staining pattern; D, diffuse staining pattern; P, peripheral staining pattern.

KA-like staining pattern (Figs. 1-3). The KA-like staining pattern was exhibited by 22/24 KA cases (92%) and by 2/27 SCC cases (7%). The SCC cases primarily exhibited a Ki-67 and CK17 DP; this was therefore named the SCC-like staining pattern (Figs. 4-6). The SCC-like staining pattern was exhibited by 16/27 SCC cases (59%) and by 0/22 KA cases (0%). All KA-like SCC cases were located on the face or head and exhibited rapid growth, similar to the KA cases. Crateriform SCC lesions were clinically characterized as long-standing nodules, and all exhibited a Ki-67 and CK17 DP. Also, CK17 was expressed in the suprabasal cells of the outer root sheath of the normal hair follicles in the examined cases (Fig. 7).

Discussion

There is currently no consensus within the fields of dermatology and dermatopathology as to whether KA is a benign tumor, a pseudomalignancy, a regressing malignancy or an SCC variant. The lesion was previously considered to be a benign neoplasm and has been described using various names, including molluscum sebaceum and self-limiting epithelioma (16). Certain KA lesions were demonstrated to not exhibit metastasis or recurrence despite the presence of perineural infiltration (17). By contrast, there have been cases of metastases purported to originate from KAs, which fulfill the diagnostic histological criteria of KA (3,18). KA may be defined as a distinct subtype of SCC that exhibits low-grade malignancy and typically regresses; however, it is able to progress into invasive SCC (2). KA-like SCC may be used to describe lesions that histopathologically resemble KA but exhibit eccentric architecture and prominent atypical cytological features (2,19).

Misago *et al* (2) attempted to standardize the terminology used to define KAs and associated neoplasms exhibiting a crateriform architecture. The following six histopathological classifications were identified: i) KA; ii) KA-like SCC; iii) KA with malignant transformation; iv) infundibular SCC (crateriform); v) crateriform SCC arising from actinic keratosis; and vi) crateriform Bowen's disease. In the present study, 6 lesions were described as KA-like SCC and 4 as crateriform SCC according to the above classification. No cases of KA with

Table II. Clinicopathological and immunohistochemical features of squamous cell carcinoma samples.

Patient no.	Age (years)	Gender	Histological subtype	Location	Duration of the lesions (months)	Size (cm)	CK17 staining pattern	Ki-67 staining pattern
1	56	M	NOS	Face	24.0	1.4	C	P
2	83	M	NOS	Neck	16.0	1.5	C	D
3	88	F	NOS	Face	11.0	1.3	D	D
4	62	M	NOS	Head	Unknown	2.0	D	D
5	78	F	NOS	Face	Unknown	2.5	D	D
6	68	M	NOS	Neck	16.0	2.5	D	D
7	88	M	NOS	Head	12.0	3.5	D	D
8	81	M	NOS	Head	14.0	1.4	D	D
9	89	F	NOS	Face	22.0	2.5	D	P
10	64	M	NOS	Face	Unknown	1.5	D	D
11	81	M	NOS	Lower extremity	Unknown	3.5	D	D
12	90	M	NOS	Head	Unknown	1.9	D	D
13	66	M	NOS	Face	11.0	1.0	C	D
14	86	M	NOS	Upper extremity	14.0	2.3	D	P
15	76	M	NOS	Upper extremity	Unknown	1.2	D	D
16	71	M	NOS	Head	Unknown	0.5	C	D
17	73	M	NOS	Head	Unknown	1.0	C	D
18	67	M	KA-like	Face	3.0	1.4	C	P
19	77	M	KA-like	Head	Unknown	2.3	D	P
20	81	F	KA-like	Face	6.0	1.6	C	D
21	69	M	KA-like	Head	3.0	1.5	D	D
22	59	M	KA-like	Head	2.5	3.5	C	D
23	67	F	KA-like	Face	5.0	1.7	D	D
24	59	M	Crateriform	Face	15.0	2.0	D	D
25	82	M	Crateriform	Face	19.0	3.0	D	D
26	67	M	Crateriform	Upper extremity	Unknown	1.0	D	D
27	86	M	Crateriform	Head	32.0	1.8	D	D

CK17, cytokeratin 17; Ki-67, proliferation marker protein Ki-67; M, male; F, female; C, central staining pattern; D, diffuse staining pattern; P, peripheral staining pattern; NOS, not otherwise specified; KA, keratoacanthoma.
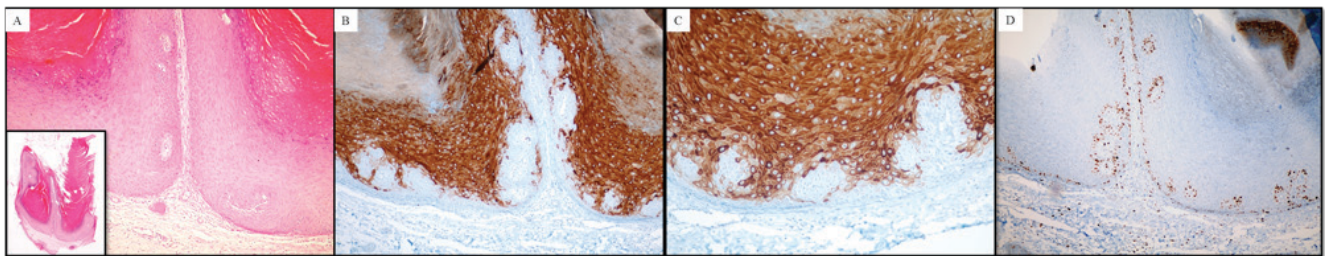

Figure 1. Representative images of (A) hematoxylin and eosin-stained KA (magnification, x100) exhibiting a central CK17 IHC staining pattern at (B) magnification, x100 and (C) magnification, x200, or (D) a peripheral proliferation marker protein Ki-67 IHC staining pattern (magnification, x100). These staining patterns constitute the KA-like staining pattern, exhibiting no basal or peripheral CK17 staining. KA, keratoacanthoma; CK17, cytokeratin 17; IHC, immunohistochemistry.

malignant transformation or infundibular SCC were identified in the present study.

Multiple immunohistochemical markers have been investigated in order to differentiate between KA and SCC. Immunoperoxidase staining has been used to demonstrate that transforming growth factor (TGF)-α primarily exhibits a DP in the one or two layers of KA samples, while exhibiting no PP (20). Similarly, TGF-α has been demonstrated to
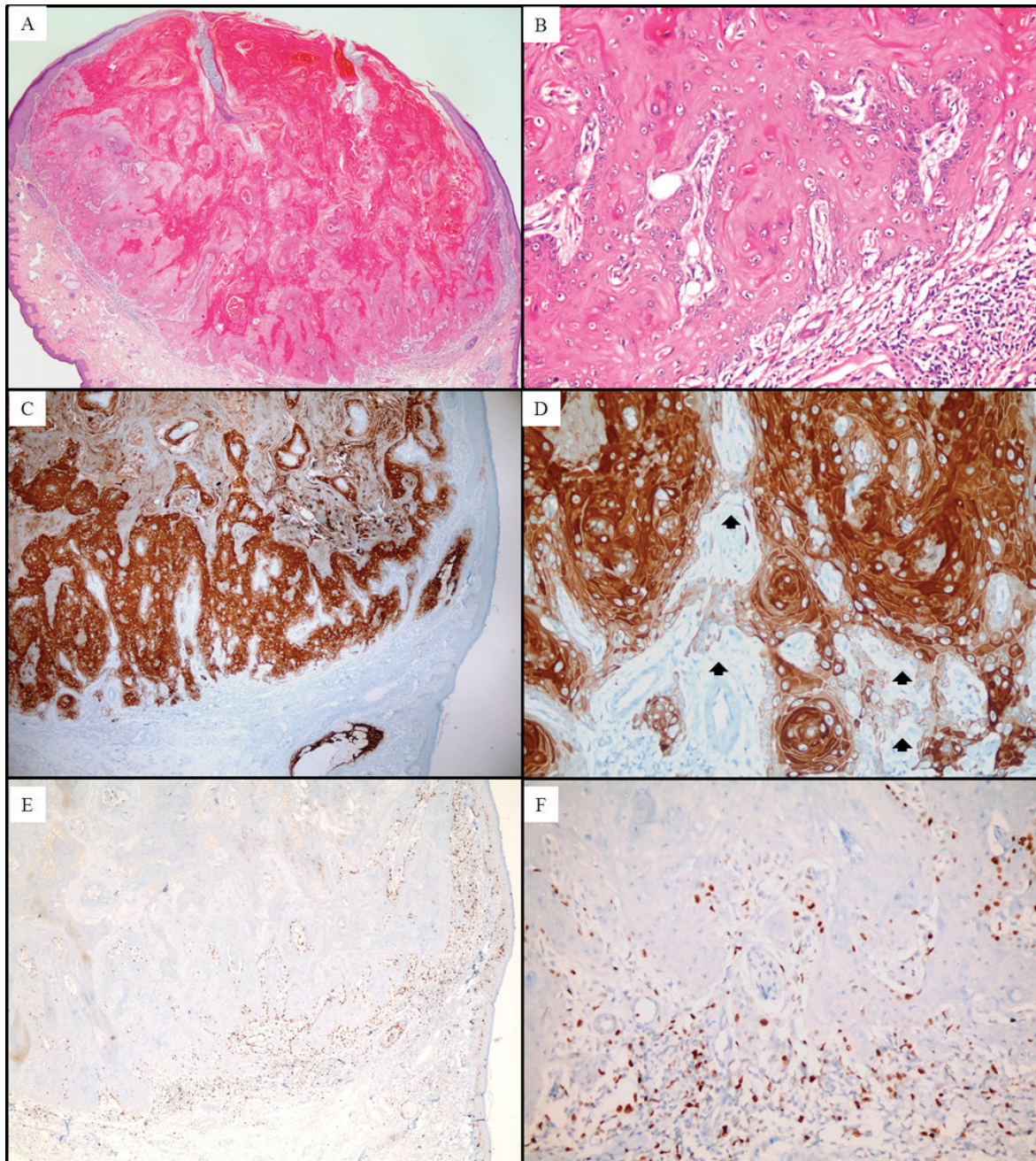
Figure 2. Representative images of hematoxylin and eosin-stained KA, at (A) magnification x20 and (B) magnification x200, exhibiting a central CK17 IHC staining pattern, at (C) magnification x40 and (D) magnification x200, or a peripheral proliferation marker protein Ki-67 IHC staining pattern, at (E) magnification x40 and (F) magnification x200. These staining patterns constitute the KA-like staining pattern, exhibiting weak basal or peripheral CK17 staining. Black arrowheads indicate weak peripheral CK17 staining. KA, keratoacanthoma; CK17, cytokeratin 17; IHC, immunohistochemistry.

exhibit a weak PP in one or two layers of SCC samples (20). Lu *et al* (5) demonstrated decreased p21 and proliferating cell nuclear antigen expression in KA samples despite increased p53 expression. Vasiljević *et al* (21) suggested that decreased expression of the anti-apoptotic protein Bcl-2-like protein 1 in KA compared with SCC is a differential marker. By contrast, evaluation of syndecan-1, E-cadherin and catenin staining revealed subtle differences in staining patterns between KA and SCC (22,23).

Multiple studies have identified significant overexpression of Ki-67 in SCC compared with that in KA (6,7,21,23-26). Scola *et al* (6) suggested that a Ki-67 fraction of <20% may therefore be indicative of KA. All SCC cases in the study by

Shimizu *et al* (24) also exhibited a positive Ki-67 fraction of >20%. In these studies, Ki-67 expression was primarily restricted to basal cells in KAs, whereas it was distributed diffusely throughout SCC samples (6,24). In addition, a study investigating subungual cases of KA and SCC evaluated Ki-67 immunohistochemical staining in a semiquantitative manner, according to the percentage of stained basal cells and the presence or absence of suprabasal cell staining (7). It was observed that subungual KA exhibited an occasional Ki-67 basal staining pattern, while subungual SCC cases exhibited a Ki-67 DP (7). A Ki-67 PP was therefore defined as the staining of basal cells only, and a Ki-67 DP was defined as the staining of basal and suprabasal/central cells, to ensure practical and
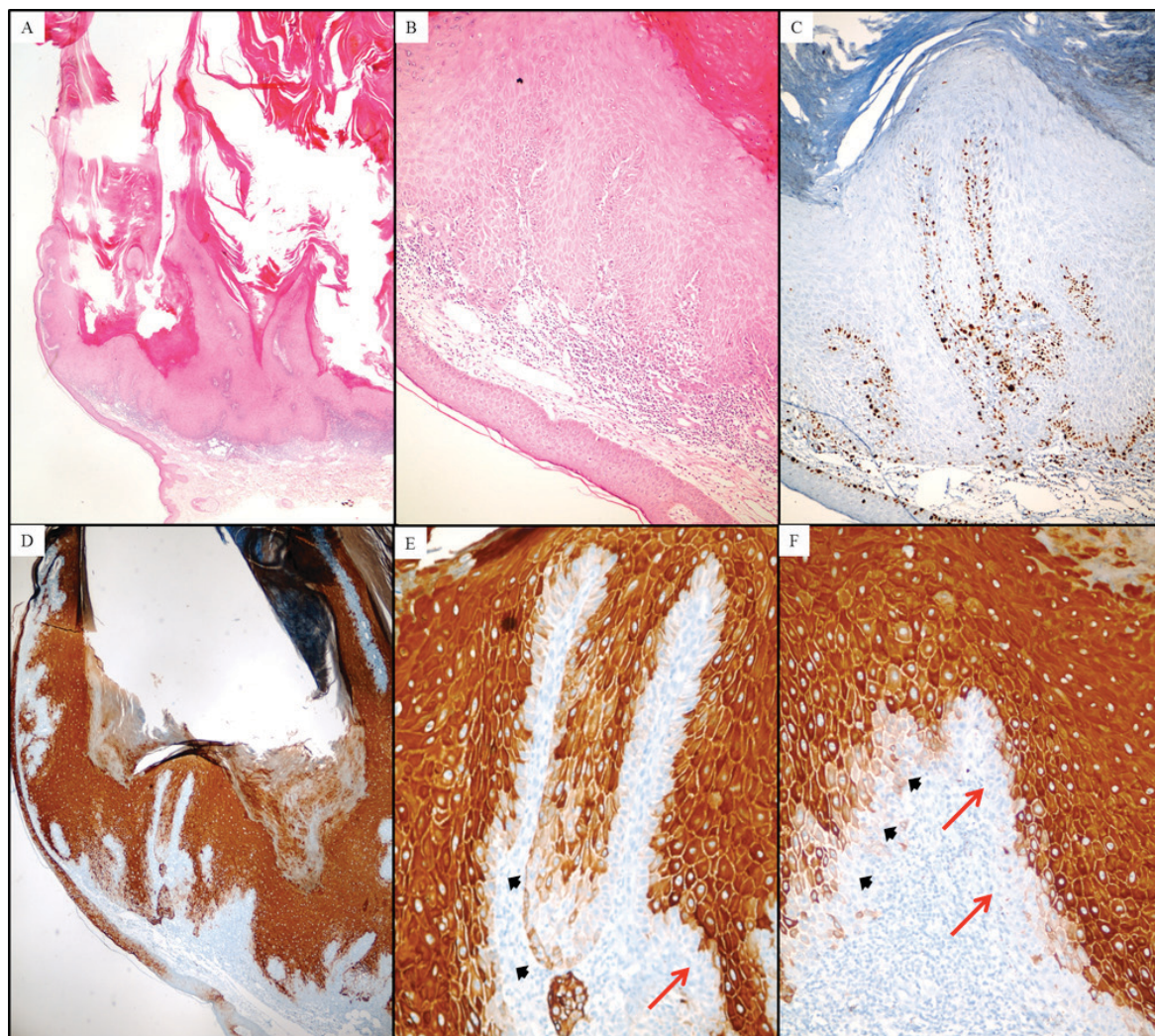
Figure 3. Representative images of hematoxylin and eosin-stained keratoacanthoma, at (A) magnification, x40 and (B) magnification, x100, exhibiting (C) a peripheral proliferation marker protein Ki-67 IHC staining pattern (magnification, x100) or a central CK17 IHC staining pattern at (D) magnification, x40, and magnification, x200 in (E) one field and vision and (F) another field of vision. Red arrowhead, no CK17 staining of basal cells; black arrowhead, weak CK17 staining of basal cells. CK17, cytokeratin 17; IHC, immunohistochemistry.

Table III. Cytokeratin 17 staining patterns in the SCC and KA cases.

Staining pattern	Tumor type (no. of cases)	
	SCC	KA
Central	8	22
Diffuse	19	2

SCC, squamous cell carcinoma; KA, keratoacanthoma.

Table IV. Summary of the statistical analysis of cytokeratin 17 central staining in keratoacanthoma cases.

Statistical analysis	No. of cases (%)
Sensitivity	22/24 (92)[a]
Specificity	19/27 (70)[a]
PPV	22/30 (73)[a]
NPV	19/21 (90)[a]

[a]$P<0.0001$ calculated using the Fisher's exact test. PPV, positive predictive value; NPV, negative predictive value.

rapid assessment (7). The Ki-67 expression percentage was not calculated (7).

CK17 was initially described within the pilosebaceous unit and basal cell carcinomas, and was considered to be a purely follicular keratin (27). CK17 is typically expressed in the suprabasal cells of the outer root sheath (ORS) of the hair follicle, the sebaceous duct, the suprabasal cells of the sebaceous gland, the basal cells of sweat glands and a few epidermal basal cells at sites of entry of the acrosyringium (15). CK17 protein expression is induced in activated keratinocytes in the suprabasal layers of the epidermis, despite the fact that the normal epidermis does not positively stain for CK17 (15). CK17 expression has been reported in cultured wild-type epidermis and under hyperproliferative conditions, including
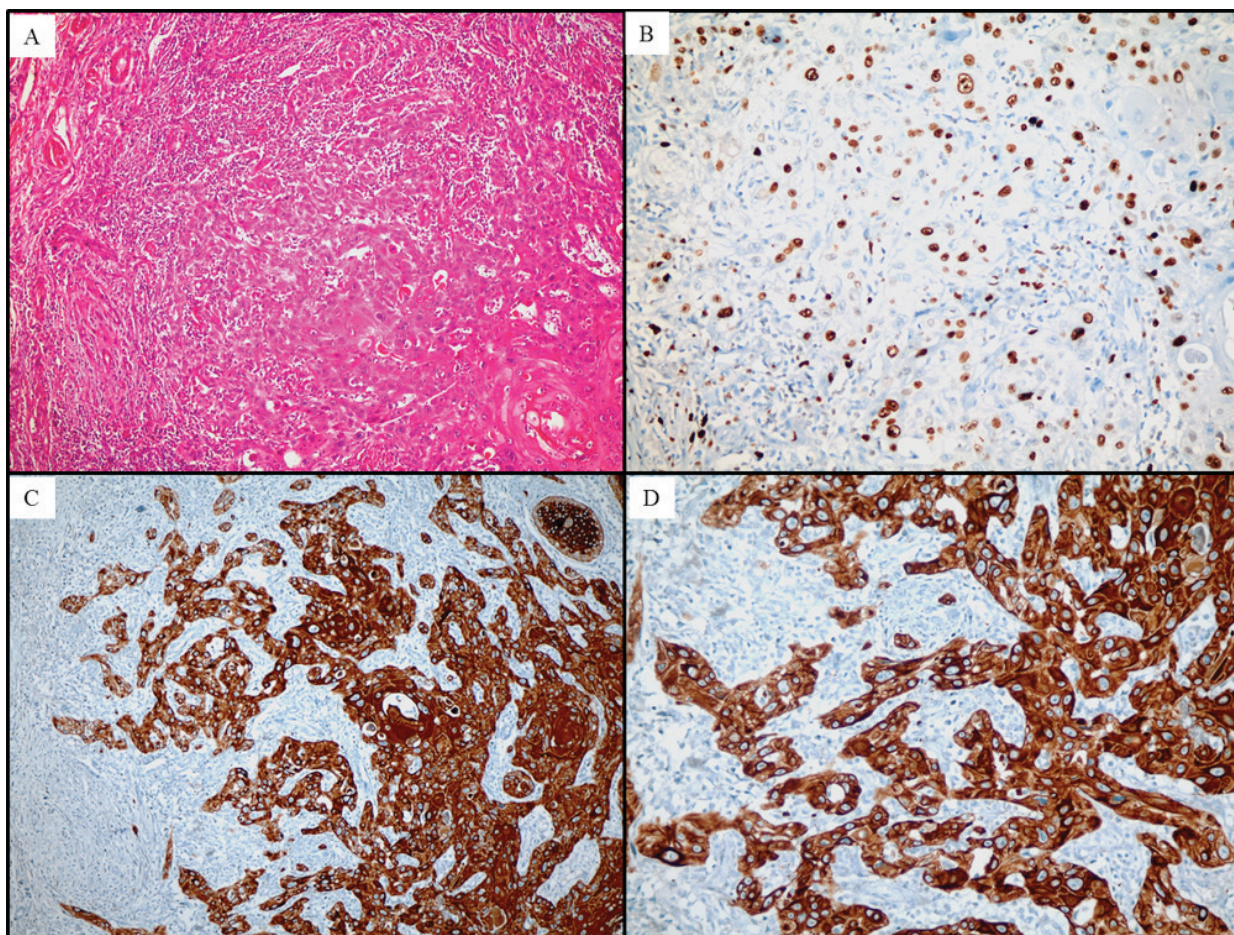
Figure 4. Representative images of (A) hematoxylin and eosin-stained SCC (magnification, 100) exhibiting (B) a proliferation marker protein Ki-67 IHC DP (magnification, x200), and a cytokeratin 17 IHC DP at (C) magnification x100 and (D) magnification x200. These staining patterns make up the SCC-like staining pattern. SCC, squamous cell carcinoma. DP, diffuse staining pattern; IHC, immunohistochemistry.

Table V. Proliferation marker protein Ki-67 staining patterns in the SCC and KA cases.

Staining pattern	Cancer type (no. of cases)	
	SCC	KA
Peripheral	5	24
Diffuse	22	0

SCC, squamous cell carcinoma; KA, keratoacanthoma.

Table VI. Summary of the statistical analysis of proliferation marker protein Ki-67 diffuse staining in squamous cell carcinoma cases.

Statistical analysis	No. of cases (%)
Sensitivity	22/27 (81)[a]
Specificity	24/24 (100)[a]
PPV	22/22 (100)[a]
NPV	24/27 (89)[a]

[a]P<0.0001 calculated using the Fisher's exact test. PPV, positive predictive value; NPV, negative predictive value.

psoriasis, warts and wound healing, and is thought to reflect a hyperproliferative cell state (9,15,28). CK17 is considered to be an early marker of keratinocyte activation following injury, and is expressed in migrating epithelial cells (29). In addition, CK17 staining has been observed in the basal cells of complex epithelial groups, including glandular epithelium containing a myoepithelial component, and transitional and pseudostratified epithelia (5). In a previous study, CK17 was reported to be a novel and interdependent regulator of hair cycling (30).

SCC CK17 expression has been reported in the skin, oral cavity, cervix, larynx, esophagus, anus and lung (9-12,31).

Divani and Kalodimos (12) observed that premalignant and malignant cells of the cervix exhibited CK17 expression, while wild-type ectocervical epithelial cells did not. Kitamura et al (11) demonstrated that CK17 expression was associated with differentiation and malignancy in oral SCC, indicating that it is a suitable biomarker of malignant transformation. Nazarian et al (10) reported that invasive anal squamous neoplastic lesions exhibited a CK17 PP or DP, in contrast to anal intraepithelial neoplasia. Proby et al (9) studied CK17 expression in skin SCC, and observed a CK17
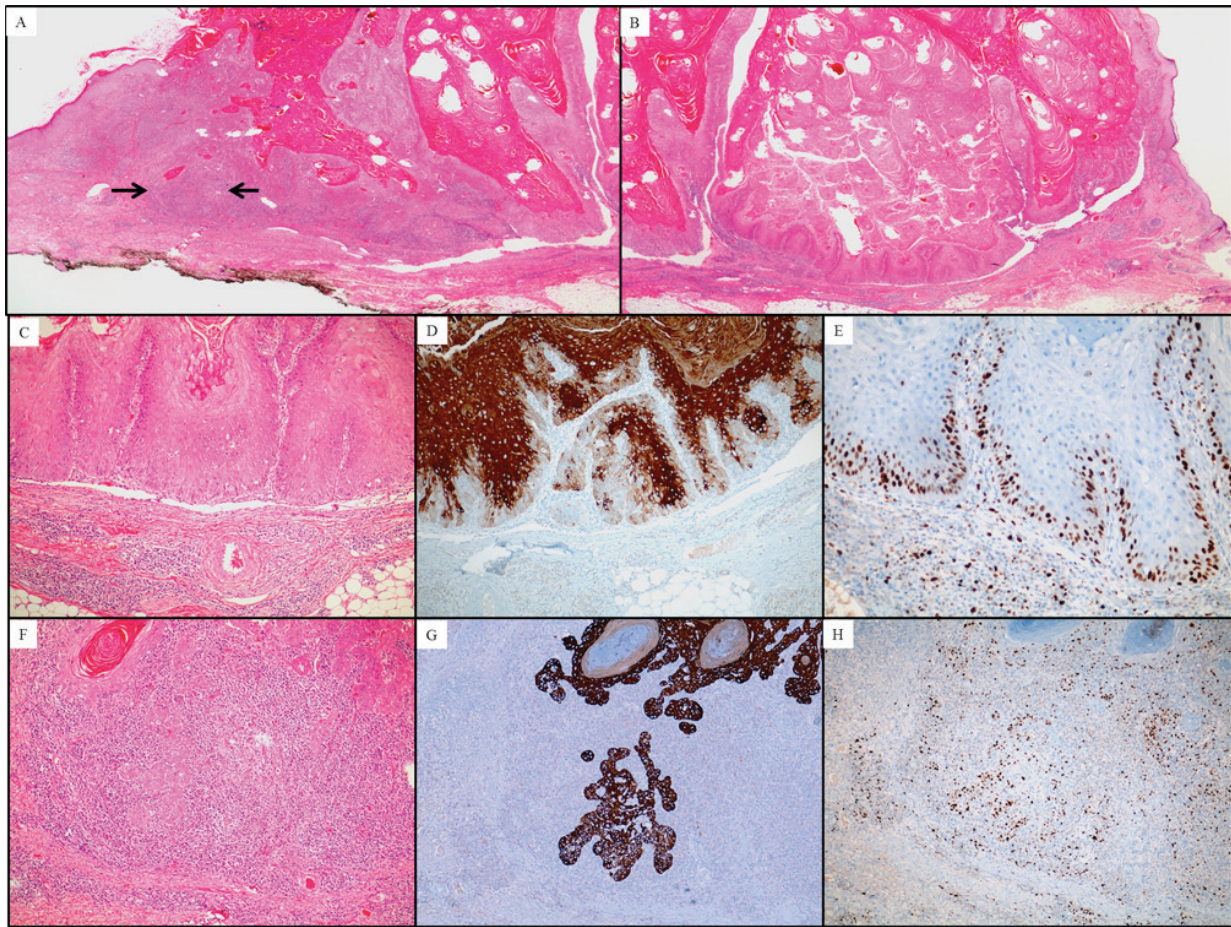
Figure 5. Representative images of H&E-stained KA-like SCC at magnification, x20 in (A) one field of vision and (B) another field of vision, and (C) magnification x100, exhibiting a KA-like (D) CK17 (magnification, x100) or (E) Ki-67 (magnification, x200) IHC staining pattern. The invasive area exhibits (F) H&E-stained cytological atypia (magnification, x100), and an SCC-like (G) CK17 (magnification, x100) or (H) Ki-67 (magnification, x100) IHC staining pattern. Black arrowhead, invasive area. KA, keratoacanthoma; SCC, squamous cell carcinoma; CK17, cytokeratin 17; Ki-67, proliferation marker protein Ki-67; H&E, hematoxylin and eosin; IHC, immunohistochemistry.
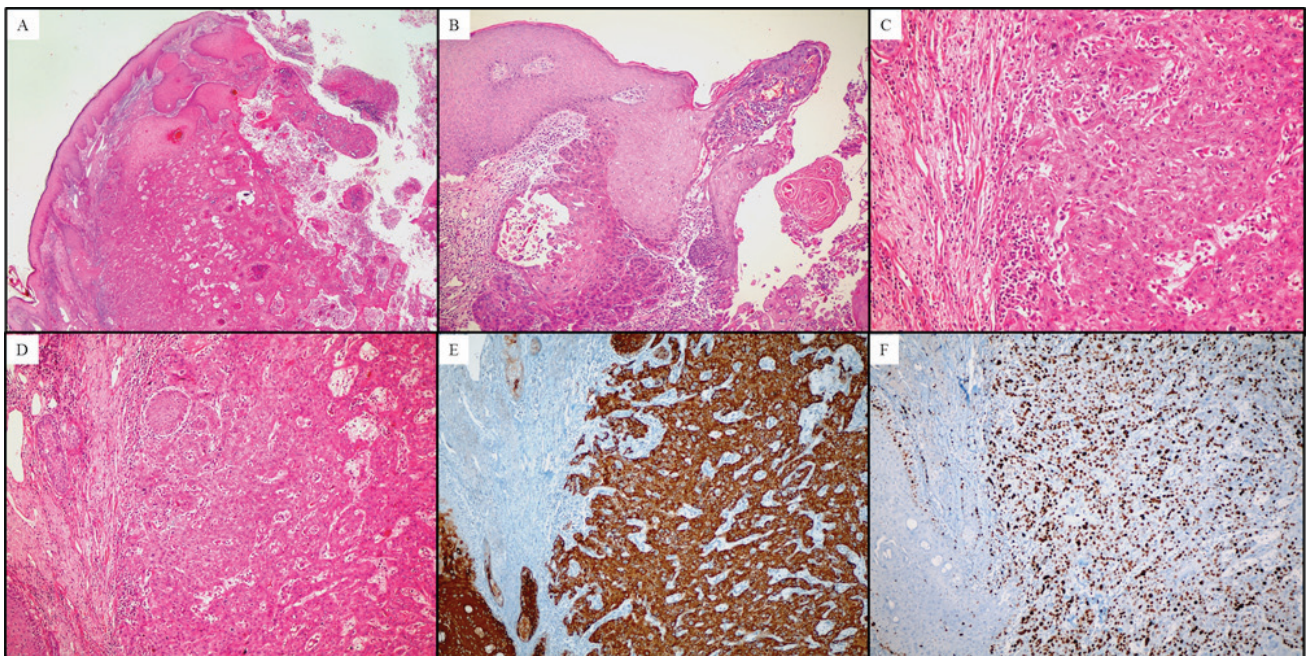

Figure 6. Representative images of crateriform SCC. Representative images of (A) the H&E-stained crateriform architecture (magnification, x40), (B) H&E-stained actinic keratosis adjacent to the lesion (magnification, x100), (C) H&E-stained cytological atypia (magnification, x200), (D) an infiltrative H&E staining pattern (magnification, x100), and an SCC-like (E) cytokeratin 17 (magnification, x100) or (F) proliferation marker protein Ki-67 (magnification, x100) immunohistochemical staining pattern. SCC, squamous cell carcinoma; H&E, hematoxylin and eosin.
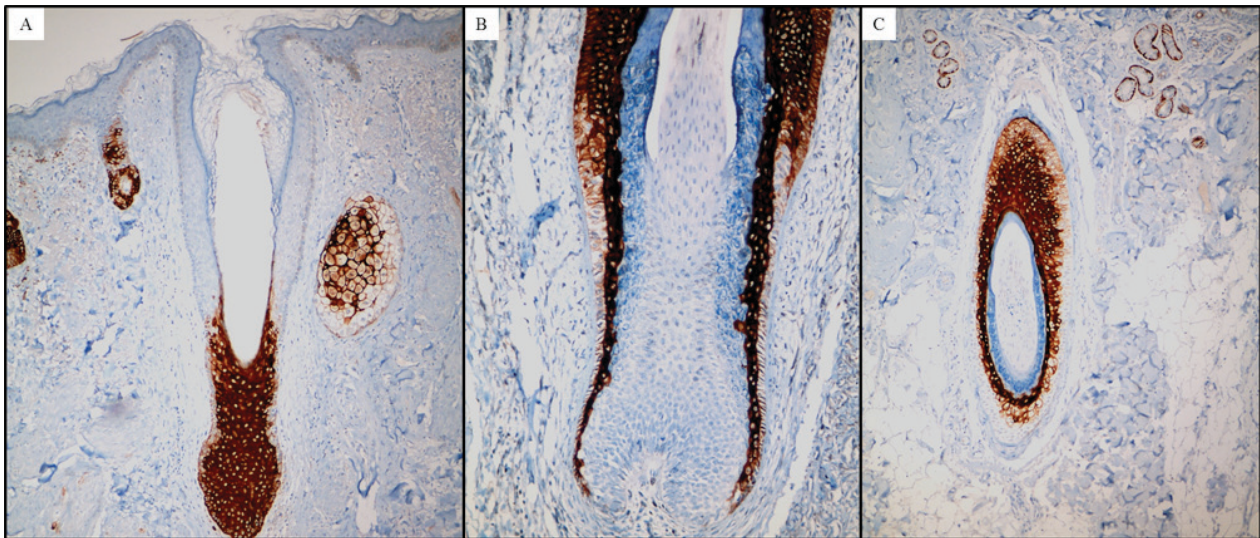
Figure 7. Representative images of the suprabasal cells of the ORS. (A) The normal hair follicle and sebaceous gland cells stain positively for CK17, while the epidermal cells do not (magnification, x100). The majority of ORS basal cells (B) stain weakly for CK17 (magnification, x200) or (C) do not stain at all (magnification, x100). Immunohistochemical staining used for all. ORS, outer root sheath.

DP in the basal and suprabasal cells of invasive SCC cases. To the best of our knowledge, no studies have been performed to evaluate the CK17 expression pattern in KA, although it is known that CK17 is expressed in hair-follicle-derived tumors, including KA.

In the present study, the utility of CK17 and Ki-67 expression in distinguishing between KA and SCC was investigated. All cases of KA exhibited a Ki-67 PP, whereas 22/27 (81%) SCC cases exhibited a Ki-67 DP. The sensitivity and specificity of a Ki-67 DP in the identification of SCC was 81 and 100%, respectively. Additionally, 22/24 (92%) KA cases exhibited a CK17 CP, while 19/27 (70%) SCC cases exhibited a CK17 DP. The sensitivity and specificity of a CK17 CP in the identification of KA was 92 and 70%, respectively. Therefore, the results of the present study suggest that the presence of a Ki-67 DP or CK17 CP may be used as a biomarker of SCC and KA, respectively.

In the present study, it was observed that the basal cells in KA tumor lobules exhibited positive Ki-67 staining and negative/weak CK17 staining, whereas the suprabasal cells exhibited positive CK17 staining and negative Ki-67 staining. This staining pattern was named the KA-like staining pattern (Ki-67 PP and CK17 CP). A total of 22/24 (92%) KA cases and 2/27 (7%) SCC cases exhibited a KA-like staining pattern. The majority of SCC cases exhibited a Ki-67 and CK17 DP, and this was named the SCC-like staining pattern. A total of 16/27 (59%) SCC cases exhibited a SCC-like staining pattern, while and no KA cases exhibited this pattern.

Lesion sections that appeared morphologically similar to KA in KA-like SCC cases exhibited a KA-like immunohistochemical staining pattern, whereas the majority of the lesions (morphologically similar to SCC) exhibited an SCC-like staining pattern. These results may be due to the difficulty in determining the characteristics of KA-like SCC and the association between KA, KA-like SCC and conventional SCC. The KA-like SCC cases exhibited rapid growth, similar to that of the KA cases, which is consistent with a previous study (2). There was potential subsequent regression in the KA-like SCC cases,

similar to that of the KA cases. Accordingly, the histological features of KA-like SCC may be simply one histopathological type observed during the natural evolution of KA and one step in the process of KA evolution. However, KA-like SCCs may also be KAs, which exhibit early malignant transformation. In a previous study, a histopathological spectrum of epithelial neoplasms induced by sorafenib, which include typical KA, KA-like SCC and invasive SCC, has been suggested (32). This supports the hypothesis that KA-like SCC's are borderline lesions between KA and invasive SCC.

The results of the present study suggest that crateriform SCC is identical to conventional SCC from an immunological and clinical aspect, as all the crateriform SCC cases (4/4) exhibited an SCC-like staining pattern and were characterized as long-standing nodules.

Consistent with previous studies (8,9,15,29,30), CK17 staining was absent in wild-type epidermal cells with the exception of focal staining adjacent to the lesions. CK17 was expressed in the suprabasal cells of the ORS of the wild-type hair follicle, which is consistent with previous studies. The CK17 CP detected in KA resembles the staining pattern of the ORS, which supports the theory that KA is a tumor of follicular origin derived from ORS cells (4).

In conclusion, a KA-like staining pattern was observed in the majority of KA cases examined in the present study, while it was rarely detected in SCC cases. By contrast, an SCC-like staining pattern was observed in the majority of SCC cases but in none of the KA cases. The CK17 CP exhibited high sensitivity and specificity for the detection of KA, while the Ki-67 DP exhibited high sensitivity and specificity for the detection of SCC. CK17 and Ki-67 may therefore be effective immunohistochemical markers for distinguishing between SCC and KA.

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