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Original Investigation

Cytokeratin 17: An Adjunct Immunohistochemical Marker of Invasion in Squamous Neoplastic Skin Lesions

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Abstract

Introduction: In some cases, it is difficult to evaluate histological invasion in squamous cell carcinomas (SCCs) and to discriminate them from in situ squamous cell carcinomas (ISSCCs). Cytokeratin (CK) 17 is induced by activated keratinocytes, and its expression is associated with disease progression in SCCs of certain organs. In this study, the utility of CK17 as an adjunct immunohistochemical marker to facilitate the diagnosis of invasion in cutaneous SCCs was investigated.

Methods: Immunohistochemical staining for CK17 was evaluated in 19 ISSCCs and 27 invasive SCCs (IVSCCs). Staining patterns were defined as diffuse (DF), patchy (PT), suprabasal/central (SC), or peripheral/basal (PB). SCCs showing only a DF pattern were interpreted as testing positive for CK17 immunoexpression. SCCs were interpreted to as testing negative if there was no CK17 expression or they showed other staining patterns.

Results: All ISSCCs tested negative for CK17 immunoexpression. While no staining was detected in 7 of the 19 ISSCCs, the PT pattern was observed in 6 and the SC pattern in 6. Twenty-two (81%) IVSCCs tested positive. Of the 5 IVSCCs that tested negative, 4 showed the PB pattern and 1 showed the SC pattern. The sensitivity and specificity of CK17 immunohistochemical staining for identifying invasion were 81% and 100%, respectively.

Conclusion: CK17 may be a useful immunohistochemical marker for identifying invasion in cutaneous SCCs and may help pathologists determine surgical margins.

Keywords: Cytokeratin 17, in situ squamous cell carcinoma, invasive squamous cell carcinoma, Bowen disease, skin, actinic keratosis

Introduction

Cytokeratin (CK) 17 is a type 1 keratin that was first described in the pilosebaceous unit and basal cell carcinoma (1). It is expressed in the basal/myoepithelial cells of complex epithelia, such as the respiratory tract, glandular epithelium, and transitional epithelium (2). Its expression has been reported in the outer root sheath of the hair follicle in the skin, in the suprabasal cells of the isthmus and sebaceous ducts, and in several basal cells located in the entrance region of the acrosyringium (3). There is no expression in the epidermis under normal conditions. However, it can be triggered by active suprabasal keratinocytes (3). Its expression has been reported in psoriasis, warts, and wound healing in epidermis cultures and is thought to reflect the hyperproliferative state of the cells (3-5). It has an important role in skin healing, development of fetal epidermis, and various inflammatory dermatitis (5, 6). It also has functions related to cell growth, mobility, and migration (6, 7).

Because of all of these features, CK 17 immunexpression has been investigated in intraepithelial and invasive neoplasms of various organs (eg, oral cavity, uterine cervix, larynx, esophagus, and anus) (4, 8-11). Divani et al (9) reported CK 17 expressions in malignant and premalignant cells of the cervix, while the normal ectocervix epithelium was negative. Kitamura et al (10) demonstrated that CK 17 expression in oral cavity squamous cell carcinomas (SCCs) was associated with tumor differentiation and malignancy. Chu and Weiss reported a positive expression in about one-third of colorectal adenocarcinoma cases (12). Kim et al (13) demonstrated a positive correlation of the immunohistochemical CK 17 expression with the tumor stage in 82 bile duct adenocarcinomas.

Cytokeratin 17 was also studied in squamous cell lesions of the skin. Fernandez-Flores et al (14) suggested that CK 17 immunexpression did not differentiate actinic keratosis and Bowen's disease, but it could reveal small atypical foci that could be missed out with hematoxylin-eosin (HE) at the first view; therefore, it could contribute to the evaluation of surgical margin. Proby et al (4) demonstrated diffuse cytoplasmic expression in CK 17 suprabasal expression as well as invasive SCC (IvSCC) in hyperproliferative conditions such as benign warts. CK 17 expressions have also been demonstrated in various epidermal malignancies, such as basal cell carcinoma, IvSCC, and basaloid variant SCCs (15).

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Nazarian et al. (8) suggested that CK 17 could be a useful marker to detect invasion in SCCs of the anus. In this study, unlike the other studies, pattern-based evaluation was performed based on the immunolocalization of CK 17 expression in the basal and suprabasal layers of the human epithelium. While superficial or suprabasal staining was considered negative, only diffuse staining was considered positive. However, in previous studies, a pattern-based study of the CK 17 immunohistochemical expression have not been performed to evaluate the invasion in cutaneous SCC.

In this study, we investigated the use of CK 17 as an adjunct marker to assess the invasion in in situ SCC (IsSCC) or IvSCCs of the skin.

Methods

Case Selection

HE preparations of the patients in whom a total excisional biopsy was performed between 2013 and 2015 in the pathology section of our hospital and who were diagnosed with IsSCC, bowenoid type actinic keratosis, Bowen's disease, and IvSCC were re-evaluated. All cases with full-layer involvement reaching the surface of the epidermis were assessed as IsSCC without any discrimination of bowenoid actinic keratosis or Bowen's disease. In IvSCC cases, the histological grade of the tumor and whether it was the precursor lesion (actinic keratosis) accompanying the tumor were noted.

The slides of 61 cases meeting the diagnosis criteria were obtained from archives and evaluated. The cases for which we could not confirm the diagnosis or appropriate slide and blocks could not be found in the archives were excluded from the study. A total of 46 cases, nineteen of which were IsSCC and 27 were IvSCC, were included in the study. Ethics committee approval and patient informed consent was obtained.

Immunohistochemistry

Immunohistochemical studies were performed on the 5-µm-thick formalin-fixed paraffin-embedded tissue sections with standard techniques in the Ventana automatic staining device using CK 17 (Ventana medical system, catalog number: SP 95) as the primary antibody. Hair follicle structures adjacent to the lesion were used as an internal positive control, and the non-neoplastic epidermis was used as a negative control. Immunohistochemical staining patterns and whether the staining occurred with CK 17 were examined microscopically in all IsSCC and IvSCC cases studied.

In the interpretation of the CK 17 staining pattern, the immunolocalization of keratin expression in the basal and suprabasal layers of the human epithelium was exemplified as reported by Troyanovsk, Sun, and Purkis (16-18). Our criteria for evaluating CK 17 staining patterns and their positivity are described below.

Diffuse pattern: Full-layer staining in the epidermis from the baseline to the surface for in situ carcinoma and full-layer strong staining from the baseline to the center of the invasive cell islands for invasive carcinoma. This staining pattern was evaluated as positive.

Suprabasal/central pattern: A limited staining to the surface of the epidermis or to the central part of the invasive cell islands (suprabasal or two-third of the inner part of the neoplasm) was noted. This staining pattern was evaluated as negative.

Patchy pattern: Along with the staining of the epidermal basal part or the periphery of invasive cell islands, the staining of the epidermal surface part or the central one-third part of invasive cell islands was interpreted as negative.

Peripheral/basal pattern: Staining only at the basal part of the epidermis or at the periphery of invasive cell islands was interpreted as negative.

If different patterns were found together, the dominant pattern that was over 90% was accepted as the pattern of that lesion.

Statistical Analysis

The statistical analysis was performed using the Statistical Package for Social Sciences version 13 (SPSS Inc.; Chicago, IL, USA). In the detection of IvSCC, the sensitivity (true positive/[true positive + false negative]), specificity (true negative/[false positive + true negative]), positive predictive value (PPV; true positive/[true positive + false positive]), and negative predictive value (NPV; true negative/[true negative + false negative]) were calculated. CK 17 expression was compared using the Fisher exact chi-square test between the IsSCC and IvSCC groups. Two-tailed p values of <0.05 were considered statistically significant.

Results

A total of 46 cases, 19 of which were IsSCC and 27 of which were IvSCC, were included in the study.

Cytokeratin 17 was evaluated as immunohistochemically negative in all cases of IsSCC (19/19; 100%). No expression was found in any of these seven negative cases (36%). The patchy (PT) pattern was found in six (32%) of them, and the suprabasal/central (SC) pattern was detected in the remaining (Table 1, Figure 1).

In IvSCC cases, twenty two (81%) patients were evaluated as CK 17 positive and five patients (19%) as negative. While the cases that were stained positive were stained in the diffuse (DF) pattern by definition, the peripheral/basal (PB) pattern was observed in four of the negative cases and SC pattern in one of the negative cases (Table 2, Figure 2).

The histological grade was 1 in 23 of the IvSCC cases, 2 in two cases, and 3 in two cases. All the IvSCC cases evaluated as CK 17 positive were grade 1. Of the five CK 17 negative cases, two had histological grade 2, two had grade 3, and one had grade 1.

The PB pattern was observed only in the high-grade (grade 2 or 3) cases of IvSCC. It was not seen in the IsSCC cases (Figure 2).

Actinic keratosis were detected around the tumor of 13 IvSCC cases (13/27; 48%). In the actinic keratosis areas, CK 17 was evaluated as immunohistochemically negative except for one case. While no staining was detected in three cases, the SC pattern was detected in nine cases (Figure 3). While CK 17 expression was observed weaker in the basal part of the only one actinic keratosis case that was positively evaluated, a strong diffuse expression was observed in the suprabasal areas and invasive tumor cells (Figure 4).

It was also found that some IvSCC foci, which could be missed out at the first view, were more easily detected with CK 17 staining (Figure 5).

To evaluate the invasion, the sensitivity and specificity of CK 17 immunohistochemical staining in skin SCCs were detected as 81% and 100%, respectively, (Table 3). In addition, CK 17 staining had a high PPV (100%) and NPV (79%). There was a significant difference in CK 17 immunostaining between IsSCC and IvSCCs ($p<0.0001$).

Discussion

In this study, we evaluated the immunohistochemical use of CK 17 in a pattern-based method to detect the invasion in the squamous cell lesions of the skin. According to the above-mentioned staining criteria, we obtained negative results in all the IsSCC cases. Positive staining was detected in 81% of the IvSCC cases.

The IsSCC of the skin is known as Bowen's disease. Although actinic keratosis is included in precancerous lesions, some authors describe actinic keratosis as "keratinocytic intraepidermal neoplasia" or "solar keratotic intraepidermal SCC" (19, 20). Bowenoid-type actinic keratoses are difficult, sometimes impossible, to distinguish microscopically from Bowen's disease because of their full-layer epidermis involvement (19). Although Bowen's disease and actinic keratosis have been suggested to develop from different cell types, the approach and treatment of the patient are similar in practice (19). Because of these reasons, Bowen's disease and bowenoid actinic keratosis were evaluated as IsSCC without any discrimination in our study.

Table 1. Results of histological and immunohistochemical evaluation of the cases with Bowen's disease

Case	CK 17 status	CK 17 pattern
1	Negative	No staining was detected
2	Negative	No staining was detected
3	Negative	No staining was detected
4	Negative	No staining was detected
5	Negative	No staining was detected
6	Negative	No staining was detected
7	Negative	No staining was detected
8	Negative	SC
9	Negative	SC
10	Negative	SC
11	Negative	SC
12	Negative	SC
13	Negative	SC
14	Negative	PT
15	Negative	PT
16	Negative	PT
17	Negative	PT
18	Negative	PT
19	Negative	PT

CK: cytokeratin; SC: suprabasal/central; PT: patchy

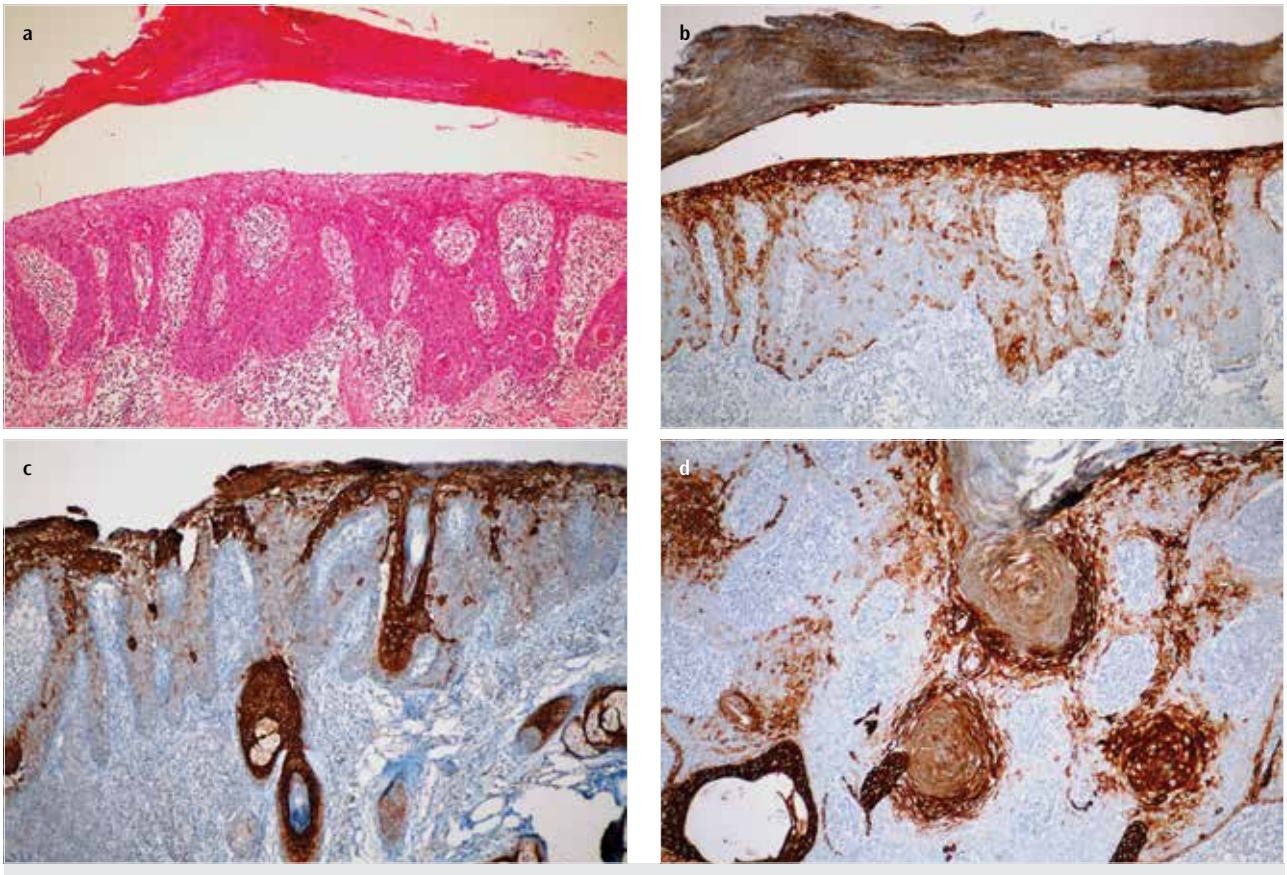

Figure 1. a-d. (a) IsSCC case; (b-d) CK 17 expressions in these cases, (b) PT pattern, (c) SC pattern, and (d) PB pattern. These staining patterns were evaluated as negative

Table 2. Histological and immunohistochemical evaluation results of the cases with IvSCC

Case	CK 17 status in tumor	CK 17 pattern in tumor	Histological grade	CK 17 status in the area of AK accompanying the tumor	CK 17 pattern in the area of AK accompanying the tumor
1	Positive	DF	1	Negative	No staining was detected
2	Positive	DF	1	Negative	No staining was detected
3	Positive	DF	1	Negative	No staining was detected
4	Positive	DF	1	Negative	SC
5	Positive	DF	1	AK was not detected	AK was not detected
6	Positive	DF	1	AK was not detected	AK was not detected
7	Positive	DF	1	Negative	SC
8	Positive	DF	1	AK was not detected	AK was not detected
9	Positive	DF	1	AK was not detected	AK was not detected
10	Positive	DF	1	Negative	SC
11	Positive	DF	1	Negative	SC
12	Positive	DF	1	AK was not detected	AK was not detected
13	Positive	DF	1	AK was not detected	AK was not detected
14	Positive	DF	1	AK was not detected	AK was not detected
15	Positive	DF	1	AK was not detected	AK was not detected
16	Positive	DF	1	AK was not detected	AK was not detected
17	Positive	DF	1	Negative	SC
18	Positive	DF	1	Negative	SC
19	Positive	DF	1	AK was not detected	AK was not detected
20	Positive	DF	1	Negative	SC
21	Positive	DF	1	AK was not detected	AK was not detected
22	Positive	DF	1	Positive	DF
23	Negative	SC	1	Negative	SC
24	Negative	PB	2	AK was not detected	AK was not detected
25	Negative	PB	2	AK was not detected	AK was not detected
26	Negative	PB	3	AK was not detected	AK was not detected
27	Negative	PB	3	Negative	SC

CK: cytokeratin; DF: diffuse; SC: suprabasal/central; PB: peripheral/basal; AK: actinic keratosis; IvSCC: invasive squamous cell carcinoma

Table 3. Summary of the statistical analysis of CK 17 immunoexpression to detect IvSCC

Sensitivity (n [%])	22/27 (81)
Specificity (n [%])	19/19 (100)
PPV (n [%])	22/22 (100)
NPV (n [%])	19/24 (79)
p value*	p<0,001

*Calculated using Fisher exact square test.
CK: cytokeratin; PPV: positive predictive value; NPV: negative predictive value; IvSCC: invasive squamous cell carcinoma

There is only one study in literature that investigates the presence and patterns of CK 17 expression in the intraepithelial neoplasms (in situ carcinomas) of the skin (14). In this study, suprabasal staining with CK 17 was observed in almost all cases of actinic keratosis and Bowen's disease, and it was shown that these two entities cannot be separated from each other through the expression pattern. Only one case was stained in the PT pattern and the other case was stained in the DF pattern. The authors report that the only Bowen's disease case in which the DF pattern was observed is composed of more mature and wide cytoplasm cells contrary to our cases. If the criteria that we determined was used, it is possible to evaluate all the cases, except for one, with actinic keratosis and Bowen's disease as negative in this study; therefore, it is possible to say that they show parallelism with our findings. In our study, we found negative staining with CK 17 in all the 19 cases we examined without any discrimination of bowenoid actinic keratosis and Bowen's disease. Moreover, in the areas of actinic keratoses accompanying invasive tumors, negative staining was detected with CK 17 in all cases, except one.

In our study, we obtained positive staining in almost all cases (22/23; 96%) with grade 1 IvSCC and negative staining in all of a few cases (4/4; 100%) with a higher degree. Although there were not sufficient cases for statistical evaluation, we observed that as the tumor grade increased, the cells became immature/basaloid, CK 17 expression decreased, and diffuse staining gave place to peripheral (PT) pattern. This observation is similar to that of Linksey's study showing that the

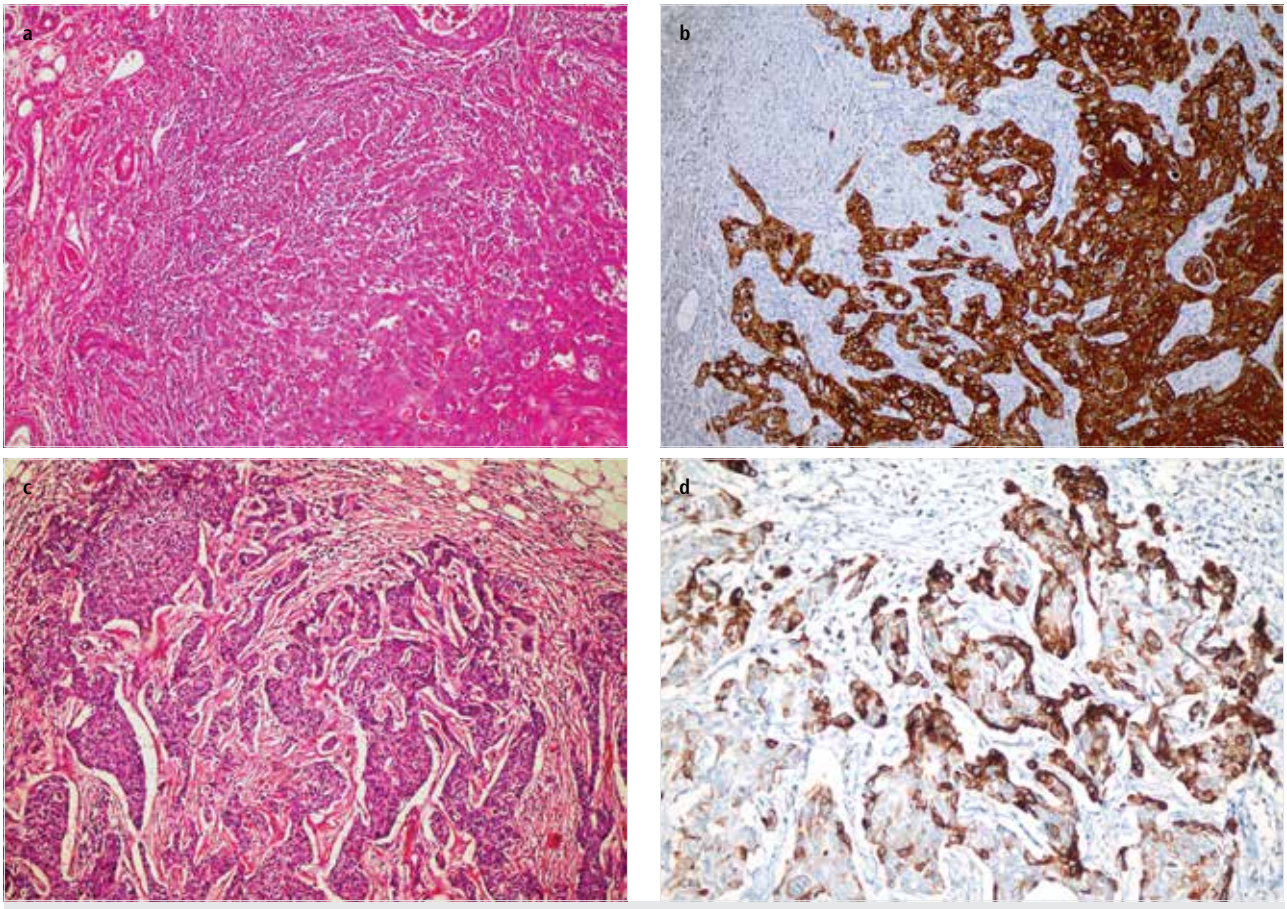

Figure 2. a-d. (a) IvSCC case; (b) diffuse CK 17 expression assessed as positive staining in this case; (c) IvSCC case; (d) CK 17 expression in the central pattern evaluated as negative in this case

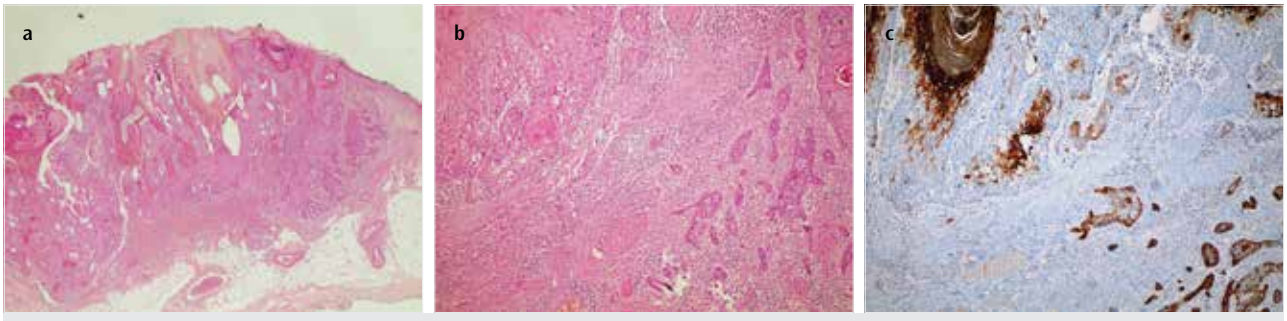

Figure 3. a-c. (a, b) IvSCC case involving areas of actinic keratosis on the surface; (c) invasive tumor is positive with CK 17. The actinic keratosis area was evaluated as negative because it was stained in the suprabasal pattern

expression decreased in basaloid SCCs (15). There is a need for different studies with a high number of cases to reveal the relationship between the grade and CK 17 expression in primary skin SCCs.

Most of IsSCC and IvSCC foci were easily detectable with HE in our cases; however, the small foci that could be missed out were easily demonstrated with CK 17 immunoexpression (Figure 5). In some cases, CK 17 expression was observed to be beneficial in the confirmation or exclusion of surgical margin involvement. Similarly, it has been reported in literature that the use of CK 17 is useful in establishing a positive surgical margin in IsSCC cases and basal cell carcinomas (14, 21).

Microscopically, distinguishing IvSCC from proliferative actinic keratoses can be difficult and problematic from time to time (22). The fact that we detected CK 17 immunoexpression as negative in all actinic keratosis cases, except one, suggests that the use of this immunohistochemical marker may be useful in distinguishing the two entities (Figure 4). In our actinic keratosis case, which is the only positive case, the presence of weak immunoexpression in the cells in the epidermal basal part may be helpful in distinguishing IvSCC (Figure 5).

Conclusion

In most of the IvSCCs, CK 17 expression was detected in the DF pattern. IsSCCs showed the SC/PT pattern staining or no staining. We suggest that CK 17 immunoexpression may help the pathologist in assessing invasion in difficult lesions, particularly in low-grade superficial IvSCCs, and help determine the surgical margins in suspicious cases.

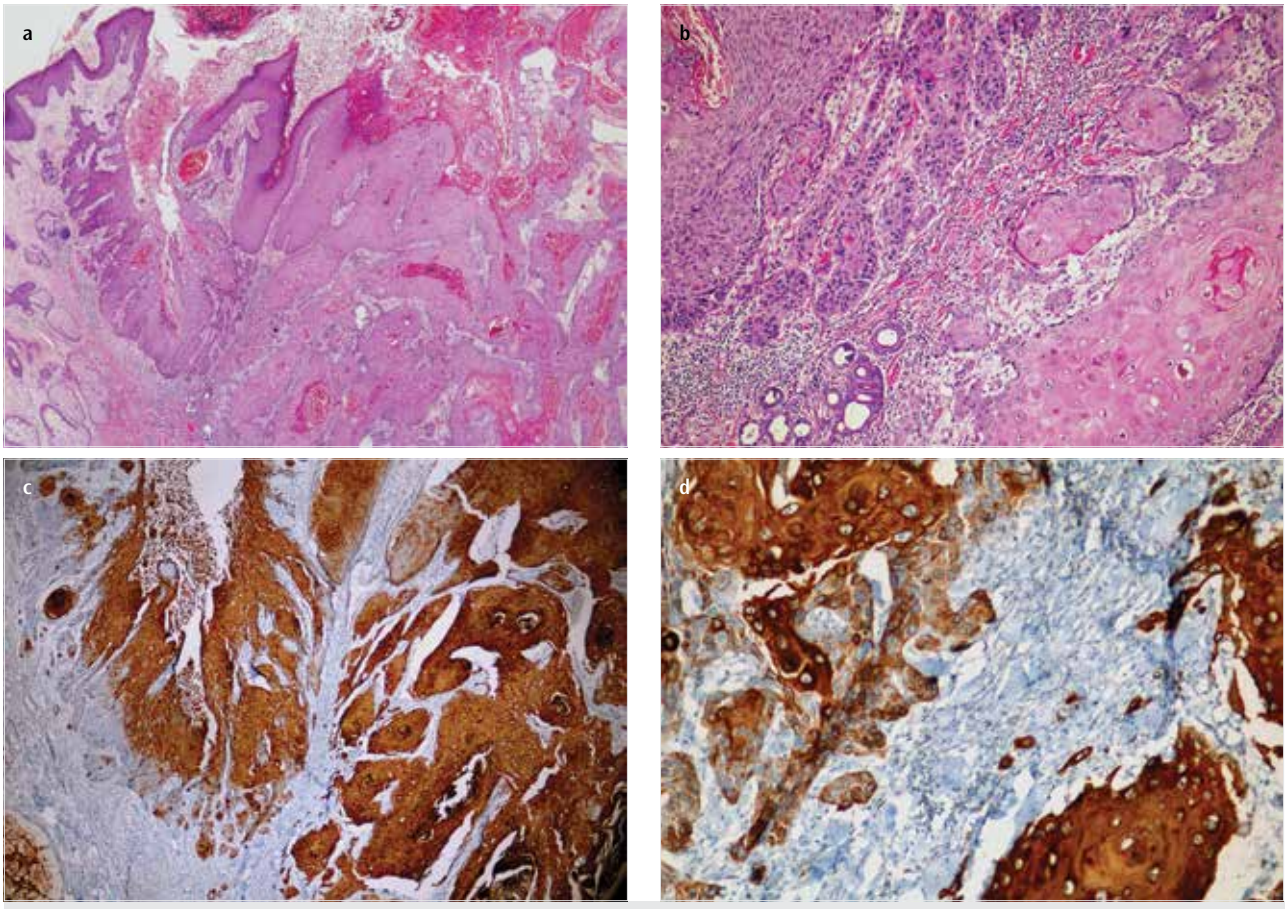

Figure 4. a-d. (a-d) Actinic keratosis is seen in the left half of the pictures, and invasive tumor is seen in the right half. In the actinic keratosis area, while CK 17 expression is observed weaker in the basal part, there is strong and diffuse CK 17 expression in the invasive tumor cells

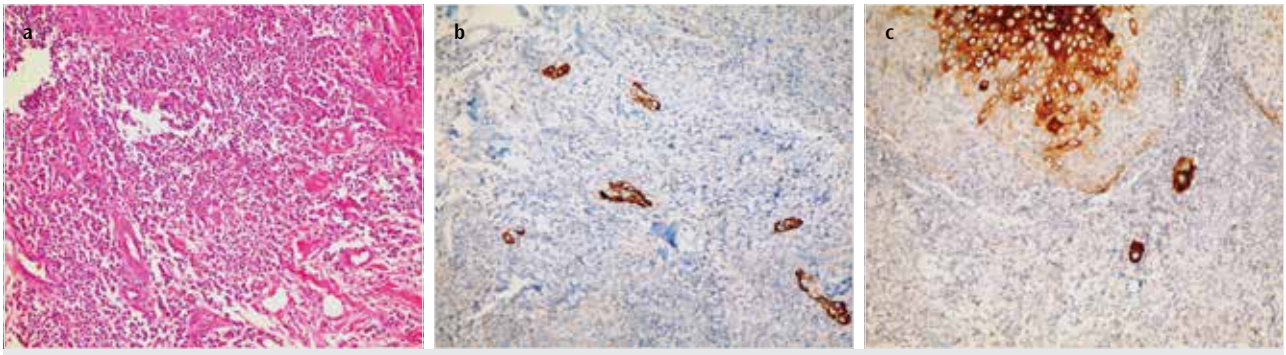

Figure 5. a-c. (a, b) Invasive focus that which was difficult to realize in the HE sections was revealed in the CK 17 staining; (c) suprabasal and focal basal staining pattern at the focus of actinic keratosis that is adjacent to the microinvasive tumor focus

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of İstanbul Training and Research Hospital.

Informed Consent: Verbal informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Conflict of Interest: No conflict of interest was declared by the author.

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References

1. Moll R, Franke WW, Volc-Platzer B, Krepler R. Different keratin polypeptides in epidermis and other epithelia of human skin: a specific cytokeratin of molecular weight 46,000 in epithelia of the pilosebaceous tract and basal cell carcinoma. J Cell Biol 1982; 95: 285-9. [CrossRef]
2. Lu S, Tiekso J, Hietanen S, Syrjänen K, Havu VK, Syrjänen S. Expression of cell-cycle proteins p53, p21 (WAF-1), PCNA and Ki-67 in benign, premalignant and malignant skin lesions with implicated HPV involvement. Acta Derm Venereol 1999; 79: 268-73. [CrossRef]
3. Yamamoto O, Asahi M. Cytokeratin expression in trichoblastic fibroma (small nodular type trichoblastoma), trichoepithelioma and basal cell carcinoma. Br J Dermatol 1999; 140: 8-16. [CrossRef]

4. Proby CM, Churchill L, Purkis PE, Glover MT, Sexton CJ, Leigh IM. Keratin 17 expression as a marker for epithelial transformation in viral warts. Am J Pathol 1993; 143: 1667-78.
5. Wilson CL, Dean D, Lane EB, Dawber RP, Leigh IM. Keratinocyte differentiation in psoriatic scalp: morphology and expression of epithelial keratins. Br J Dermatol 1994; 131: 191-200. [CrossRef]
6. McGowan KM, Coulombe PA. Onset of keratin 17 expression coincides with the definition of major epithelial lineages during skin development. J Cell Biol 1998; 143: 469-86. [CrossRef]
7. Tong X, Coulombe PA. Keratin 17 modulates hair follicle cycling in a TNF alpha-dependent fashion. Genes Dev 2006; 20: 1353-64. [CrossRef]
8. Nazarian RM, Primiani A, Doyle LA, Linskey KR, Duncan LM, Odze RD, et al. Cytokeratin 17: an adjunctive marker of invasion in squamous neoplastic lesions of the anus. Am J Surg Pathol 2014; 38: 78-85. [CrossRef]
9. Divani S, Kalodimos G. Expression of cytokeratins 8 and 17 as a diagnostic marker of cervical intraepithelial neoplasia. Arch Oncol 2010; 18: 88-90. [CrossRef]
10. Kitamura R, Toyoshima T, Tanaka H, Kawano S, Kiyosue T, Matsubara R, et al. Association of cytokeratin 17 expression with differentiation in oral squamous cell carcinoma. J Cancer Res Clin Oncol 2012; 138: 1299-310. [CrossRef]
11. Arnault JP, Wechsler J, Escudier B, Spatz A, Tomasic G, Sibaud V, et al. Keratoacanthomas and squamous cell carcinomas in patients receiving sorafenib. J Clin Oncol 2009; 10;27: 59-61. [CrossRef]
12. Chu PG, Schwarz RE, Lau SK, Yen Y, Weiss LM. Immunohistochemical staining in the diagnosis of pancreatobiliary and ampulla of Vater adenocarcinoma: application of CDX2, CK17, MUC1, and MUC2. Am J Surg Pathol 2005; 29: 359-67. [CrossRef]
13. Kim K, Lee HW, Chae SW, Kim DH, Do IG, Lee HJ, et al. Cytokeratin 17 Expression is Associated With Poor Prognosis in Gallbladder Adenocarcinoma. Appl Immunohistochem Mol Morphol 2017; 25: 346-50. [CrossRef]
14. Fernandez-Flores A. Cytokeratin 17 immunoexpression in actinic keratosis bowenoid and nonbowenoid) and in Bowen disease. Ann Diagn Pathol 2016; 20: 1-6. [CrossRef]
15. Linskey KR, Gimbel DC, Zukerberg LR, Duncan LM, Sadow PM, Nazarian RM. BerEp4, cytokeratin 14, and cytokeratin 17 immunohistochemical staining aid in differentiation of basaloid squamous cell carcinoma from basal cell carcinoma with squamous metaplasia. Arch Pathol Lab Med 2013; 137: 1591-8. [CrossRef]
16. Troyanovsky SM, Guelstein VI, Tchipysheva TA, Krutovskikh VA, Bannikov GA. Patterns of expression of keratin 17 in human epithelia: dependency on cell position. J Cell Sci 1989; 93: 419-26.
17. Sun TT, Eichner R, Nelson WG, Tseng SC, Weiss RA, Jarvinen M, et al. Keratin classes: molecular markers for different types of epithelial differentiation. J Invest Dermatol 1983; 81: 109-15. [CrossRef]
18. Purkis PE, Steel JB, Mackenzie IC, Nathrath WB, Leigh IM, Lane EB. Antibody markers of basal cells in complex epithelia. J Cell Sci 1990; 97: 39-50.
19. Kessler GM, Ackerman AB. Nomenclature for very superficial squamous cell carcinoma of the skin and of the cervix: a critique in historical perspective. Am J Dermatopathol 2006; 28: 537-45. [CrossRef]
20. Cockerell CJ. Histopathology of incipient intraepidermal squamous cell carcinoma ("actinic keratosis"). J Am Acad Dermatol 2000; 42: 11-7. [CrossRef]
21. Baum CL. Commentary: diagnostic utility of cytokeratin 17 immunostaining in morpheaform basal cell carcinoma and for facilitating the detection of tumor cells at the surgical margins. Dermatol Surg 2012; 38: 1367-8. [CrossRef]
22. Filosa A, Filosa G. Actinic keratosis and squamous cell carcinoma: clinical and pathological features. G Ital Dermatol Venereol 2015; 150: 379-84.

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