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ORIGINAL PAPER

Fibroblast-activation protein: valuable marker of cutaneous epithelial malignancy

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Abstract Fibroblast-activation protein (FAP) is a key protein that is characteristically expressed by carcinoma-associated fibroblasts (CAFs). It has been shown to be expressed in CAFs of 90 % of internal epithelial cancers as well as cutaneous epithelial malignancies. We have recently shown that this marker is useful in differentiating between morpheaform/infiltrative BCC from desmoplastic trichoepithelioma (TE). Given this, we sought to assess FAP expression in both benign and malignant cutaneous epithelial entities. Immunohistochemical FAP staining was performed on BCC ($n = 26$), SCC ($n = 26$), porocarcinoma ($n = 10$), metastatic adenocarcinoma ($n = 12$), keratoacanthoma (KA) ($n = 16$), TE ($n = 14$), pseudoepitheliomatous hyperplasia ($n = 15$), poroma ($n = 15$), syringoma ($n = 10$), and chondroid syringoma ($n = 6$). Control group consisted of scars ($n = 10$). FAP expression was observed in all scars and all malignant entities, but not in any of the benign cases. Interestingly, ten KA cases exhibited positivity, whereas six were negative. In summary, FAP is a reliable marker of cutaneous epithelial malignancy.

Keywords Fibroblast-activation protein · Trichoepithelioma · Basal cell carcinoma · Pseudoepitheliomatous hyperplasia · Squamous cell carcinoma · Keratoacanthoma · Eccrine tumors

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Introduction

The work of a dermatopathologist can be challenging at times, as one may be faced with the daunting task of differentiating entities that may look alike, but have a different biological behavior and that is where the distinction between them is important. For instance, trichoepithelioma (TE) and basal cell carcinoma (BCC) are two cutaneous neoplasms that resemble each other microscopically. The distinction between them may be problematic especially if the biopsy specimen is small or superficial [7, 35, 36]. The same can be said of other entities that are regularly encountered by dermatopathologists, such as squamous cell carcinoma (SCC), keratoacanthoma (KA) [24, 28], and pseudoepitheliomatous hyperplasia (PEH), as well as benign and malignant epithelial neoplasms of glandular origin (eccrine, apocrine, etc.) [2, 3, 13, 15, 16, 25, 29, 31, 38–41].

Fibroblast-activation protein (FAP), a type II membrane-bound glycoprotein belonging to the serine protease family with both a dipeptidyl peptidase and a collagenolytic activity, is selectively expressed in peritumoral stromal fibroblasts of multiple epithelial cancers including breast, pancreatic, colorectal, and lung carcinomas, and in the granulation tissue of healing wounds [4, 8, 12, 14, 18, 34]. Its expression pattern has made it a valuable marker of malignancy [14, 34]. Similarly, FAP expression has also been shown to be upregulated in the stromal fibroblasts of cutaneous epithelial malignancies such as BCCs and SCCs as well as benign and malignant melanocytic lesions [21, 22]. We have recently shown that this marker is useful in differentiating between morpheaform/infiltrative BCC from desmoplastic TE [1]. Given this, we sought to assess FAP expression in both benign and malignant cutaneous epithelial entities, to verify if it can be used as a marker of epithelial malignancies in the skin and to evaluate its

usefulness in distinguishing between benign and malignant cutaneous epithelial entities, in which difficulties may exist in their differentiation on regular microscopy.

Materials and methods

The study was approved by the institutional review board of the American University of Beirut Medical Center. Archival materials with a diagnosis of BCC, SCC, eccrine porocarcinoma, cutaneous metastatic adenocarcinoma, KA, TE, PEH, eccrine poroma, syringoma, and chondroid syringoma were retrieved from the database of the dermatology department at the American University of Beirut Medical Center. A total of 26 cases of BCC (16 nodular, 5 superficial, 5 infiltrative), 14 cases of TE, 26 cases of SCC (16 well-differentiated, 6 moderately differentiated, 4 poorly differentiated), 16 cases of KA, 15 cases of PEH (7 associated with cutaneous leishmaniasis, 4 with atypical mycobacterial infection, 3 with deep fungal infection, and 1 with granular cell tumor), 10 cases of eccrine porocarcinoma, 12 cases of metastatic adenocarcinoma to the skin (5 from primary breast, 4 from primary gastrointestinal, 2 from primary lung, and 1 from primary prostate), 15 cases of eccrine poroma, 10 cases of syringoma, and 6 cases of chondroid syringoma fit inclusion criteria for the study. The histologic sections of all cases were reviewed and the diagnoses confirmed by the dermatopathologists (A.G.K. and O.A.). Only cases with a straightforward histopathologic diagnosis were included in the study. Ten scars from re-excision specimens served as controls (ranged in age from 2 to 12 weeks). Clinical information was obtained. All patient data were de-identified.

Immunohistochemical staining

Sections (5 mm thick) were obtained for immunohistochemical studies, which were performed on formalin-fixed, paraffin-embedded tissue. The avidin–biotin complex immunoperoxidase method for FAP was done as previously described [4, 8, 18]. In brief, clone D8 (FAP/seprase antibody, 1:200; SUNY, Stony Brook, NY, USA) was applied to sections pretreated with microwave (10 min) in 0.01 M Tris–EDTA buffer (pH 9.0). After incubation with the primary antibody, endogenous peroxidase activity was blocked by treating the sections for 5 min with 3 % hydrogen peroxide in Tris-buffered saline. As the secondary antibody, we used a biotinylated anti-mouse IgG (1:30; Biogeneics Laboratories, USA). Chromogen 3,30-diaminobenzidine was used for the visualization of the final reaction product. Sections were counterstained with Harris' hematoxylin. Appropriate positive and negative controls were included. All stained slides were reviewed and scored by the dermatopathologists (A.G.K. and O.A.) in a masked manner to ensure consistency of interpretation. Stained sections were scored as positive or negative.

Statistical analysis

The statistical association of FAP expression was analyzed using the Fisher's exact test to determine whether there were differences of significance in expression between the entities tested. A two-tailed p value of <0.05 was considered to be statistically significant.

Results

The clinical features of cases are summarized in Table 1. A positive stain was noted when cytoplasmic FAP positivity was seen within stromal fibroblasts. Any nuclear staining was considered background artifact. All control cases of scar showed positive expression of FAP within fibroblasts. Positive staining of mature sebocytes within sebaceous glands was also noted in some cases.

Positive cytoplasmic FAP expression by the peritumoral stromal fibroblasts was seen in all BCC cases (26 of 26, 100 %), all SCC cases (26 of 26, 100 %), all eccrine porocarcinoma cases (10 of 10, 100 %) (Fig. 1), and all cases of metastatic adenocarcinoma to the skin (12 of 12, 100 %), but not in any cases of TE (0 of 14, 0 %), PEH (0 of 15, 0 %), poroma (0 of 15, 0 %), syringoma (0 of 10, 0 %) (Fig. 2), and chondroid syringoma (0 of 6, 0 %). Moreover, a gradient of FAP expression was noted in the positively staining cases in which the strongest FAP cytoplasmic staining was observed in fibroblasts that were most adjacent to the tumor and the intensity of staining became progressively weaker with increasing distance from the tumor. Interestingly, the cases of KAs did not reveal uniform results as ten cases exhibited positivity, whereas six cases were negative. Notably, the heavy dermal inflammatory infiltrate that was sometimes observed in cases of PEH and KA made the interpretation of the immunohistochemical staining in the peritumoral fibroblasts more difficult as the fibroblasts were hard to discern. However, careful evaluation, especially looking for areas with less inflammatory infiltrate, made it possible to interpret those cases.

Taking all the malignant epithelial entities together versus all the benign entities, the difference between the two groups was statistically significant ($p < 0.05$). In addition, the difference in FAP expression between BCC and TE as well as between SCC and PEH was statistically significant ($p < 0.05$).

Table 1 Demographic data of all cases included in the study

	Number of cases	Age (years)	Gender	Location	
Basal cell carcinoma	26	39–86	19 M; 7 F	Head and neck	19
				Trunk	6
				Extremities	1
Squamous cell carcinoma	26	40–94	15 M; 11 F	Head and neck	17
				Trunk	3
				Extremities	6
Eccrine porocarcinoma	10	43–75	7 M; 3 F	Head and neck	4
				Trunk	1
				Extremities	5
Metastatic adenocarcinoma	12	40–81	5 M; 7 F	Head and neck	5
				Trunk	7
Keratoacanthoma	16	29–68	11 M; 5 F	Head and neck	10
				Trunk	1
				Extremities	5
Trichoepithelioma	14	18–69	6 M; 8 F	Head and neck	14
Pseudoepitheliomatous hyperplasia	15	3–66	8 M; 7 F	Head and neck	6
				Trunk	1
				Extremities	8
Eccrine poroma	15	28–78	8 M; 7 F	Head and neck	7
				Trunk	2
				Extremities	6
Syringoma	10	24–75	4 M; 6 F	Head and neck	6
				Trunk	3
				Extremities	1
Chondroid syringoma	6	35–78	3 M; 3 F	Head and neck	3
				Extremities	3

Discussion

FAP, also known as seprase, is a key protein that is characteristically expressed by carcinoma-associated fibroblasts (CAFs) [14, 32]. These cells reside in the stroma surrounding a tumor and play a central role in regulating the dynamics between the malignant cells, the extracellular matrix, and the numerous non-malignant cells that usually accompany the tumor [12]. The FAP protein is a type II integral membrane serine that has the ability to cleave a protein at sites following a proline residue [11]. Its unique pattern of expression has made it a useful marker and target of certain epithelial cancers. FAP is not detected in normal adult tissues. It is upregulated in fibroblasts at sites of tissue remodeling and wound healing in normal adults [14]. Moreover, FAP-positive fibroblasts are detected in keloids, which are benign dermal fibroproliferative tumors specific to humans [9]. The role of FAP in cancer biology has not been fully elucidated. It has been found to be expressed in CAFs of 90 % of epithelial cancers such as breast, colorectal, pancreatic and lung carcinomas as well as cutaneous epithelial malignancies such as BCCs, SCCs and malignant melanoma [1, 4, 8, 14, 18, 21, 22, 34]. The FAP serine protease has been shown to exhibit both tumor promoting and tumor suppressing behavior depending on the type of cancer [10, 17, 20, 37]. FAP exhibits an endopeptidase activity and is able to degrade gelatin therefore it plays a role in peritumoral stromal digestion and the promotion of invasiveness [5]. Moreover, evidence has shown that the cleavage products of FAP promote angiogenesis and explain in part the increased vascularity of FAP rich tumors [6, 20, 23, 27]. Another mechanism by which FAP promotes tumor growth is through the suppression of antitumor immunity [26]. Moreover, FAP also acts through non-enzymatic functions to promote tumor growth as described by Huang et al. where the authors show that breast cancer cells expressing a catalytically inactive mutant of FAP (FAP^{S624A}) produced tumors that grew rapidly [19].

The aim of our study was to examine FAP expression in benign and malignant epithelial tumors in the skin. Our results confirmed and expanded on previous findings that FAP is mainly expressed in the peritumoral fibroblasts of epithelial malignancies including cutaneous cancers such as BCC and SCC [1, 22]. We have shown positive FAP

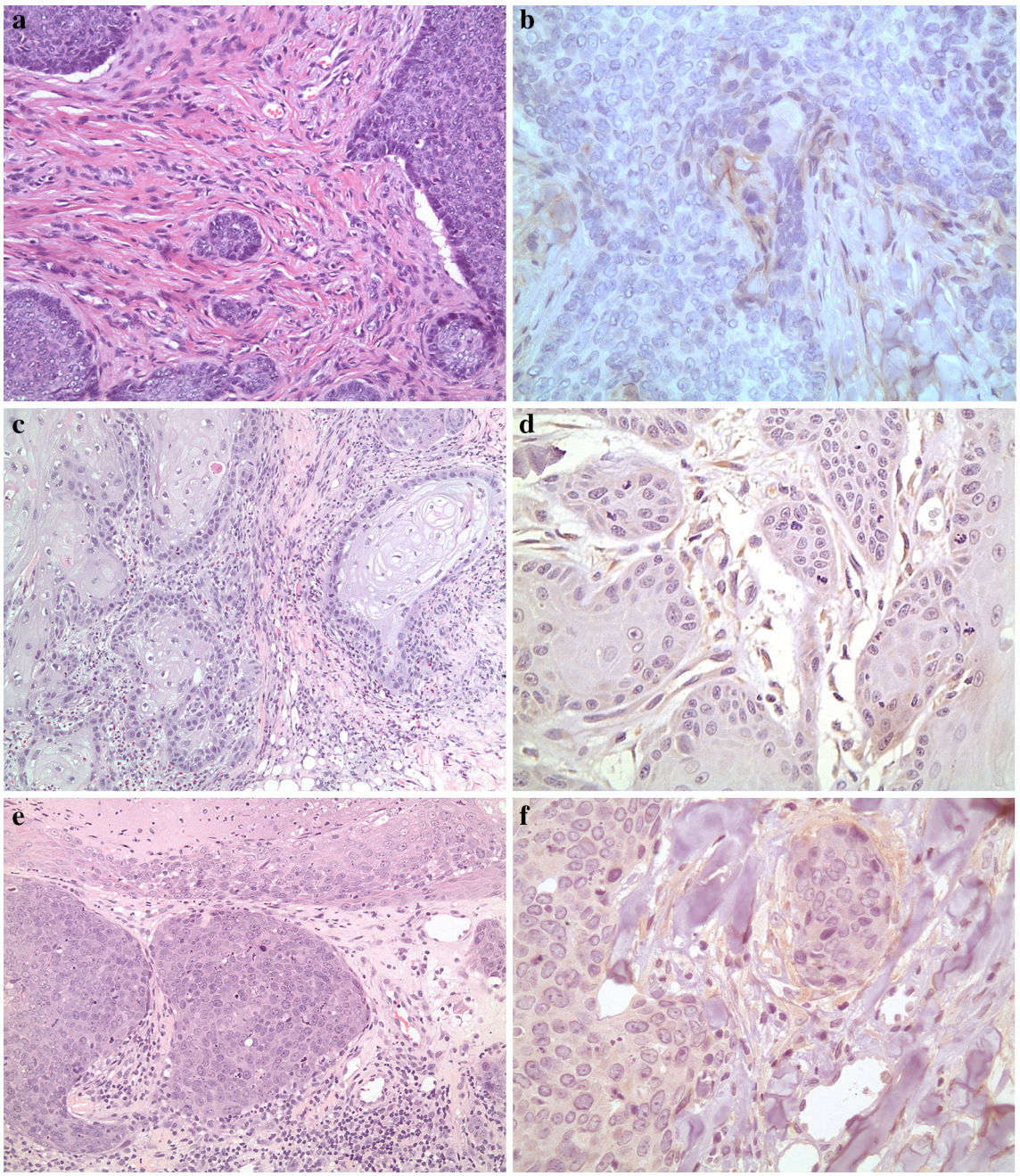

Fig. 1 **a** Representative BCC example; H&E, ×20. **b** Representative BCC example; FAP, ×20. **c** Representative SCC example; H&E, ×20. **d** Representative SCC example; FAP, ×20. **e** Representative eccrine porocarcinoma example; H&E, ×20. **f** Representative eccrine porocarcinoma example; FAP, ×20

staining in all epithelial malignancies including primary cutaneous (BCC, SCC, and eccrine porocarcinoma) and metastatic adenocarcinomas to the skin, while all benign cutaneous epithelial entities stained negatively for FAP. These findings thus confirm that FAP represents a valuable marker for cutaneous epithelial malignancy.

In addition, our study also highlighted the importance of this marker in differentiating between malignant and benign cutaneous entities that may share overlapping features such as BCC vs. TE and SCC vs. PEH. The results from the current study reinforce our previous findings that morpheaform BCC can be differentiated from desmoplastic TE by FAP expression in the stromal fibroblasts, making this marker a more reliable marker in the differentiation of BCC and its variants from TE and its variants than other markers that have been studied such as CD34, bcl2, androgen receptor, and CK20 [1]. Similarly, PEH may easily be confused with SCC, and the distinction is

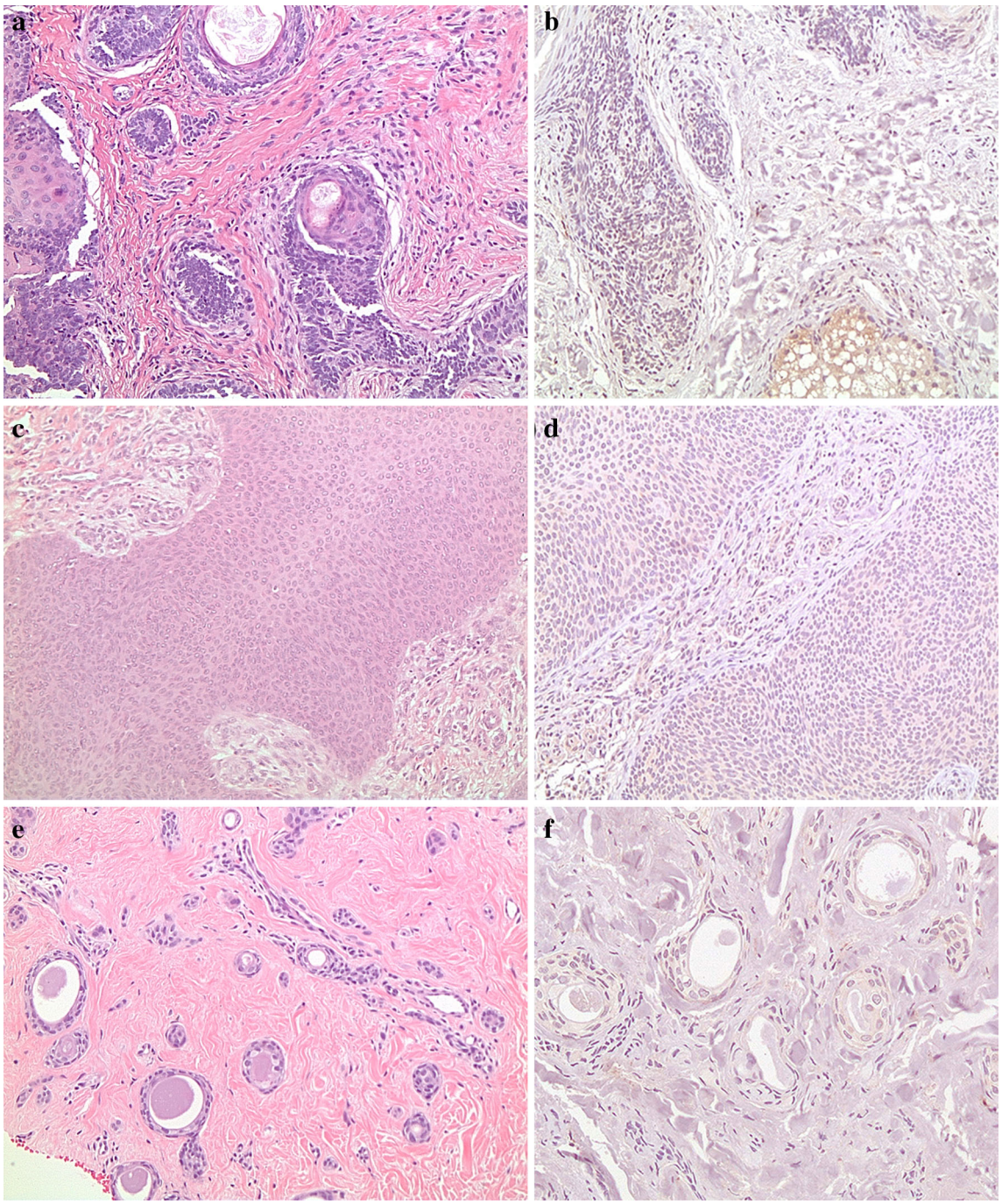

Fig. 2 **a** Representative TE example; H&E, ×20. **b** Representative TE example; FAP, ×20. **c** Representative eccrine poroma example; H&E, ×20. **d** Representative eccrine poroma example; FAP, ×20. **e** Representative syringoma example; H&E, ×20. **f** Representative syringoma example; FAP, ×20

particularly difficult when faced with a superficial shave biopsy. There are a few clues that may help in the distinction between the two entities on histopathology: namely, in SCC, the presence of numerous mitosis, individual necrotic keratinocytes and the invasion beyond the basement membrane, whereas in PEH, the absence of the above criteria for SCC diagnosis and the presence of an underlying infection, inflammation or tumor. Several markers including p53, Ki67, E-cadherin, MMP-1, EGFR, TGF-α, cyclin D1, collagen IV, Langerhans cells and AgNOR have been studied with respect to the differentiation of these two entities, none of which has been shown to be highly reliable in their distinction [3, 13, 25, 28, 39–41]. In our study, all cases of SCC expressed FAP, whereas all

cases of PEH were negative. This is despite the fact that many cases of PEH exhibit some degree of dermal fibrosis/scarring. This may be explained by the age of the scarring process. While all the ten control scar cases were relatively fresh (ranged from 2 to 12 weeks in age), it may be that the fibrosis/scarring seen in PEH cases is older. Another explanation may be related to the fibrotic process itself. Not all fibrotic or desmoplastic processes are mediated by converting fibroblasts to the phenotype seen in malignancies as we did demonstrate in cases of desmoplastic TE, which were also negative for FAP staining [1]. Thus, FAP offers the advantage of having a consistent staining pattern that allows us to differentiate between the two entities as compared with p53. The only limitation of FAP use in PEH is that the fibroblasts may be masked by the heaviness of the stromal inflammatory cell infiltrate. Moreover, evidently, the sample of tissue received for interpretation should include a dermal component or else FAP stromal staining loses its diagnostic value.

As for KA, there is still controversy as to its nature and whether or not it represents a well-differentiated SCC. Many believe that KA, though sharing some histopathological features with well-differentiated SCC, is a benign crateriform epithelial neoplasms that are notorious for their spontaneous regression after 3–6 months. To date, no reported criteria are sensitive enough to discriminate between the two entities [24, 30, 33]. Our results concerning the expression of FAP in KAs are interesting and inconclusive. In more than half of the cases ($n = 10$) there was positive FAP expression within peritumoral fibroblasts, whereas the rest of the samples ($n = 6$) were negative. Thus, FAP may not be an appropriate marker for the distinction between KAs and well-differentiated SCC. While the FAP expression within the peritumoral stroma may indicate malignancy, it may simply indicate tissue remodeling in this rapidly growing tumor comparable to tissue remodeling in scar tissue [4, 8, 14, 18, 34].

In conclusion, our study emphasized FAP as a marker for cutaneous epithelial malignancy and confirmed its diagnostic usefulness in the distinction of BCC from TE, and SCC from PEH.

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Conflict of interest None declared.

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