Expression of pigment epithelium-derived factor in psoriasis, verrucae, squamous cell carcinoma and normal skin: An immunohistochemical study

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Abstract

Background: Preservation of homeostasis status in the skin needs an equilibrium of keratinocyte proliferation, differentiation, necrosis and apoptosis. Disturbance of these regulatory mechanisms may lead to keratinocyte neoplastic and hyperproliferative diseases. Pigment epithelium-derived factor is a glycoprotein that is endogenously produced in different tissues and has a variety of biological effects in different diseases.

Objective: To evaluate the keratinocyte expression of pigment epithelium-derived factor in normal skin and three epidermal hyperproliferative diseases, namely, psoriasis, verrucae and squamous cell carcinoma.

Methods: This study included skin biopsy samples from 80 participants who were divided into four equal groups; each containing 20 samples. The first group included skin biopsies from normal skin, the second group from psoriatic lesions, the third group from verruca vulgaris and the fourth group from squamous cell carcinoma. All tissue samples were stained with hematoxylin and eosin stain and later immunohistochemically for pigment epithelium-derived factor expression.

Results: Scores of pigment epithelium-derived factor expression were lower in squamous cell carcinoma and verruca and psoriasis than normal skin with a significant difference ($P = 0.04$). In addition, the pattern of pigment epithelium-derived factor expression was mainly cytoplasmic in normal skin with a significant difference with that seen in psoriasis, squamous cell carcinoma and verruca vulgaris ($P = 0.001$).

Conclusion: Pigment epithelium-derived factor may play a role in keratinocyte differentiation.

Key words: Keratinocyte, pigment epithelium-derived factor, psoriasis, squamous cell carcinoma, verrucae

Introduction

The epidermis is characterized by a self-renewing ability which is maintained by the delicate regulation of keratinocyte proliferation, migration, differentiation, necrosis and apoptosis.[1] A precise balance is needed to control cellular proliferation. This balance is critical, as the excess proliferation leads to disease process while excess cell loss can lead to ulceration.[2]

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The epidermis and hair follicles constantly renew, thanks to keratinocyte stem cells located in the basal layer of the epidermis and in the bulge area.[3] Keratinocyte stem cells are long-lived residents in the epidermis, do not protect their genome by asymmetric chromosome segregation and are highly resistant to apoptosis.[4] It is thus likely that they accumulate several oncogenic mutations and induce skin cancer formation. It has been shown that the same cell subset is characterized by high clonogenic potential and by great resistance to apoptosis.[5] Psoriasis, verruca and squamous cell carcinoma are dermatological diseases characterized by both hyperproliferation and neoangiogenesis.[6]

Pigment epithelium-derived factor is a neurotrophic factor and is known to have antiangiogenesis effect.[7]

Aim of the work

This study aimed to evaluate the keratinocyte expression of pigment epithelium-derived factor in normal skin and three epidermal hyperproliferative disorders characterized by both cellular hyperproliferation and new angiogenesis: plaque psoriasis, verruca vulgaris and squamous cell carcinoma.

Methods

Ethical approval

Approval of the Research Ethical Committee in the Faculty of Medicine, Benha University, Egypt was obtained before starting this study. All patients and control persons signed written informed consent. None of the participants were subjected to any harmful effect and their personal data were kept confidential.

Type of the study

This was a retrospective case-control comparative study.

This study was conducted from January 2017 to January 2018 and included skin tissue samples from 80 individuals, subdivided into four equal groups, each containing 20 skin biopsies from plaque psoriatic lesions, verrucae vulgaris, squamous cell carcinoma and normal skin. Normal skin samples were obtained from surgical pathology specimens choosing a non-sun-exposed site. The squamous cell carcinoma tissue samples used in this study were obtained after surgical excision at the surgery Department in Benha University Hospitals.

The psoriatic patients were instructed to stop topical or systemic treatment for 1 month before skin biopsy. Tissue samples from psoriatic patients were taken using a 4 mm sterile punch while verruca vulgaris biopsies were taken with surgical excision.

Histological evaluation

Tissue samples were formalin-fixed, paraffin-embedded, they were divided into two parts. The first part was stained with hematoxylin and eosin for confirmation of clinical diagnosis and grading in the case of squamous cell carcinoma while the immunohistochemical evaluation was done using the second part.

Immunostaining procedure

The immunohistochemical staining was done using the streptavidin-biotin peroxidase technique.[8] For immunostaining, 4 μm sections were dewaxed in xylene, later, rehydration in descending concentrations of ethanol was followed. Blockage of endogenous peroxidases (by incubation in 0.3% H2O2 for 30 min) was tracked by microwave treatment (15 min in 10 mmol/L sodium citrate buffer pH 6.0) for antigen retrieval. Slides were then incubated for 30 min at 25°C with mouse IgG2b monoclonal antihuman antibody to pigment epithelium-derived factor (R and D Systems, Boston, MA, USA) at 1: 80 dilution. Using an available commercial kit (Biogenex, San Ramon, CA, USA) and according to the manufacturer's instruction, specific binding was identified. Sections were rinsed in phosphate buffer saline after each step. Phosphate buffered saline was used in control specimens, instead of the primary antibody.

Interpretation of immunostaining slides

Slides were examined by an independent pathologist blinded to the participants' clinical data. Examination of negative and positive control slides was performed first to rule out nonspecific staining and to judge the effectiveness of the technique and the reagents, respectively. Examination and evaluation of pigment epithelium-derived factor staining for both patterns and scores of expression were done.

The pattern of pigment epithelium-derived factor immunoreactivity was observed as cytoplasmic and/or membranous brown staining. Immunoreactivity for pigment epithelium-derived factor was evaluated semiquantitatively. In this semiquantitative grading method, the overall score of staining was calculated by multiplying the percentage of positive cells stained in 10 microscopic fields and the grade of pigment epithelium-derived factor expression was scored into three grades based on the percentage of epidermal expression as follows: low, moderate and high (1–10%, 11–50%, >50%, respectively).

Statistical analysis

The data were analyzed by mean ± SD, number and percentage of expression in each group. As both variables in this study are of a qualitative type, their analysis needed a chi-square test or its correction (the Fischer exact test). Chi-square test was used in comparing tow outcomes while Fischer exact test is used with three outcomes. A *P* value of less than 0.05 was considered significant. Statistical analysis was achieved using the statistical package for the social sciences program (v19; SPSS Inc., Chicago, IL, USA) for Microsoft Windows 7®.

Results

Skin biopsy samples from 80 subjects with a mean age of 45.16 ± 14.86 years were used in this study. Their data is summarized in Table 1.

The results of this study revealed that the pattern of pigment epithelium-derived factor expression was mainly cytoplasmic in control samples with a significant difference than other included diseases (*P* value = 0.001). Moreover, there was a significant difference regarding the expression score of pigment epithelium-derived factor between the studied groups (*P* value = 0.04) [Table 2]. Nevertheless, squamous cell carcinoma and verrucae vulgaris showed a lower score of expression followed by psoriasis while there was no low score observed in the control group [Table 2 and Figures 1-4].

By comparing the studied groups, results showed significant differences regarding the pattern of pigment epithelium-derived factor expression between control and both verruca vulgaris and psoriasis (*P* value = 0.008, 0.001, respectively). In addition, there was a significant difference found between psoriasis and squamous cell carcinoma (*P* value = 0.002). However, there were no significant differences between verruca vulgaris and both psoriasis and squamous cell carcinoma [*P* value = 0.46, 0.058, respectively, Table 3].

The results of this study showed that there was no significant difference between all SCC cases and control regarding the expression pattern of pigment epithelium-derived factor (*P* value = 0.06) [Table 3]. However, comparing the subtypes of squamous cell carcinoma included in this study and control, there was a significant difference (*P* value = 0.036) [Table 4].

The results of this study showed that the expression score of pigment epithelium-derived factor was high in all squamous

Table 1: Age, sex, site of skin lesion and pigment epithelium-derived factor expression

Variable	Control (*n*=20), *n* (%)	Psoriasis (*n*=20), *n* (%)	Squamous cell carcinoma (*n*=20)				Verruca vulgaris (*n*=20), *n* (%)
			Well-differentiated (*n*=4), *n* (%)	Moderately-differentiated (*n*=13), *n* (%)	Poorly-differentiated (*n*=3), *n* (%)	Total (*n*=20), *n* (%)	
Age (year±SD)	49.45±6.81	47.8±12.51	46±3.37	58.3±11.56	58.67±16.77	55.9±11.90	37.5±21.89
Sex							
Man	11 (55)	12 (60)	3 (75)	8 (61.5)	1 (33.3)	12 (60)	9 (45)
Woman	9 (45)	8 (40)	1 (25)	5 (38.5)	2 (66.7)	8 (40)	11 (55)
Site of skin lesion							
Head	0 (0)	0 (0)	2 (50)	10 (77)	2 (66.7)	14 (70)	0 (0)
Trunk	16 (80)	10 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Upper limb	1 (5)	5 (25)	1 (25)	1 (7.5)	0 (0)	2 (10)	12 (60)
Lower limb	3 (15)	5 (25)	1 (25)	2 (15.5)	1 (33.3)	4 (20)	8 (40)
PEDF expression							
Pattern							
Cytoplasmic	18 (90)	5 (5)	4 (100)	11 (84.5)	1 (33.3)	16 (80)	9 (45)
Membranous	2 (10)	10 (50)	0 (0)	0 (0)	2 (66.7)	2 (10)	8 (40)
Combined	0 (0)	5 (25)	0 (0)	2 (16.5)	0 (0)	2 (10)	3 (15)
Score							
Low	0 (0)	2 (10)	0 (0)	1 (7.5)	3 (100)	4 (20)	4 (20)
Moderate	14 (70)	6 (30)	0 (0)	5 (38.5)	0 (0)	5 (25)	7 (35)
High	6 (30)	12 (60)	4 (100)	7 (54)	0 (0)	11 (55)	9 (45)

PEDF: Pigment epithelium-derived factor

Table 2: Comparison between different groups regarding pattern and score of pigment epithelium-derived factor expression

Group	PEDF expression					
	Pattern			Score		
	Cytoplasmic (%)	Membranous (%)	Combined (%)	Low (%)	Moderate (%)	High (%)
SCC	16 (40)	2 (10)	2 (10)	4 (20)	5 (25)	11 (55)
Verrucae	9 (45)	8 (40)	3 (15)	4 (20)	7 (35)	9 (45)
Psoriasis	5 (25)	10 (50)	5 (25)	2 (10)	6 (30)	12 (60)
Control	18 (90)	2 (10)	0 (0)	0 (0)	14 (70)	6 (30)
FET		23.63			12.4	
P		0.001			0.04	

P≤0.05 is significant. The pattern of expression was determined according to cytoplasmic and/or membranous brown staining. The score of expression was determined according to the percentage of epidermal expression as follows: low, moderate and high (1%–10%, 11%–50%, >50%, respectively). FET: Fischer exact test, PEDF: pigment epithelium-derived factor, SCC: squamous cell carcinoma

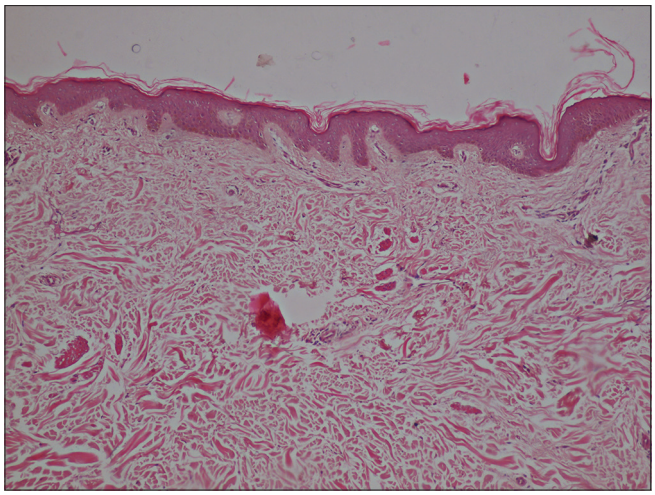

Figure 1a: Normal skin stained by hematoxylin and eosin (×200)

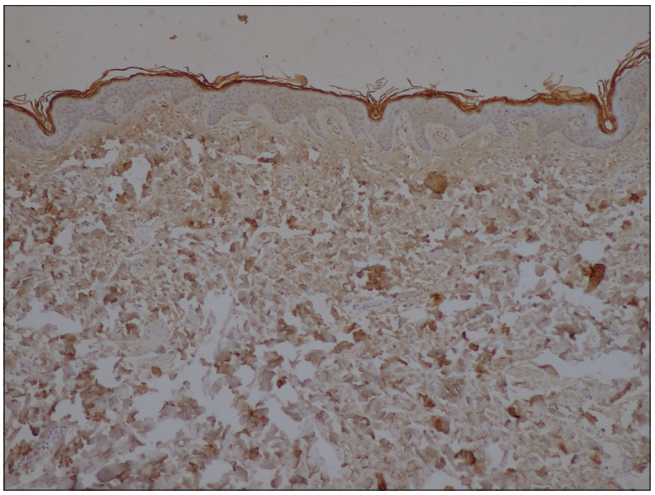

Figure 1b: Normal skin stained immunohistochemically showing pigment epithelium-derived factor moderate expression score with cytoplasmic pattern (×200)

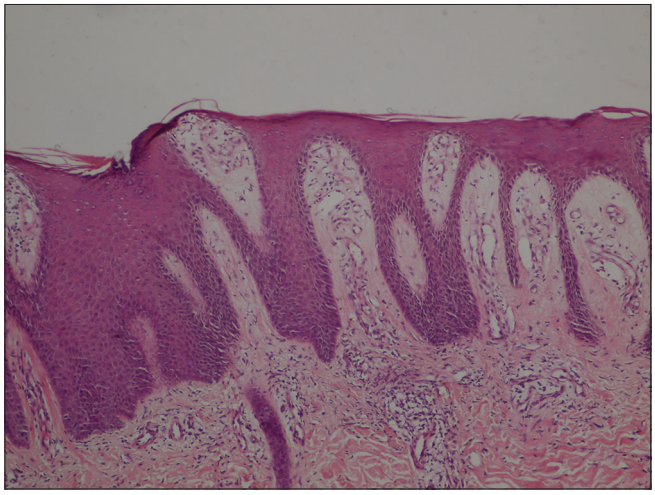

Figure 2a: Psoriasis stained by hematoxylin and eosin (×200)

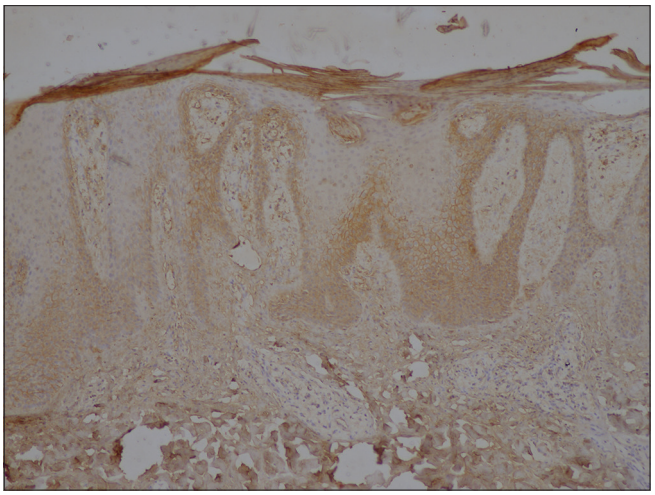

Figure 2b: Psoriasis stained immunohistochemically showing pigment epithelium-derived factor high expression score with membranous pattern (×200)

cell carcinoma cases with a high grade of differentiation, a higher score in seven cases with a moderately differentiated and low score in poorly differentiated squamous cell carcinoma cases. Comparing between different grades of the squamous cell carcinoma and control group, there was a significant difference regarding the expression score of pigment epithelium-derived factor (*P* value = 0.036) [Table 4].

There were significant differences between the control and verruca vulgaris, psoriasis and squamous cell carcinoma regarding expression scores of pigment epithelium-derived factor (*P* value = 0.029, 0.035, 0.006, respectively). However, there were no significant differences between psoriasis and both verruca vulgaris and squamous cell carcinoma (*P* value = 0.69, 0.82, respectively). In addition, there was no significant difference between the verruca vulgaris and SCC (*P* value = 0.91) [Table 5].

Discussion

A common feature of psoriasis, skin cancer and verrucae is the expression of antiapoptotic markers with different patterns of staining.[9]

Pigment epithelium-derived factor is a glycoprotein that is endogenously produced.[10] As a noninhibitory member of the serine protease inhibitor gene family[11] and a protein secreted by nearly all normal cells, bioactivities for pigment epithelium-derived factor have been expanded to include tumor suppression, cell growth and metabolism.[12] pigment epithelium-derived factor is a multifunctional, pleiotropic protein which is known for its antiangiogenic, antiproliferative, anti-inflammatory, antioxidant, neuroprotective and antithrombotic effects.[13]

Pigment epithelium-derived factor expression pattern in this study was mainly cytoplasmic in cases of low proliferation

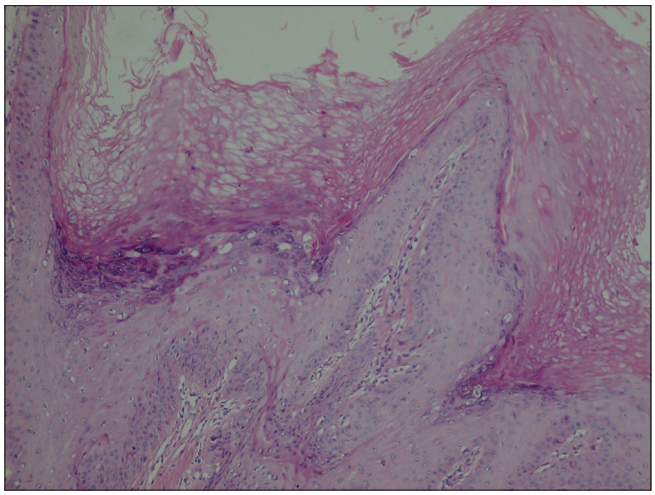

Figure 3a: Verrucae stained by hematoxylin and eosin (×200)

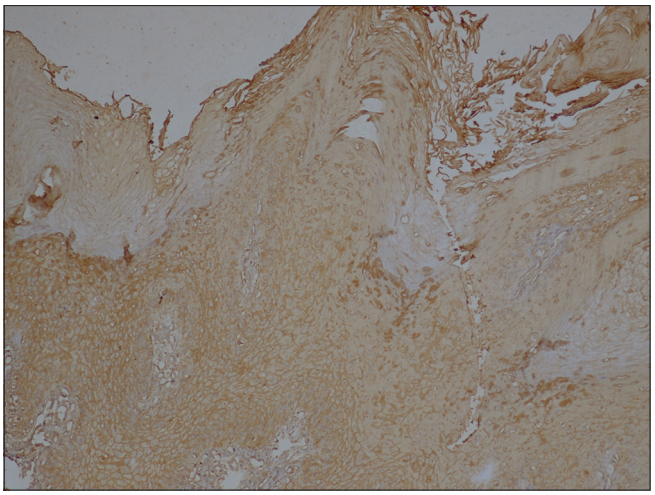

Figure 3b: Verrucae stained immunohistochemically showing pigment epithelium-derived factor high expression score with membranous pattern (×200)

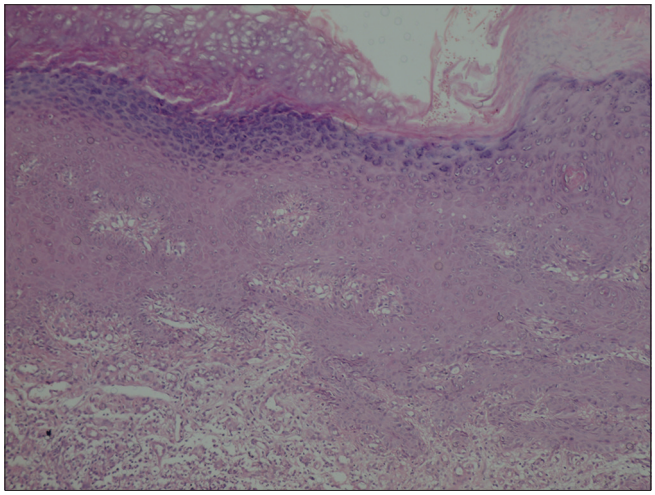

Figure 4a: Moderately differentiated squamous cell carcinoma stained by hematoxylin and eosin (×200)

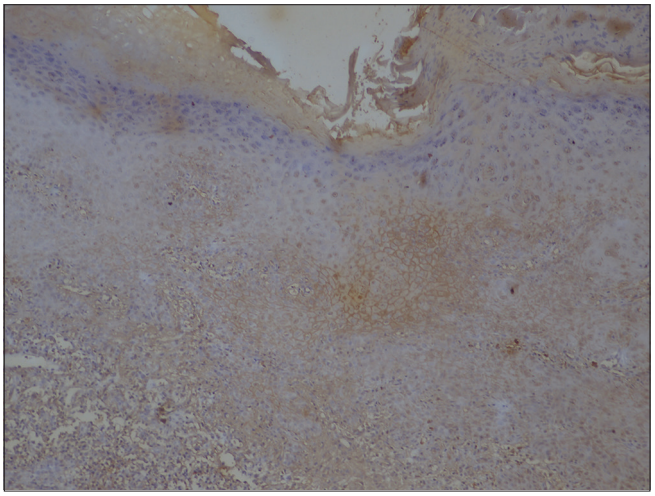

Figure 4b: Moderately differentiated squamous cell carcinoma stained immunohistochemically showing pigment epithelium-derived factor low expression score with membranous pattern (×200)

rate and highly differentiated keratinocyte (normal skin and well-differentiated squamous cell carcinoma). In contrast, pigment epithelium-derived factor expression pattern was mainly membranous in cases of high proliferation rate (psoriasis and poorly-differentiated squamous cell carcinoma). Although the score of expression differed, this was in accordance with Bowen *et al*., who stated that the proliferative index of psoriasis is more than squamous cell carcinoma and verruca.[14] Pigment epithelium-derived factor is strongly immunolocalized in the nucleus of many mammalian cells, suggesting that pigment epithelium-derived factor could migrate to the nuclear compartment to perform a specific function such as regulation of cell cycle.[15] The presence of cytoplasmic pigment epithelium-derived factor may control the cell cycle and prevent proliferation through interaction with the nuclear receptor corepressor 1[16] that can repress transcription factors as activator protein (AP)-1,[17] nuclear factor-kappa β (NF-κβ)[18] and retinoid receptors (retinoic acid receptor, retinoid X receptor),[19] which have roles in cellular inflammation[20] proliferation, differentiation and apoptosis.[21]

The membranous expression of pigment epithelium-derived factor may be required to exert its antiangiogenic potential which is counteracted by other angiogenesis promoting factors, upregulated in hyperproliferative keratinocyte disorders.[22] Pigment epithelium-derived factor appears to be a selective inhibitor of angiogenesis, sparing the preexisting vasculature and targeting de novo vasculature growth only.[23] The pathological angiogenesis occurs in conditions as tumor growth and chronic inflammation such as psoriasis, hence, pigment epithelium-derived factor is expected to have a role in these diseases.

These pleiotropic effects of pigment epithelium-derived factor are due to different target receptors and each has a distinctive function. The first receptor is pigment epithelium-derived

Table 3: Comparison between the studied groups regarding the expression pattern of pigment epithelium-derived factor

Number	Study group	Expression pattern of PEDF			FET	*P*
		Cytoplasmic	Membranous	Combined		
1	Control	18 (90)	2 (10)	0 (0)	9.05	0.008
	Verruca	9 (45)	8 (40)	3 (15)		
2	Control	18 (90)	2 (10)	0 (0)	17.63	0.001
	Psoriasis	5 (25)	10 (50)	5 (25)		
3	Control	18 (90)	2 (10)	0 (0)	1.87	0.54
	SCC	16 (80)	2 (10)	2 (10)		
4	Psoriasis	5 (25)	10 (50)	5 (25)	1.85	0.46
	Verruca	9 (45)	8 (40)	3 (15)		
5	Psoriasis	5 (25)	10 (50)	5 (25)	12.3	0.002
	SCC	16 (80)	2 (10)	2 (10)		
6	SCC	16 (80)	2 (10)	2 (10)	5.68	0.058
	Verruca	9 (45)	8 (40)	3 (15)		

P≤0.05 is significant. The pattern of expression was determined according to cytoplasmic and/or membranous brown staining. FET: Fischer exact test, PEDF: pigment epithelium-derived factor, SCC: squamous cell carcinoma

Table 4: Comparison between expression pattern and score of pigment epithelium-derived factor in different grades of squamous cell carcinoma and control group

Group	PEDF expression					
	Pattern			Score		
	Cytoplasmic (%)	Membranous (%)	Combined (%)	Low (%)	Moderate (%)	High (%)
Well-differentiated SCC	4 (100)	0 (0)	0 (0)	0 (0)	0 (0)	4 (100)
Moderately differentiated SCC	11 (84.6)	0 (0)	2 (15.4)	1 (7.69)	5 (38.46)	7 (53.85)
Poorly differentiated SCC	1 (33.3)	2 (66.7)	0 (0)	3 (100)	0 (0)	0 (0)
Control	18 (90)	2 (10)	0 (0)	0 (0)	14 (70)	6 (30)
FET		10.97			15.07	
P		0.036			0.001	

P≤0.05 is significant. The pattern of expression was determined according to cytoplasmic and/or membranous brown staining. The score of expression was determined according to the percentage of epidermal expression as follows: low, moderate and high (1%–10%, 11%–50%, >50%, respectively). FET: Fischer exact test, PEDF: pigment epithelium-derived factor, SCC: squamous cell carcinoma

Table 5: Comparison between the studied groups regarding expression score of pigment epithelium-derived factor

	Group	Expression score of PEDF			FET	*P*
		Low (%)	Moderate (%)	High (%)		
1	Control	0 (0)	14 (70)	6 (30)	6.58	0.029
	Verruca	4 (20)	7 (35)	9 (45)		
2	Control	0 (0)	14 (70)	6 (30)	6.72	0.035
	Psoriasis	2 (10)	6 (30)	12 (60)		
3	Control	0 (0)	14 (70)	6 (30)	9.35	0.006
	SCC	4 (20)	5 (25)	11 (55)		
4	Psoriasis	2 (10)	6 (30)	12 (60)	1.19	0.69
	Verruca	4 (20)	7 (35)	9 (45)		
5	Psoriasis	2 (10)	6 (30)	12 (60)	0.84	0.82
	SCC	4 (20)	5 (25)	11 (55)		
6	SCC	4 (20)	5 (25)	11 (55)	0.62	0.91
	Verruca	4 (20)	7 (35)	9 (45)		

P≤0.05 is significant. The score of expression was determined according to the percentage of epidermal expression as follows: low, moderate and high (1%–10%, 11%–50%, >50%, respectively). FET: Fischer exact test, PEDF: pigment epithelium-derived factor, SCC: squamous cell carcinoma

factor receptor A which is located intracellularly and responsible for cell differentiation, whereas the other receptor is pigment epithelium-derived factor receptor A which is detected in plasma membranes and involved in cellular adhesion, apoptosis and angiogenesis.[24]

Pigment epithelium-derived factor expression score in this study was correlated with keratinocyte differentiation. Our results were in accordance with previous studies regarding verrucae[25] and squamous cell carcinoma.[26]

Regarding psoriasis, a study by Abe *et al*. showed that the pigment epithelium-derived factor expression in uninvolved lesions was observed to be much higher than that in psoriatic lesions and topical application of peptide mimetics of pigment epithelium-derived factor led to reducing both epidermal thickness and angiogenesis in a mouse model of psoriatic disease.[27] Keratinocytes can produce pigment epithelium-derived factor with its functions and wide pattern of expression; pigment epithelium-derived factor has been suggested to be a factor that promotes the return to tissue homeostasis following a pathologic insult.[28]

Furthermore, pigment epithelium-derived factor can be used as a marker of both cellular differentiation and proliferation

in cases of malignancy.[29] *In vitro*, the addition of pigment epithelium-derived factor in cellular culture can inhibit proliferation and promote apoptosis of tumor cells.[30] While *in vivo*, measuring pigment epithelium-derived factor's concentration within the tissue or fluid of a cancer patient may define whether the tumor is in early or advanced stages of tumorigenesis.[31]

Conclusion

Pigment epithelium-derived factor plays a role in keratinocyte differentiation, proliferation, skin angiogenesis and inflammation-related disease processes. The expression pattern of pigment epithelium-derived factor may be used as a marker of keratinocyte differentiation.

Recommendation

Large-scale studies to evaluate the role of pigment epithelium-derived factor as a targeted therapy in skin proliferative lesions are recommended and topical application of pigment epithelium-derived factor may be used in hyperproliferative skin diseases.

Limitations

Poorly differentiated squamous cell carcinoma and squamous cell carcinoma *in situ* were not included in this study as only four samples of poorly differentiated squamous cell carcinoma were available.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity but anonymity cannot be guaranteed.

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Nil

Conflicts of interest

There are no conflicts of interest.

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