EXPRESSION OF STEM CELL IN BASAL CELL CARCINOMA

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ABSTRACT

Background: Emerging evidence is implicating stem cells in the pathogenesis of different cutaneous neoplasms. The immunohistochemical use of stem cell markers has facilitated stem cell identification.

Aim: This study was designed to determine the expression of stem cell markers ck19 and p63 in BCC and their link to proliferation marker ki67.

Subjects: Thirty patients with BCC were the subjects of this study. They were classified according to the hitopathological types into 12 patients solid circumscribed type, 3 patients solid infiltrative type, 8 patients mixed type, 3 patients superficial type, 2 patients adenoid type, one patient morpheaform type and one patient basosquamous type.

Methods: The thirty paraffin-embedded BCC skin lesions were examined histopathologically to confirm the diagnosis and to determine histological type of the tumour and also, were examined immunohistchemically for CK19, p63 and Ki-67.

Results: Cytokeratin 19 immunoreactivity was observed in 30% (9 of 30) of BCC cases while P63 immunoreactivity was observed in 70% (21 of 30) of BCC cases and Ki67 immunoreactivity was observed in 70% (21 of 30) of BCC cases. Significant correlation between p63 and ck19 expression in BCC does actually exist (P 0.006) and also strong correlation between expression of p63 and ki76 in BCC exist (P 0.006) while insignificant correlation between ck19 and ki67 expression in BCC was found (P .45).

Conclusion: Our study described the expression profiles of stem cell markers CK19 and p63 and also proliferation marker ki67 in BCC. In addition to confirming results of the previous reports, our study also showed that stem cells and cancer stem cells are found in BCC and may be the origin of this tumour. In addition, Ck19 is a more specific marker for stem cells than p63 which can stain transient amplifying cells as stem cells.

INTRODUCTION

The development of cancer is a complex multistep process that requires the accumulation of mutations resulting in a cell acquiring the essential hallmarks of cancer: evasion of apoptosis, self-sufficiency in growth signals, insensitivity to antigrowth signals, invasive and metastatic abilities, limitless replicative potential and sustained angiogenesis. Given that normal adult stem cells already exhibit limitless replicative potential, it is hypothesized that transformed stems cells may be the cells of origin for many cancers.[1]

In addition to their high potentiality for replication, long-lived stem cells have the opportunity to accumulate oncogenic mutations over years or decades from common mutagenic sources like inflammation, radiation, chemicals, or infection, unlike shorter-lived transit amplifying (TA) cells that rapidly proliferate and differentiate.[2] Like healthy adult stem cells, transformed stem cells are expected to be able to generate oncogenic TA cells. These TA cells would be capable of driving tumor formation and generating the heterogeneous combination of populations commonly seen in cancer. Transformed stem cells have been termed cancer stem cells (CSCs), also known as cancer initiating cells, and are defined as the fraction of cells within a tumor that are long lived, possess the potential to proliferate indefinitely, and can generate all heterogeneous lineages of the original tumor in xenograft models.[3]

Cancer stem cells (CSCs) are expected to utilize characteristics commonly found in stem cell populations such as differential metabolic activity, specific signaling pathway activity, and regulation of cell cycling characteristics, albeit with aberrant regulation.[4] Importantly, CSCs that survive treatment could account for tumor recurrence as a result of reactivation of proliferation in surviving CSCs. Traditional chemotherapy regimens target proliferating cells, potentially missing slower dividing CSCs that must be eradicated to provide long-term disease-free survival. A better understanding of CSCs is essential in understanding the biological and clinical consequences of existing regimens and designing new therapies to improve patient outcome.[5] In contrast to the differentiated tumour cells, cancer stem cells are undifferentiated primitive cells which maintain their proliferative potential. While cancer stem cells have already been described in leukaemias[6] brain tumours[7] and breast cancer[8], they are only recently being explored in cutaneous malignancies.[9] The identification of stem cell populations within normal skin and cutaneous malignancies has been facilitated by the immunohistochemical use of different stem cell markers. This, in turn, helped uncover some of the mechanisms underlying tumorigenesis.[10] Basal cell carcinoma is the most common cutaneous malignancy that tends to be mostly localized in sun-exposed hair-bearing areas, especially the

face. Several theories exist on the origin of BCC including basal epidermal cells, hair matrix cells, or stem cells in the bulge region or outer root sheath of the hair follicle. The histogenetic considerations about the origin of dermatological tumours rely largely on morphological similarities of a given tumour to its perceived normal counterpart. In the case of BCC, the epidermal basal cell layer has historically been considered to represent the site of tumour origin, whereas other studies favoured a follicular derivation. With the exponentially evolving impact of stem cell biology on contemporary medicine, newer approaches to the potential origin of a tumour make use of stem cell markers.[11]

Cytokeratin 19 (CK19) is an intermediate filament with a molecular weight of 44 kDa belonging to the acidic type of cytokeratins. It has been proposed that CK19 is an indicator of the stem cell population. In adult hair follicles, CK19 can be found in the outermost cells of the outer root sheath at the isthmus and in some cells of the lower outer root sheath.[12]In vivo and in vitro studies have shown that CK19 may be important in the commitment of stem cells to an epidermal cell fate anddifferentiation.[13]

The p63 gene, a member of the p53 gene family, is located on chromosome 3q27–29. The gene contains 2 separate promoters that lead to the expression of at least 6 major transcripts, resulting in 2 fundamentally different classes of proteins. Three of the p63 isoforms (TAp63) encode proteins with roles similar to p53 (ie, transactivation and induction of apoptosis); the other 3 isoforms (DNp63) exert inhibitory effects on p53 activity and lack the acidic amino (N)-terminal transactivation domain.[14] P63 is both highly expressed in the basal cells of human epithelial tissues and plays an essential role in epithelial development by helping to maintain the proliferative capacity of basal/progenitor cells.[15]

Although in vitro studies have initially identified p63 as a keratinocyte stem cell marker, more recent in vivo studies have shown that p63 expression is not restricted to epidermal stem cells but also involves basal and suprabasal epidermal cells as well as the outer root sheath and hair matrix of hair follicles. Thus, it may be a marker of transient amplifying cells rather than of stem cells. Compared to stem cells, transient amplifying cells are more differentiated and committed unipotent cells that have lost the ability to self-renew and can only go through limited rounds of proliferation before undergoing terminal differentiation. However, despite the promiscuous distribution of p63, this marker is still being used as stem cell marker, based on irrefutable evidence that a proportion of the cells in the skin that stain positively with this marker have stem cell characteristics of self-renewal and multipotency.[16]

Ki-67 is a non-histone protein located predominantly in the nucleolus. During mitosis, Ki-67 is associated with surfaces of condensed chromatin and the chromosomes, and after cell division, it is located in the nucleoplasm before localizing in the nucleoli .[17]Characterization of the Ki-67 antibody revealed an interesting staining pattern. The antibody was reactive with a nuclear structure present exclusively in proliferating cells. A detailed cell cycle analysis revealed that the antigen was present in the nuclei of cells in the G1, S, and G2 phases of the cell division cycle as well as in mitosis. Quiescent or resting cells in the G0 phase did not express the Ki-67 antigen.[18] Because the Ki-67 antigen was present in all proliferating cells (normal and tumor cells), it soon became evident that the presence of this structure is an excellent operational marker to determine the growth fraction of a given cell population. For this reason, antibodies against the Ki-67 protein were increasingly used as diagnostic tools in different types of neoplasms.[19]

Thus, as Ki-67 protein is expressed during all active phases of the cell cycle (G1, S, G2 and mitosis) but is not detectable in resting cells (G0), so this adds more to its application to the diagnosis and prognostication of neoplasms. If cells are dividing slowly or if Ki-67 expression is undetectable, we find that reassuring. If cells are dividing lickety-split, we find that of concern. If we identify an intermediate proliferation rate, the significance remains ambiguous.[20]

AIM OF WORK

This study was designed to determine the expression of stem cell markers ck19 and p63 in BCC and their link to proliferation marker ki67.

SUBJECTS

This study included thirty patients with BCC presented to the Dermatology outpatient clinic, Zagazig university hospitals, over a period of 2 years (January 1, 2010 to December 31, 2011). After clinical diagnosis, skin biopsy specimens were obtained from all patients for routine histopathological and Immunohistochemical study. Basal cell carcinoma was diagnosed clinically and confirmed hitopathologically.

METHODS A-Immunohistochemistry

All samples were fixed with 10% buffered formalin. The paraffin-embedded tissue blocks were cut into 4μm-thick serial sections and mounted on silanated slides. The sections were deparaffinized with xylene for 10 min and rehydrated through graded ethanol–water

solutions. Antibody-binding epitopes were retrieved by pressure cooking the tissue sections in 1 mmol L) 1 ethylenediamine tetraacetic acid buffer (pH 9.0) (Nichirei, Tokyo, Japan) for 10 min. The sections were then incubated overnight at 4ºC with antibodies against CK19 (1:100 dilution; Antibody Keratin 19 Ab-1 (Clone A53-B/A2.26, same as Ks 19.1, Thermo scientific, UK) and Ki67 (MIB-1, ready to use; Thermo scientific, UK.) and p63 (Ab-1, Clone 4A4, Thermo scientific, UK). Immunodetection was conducted with an alkaline phosphatase detection system according to the manufacturer's instructions. After immunohistochemical staining for CK19, p63 and Ki-67, three high-power field images (HPFs, x 200) were randomly selected in each specimen, 100 tumour cells were counted in each field, and the average percentage of positive cells for each staining from the three HPFs was calculated. Expression levels of CK19 and Ki-67 labelling indices were graded semiquantitatively as negative (-), weak (+), moderate (2+) or strong (3+), when tumour cells were positive at <5%, 6–25%, 26–50% or > 50%, respectively. P63 expression was graded semiquantitatively as negative (-), weak (+), moderate (2+) or strong (3+), when tumour cells were positive at 0%, <10%, 10–50% or > 50%, respectively.

B- Degree of intensity of staining

The intensity of staining was determined by the intensity of staining of the majority of the positive cells. Cells with light staining were not counted. To be judged as moderately intense staining, the staining had to be clearly more intense than any background staining on the slide with sharp demarcation of the nucleus. The intensity of staining was graded as 0: no staining, +1: faint staining, +2: chestnut brown, +3: deep brown. The sum of the two scores was taken as representative of the level of expression.

Statistical analysis:

SPSS for Windows 10.0 statistical package program was used for the evaluation of the data. Statistical analysis using the Student's t-test was performed to determine expression of CK19, p63 and ki67 among different types of BCC. Correlations among CK19, p63 and Ki-67 expression levels were considered to be assessed using Pearson's correlation coefficient analysis. A P-value < 0.05 was considered to be statistically significant.

RESULTS

The immunohistochemical markers expression in all the cases is shown in table 1 and summarized in table 2. **Cytokeratin 19:**

Positive CK19 staining was noted by ascertaining cytoplasmic expression. Any nuclear staining was considered background artifact. In each case, CK19 was expressed in the bulge area of the hair follicle, the outer root sheath of the follicle just above and below the bulge area, and eccrine glands and this served as positive internal control. Cytokeratin- 19 is not normally expressed in the normal epidermis (Fig.1).

Nine of 30 (30%) BCCs specimens showed CK19 immunoreactivity [weak (n = 6), moderate (n = 3) and no strong positivity detected (n = 0)] (Fig. 2-7). These positively stained specimens were not specific to the type of basal cell carcinoma (four were of the solid circumscribed type, four were of mixed type and one was superficial type basal cell carcinomas) (Table 3).

P63: P63 staining was considered positive by ascertaining only nuclear expression. Any cytoplasmic staining was considered background artifact. In each case, p63 was expressed in the normal epidermis in the nuclei of epidermal basal cells with a gradient i.e. a gradual decrease in expression of p63 in the more differentiated or superficial epidermal layers. Staining of the germinative layer of mature sebaceous glands, germinative hair matrix cells and the external root sheath of the hair follicles served as the positive internal controls .21 of 30 (70%) BCCs specimens showed p63 immunoreactivity [weak (n = 3), moderate (n = 8) and strong (n = 10)] (Fig. 8-11). These positively stained specimens were not specific to the type of basal cell carcinoma (eight were of the solid circumscribed type, eight were of mixed type, one was superficial type basal cell carcinomas, two were of adenoid type, one was of solid infiltrative type and one was basosquamous type). Non significant relation between BCC subtype and staining characteristics of p63 (p=0.094) (Table 4).

Ki67: Ki67 staining was considered positive by ascertaining only nuclear expression.Anycytoplasmic expression was considered background artifact. The cells of basal and suprabasal layers of the epidermis in normal skin adjacent to tumor masses served as positive internal control. 21 of 30 (70%) BCCs specimens showed ki-67 immunoreactivity [weak +1 (n = 6), moderate +2 (n = 7) and strong +3 (n = 8)] (Fig. 12-14). These positively stained specimens were not specific to the type of basal cell carcinoma (ten were of the solid circumscribed type, eight were of mixed type, one was superficial type basal cell carcinomas, one was of adenoid type, and one was basosquamous type). Non significant relation between BCC subtype and staining characteristics of p63 was found (p=0.105) (Table 5).

Table(1): Histopathological and immunohistochemical staining characteristics of CK19, Ki-67 and P63 in BCC patients

. No.	Histological type	CK19		Ki-67		P63	
		positively stained cells	staining intensity	positively stained cells	staining intensity	positively stained cells	staining intensity
1	Solid circumscribed	-ve	-ve	++	++	++	+++
2	Superficial	-ve	-ve	-ve	-ve	-ve	-ve
3	BCC-MH	++	++	++	++	+++	+++
4	Solid circumscribed	-ve	-ve	+	+	-ve	-ve
5	Morpheaform	-ve	-ve	-ve	-ve	-ve	-ve
6	Solid circumscribed	-ve	-ve	-ve	-ve	-ve	-ve
7	Solid infiltrative	-ve	-ve	-ve	-ve	-ve	-ve
8	Adenoid	-ve	-ve	++	++	++	++
9	Solid circumscribed	+	+	-ve	-ve	-ve	-ve
10	BCC-MH	++	++	++	++	+++	+++
11	BCC-MH	-ve	-ve	++	++	+++	+++
12	BCC-MH	-ve	-ve	+++	+++	+++	+++
13	Superficial	+	+	+++	+++	++	+++
14	BCC-MH	-ve	-ve	+++	++	+++	+++
15	BCC-MH	-ve	-ve	+	+	++	++
16	Solid circumscribed	+	+	+	+	++	++
17	Adenoid	-ve	-ve	-ve	-ve	+++	+++
18	Solid circumscribed	-ve	-ve	+++	+++	+++	+++
19	BCC-MH	++	++	+++	+++	+++	+++
20	BCC-MH	+	+	++	++	++	++
21	Solid circumscribed	-ve	-ve	+	+	+	+
22	Solid circumscribed	-ve	-ve	+++	+++	+++	+++
23	Solid circumscribed	+	+	+++	+++	++	++
24	Solid circumscribed	-ve	-ve	++	++	+++	+++
25	Basosquamous	-ve	-ve	+++	+++	+	+
26	Solid infiltrative	-ve	-ve	-ve	-ve	-ve	-ve
27	Solid infiltrative	-ve	-ve	-ve	-ve	+	+
28	Solid circumscribed	+	+	+	+	++	++
29	Solid circumscribed	-ve	-ve	+	+	-ve	-ve
30	Superficial	-ve	-ve	-ve	-ve	-ve	-ve

Table (2): Summary of the Immunohistochemical staining characteristics of CK19, Ki-67 and P63 in BCC patients

	Percentage of positively stained cells	staining Intensity
Ck19	-ve 21 (70%) + 6 (20%) ++ 3 (10%)	-ve 21 (70%) + 6 (20%) ++ 3 (10%)
Ki67	-ve 9 (30%) + 6 (20%) ++ 7 (23.3%) +++ 8 (26.7%)	-ve 9 (30%) + 6 (20%) ++ 8 (26.7%) +++ 7 (23.3%)
P63	-ve 9 (30%) + 3 (10%) ++ 8 (26.66%) +++ 10 (33.33%)	-ve 9 (30%) + 3 (10%) ++ 6 (20%) +++ 12 (40%)

Table (3): Relation of Ck19 staining percentage and histopathological types of basal cell carcinoma

	-ve	+	++
1-Solid circumscribed	8	4	
2-Solid infiltrative	3		
3-Superficial	2	1	
4-Morpheaform	1		
5-Adenoid	2		
6-BCC-MH	4	1	3
7-Basosquamous	1		
P = 0.4 (insignificant)			

Table (4): Relation of P63 staining percentage and histopathological types of basal cell carcinoma:

	-ve	+	++	+++
1-Solid circumscribed	4	1	4	3
2-Solid infiltrative	2	1		
3-Superficial	2		1	
4-Morpheaform	1			
5-Adenoid			1	1
6-BCC-MH			2	6
7-Basosquamous		1		
P = 0.061 (insignificant)				

Table (5): Relation of Ki-67 staining percentage and histopathological types of basal cell carcinoma.

	-ve	+	++	+++
1-Solid circumscribed	2	5	4	3
2-Solid infiltrative	3			
3-Superficial	2	1		
4-Morpheaform	1			
5-Adenoid	1		1	
6-BCC-MH		1	4	3
7-Basosquamous			1	
P = 0.105 (insignificant)				

Table (6): Relation between Ck19 and P63 staining percentage in BCC

		P63			
		-ve	+	++	+++
Ck19	-ve	8	3	3	7
	+	1		5	
	++				3
P = 0.006 (significant)					

Table (7): Relation between Ki-67 and P63 staining percentage in BCC

		P63			
		-ve	+	++	+++
Ki-67	-ve	7	1		1
	+	2	1	3	
	++			3	4
	+++		1	2	5
P = 0.006 (significant)					

Table (8): Relation between Ck19 and Ki-67 staining percentage in BCC

		Ki-67			
		-ve	+	++	+++
Ck19	-ve	8	4	4	5
	+	1	2	1	2
	++			2	1
P = 0.45 (insignificant)					

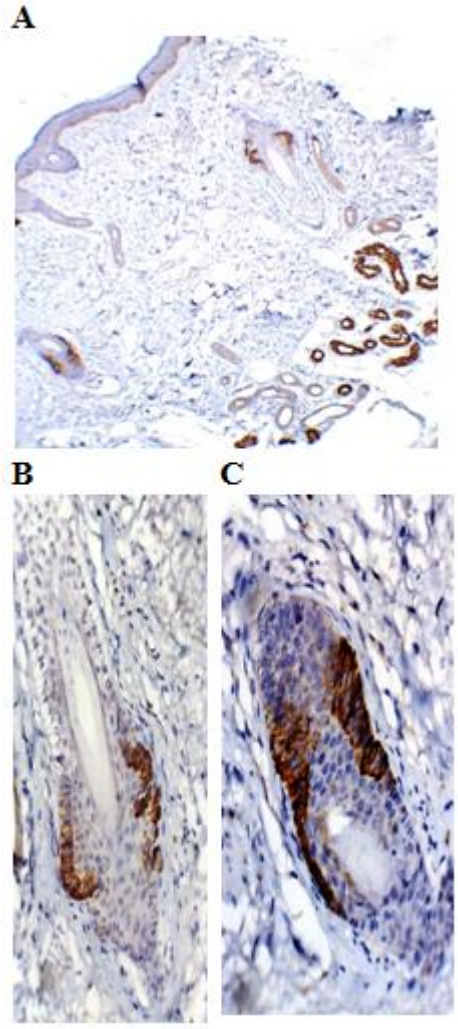

Figure (1): internal control positivity for cytokeratin 19 staining in nearby tissue. Note ck19 stains eccrine glands and hair follicle bulge region. (Ck19 stain original magnification, x 100). Expression of ck19 in hair follicle bulge region. Cytokeratin 19 stains the lower bulge area and displays a discontinuous expression in the outer layer of the outer root sheath extending up to the level of the hair matrix (B) and (C). (Ck19 stain original magnification x 400)

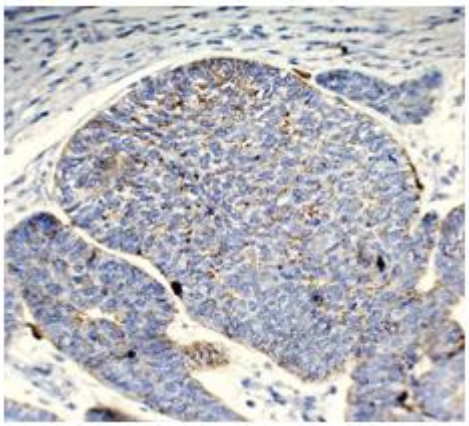

Figure (2): Weak expression of CK19 in tumour islands with weak positivity in patient 9. (Ck19 stain, original magnification x 100)

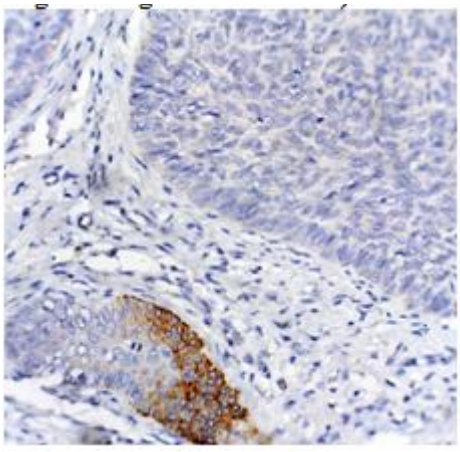

Figure (3): Negative expression of CK19 in tumour islands with positive internal control in the bulge region of nearby hair follicle. (ck19 stain, original magnification x 400)

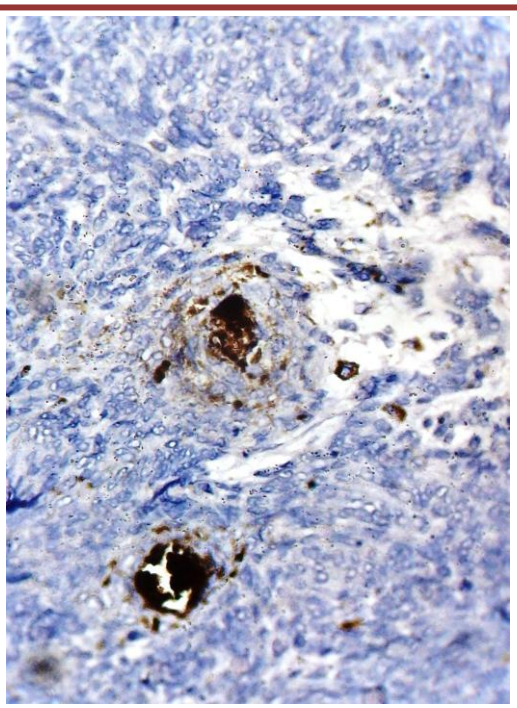

Figure (4) : Negative expression of CK19 in tumour islands but positive in squamous foci (ck19 stain, original magnification x 100)

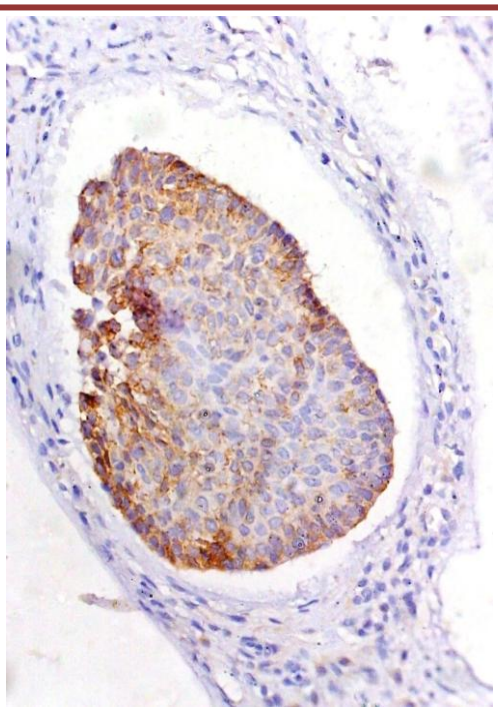

Figure (5): Moderate expression of CK19 in tumour islands in patient No. 10. (ck19 stain, original magnification x 400)

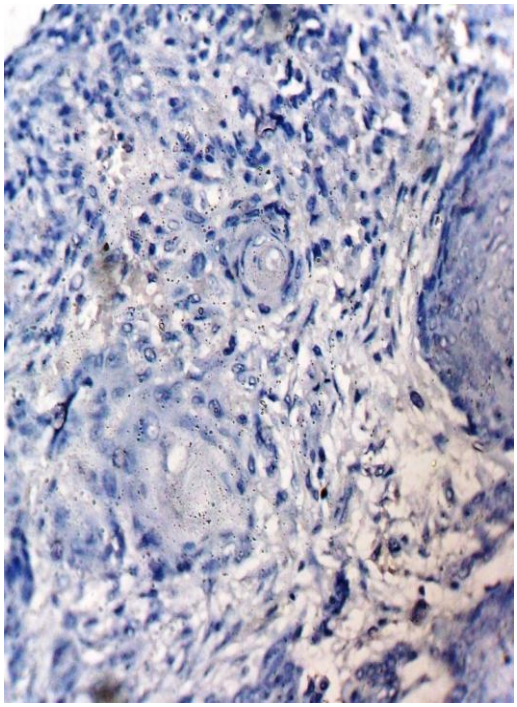

Figure (6): Negative expression of CK19 in tumour islands and also in squamous foci (ck19 stain, original magnification x 100)

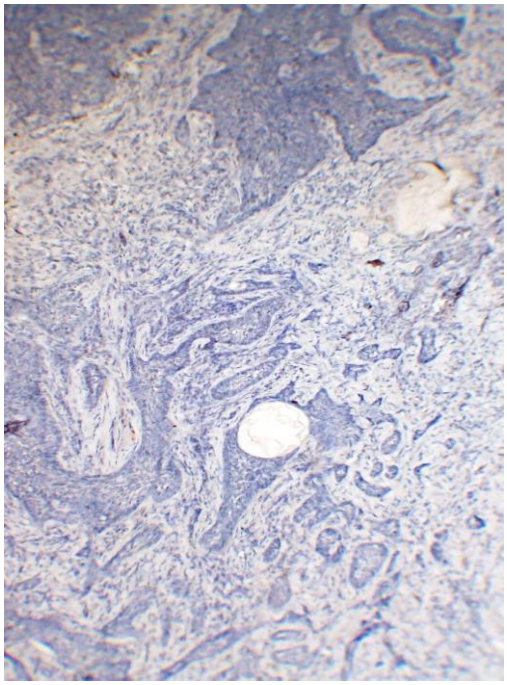

Figure (7): Negative expression of CK19 in tumour islands in infiltrative BCC. (ck19 stain, original magnification x 100)

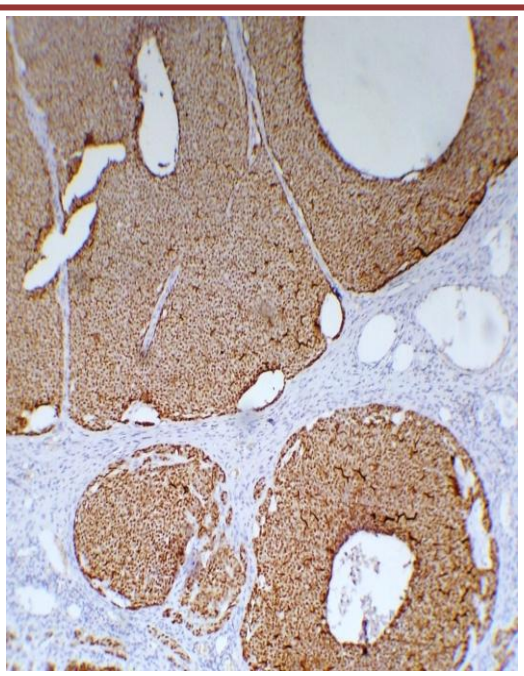

Figure (8): Strong p63 expression in tumour islands with high positivity in patient No. 24 (solid circumscribed type). Note that all cells, including those in a palisade arrangement, are decorated by p63. (p63 stain, original magnification x 100)

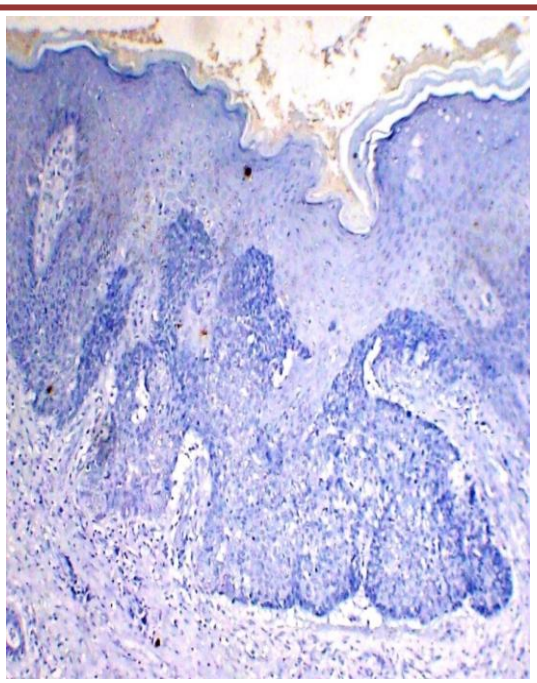

Figure (9): Negative p63 expression in tumour islands in patient No. 2 (Superficial type). (p63 stain, original magnification x 100)

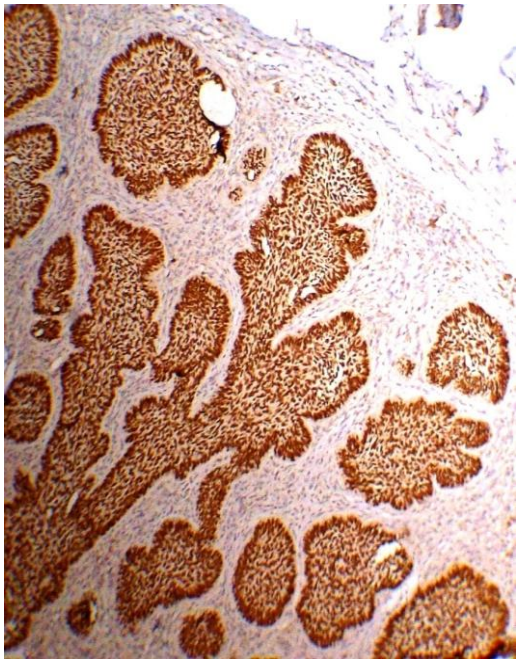

Figure (10): Strong p63 expression in tumour islands with high positivity in patient No. 19 (Mixed type). Note that all cells, including those in a palisade arrangement, are decorated by p63. (p63 stain, original magnification x 200)

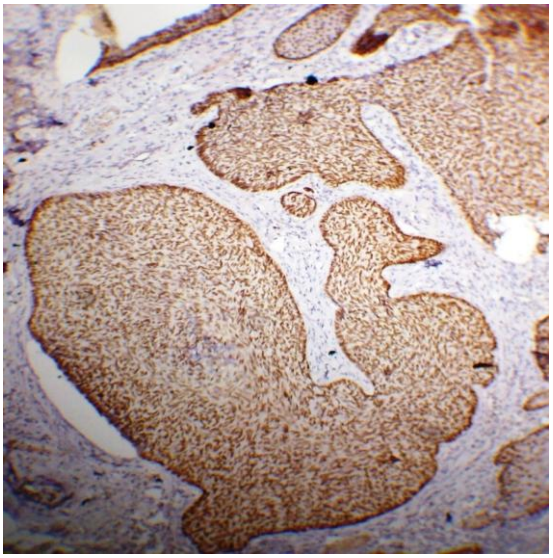

Figure (11): Strong p63 expression in tumour islands with high positivity in patient No. 19 (Mixed type). Note that all cells, including those in a palisade arrangement, are decorated by p63. (p63 stain, original magnification x 200)

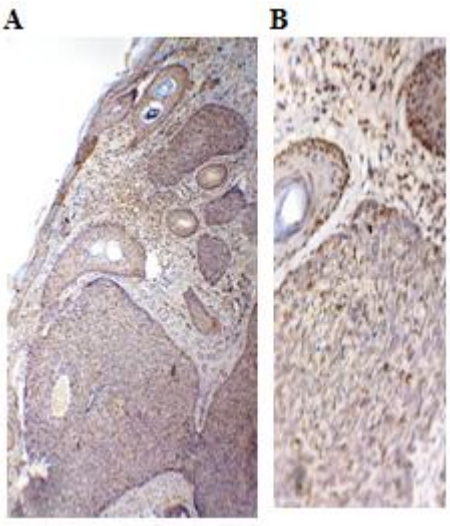

Figure (12): Moderate nuclear immunostaining for Ki67 in the central region and periphery of neoplastic islands in patient No.3. (Mixed type). A (ki-67 stain, original magnification x 100), B (ki-67 stain, original magnification x 200)

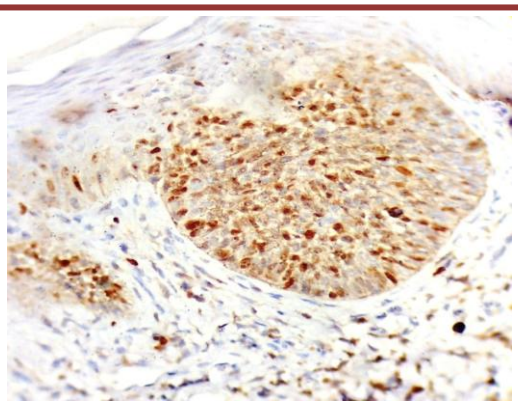

Figure (13): Strong nuclear immunostaining for Ki67 in the central region and periphery of neoplastic islands in patient No.13. (Superficial type). (ki-67 stain, original magnification x 440)

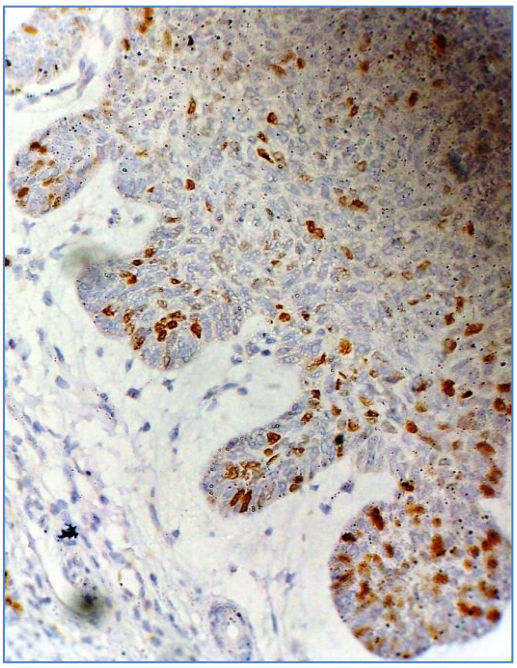

Figure (14): Mild nuclear immunostaining for Ki67 mainly in the central region neoplastic islands in patient No.16. (Solid circumscribed type). (ki-67 stain, original magnification x 200)

DISCUSSION

The cell of origin of most cancers has yet to be established. The majority of data thus far accumulated favor either tissue stem cells or progenitor cells as the cells that sustain the oncogenic mutation.[17]

Basal cell carcinoma is the most common cutaneous malignancy that tends to be mostly localized in sun-exposed hair-bearing areas, especially the face. Several theories exist on the origin of BCC including basal epidermal cells, hair matrix cells, or stem cells in the bulge region or outer root sheath of the hair follicle. Newer approaches to the potential origin of this tumour make use of stem cell markers especially stem cell markers CK15 and CK19.[11] These cytokeratins are usually conserved during malignant transformation, so that their identification can

help establishing the origin and degree of differentiation of this malignancy.[18] Positive CK15 expression in a subset of BCCs raised the possibility that these tumours are related to the hair follicle bulge stem cells.[8] Also, several studies showed increased CK19 expression in BCC with more positive BCCs for CK19 than for CK15. These studies suggested possible BCC origin from or probably its differentiation towards the follicular bulge region and/or the cells of the outer root sheath, which also express CK19.[11]

As regards to the present study, nine of 30 (30%) BCCs specimens showed CK19 immunoreactivity with weak CK19 immunoreactivity in patients and moderate immunoreactivity in 3 patients while no strong positivity detected. These positively stained specimens were not specific to the type of basal cell carcinoma. As was the case in our study, different reports have shown that positive CK19 expression in BCC ranges between 27% and 70% of the cases, suggesting possible BCC origin from stem cells.[20-22]In many of the previous studies as well as the present study, weak expression was demonstrated for CK19. This is not contradictory to a proposed stem cell origin of BCC. As tumours are heterogeneous and are composed mostly of differentiated malignant cells, it would be an expected finding that only a minority of tumour cells represents stem cells. So, this heterogeneity of CK 19 expression in BCC, as demonstrated in this study and other studies is part of the process of cell transformation and tumor development with preferred expression of low molecular weight cytokeratins. On the other hand, it could possibly be due to the heterogeneity of the tumors themselves, with various subpopulations showing different antigenic epitopes.

The p63 gene, a member of the p53 gene family, is highly expressed in the basal cells of human epithelial tissues and plays an essential role in epithelial development by helping to maintain the proliferative capacity of basal/progenitor cells.[21] **Kai-Hong et al., (2007)**[22] demonsrated that p63 is the most promising gene product which is capable of distinguishing stem cells from their transient amplifying progeny and P63 transcription factor may be a specific marker for keratinocyte progenitor cells of rat epidermis.

Although in vitro studies have initially identified p63 as a keratinocyte stem cell marker, other in vivo studies have shown that p63 expression is not restricted to epidermal stem cells but also involves basal and suprabasal epidermal cells as well as the outer root sheath and hair matrix of hair follicles.[23] Thus, it may be a marker of transient amplifying cells rather than of stem cells. Compared to stem cells, transient amplifying cells are more differentiated and committed unipotent cells that have lost the ability to self-renew and can only go through limited rounds of proliferation before undergoing terminal differentiation. However, despite the promiscuous distribution of p63, this marker is still being used as stem cell marker, based on irrefutable evidence that a proportion of the cells in the skin that stain positively with this marker have stem cell characteristics of self-renewal and multipotency.[16]

The results of the current study confirmed the high expression of p63 in BCC. P63 was strongly and diffusely expressed in the most of tumoral cells. These results were in agreement with those reported by **Bircan et al. (2006)**[24]**, Sakiz et al. (2009)**[25]**, Vidal et al. (2010)**[26] **and Plaza et al. (2010)**[27] who have demonstrated positive staining of p63 in most of their BCC studied specimen. Interestingly, in the current study, in some BCC cases, mature squamous nests embedded in tumoral cells were negative for p63 immunoreactivity while the rest was positive. These results confirmed the loss of p63 expression with more differentiation as the case in keratotic and squamous foci in BCC. Similar findings have also been reported by **Bircan et al. (2006).**[24]

Thus, according to the results of this study, overexpression of p63 in most histological subtypes of BCC may reflect that basaloid progenitor cells of squamous lineage are linked tumor-cell lineage in BCCs. Moreover, we observed that some squamous nests embedded in the BCCs were exactly negative for p63, which confirms p63 expression is tightly lost with terminal differentiation. These findings may suggest that p63 is a keratinocyte stem cell marker not only in normal epidermis but also in BCC, and it is highly expressed in BCCs independent from histological differentiation. Its overexpression in all subtypes may confirm that tumoral cells are derived from basaloid progenitor cell and have a role in the tumorigenesis of BCC.

Regarding Ki-67 immunohistochemical expression in BCC, the present work revealed positive immunoreactivity in 21 of 30 (70%) of patients [weak (n = 6), moderate (n = 7) and strong (n = 8)]. No statistically significant relationship was found between ki-67 expression and the histological subtypes of BCC. This high proliferative potential of BCC marked by high expression of proliferative marker ki67 is in contrast with the clinical observation that BCC is an extremely slowly growing tumor, which may

take months or even years to double in size. It has therefore been suggested that only a small percentage of all tumor cells is actively proliferating and it was found that these cells are mainly located at the periphery of the tumor nests. In addition, an increase of the apoptotic potential of BCC cells may be another explanation for the indolent growth behavior of BCC.[28]

To the best of our knowledge; this is the first study that correlates between the expression of proliferative marker ki67 and stem cell markers p63 and ck19. Significant correlation does actually exist between p63 and ki67 expression in BCC (p 0.006). Many specimens which show highly positive staining for ki67 also show high positive staining for p63. Now, one question arises is there any conflict between these results? If stem cells in BCC are quiescent in resting stage or actively dividing. BCC is hyperproliferative tumour regarding its high expression of ki67. At the same time BCC is a tumour expressing stem cells which are resting quiescent cells. This can be explained by hetrogenous nature of BCC. This tumour indeed contains stem cells which are suspected to be putative cancer stem cells and the origin of the tumour cells and also actively dividing cells at different stages of differentiation. These results are consistent with the results of **Glauche et al, (2009)**[29] who concluded that, stem cell proliferation and quiescence are two sides of the same coin. Quiescence of stem cells is regularly associated with the affiliation to stem cell niches. These particular, spatial environments exert a protective action in which stem cells are held in a rather inactive state while they maintain their full repopulation ability. Thus, there is an overlap of staining with strong correlation between expression of p63 and ki76 in BCC indicating that p63 is not a specific marker for stem cells and may also stain transient amplifying cells. These results are in agreement with the results of **Koster et al, (2005)**[23] who concluded that p63 expression is not restricted to epidermal stem cells but also involves basal and suprabasal epidermal cells as well as the outer root sheath and hair matrix of hair follicles. Thus, it may be a marker of transient amplifying cells rather than of stem cells. In the current study, significant correlation between p63 and ck19 expression in BCC does actually exist (P .006). This correlation supports the hypothesis of stem cell origin for BCC and the cancer stem cell hypothesis in general. Another point of importance is the significant relationship between ki-67 and p63 (not ck19) where p63 would be considered as a marker for progenitor cells in the phase of differentiation and not a marker for completely stable stem cells. These results add to the theory that ck19 is a specific marker for stem cells in contrast to p63 which can mainly express transient amplifying cells. These results when taken together point to heterogeneity of BCC as a tumour originating mainly from stem cells either follicular or interfollicular. These stem cells stepped out of their niches by sustained aberrant hedgehog pathway leading to their proliferation expressing ki67 and p63.

CONCLUSION

-Stem cells and cancer stem cells are found in BCC and may be the origin of this tumour. - Ck19 is more specific marker for stem cells than p63. - P63 can stain transient amplifying cells as well as stem cells. - Stem cell research is still growing and more work is needed to discover more specific stem cell markers. - No single stem cell marker that can be used solely for stem cell, instead a battery of markers should be used.

RECOMMENDATIONS

-We recommend further studies to explore more specific stem cell markers than the available markers. - We also recommend future research for differentiatin of cancer stem cells and somatic stem cells.

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إظهار الخلية الجذعية في سرطان الخلية القاعدية

***مقدمة**

سرطان الخلية القاعدية هو الشكل الأكثر شيوعا في سرطان الجلد وعلى الرغم من انه نادرا ما يكون قاتلا ، فان ارتفاع معدل وتكرار وقوعه في الأفراد المتضررين يمكن أن يشكل أثرا كبيرا كما انه من أكثر انواع السرطان تكلفة في العلاج والرعاية الطبية. وعلي الرغم من ان سرطان الخلية القاعدية يصنف باعتباره نوعا من انواع الاورام المنبثقة من سطح الجلد فإن اظهار الكيراتين فيه مماثل تقريبا لذلك الذي في الطبقة الخارجية لجذرالشعرة و تبين الدراسات الحديث أدلة تظهر تسبب الخلايا الجذعية في مختلف الأورام الجلدية ومن اهمها سرطان الخلية القاعدية . ولقد سهل استخدام مظهرات الخلايا الجذعية المناعية في تحديد هوية هذه الخلايا بداخل سرطان الخلية القاعدية .

***الغرض من الدراسة**

أجريت هذه الدراسة لتحديد اظهار اثنين من مظهرات الخلايا الجذعية وهما السيتوكيراتين- 19 وp63 في سرطان الخلية القاعدية وكذلك تحديد صلتهما بمظهر الانتشار وهو ki67 في سرطان الخلية القاعدية.

***طرق البحث:**

اشتملت هذه الدراسة على ثلاثين مريضا يعانون من سرطان الخلية القاعدية تم تصنيفهم وفقا للنوع الهستوباثولوجي . تم فحص الثلاثين عينة الماحوذة من هؤلاء المرضي ميكروسكوبيا لتأكيد التشخيص وتحديد النوع الهستوباثولوجي لسرطان الخلية القاعدية لكل مريض علي حدة. وكذلك تم فحصهم باستحدام اثنين من مظهرات الخلايا الجذعية المناعية وهما السيتوكيراتين- 19 و p63 وكذلك مظهر الانتشار.ki67

***النتائج:**

وجد أن نسبة اظهار السيتوكيراتين- 19 في مرضي سرطان الخلية القاعدية 30٪ (9 من 30) من الحالات في حين ان نسبة اظهار P63 في مرضي سرطان الخلية القاعدية 70٪ (21 من 30) من الحالات وكذلك نسبة اظهار Ki67 في مرضي سرطان الخلية القاعدية 70٪ (21 من 30). كما وجدت علاقة طردية ذات قيمة إحصائية بين p63 و ck19وكذلك علاقة طردية ذات قيمة إحصائية بين p63 و ki76في حين انه لا توجد علاقة ذات قيمة إحصائية بين ki67 , ck19 .

***ملخص ما سبق:**

لقد عضدت نتائج هذه الدراسة نتائج مؤكدة من السابق عن مدي اظهار مظهرات الخلايا الجذعية السيتوكيراتين- 19 و p63وأيضا مدي اظهار ki67في سرطان الخلية القاعدية. بالإضافة إلى تاكيد المفهوم المستند الي كون الخلايا الجذعية مصدر نشوء سرطان الخلية القاعدية.. وبالإضافة إلى ذلك اكدت هذه الدراسة ان السيتوكيراتين- 19 أكثر تحديدا للخلايا الجذعية منp63.

***التوصيات:** ننصح بعمل دراسات أكثر حتى

تكتشف مظهرات اكثر دقة في تحديد الخلايا الجذعية. كما ننصح بعمل دراسات أكثر حتى تكتشف طرق للتمييز بين الخلايا الجذعية السرطانية وتلك الطبيعية بداخل الجسم البشري.