

Immunohistochemical Expression of IGF-1R in Cutaneous and Mucocutaneous Squamous Cell Carcinoma

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ABSTRACT

Background: IGF-1R is a transmembrane receptor belongs to class of tyrosine kinase receptors, found in many tumors and is often associated with an aggressive phenotype. IGF-1R is important for regulation of growth, differentiation and apoptosis, as such, could be a preferred target for therapeutic interventions.

Aim: Investigate the expression pattern of IGF-1R in different histological grades of cutaneous and mucocutaneous squamous cell carcinoma and correlate with clinicopathological prognostic factors.

Materials: Forty-eight specimens of cSCC were stained immunohistochemically with IGF-1R monoclonal antibody and correlated with different clinicopathologic factors.

Results: All tumors showed a degree of positivity of IGF-1R cytoplasmic and membranous expression, however; with variable degrees. High grade tumors had significantly higher IGF-1R expression than low grade tumors. IGF-1R expression showed a strong positive correlation with the tumor grade ($P < 0.001$). Also, IGF-1R expression showed a significant relation with the depth of invasion of SCC ($P < 0.001$).

Conclusion: IGF-1R expression is significantly associated with high grade in cutaneous and mucocutaneous squamous cell carcinoma and also associated with tumors showing deeper invasion; therefore, IGF-1R may have a role pathogenesis and progression of cutaneous and mucocutaneous squamous cell carcinoma.

Keywords: Squamous Cell Carcinoma, Insulin-like Growth Factor-1 Receptor, Immunohistochemistry, Cancer, Tyrosine Kinase.

Introduction

Squamous cell carcinoma (SCC) is ranked as the second most common type of skin cancer. It represents about 20% of cutaneous malignancies. Annual incidence estimates range from 1 million to 3.5 million cases in the united states.

^[1]In Egypt, according to the registry of National Cancer Institute, malignant skin tumors represented 4.76 % of the malignant tumors of the entire body, SCC represent 44.9% of total malignant skin cancer during the period 2000-2011.

^[2]Chronic Sunlight exposure and immunosuppression are the strongest risk factors for SCC of the skin.^[3]

The insulin like growth factor 1 receptor (IGF-1R) is a transmembrane receptor which belongs to the tyrosine kinase receptors large class.^[4] IGF-1R is expressed in many cell types and tissues, and mediates the effect of insulin like growth factor 1 (IGF-1) which is similar in molecular structure to insulin. IGF-1R regulates growth, differentiation and apoptosis.^[5] IGF-1R over expression is has been found in many tumors like breast, colon, lung, ovary, prostate cancer. It is associated with an aggressive phenotype. In some tumors like cervical, non-small cell lung, pediatric and gastrointestinal cancers, its role is especially crucial.

^[6]In this study, we will investigate the role of IGF-1R in the pathogenesis in cutaneous and mucocutaneous SCC and try to correlate its immunoexpression with various clinicopathological features.

Materials and Methods

This study included 48 specimens of cutaneous squamous cell carcinoma (CSCC) that was diagnosed by excisional (42), incisional (1) and wedge (5) biopsies. The formalin fixed, paraffin embedded blocks of those specimens were retrieved from the archives of Pathology Department in Suez Canal University Hospital in the period from 2008 to 2015. Clinical data of the patients from which the specimens were taken (e.g. age and gender) were obtained from the pathology department records in Suez Canal University Hospital. One section from each formalin fixed paraffin embedded tissue block was cut at 5µm and mounted on super frost plus slides. The slide was immune-stained with IGF-1R monoclonal antibody 0.1 ml (Biocare Medical) and detection kit CRF™ Anti-Polyvalent HRP Polymer (DAB) Stain Kit were used. The procedure was performed according to the manufactures' instructions.

Tumor cells showing cytoplasmic and membranous staining of IGF-1R were assessed using the Histochemical scoring system (H Score). Staining intensity was scored as: 0 (no staining), +1 (weak staining), +2 (moderate staining) and +3 (strong staining). Percentage of staining of each intensity was assessed on scale from 0 to 100. Then the final H score was obtained by multiplying the intensity by the percentage on scale from 0 to 300. [6] Cut off value for positivity of the slides for IGF-1R immunostaining was determined at 100 or more for H score. [7] Sections from normal colonic tissue served as positive control. Negative control was obtained by replacing the primary antibody with phosphate buffered saline (PBS).

Statistical Analysis: Data were analyzed using IBM SPSS (Version 19.0). Quantitative data were presented as Mean ± SD (Standard deviation), while qualitative data were presented as frequency (%). Tables & graphs were used as appropriate to summarize & present data.

ANOVA test was used to test the statistical significance of difference in means between groups. Chi-square test was used to test statistical significance of association between qualitative variables; Pearson correlation was used to evaluate the correlation between quantitative variables. Statistical significance was set at P value <0.05.

Results

The histological grade of the studied specimens was as follows; twenty-one cases were well differentiated (Grade I) SCC, (43.8%) (Figure 1), 25 cases were moderately differentiated (Grade II) SCC (52.1%) (Figure 2) and two cases were poorly differentiated (Grade III) SCC (4.2%) (Figure 3). Assessment of depth of invasion in the studied specimens revealed that twenty-seven cases showed invasion < 2 mms (56.3%), twenty cases showed invasion between 2 to 4 mms (41.7%) and 1 cases showed invasion >4 mms (2.1%). Among the grade I cases, fifteen (31.3%) cases showed an invasion < 2 mms and among the grade II cases, thirteen (27.1%) cases showed invasion between 2 to 4 mms while the 2 cases of

poorly differentiated SCC showed also invasion between 2 to 4 mms. However, we did not find significant relation between the depth of invasion and the grade of the tumor (Table 1). Regarding the immunostaining intensity of IGF-1R in relation to the histologic grade of the studied cases, we found a statistically significant relation and the results were as follows; among SCC grade I; 10 cases showed weak intensity, 8 cases show moderate intensity and 3 cases show strong intensity (Figure 4). Among SCC grade II, ten specimens show moderate intensity (Figure 5), one specimen showed weak intensity (Figure 6), and 14 specimens show strong intensity while in SCC grade III one specimen showed moderate intensity, and one specimen showed strong intensity (Table 2). Concerning the percentage of stained cells by IGF-1R in each grade of SCC, a highly statistically significant relation was found. Grade I SCC cases showed the least percentage of stained cells for IGF-1R with mean of 33.8± 18.29 (Mean± SD) while grade III SCC cases showed the highest percentage of stained cells for IGF-1R with mean of 75± 70.71 (Mean± SD). So, the higher the grade of the tumor, the more the percentage of stained cells for IGF-1R (Table 3).

The final H score (percentage of stained cell x intensity of staining) showed statistically significant variations among the studied groups; among grade I SCC cases; 6 cases were positive for IGF-1R and 15 cases were negative. On the other hand, twenty-one cases of grade II cases were positive for IGF-1R and 4 cases were negative. While the 2 cases of grade III SCC were positive for IGF-1R (Table 4).

We found a highly statistically significant relation between the depth of invasion and the final H score. Among the cases of SCC with invasion < 2 mms (27 cases); 9 cases were positive for IGF-1R expression while almost all cases (19 cases out of 20) of SCC with invasion between 2-4 mms were positive for IGF-1R expression and the one case with invasion >4 mms was positive for IGF-1R (Table 5).

Table 1: Relation between the histologic grade and the depth of invasion

Tumor Grade	Depth of invasion			Total	P Value
	< 2mm	2 – 4mm	>4mm		
Grade I	15 (31.2%)	5 (10.4%)	1 (2.1%)	21	0.1137*
Grade II	12 (25%)	13(27.1%)	0 (0%)	25	
Grade III	0 (0%)	2 (4.2%)	0 (0%)	2	
Total	27 (56.2%)	20 (41.7%)	1 (2.1%)	48	

*Statistically significant at p< 0.05, highly statistically significant at p<0.01; Chi-Square test.

Table 2: Relation between the Immunostaining intensity for IGF-1R and histologic grade

Tumor Grade	Immunostaining Intensity			Total	P Value
	weak	moderate	strong		
Grade I	10(20.8%)	8 (16.7%)	3 (6.2%)	21(43.7%)	0.0039*
Grade II	1 (2.1%)	10(20.8%)	14 (29.2%)	25 (52.1%)	
Grade III	0 (0%)	1 (2.1%)	1 (2.1%)	2 (4.2%)	
Total	11(22.9%)	19(39.6%)	18 (37.5%)	48 (100%)	

*Statistically significant at $p < 0.05$, highly statistically significant at $p < 0.01$; Chi-Square test.

Table 3: percentage of stained cells with IGF-1R in relation to tumor grade.

Tumor Grade	Percentage of stained cell		P Value
	Mean± Std. Deviation	Range	
G I	33.8 ± 18.29	10-70	0.0001*
G II	62.8 ± 16.46	10-90	
G III	75 ± 70.71	70-80	
Total	50.62 ± 22.63	10-90	

*Statistically significant at $p < 0.05$, highly statistically significant at $p < 0.01$; ANOVA test.

Table 4: Final H score for IGF-1R in relation to tumor grade.

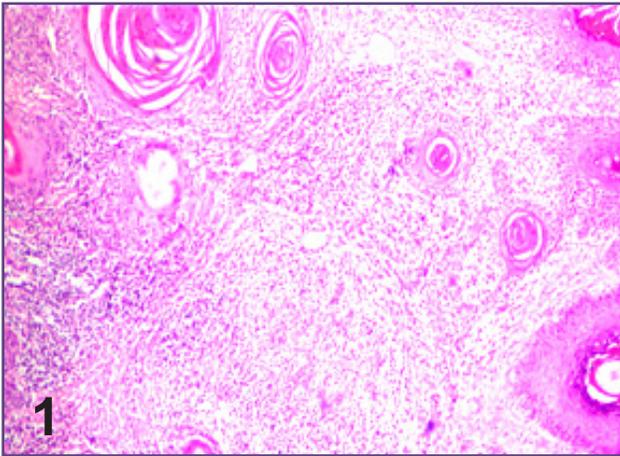
Tumor Grade	H score			P value
	< 100	>100	Total	
Grade I	15	6	21	0.0003*
Grade II	4	21	25	
Grade III	0	2	2	
Total	19	29	48	

*Statistically significant at $p < 0.05$, highly statistically significant at $p < 0.01$; Chi-Square test.

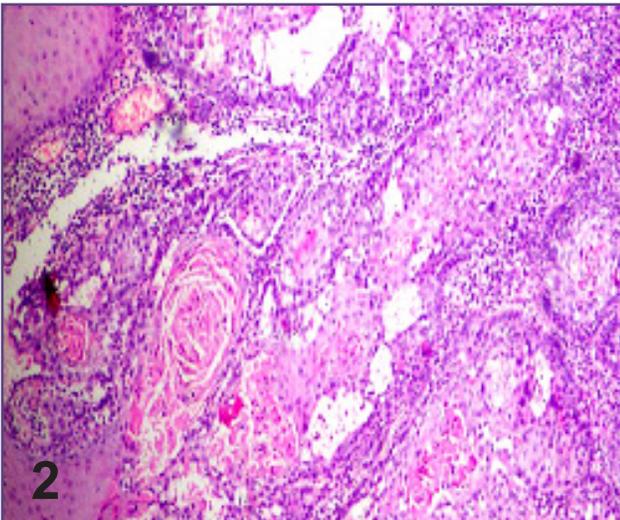
Table 5: Relation between the depth of invasion and H score for IGF-1R

Depth of invasion	H Score			P Value
	Negative	Positive	Total	
< 2mm	18 (37.5%)	9 (18.8%)	27 (56.2%)	0.0001*
2 – 4mm	1 (2.1%)	19 (39.6%)	20 (41.7%)	
>4mm	0 (0.0%)	1 (2.1%)	1(2.1%)	
Total	19 (39.6%)	29 (60.4%)	48 (100%)	

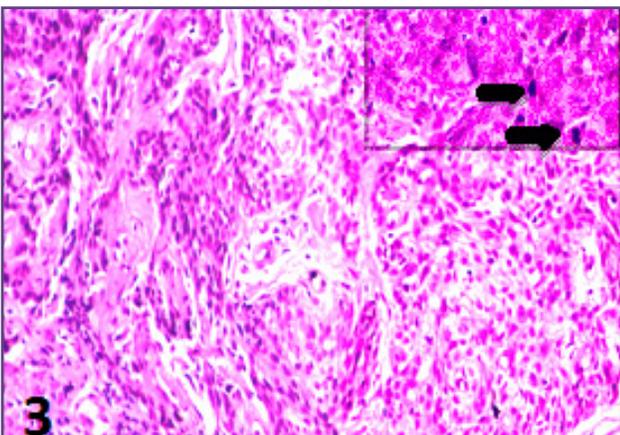
*Statistically significant at $p < 0.05$, highly statistically significant at $p < 0.01$; Chi-Square test.



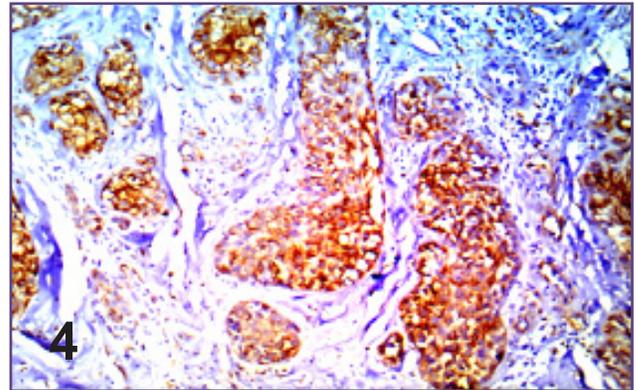
1
Fig.1: Well differentiated (Grade I) Squamous cell carcinoma, [H&E, 40x]



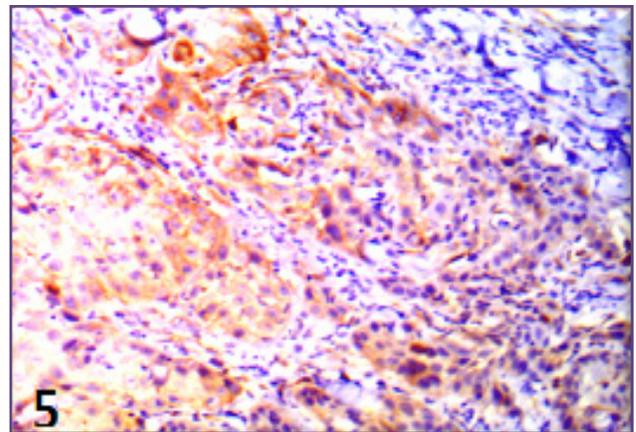
2
Fig.2: Moderately differentiated (Grade II) Squamous cell carcinoma, [H&E, 100x]



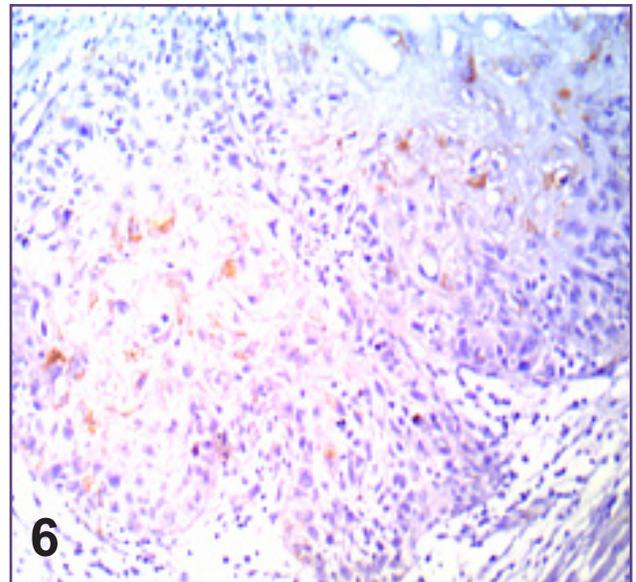
3
Fig.3: Poorly differentiated (Grade III) Squamous cell carcinoma, [H&E, 100x]. Inset: mitotic figures (arrows).



4
Fig. 4: Strong cytoplasmic intensity staining with IGF-1R, [immunostaining, X100]



5
Fig. 5: Moderate cytoplasmic intensity staining with IGF-1R, [immunostaining, X100]



6
Fig. 6: Weak cytoplasmic intensity staining with IGF-1R, [immunostaining, X100]

Discussion

The skin is considered as a major target organ for growth hormone (GH) which exerts its proliferative and anabolic effects through its receptor. Growth hormone receptor (GHR) has been found to be expressed in the stratum basale and spinosum of epidermis, dermal fibroblasts, adnexal structures, schwann, adipocytes and muscle cells.^[8]

Growth hormone triggers the synthesis of insulin like growth factor-1 (IGF-1), The action of which is mainly mediated through the IGF-1 receptor (IGF-1R) which is overexpressed by various tumor cell lines.^[9] Many studies have proved the overexpression of IGF-1R in malignant cutaneous melanoma.^[10,11] However, few studies have investigated IGF-1R expression in CSCC.^[12,13] Our study showed that the immunorexpression of IGF-1R varied significantly according to the differentiation of CSCC. Most of grade I SCC were negative for IGF-1R while almost all grade II cases and all grade III cases were positive for IGF-1R. These findings concur with Alcides et al^[14] who found a strong association between IGF-1R overexpression with histologic grade where grade III SCC showed the highest percentage 69% (53/77 cases). However, in their study, they included only penile SCC and combined grade I and grade II SCC into one category (low grade SCC). Our findings also agreed with Mark et al^[15] who found that overexpression of IGF-1R was significantly associated with increasing histologic grade. In accordance with our results, Oh et al^[16] found strong immunoreactivity for IGF-1R in grade II SCC (67%) and grade III SCC (100%). While only 40 % of grade I SCC showed strong immunoreactivity for IGF-1R. On the other hand, Gündüz et al^[17] could not prove that IGF-1R expression is related to grade of SCC. In their study, a high number of cases showed IGF-1R immunorexpression in SCC (87%) irrespective to histological grade. Similarly, Daniel et al^[18] and Boby & Devipriya^[19] also found overexpression of IGF-1R in CSCC is irrespective to histological grade.

Our study showed a statistical significant correlation between the IGF-1R expression and depth of invasion with p value = 0.0001, as the expression of IGF-1R increased as the depth of invasion increased. These findings agree with the study performed by Imsumran et al^[20] on esophageal squamous cell carcinoma, they found that IGF-1R expression showed a statistically significant relation with the depth of invasion were the expression increased in cases with deeper invasion. Results of our current study reveal IGF-1R higher expression with rising tumor grade of cutaneous squamous cell carcinoma. Follow up studies may help in further clarification of the recurrence and grade

progression or undifferentiation especially with larger number of specimens .Whether IGF-1R can be a potential prognostic marker or as a potential therapeutic target in cutaneous and mucocutaneous squamous cell in carcinoma may also be an issue to address in further studies.

Conclusions

Head & neck squamous cell carcinoma attracts great interest compared to other areas as limbs, trunk & genitourinary area. The statistically significant relation between IGF-1R expression and the grade and the depth of invasion of the tumor could suggest that IGF-1R may have a crucial role in the pathogenesis and progression of cutaneous squamous cell carcinoma and that IGF-1R expression is related to the degree of CSCC differentiation. Thus, anti-IGF-1R drugs may be an option for the treatment of inoperable cSCC.

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