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Immunohistochemical evaluation of actin expression in basal cell carcinoma and oral squamous cell carcinoma

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Key words

Basal Cell Carcinoma, Oral Squamous Cell Carcinoma, a-SMA

Abstract

Background: Basal cell carcinomas (BCCs) are generally slow-growing tumours. They have been classified as aggressive (A-BCC) and non-aggressive (NA-BCC).Oral squamous cell carcinoma (OSCC) is a major cause of cancer morbidity worldwide, this is due to the characteristics of invasion. The microenviroment or stroma of neoplastic tissues plays an active role in tumour progression. Trans-differentiation of fibroblast to myofibroblast is a crucial and early event in tumourigensis. Alterations of contractile tension generated by the actin–myosin complex are of central importance in the development of the phenotype of morphologically transformed neoplastic cells with invasive behavior. Actin is the predominant component of contractile microfillament and it may be associated with increase contractility and invasiveness of tumour cells.

Objective: This study aimed to investigate the presence of myofibroblasts in the stroma of basal cell carcinoma and oral squamous cell carcinoma, evaluated by the immunohistochemical expression of actin.

Materials and methods: Twenty four formalin –fixed, paraffin - embedded tissue blocks (14 cases basal cell carcinoma, 10 cases oral squamous cell carcinoma) were included in this study. An immunohistochemical analysis was performed using anti alpha - smooth muscle actin (α- SMA) monoclonal antibody.

Results: All cases of OSCC, BCC and normal oral mucosa showed positive reaction of actin in the smooth muscles surrounding blood and lymphatic vessels. All OSCC and BCC cases demonstrated stromal immunostaining for actin with different scores indicating the presence of myofibroblasts. There were no myofibroblasts in the stroma of normal mucosa indicated by negative α- SMA expression in it.

Conclusions: Immunohistochemical examination of BCC and OSCC for this marker may help clinicians in predicting tumour behaviour.

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Introduction

Basal cell carcinoma (BCC) is the most commonly diagnosed malignant skin tumour in white races [1, 2] .BCCs are generally slow-growing tumours that require months to years to double in size, despite high mitotic rate[2,3,4].BCC has

been classified as aggressive (A-BCC) and non-aggressive (NA-BCC) by well-described clinicopathological criteria. Immunohistochemical markers such as actin have received significant interest in connection with BCC. Actin is the predominant component of contractile microfilaments [5]. Alpha-smooth muscle actin (α-SMA) is found exclusively in contractile muscle cells, myoepithelial cells and myofibroblasts([5, 6, 7]. It has been suggested that an altered expression of α-SMA in BCC might be predictive of aggressive invasion [5, 6].

Oral squamous cell carcinoma (OSCC) is a major cause of cancer morbidity worldwide [7]. The 5-year survival rate for OSCC is about 40% and has improved only poorly over the past decades [8]. OSCC is highly correlated with metastasis and treatment depends on the anatomical site of the disease [9,10] Conventional treatment includes surgery and ⁄ or radiation and ⁄ or chemotherapy is associated with significant morbidity, affects speech, swallowing and overall quality of life. Despite these interventions a recurrence of the disease is observed in about 50% and is associated with high rates of mortality. During the last years, important advances have been made in understanding carcinogenesis, resulting in improved diagnosis and treatment [11, 12].

One of the characteristics of malignancy is invasion. Cytoskeletal reorganizations, especially alterations of contractile tension generated by the actin–myosin complex, are of central importance in the development of the phenotype of morphologically transformed neoplastic cells with invasive behavior. Actin may be associated with increased contractility and invasiveness of tumour cells, and have been identified in infiltrative basal cell carcinoma [13, 14, 15]. However, the number of studies evaluates the role of myofibroblasts in OSCC remained limited.

The present study investigated the presence of myofibroblasts in the stroma of oral squamous cell carcinoma and basal cell carcinoma evaluated by the immunoreactivity of actin in them.

MATERIALS AND ETHODS:

The study was conducted on twenty four formalin-fixed paraffin embedded tissue blocks of which, 14 cases were diagnosed as basal cell carcinoma obtained from the archives of Al-Shaheed Ghazi Hospital, Teaching Laboratory Department/ Baghdad Medical City/, and 10 cases were diagnosed as squamous cell carcinoma, obtained from the archives of the department of Oral & maxillofacial Pathology/ College of Dentistry/ Baghdad University.

Tumour histology was reviewed blindly by two pathologists, and representative paraffin blocks were selected. Data concerning patients' age, sex, clinical presentation and tumours site were obtained from the associated surgical reports.

Immunochistochemistry was performed on 4 µm, formalin-fixed, paraffin-embedded serial sections of tumour blocks using anti α - SMA monoclonal antibody (US Biological/Catalogue No A0760-26).

Negative and Positive tissue controls were included into each immunohistochemical run. Positive control for SMA was obtained from colon tissue that had acute appendicitis according to the manufacturer (Fig. 1).

Normal oral mucosa was obtained from patients undergoing tooth extraction for orthodontic purposes who have no sign of inflammatory gingival or periodontal disease to compare the immunoreaction of actin to that of the studied lesions.

Slides were put in hot air oven at 65°C overnight. Sections were sequentially dewaxed and rehydrated through a series of xylene, graded alcohol and water immersion steps. Then endogenous peroxidase activity was blocked followed by blocking the non- specific staining. Anti SMA monoclonal antibody (100 ml) at a dilution (1-200) was applied for each section. The samples were then incubated at 4°C overnight in a humid chamber. After washing with phosphate buffered solution (PBS), secondary Ab was applied to the sections, incubated and rinsed with a stream of PBS. Primary Ab was visualized with diaminobenzidine (DAB) chromogen. Sections were counterstained

with Mayer's hematoxyline for 30 seconds, dehydrated and mounted.

SMA was subjectively scored, according to the extent of stromal positivity Deihimy et al, 2006[16] as follows:
0: Negative or non-reactive.
1- +: Scattered spotty staining.
2- ++: 25% positive tumour cell.
3- +++: 25-50% positive tumour cell.
4- ++++: More than 50% positive tumour cell.

RESULTS:

Out of 14 cases of BCC, 12 were males and 2 were females, their age was from (30-83) years. Clinically the lesions ranged from small papular lesion to big nodular lesions, some with crustation or appeared as ulcerated areas. These lesions were scattered on the skin of the face and scalp.
Out of 10 cases of SCC, 3 were females and 7 were males with an age range of (35-75) years. Four of the cases were located on the tongue;3 on the mandibular area; 2 on the maxilla and one case on the buccal mucosa.

Histology and immunohistochemistry:
The histological grading of OSCC cases was as follows: - 6 cases were well differentiated SCC, 3 cases were moderately differentiated and one case was poorly differentiated SCC.
Blood vessels present within the connective tissue of the immunostained sections served as positive internal control. All cases of SCC, BCC and normal oral mucosa showed positive reaction of actin in the stromal smooth muscles surrounding blood and lymphatic vessesl (Fig.2, 3, 5, 6). All SCC and BCC cases demonstrated stromal immunostaining for actin with different scores indicating the presence of myofibroblasts(Fig.3,4, 5, 6). There were no myofibroblasts in the stroma of normal mucosa indicated by negative α- SMA immunostaining (Fig.2).
Regarding the immunostaining of the tumour cell itself, out of ten cases of SCC, only two cases showed nuclear staining for actin that is score 1. While out of fourteen cases of BCC, positive tumour cells were recorded only in three cases, one with score 1, one with score 2 and the third with score 4.

DISCUSSION

Basal cell carcinoma and squamous cell carcinoma are two of the most common tumours seen by pathologists. Interestingly, smooth muscle actin (SMA) has been found to be expressed in a significant number of basal cell carcinomas of the skin (13 of 17 cases in one study) [17]. Moreover, stromal (SMA) expression was found to be restricted to the aggressive BCCs, in addition ,a significant difference of this expression was found between aggressive and non-aggressive tumours, these findings suggested that stromal (SMA) expression is an accurate and reliable marker of aggressiveness in BCC[18,19]
In this study statistical analysis was not performed because the study concerned to demonstrate the presence or absence of myofibroblasts in the stroma of both BCC and OSCC samples indicated by actin immunoexpression. Indeed, in the current study SMA reactivity was observed in a number of basal cell carcinomas which have been stained(both tumour cell expression (3 cases) and stromal α- SMA expression (all cases)) , although the frequency of reactivity is not as high in our hands as in some published series .It has been reported that actin within the stroma surrounding BCC nests is a marker for myofibroblasts that play a significant role in invasion, as they secrete stromolysin-3, a metalloproteinase that degrades the stromal matrix [20, 21].Degradation of the stromal matrix may enhance the stromal-tumour communication that is essential for invasion. Although the pathophysiologic

mechanism responsible for the myofibroblastic reaction is obscure, it has been proposed that the induction of cytokines from BCC cells (such as basic fibroblast growth factor) may be responsible for stromal α- SMA expression. The same BCC-derived cytokines that induce the stromal myofibroblastic response have an autocrine effect on the individual BCC cells, increasing tumoural actin synthesis and leading to enhanced cellular motility and invasion [22, 23].

An increased expression of stromal α-SMA might reflect increased aggressiveness in BCC and immunohistochemical examination of BCCs for this marker may help clinicians in predicting tumour behaviour.

It has become increasingly apparent, however, that the 'normal' components of the tumour stroma (including fibroblasts, inflammatory cells, and endothelial cells) play an important role in promoting tumour progression [24, 25]. Many types of solid tumours appeared to contain smooth muscle actin positive myofibroblasts('activated' fibroblasts, peritumour fibroblasts, carcinoma-associated fibroblasts) within the stroma. Myofibroblastic trans- differentiation is modulated mainly through TGF-β1 signaling and may be induced in a number of different cell types, including fibroblasts, pericytes, circulating fibrocytes, and mesenchymal cells [25].

Additionally, in recent years the concept of epithelial mesenchymal transition (EMT) has received much attention, with suggestions that apparent stromal cells actually may be derived from epithelial tumour cells [26, 27, 28]. Functional assays showed that OSCC cells can promote fibroblast-to-myofibroblast trans-differentiation through αvβ6-dependent activation of TGF-β1 and that myofibroblasts, in turn, promote OSCC invasion [28].High Myofibroblasts have been reported to be associated with poor prognosis in several carcinoma types [29, 30].

It have shown previously that myofibroblasts promote the invasion of OSCC cells and that aggressive variants of basal cell carcinoma of the skin contain a prominent, myofibroblastic stroma, which modulates keratinocyte motility through secretion of hepatocyte growth factor (HGF) [31].

Moreover, the strongest independent risk factor of early OSCC death has been reported to be a feature of the stroma rather than tumour cells and the high stromal SMA expression produced the highest hazard ratio and likelihood ratio of any feature examined, and were strongly associated with mortality, regardless of disease stage [32]. Furthermore Stromal SMA expression revealed to be used to identify aggressive OSCC, regardless of disease stage, and might be important for the treatment and follow-up of OSCC patients. [33]

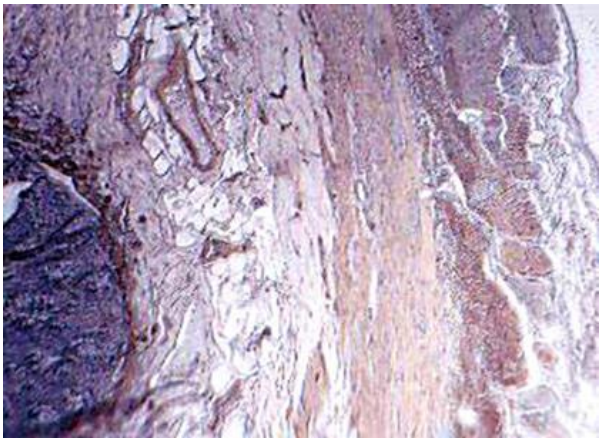

Figure 1: Positive actin immunostaining in acute appendicitis (positive control) (x200

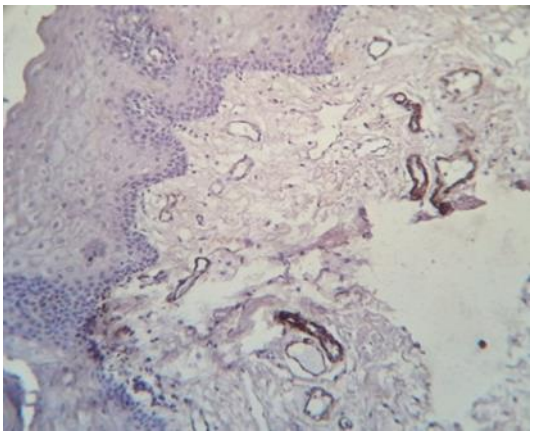

Figure 2: Positive actin immunostaining of the smooth muscles surrounding vascular and lymphatic vessels in normal oral mucosa (X400**)**

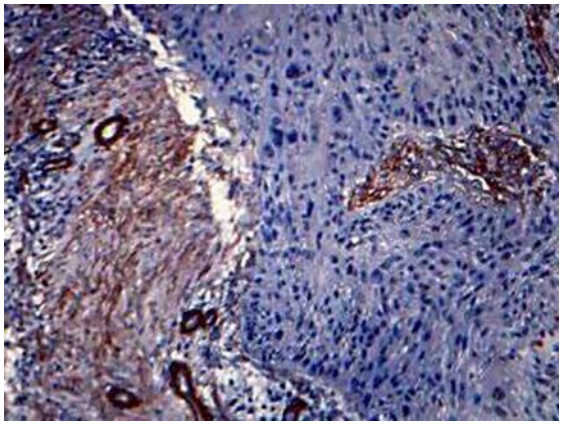

Figure 3: Positive immunostaining of actin in the stroma as well as the smooth muscles surrounding vascular and lymphatic vessels in well differentiated SCC (X200)

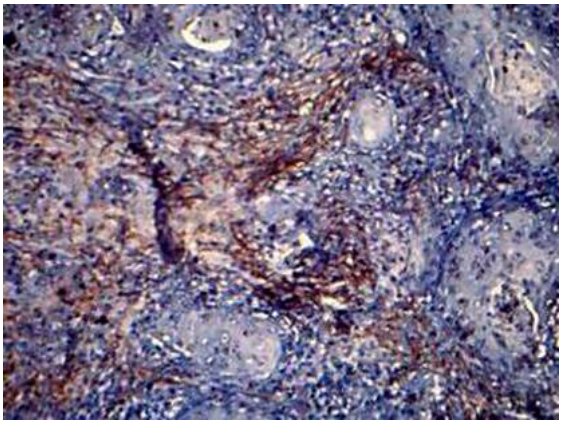

Figure 4: Positive stromal actin immunostaining in well differentiated SCC (X200)

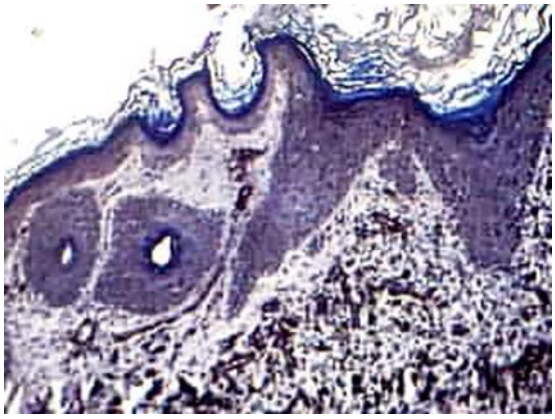

Figure 5: Positive immunostaining of actin in the stroma as well as the smooth muscles surrounding vascular and lymphatic vessels in BCC (X200)

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