

Differences between Keratoacanthoma and Squamous Cell Carcinoma Using TGF- α

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ABSTRACT

Squamous cell carcinoma (SCC) and keratoacanthoma (KA) are skin neoplasms of epithelial origin. In contrast to clearly malignant skin neoplasm SCC, KA is an unusual cutaneous neoplasm with a tendency to regression. The distinction between these two neoplasms, on histological grounds only, is still a challenge. In order to investigate further and to assess the possible differences in transforming growth factor-alpha (TGF- α) expression between SCC and KA, 40 of skin tumor specimens, 20 cases of each SCC and KA were analyzed immunohistochemically. We have found a significant difference in staining patterns between KA and SCC. In KAs we have detected TGF- α staining mainly diffusely (90% of cases) and without peripheral staining of cells in 1–2 layers (60% of cases). Contrary, there was a mostly patchy staining (55% of cases) with peripheral staining of cells in 1–2 layers (100% of cases) in SCCs. Generally, differentiation between KA and SCC can be based on clinical and histological ground, but the distinction between these two skin tumors could sometimes be difficult. We have shown that these skin neoplasms could be differentiated based on staining patterns of TGF- α expression, thus this method could aid in differentiation between these two closely related entities in clinical practice.

Key words: keratoacanthoma, squamous cell carcinoma, TGF- α

Introduction

Squamous cell carcinoma (SCC) and keratoacanthoma (KA) are skin neoplasms of epithelial origin. Skin SCC is a malignant tumor with ability to metastaze, while KA is an unusual cutaneous neoplasm, previously considered a precancerous lesion, that is destructive locally, but has tendency to regress and, generally, no ability to metastaze. Usually, the differentiation of these tumors can easily be done clinically because of their fast growth and characteristic appearance of KA. KA presents in a shape of molluscoid nodule with a keratin plug in its centre, while SCC presents in the shape of nodule or ulcer that does not heal during one-month-period or longer. The problem arises within pathohistological analysis of these tumors that may have similar pathohistological characteristics. Pathohistological analysis of cutaneous SCC revealed tumor lesion consisted of irregular masses of epidermal cells that infiltrate epidermis and proliferate into dermis. Tumor masses consist of cells that remind on spinous, keratinic and undifferentiated (anaplastic, atypic) spinous cells. Anaplasticity of

these cells could be seen in the difference of cell size and shape, hyperplasia of cells and hyperchromasia of nuclei, absence of intercellular connections, keratinization of individual cells forming keratinized pearls and presence of atypical mitoses. Keratinized pearls consist of concentric layers of cells that show gradual improvement of keratinization towards the centre of a pearl. The more the tumor is malignant, the more anaplastic cells are found in it. Only a correctly taken biopsy of a tumor can guarantee appropriate diagnosis. Marginal biopsy of KA can not present enough data for exclusion of SCC. Correct diagnosis of KA needs »architecture« of the lesion along with cell characteristics. The early, proliferative stage of KA is characterized by the presence of exoendophytic squamous proliferation with a central keratin-filled crater and the overlying epidermis extended around the crater. In this stage, epidermal cells have an eosinophilic glass-like shine, while in the dermis there was a strong inflammatory infiltrate. In developed lesions, epidermis is lying around the zone of keratinization in a shape of

papillomatous proliferation. Anaplasticity of cells could be seen on the periphery in 1 or 2 layers of basophilic cells, while eosinophilic cells are situated at the base of crater centre. In the involutive stage, proliferation is diminished and the proliferating epithelium tended to flatten out, with diminished inflammation and fibrosis underlying the base.

Therefore, immunohistochemical staining techniques are more presents in their analysis today.

Transforming growth factor alpha (TGF- α) is a polypeptid found in the culture of retrovirally neoplastic altered fibroblasts. The term TGF- α originates from a fact that this polypeptid can reversibly transform normal fibroblasts of rat kidney. TGF- α has a molecular weight that varies among 5 to 20 kDa. The structure of TGF- α is a shape of triple running loops, with 3 disulphid connections that connect 2 molecules of cistein. The final loop shows strong homologation with the epidermal growth factor (EGF). TGF- α has been sintetized in normal keratinocytes, and in other cell populations such as: cutaneous adnexes, muscles and in vascular walls, also its expression has been established in many tumors, mostly in those of malignant potential¹⁻⁶. The expression of TGF- α in cutaneous keratinocytes and its effect on mytosis have been established in psoriasis. The role of TGF- α in proliferation of tumor cells does not depend on high level of expression of endogen TGF- α , but transformed cells can show stronger answer on TGF- α because of higher number of receptors on cell surface or because of the activation of factors that play role in transduction of the signal.

To investigate further and to assess the possible differences in TGF- α expression between SCC and KA immunohistochemical analyses were performed.

Materials and Methods

Patients and skin specimens

40 of skin tumor specimens, 20 cases of each SCC and KA, were obtained from patients at Department of Dermatovenereology, Rijeka University Hospital Center, by probatory or total excision of skin changes performed in

local anesthesia with 1–2% xylocaine solution. Obtained samples were fixed with 10% buffered formaldehyde and embedded in paraffin. 4 mm-thick sections were stained with hematoxylin-eosin and two pathologists examined each slide independently.

Immunohistochemical staining

The primary antibody solution was a 1:50 dilution of monoclonal antibody TGF- α (Merck Biosciences, Calbiochem, catalogue number HCS05 Calbiochem, clon 213-4.4). TGF- α immunostaining was performed as follows: paraffin-embedded tissue sections were deparaffinized in xylene and rehydrated by washing in absolute and diluted ethyl alcohol and distilled water. Staining was carried out after sections were treated for antigen retrieval according to manufacturer's instructons. This was followed by standard ABC (avidin-biotin complex) procedure for 2 hours and 10 minutes in DAKO Techmate Immunostainer (Techmate Horizon, serial No. 30097, LjL Biosystems Inc., USA).

The sections were examined at high power in order to determine the type of staining as follows: diffuse, patchy, negative-without peripheral staining, without peripheral staining of cells in 1–2 layers, with peripheral staining of cells, weak staining.

Statistics

Obtained results were statistically analysed using χ^2 -test. P values of less than 0.05 were considered statistically significant.

Results

Results of immunohistochemical staining by TGF- α were obtained from material consisted of previously diagnosed skin neoplasms, 20 of each KA and SCC.

TGF- α staining could be detected mainly diffusely and without peripheral staining of cells in 1–2 layers in KA as shown in Figure 1. Contrary, we have detected mostly patchy staining in SCCs examined with peripheral staining of cells in 1–2 layers as shown in Figure 2.

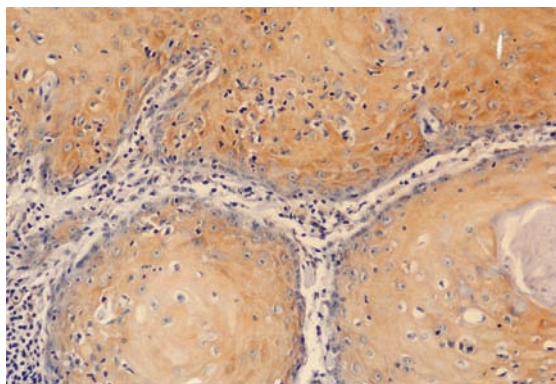


Fig. 1. Immunohistochemical staining for TGF- α in keratoacanthoma. TGF- α staining could be detected mainly diffusely and without peripheral staining of cells in 1-2 layers (x400).

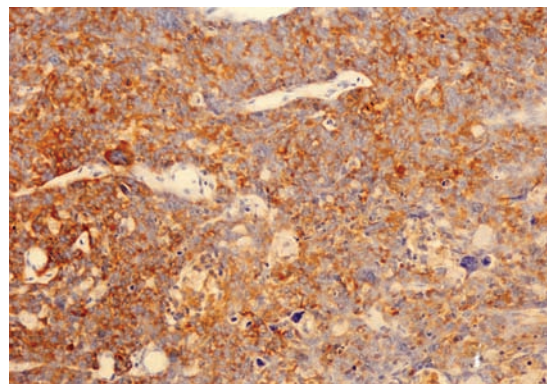


Fig. 2. Immunohistochemical staining for TGF- α in squamous cell carcinoma. TGF- α detection was mostly patchy in peripheral part of cells in 1-2 layers (x400).

TABLE 1
EXPRESSION OF TGF- α IN KERATOACANTHOMA AND SQUAMOUS CELL CARCINOMA

Staining type	Keratoacanthoma	Spinocellular carcinoma
Diffuse	18 (90%)	11 (55%)
Patchy	1 (5%)	9 (45%)
Negative – without peripheral staining	1 (5%)	0 (0%)
Without peripheral staining of cells in 1–2 layers	12 (60%)	0 (0%)
With peripheral staining of cells	7 (35%)	20 (100%)
Weak Staining	3 (1%)	1 (5%)

The frequency of different staining patterns in KA and SCC was summarized and shown in Table 1.

There was a significant difference in TGF- α staining between KA characterized by diffuse staining as compared with SCC that was more often stained focally ($\chi^2=6.12$, $p=0.01$). We have detected a significant difference in frequency of TGF- α staining between KA and SCC depending on peripheral staining in 1–2 layers, significantly more common in SCC as compared with KA ($\chi^2=15.4$; $p=0.001$).

Discussion

In literature, there are many controversies about the question of whether KA is a variant of SCC or a unique lesion. The distinction of KA from SCC on histological grounds has been a matter of convention. KA is an unusual cutaneous neoplasm characterized by self-involution. Many studies have been undertaken in order to develop a simple method for differentiation between KA and SCC in everyday practice.

Coffey et al. established that normal human keratinocytes produce TGF- α ¹. Gottlieb et al. found the presence of TGF- α in normal, hyperproliferative and malignant keratinocytes². Christensen et al. detected the expression of TGF- α in SCC of oral cavity. The authors detected TGF- α expression in all cases of primary SCCs and three out of four metastases were also positive on TGF- α . These investigations established the presence of TGF- α in oral SCC and its metastases. The immunoreactivity of TGF- α on endothelial and mucoserous glandular cells was also proven^{3,4}. Ho et al. demonstrated that the majority of KA could be differentiated from SCC by different arrangement of expression of TGF- α in their cells. In 90% of tumor samples, there was a lack of the expression of TGF- α in 1–2 layers of cells of KA tumor lobules, there was 0% expression in SCC, while we found the expression of 60% in KA. Ho et al. also demonstrated focal expression of TGF- α in 40% of SCC, while focal expression in KA was 0%⁶.

Obtained results showed focal expression of TGF- α in 45% cases of SCC and in 5% cases of KA. Other authors established expression of TGF- α in SCC of head and neck, in contrast to examined control group^{5,7,8}. Previously, Grant et al. demonstrated strong membranal and cytoplasmatic expression of TGF- α in 12 from 20 cases

(60%) of solar keratosis, and in 13 from 15 cases (86%) of Bowen disease⁹. Today the differentiating KA from SCC is being investigated using many immunohistochemical techniques of staining. Tan et al. compared SCC and KA using Bcl-x that is important anti-apoptotic member of Bcl-2 family. The activity of Bcl-x was diffuse in 75% of SCC, while in 95% of cases of KA the activity was typically situated in border zone of the middle and the upper spinous keratinocytes. Their conclusion was that generally diffuse expression of Bcl-x in SCC signified its aggressivity, while it was less expressed in the middle and the upper keratinocytes of KA, and its action was reduced in regressive forms of KA¹⁰. Melendez et al. were examining the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) in KA and SCC. ICAM-1 was more expressed in developed KA, while in regressive forms the expression was not marked. It was considerably expressed in poorly differentiated SCC, while only focally in well differentiated SCC¹¹. Some articles demonstrated the connection between KA and SCC. Tran et al. demonstrated that KA was a variant of SCC using immunohistochemical staining with oncostatin (OSM – glycoprotein that stimulates macrophages and T-lymphocytes)¹². Mukunyadzi et al. used syndecan-1 (proteoglycan important for intercellular and cellular adhesion of matrix), and they demonstrated that it was similarly expressed in KA and *in situ* SCC, but was differently expressed in invasive SCC¹³. Takeda et al. demonstrated that KA and SCC were different tumors using angiotensin II receptors in differentiating these two tumors¹⁴. Asch et al. were using stromelysin 3 (ST3- a member of metalloproteinase family) and demonstrated its stronger presence in SCC *versus* KA¹⁵. Conolly et al. demonstrated the difference between SCC and subungual KA using p53 and Ki67 markers¹⁶. Slater et al. demonstrated that KA and SCC were biochemically and pathogenetically differing, and they established that these tumors were individual entities using anti-P2x7 immunohistochemical staining¹⁷. Batinac et al. showed that apoptosis as assessed by M30 expression is related to malignant potential of KA and SCC and cell proliferation and could also aid in differentiation between these two closely related neoplasms¹⁸. It has been previously suggested that new apoptosis-based therapeutics regulating apoptotic mechanisms in keratinocytes, mostly through enhancement of apoptosis, could

aid conventional therapy procedures undertaken in these skin tumors¹⁹.

Today, differentiation between KA and SCC on histological ground is still a challenge, since the distinction between these two skin tumors could sometimes be difficult. We have shown that these skin neoplasms could be

generally differentiated based on staining patterns of TGF- α expression, thus this method, as well as other previously suggested routine immunohistochemical staining techniques, could surely aid in differentiation between these two closely related entities in clinical practice.

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RAZLIKOVANJE KERATOAKANTOMA I SPINOCELULARNOG KARCINOMA KOŽE KORISTEĆI TGF- α

SAŽETAK

Spinocelularni karcinom (SCC) i keratoakantom (KA) su kožni tumori podrijetla iz epitela. Za razliku od jasno malignog tumora SCC, KA je neobična maligna tumorska tvorba kože s tendencijom regresije. Diferencijacija ova dva tumora, na temelju histološke analize predstavlja izazov. Cilj ove studije bio je istražiti i utvrditi izražajnost i moguće razlike u pojavnosti transformirajućeg čimbenika rasta-alpha (TGF- α) u SCC-a i KA. Imunohistokemijski smo analizirali 40 uzoraka tumora kože, po 20 uzoraka SCC i KA. Utvrđena je značajna razlika u izražajnosti TGF- α između KA i SCC. Imunohistokemijsko bojenje u KA bilo je, uglavnom difuzno (90% slučajeva) i bez perifernog bojenja stanica u jednom do dva sloja (60% slučajeva). Suprotno, bojenje u SCC bilo je većinom krpičasto (55% slučajeva) s perifernim bojenjem stanica u 1-2 sloja stanica (100% slučajeva). Diferencijacija između KA i SCC većinom je moguća na temelju kliničke slike i histološke analize, ali je ponekad otežano razlikovanje ova dva kožna tumora. Pokazali smo da je diferencijacija ove dvije skupine tumora kože moguća analizom uzoraka izražajnosti TGF- α , te smatramo da ova histološka metoda može pridonijeti u kliničkoj praksi razlikovanja ova dva usko povezana kožna tumora.