Value of P53, Ki-67 and CD10 in Differentiation between Keratoacanthoma and Squamous Cell Carcinoma

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Abstract

Purpose: Keratoacanthoma (KA) is a rapidly growing cutaneous tumor and may be difficult to distinguish from squamous cell carcinoma (SCC) on histomorphology alone. There is a major controversy over the natural behavior of keratoacanthoma. KAs have been described as benign lesions, but also as variants of squamous cell carcinoma. It is important to distinguish these neoplasms because they have different clinical behavior and different therapeutic planning. The present study aims at investigating the role of P53, Ki67, and CD10 in differentiation between KA and SCC.

Patients and Methods: This retrospective study includes 18 cases of SCC, 12 cases of Keratoacanthoma, and 5 cases of normal skin as a control. Cases were collected from Pathology Department of Faculty of Medicine, Benha University, in the period 2010-2013. P53, Ki67, and CD10 immunohistochemical staining were performed in all cases and the pattern of expression was analyzed.

Results: 83.3% of the examined SCC cases and 41.7% of the examined KA cases showed nuclear expression of Ki-67 antigen and this was statistically significant (p<0.05). In KA, positive cells were usually located in the basal layers at the periphery of the lesion. SCC displayed positive cells in a diffuse pattern. CD 10 immunopositivity was detected in the stroma of 100% of SCC and in 16.7% of KA cases, and these was statistically highly significant (p<0.01). Nuclear expression of P53 antigen was detected in 88.9% of examined SCC cases and in 66.7% of examined KA cases. This was a statistically insignificant correlation (p>0.05). The pattern of p53 expression was the same of Ki-67. The expression of Ki-67 was statistically significantly correlated to p53 nuclear expression and CD10 stromal expression.

Conclusion: The results of this study suggest that Ki-67 and p53 have similar distribution patterns, but the former is more precise in differentiation between KA and SCC. Pattern and extent of expression of both Ki-67 and CD10 are valuable in differential diagnosis between KA and SCC. Combination between both markers (Ki-67 and CD10) will guide towards more precise differentiation.


Introduction

KERATOACANTHOMA (KA) is a common cutaneous neoplasm that most often occurs on sun-exposed sites in light-skinned persons of middle age or older. Almost all keratoacanthomas arise from hair follicle [1]. Occasionally, it is seen on hairless areas such as nail beds or oral cavity. It is characterized by rapid growth with a histologic pattern often suggestive of squamous cell carcinoma. Usually considered the prototype of cutaneous pseudo-malignancies for its histologic resemblance to squamous cell carcinoma (SCC), it has alternatingly been viewed as possibly pseudo-benign, or a cancer that resembles a benign neoplasm. Distinguishing between KA and SCC is not an uncommon histologic diagnostic dilemma [2].

Worldwide, squamous cell carcinoma (SCC) is the second most frequent skin cancer and occurs most frequently in the sun-exposed regions of the skin and in immunocompromised patients [3]. In Egypt, the NCI registry data of years 2000 to 2011 revealed that SCC constitutes 35.5% of non-melanoma skin cancers [4]. SCC harbors significant risk of metastasis that can eventually lead to death. However, KA usually undergoes spontaneous regression as part of its natural history. Clinically, rapid tumor growth may suggest a de novo cutaneous SCC, a relatively rare, aggressive tumor that produces regional or distant metastases in about 8% of patients [5]. Sometimes a well differentiated SCC can be difficult to distinguish from a KA without clinical history. Attempts to distinguish SCC and KA based on the findings of intra-lesional elastic fibers, intracytoplasmic glycogen, DNA changes and mutant p53 oncogene expression have shown different results but the majority failed to show significant difference [6].

P53, also known as TP53 is a gene that codes for a protein that regulates the cell cycle and hence
functions as tumor suppression. It is very important for cells in multicellular organisms to suppress cancer. P53 has been described as "the guardian of the genome", referring to its role in conserving stability by preventing genome mutation. The name is due to its molecular mass: It is in the 53 kilodalton fraction of cell proteins. The tumor-suppressor gene p53, located on the short arm of chromosome 17 (17p 13. 1), encodes for a nuclear protein which regulates cell proliferation by inhibiting cells entering S-phase. The p53 mutations are alleged to be the commonest genetic abnormality in human cancer [7].

The Ki-67 protein (also known as MKI67) is a cellular marker for proliferation. It is strictly associated with cell proliferation. During interphase, the Ki-67 antigen can be exclusively detected within the cell nucleus, whereas in mitosis most of the protein is relocated to the surface of the chromosomes. Ki-67 protein is present during all active phases of the cell cycle (G1, S, G2, and mitosis), but is absent from resting cells (G0) [8]. Antigen Ki-67 is a nuclear protein that is associated with and may be necessary for cellular proliferation. Furthermore it is associated with ribosomal RNA transcription. Inactivation of antigen Ki-67 leads to inhibition of ribosomal RNA synthesis. Ki-67 is an excellent marker to determine the growth fraction of a given cell population. The fraction of Ki-67-positive tumor cells (the Ki-67 labeling index) is often correlated with the clinical course of cancer [9].

CD10 is a 100-kd transmembrane glycoprotein initially identified as the common acute lymphoblastic leukemia antigen, or CALLA. CD 10 expression exhibits a link with the growth rate of the cells. Its expression is increased in malignant tumors and regenerating tissues, but it is not lineage specific. Furthermore, CD10 expression can be detected in the peritumoral fibroblast-like stromal cells within the invasive area of various cancers such as prostate, breast, colorectal, and lung carcinomas. Within normal adult skin, CD 10 immunopositivity has been noted in the inner sheath of hair follicles, hair matrix, and perifollicular fibrous sheath. In tumors of the skin, CD10 is expressed in dermatofibroma, dermatofibrosarcoma protuberans, and melanoma [10,11].

This work aims to determine if p53 and ki67 and CD10 expression is useful in the differential diagnosis of SCC and KA.

**Patients and Methods**

This retrospective studied group included random 18 cases of SCC (8 cases of grade 1 and 10 cases of grade 2), 12 cases of KA, and 5 cases of normal skin tissue from plastic surgery were taken as control. The cases were collected from histopathologic archive of files of Pathology Department, Faculty of Medicine, Benha University in the period 2010-2013. Paraffin-embedded tissue sections were obtained from archival tissue blocks of the hospital. Hematoxylin and Eosin sections were reviewed to confirm diagnosis.

**Immunohistochemical staining:** Tissue sections were mounted on positively-charged slides, steps of staining followed the standard ABC (avidin-biotin complex) procedure in the Ultra Vision Detection System (Anti-polyvalent, HRP/DAB, ready-to-use, Lab Vision corporation). Antigen retrieval with microwave treatment in 1 mM citrate buffer (Neo-Markers, Cat. # AP-9003), pH 6.0, Sections were incubated with mouse monoclonal antibody against p53 (DO-7), rabbit monoclonal ki-67 and mouse monoclonal CD 10 (Table 1). The freshly prepared DAB-substrate-chromogen solution was applied. Negative controls were performed by omitting the primary antibody step.

**Table (1): Antibodies used in this study.**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Source</th>
<th>Cat No.</th>
<th>Dilution</th>
<th>Incubation period (at room temperature)</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td>P53</td>
<td>Lab Vision, # MA5-12557</td>
<td>1:100</td>
<td>60 min.</td>
<td>Invasive ductal breast carcinoma</td>
<td></td>
</tr>
<tr>
<td>Ki-67</td>
<td>Thermo scientific, # RM-9106-R7</td>
<td>Ready to use</td>
<td>60 min.</td>
<td>Verruca vulgaris</td>
<td></td>
</tr>
<tr>
<td>CD 10</td>
<td>USA # MS-363-R7</td>
<td>Ready to use</td>
<td>90 min.</td>
<td>Normal intestinal epithelium</td>
<td></td>
</tr>
</tbody>
</table>

**Immunostaining interpretation:** Sections were evaluated under a light microscope and only nuclear staining was regarded as positive for p53 and ki-67. P53 staining reactions and Ki67 labeling index were determined by counting 1,000 cancer cells in each sample (original magnification, x400) and assessing the percentage of positive cells. Negative expression was considered: 0 to 9% of...
the neoplastic cells having antibody expression. Positive expression: 10 to 100% of the neoplastic cells having antibody expression [7,12,13].

CD 10 positivity was considered as brown cytoplasmic and/or membrane staining. Ten high power fields (x400) were examined for each case and mean percentage of positive cells were calculated as follow: <10% as negative and >10% as positive. Stromal or tumoral cells CD10 immunostaining was determined for each case [14].

Statistical analysis: Data analysis was performed with the statistical package for social sciences (version 16.0.1; SPSS Inc., Chicago, Illinois, USA). Descriptive analysis of the variables and statistical significance of the tests were expressed in p-value.

p-value less than 0.05 (<0.05) was considered significant and <0.01 was highly significant.

Results
Histologically, KA was defined by the presence of a symmetrical cup-shaped proliferation of clear-to-glassy-appearing epithelium associated with strands of cells protruding into dermis. The lesional cells display mild nuclear atypia with rare dyskeratotic cells (Fig. 1-A).

Squamous cell carcinomas are generally defined by lack of keratin-filled central crater, presence of stromal desmoplasia, atypical squamous cells, lack of sharp outline between tumor nests and stroma, and relatively slower onset of growth. Well-differentiated SCCs are characterized by the presence of several horn pearls, intercellular junctions, mature squamous cells, and rare atypicity. Moderately differentiated SCC is defined by the presence of fewer horn pearls and conspicuous atypical cells (Fig. 1-B).

A- Immunohistochemical results of p53 staining:
In normal skin, p53 stains the basal and suprabasal layers (Fig. 2). Positive cells in KA were usually located in the basal layers at the periphery of the lesion. SCC displayed positive cells in a diffuse pattern (Fig. 4). Nuclear expression of Ki-67 antigen was detected in 83.3% of examined SCC cases and in 41.7% of examined KA cases. This was a statistically significant correlation (p<0.05).

B- Immunohistochemical results of ki-67 staining:
Ki-67-positive cells could be assessed in the same distribution pattern as p53-positive cells in different cases. In KA, positive cells were usually located in the basal layers at the periphery of the lesion. SCC displayed positive cells in a diffuse pattern (Fig. 4). Nuclear expression of Ki-67 antigen was detected in 83.3% of examined SCC cases and in 41.7% of examined KA cases. This was a statistically significant correlation (p<0.05).

C- Immunohistochemical results of CD10 staining:
Normal epidermis was negative for CD 10 expression. Adnexal structures, vessels, and adipose tissue did not exhibit CD10 staining. In all SCC cases (100%), CD10 immunopositivity was detected in stromal cells. CD10 was negative in tumoral cells of 16 of 18 cases (88.9%) (Fig. 5-B). Only in 2 cases (11.1%), peripheral tumoral cells next to stroma were positive although central tumoral cells were negative. Weak CD 10 expression was observed in only 2 of 12 (16.7%) cases in KA at the basal layer in the periphery of the lesion. (Fig. 5-A) (Table 2).

Table (2): Immunohistochemical expression of P53, Ki-67 and CD 10 in KA and SCC.

<table>
<thead>
<tr>
<th>Overall expression</th>
<th>KA (n=12)</th>
<th>SCC (n=18)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>P53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–ve</td>
<td>4 (33.3%)</td>
<td>2 (11.1%)</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>+ve</td>
<td>8 (66.7%)</td>
<td>16 (88.9%)</td>
<td></td>
</tr>
<tr>
<td>Ki-67</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–ve</td>
<td>7 (58.3%)</td>
<td>3 (16.7%)</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>+ve</td>
<td>5 (41.7%)</td>
<td>15 (83.3%)</td>
<td></td>
</tr>
<tr>
<td>CD 10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–ve</td>
<td>10 (83.3%)</td>
<td>0</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>+ve</td>
<td>2 (16.7%)</td>
<td>18 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

KA: Keratoacanthoma. SCC: Squamous cell carcinoma.

Grades of SCC were not significantly correlated to any of the used antibodies (p>0.05). The eight cases (100%) of G1 were positive to p53 and CD 10, while 7 cases out of 8 (87.5%) were positive to Ki-67. Furthermore, Ki-67 labeling index was statistically significantly correlated to the nuclear expression of p53 (p<0.01) and to the stromal expression of CD10 (p<0.05). (Table 3).

Table (3): Correlations between markers of this study.

<table>
<thead>
<tr>
<th>Ki-67 expression</th>
<th>p value</th>
</tr>
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</table>

<table>
<thead>
<tr>
<th>P53</th>
<th>+ve</th>
<th>–ve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6 (60%)</td>
</tr>
<tr>
<td>+ve</td>
<td>4 (40%)</td>
<td>20 (100%)</td>
</tr>
<tr>
<td>CD 10</td>
<td>–ve</td>
<td>6 (60%)</td>
</tr>
<tr>
<td></td>
<td>+ve</td>
<td>4 (40%)</td>
</tr>
</tbody>
</table>

Total 10 20
Fig. (1): (A) A case of KA revealed central cup-shaped crater surrounded by proliferation of large keratinocytes with abundant glassy cytoplasm and minimal cytologic atypia (H&E x100). (B) A case of SCC revealed malignant squamous epithelium forming cell nests with keratin pearl formation. Neoplastic cells are characterized by moderate amounts of eosinophilic cytoplasm, nuclear enlargement, and hyperchromasia (H&E x200).

Fig. (2): (A) Expression of p53 in normal skin, nuclear expression was detected in the basal and subrabasal layers of epidermis. (B) Expression of Ki-67 in normal skin with same distribution pattern of p53 (IHC, DAB x200).

Fig. (3): (A) KA with +ve nuclear p53 expression. Positive cells were located in the basal layers at the periphery of the lesion. (B) SCC showing diffuse pattern of nuclear p53 expression (IHC, DAB x200).
Discussion

Keratoacanthomas (KA) and squamous cell carcinomas (SSC) are epithelial skin tumors exhibiting distinctive clinical and histologic features. However, the differential diagnosis between them in individual cases may be difficult or even impossible [15]. It is believed that molecular events regulating cell survival, apoptosis, growth arrest, and differentiation play an important role in the development, progression, and regression of benign and malignant cell growth [3]. Neoplastic disorders are characterized by uncontrolled cell growth. Activation of proto-oncogenes and inactivation of tumor-suppressor genes are critical molecular events that lead to neoplastic transformation [13].

The p53 tumor-suppressor gene is the classic example of these genes, as it is found mutated in 50-90% of human malignant tumors including skin cancers [3].

In this study, 88.9% of SCC cases were positive to p53 expression in a diffuse pattern and also in dysplastic epidermis adjoining lesional nests. On the other hand, 66.7% of KA cases showed positive nuclear expression of p53. Positive cells were located in the basal layers at the periphery. This pattern of p53 expression in SCC and KA was statistically insignificant ($p>0.05$). Parallel to such results, Cain et al., [16] found that the p53 staining showed basal, patchy, or diffuse patterns. These patterns were present in all cases of KA, well differentiated SCC, and there were no statistical differences among the examined groups. Also, Batinac et al., [7] found that p53 immunostaining of KA, and SCC was detected in 66.7% and 86.7% of cases, respectively. P53 positive cells were
present not only in cancer nests of KA and SCC but also in dysplastic and even morphologically normal epidermis adjoining cancers. Sakiz et al., [2] found that no statistically significant difference of mean p53 staining in SCC and KA.

In contrast to the current results, Chon et al., [1] reported that, there was a significant difference in the p53 expression between KA and SCC. Also, Ruhoy et al., [8] found that SCC:KA showed a decreased p53 (p=0.0096) immunoeexpression. Khodaeiani et al., [3] reported that the expression rate of p53 was 50.20% for the SCCs, and null for the KAs. Lucia et al., [18] found that p53 protein was expressed in the cells at the periphery of both KA and SCC. Lu et al., [8] reported that in keratoacanthomas intense p53 expression was detected; however in squamous cell carcinomas, it was heterogeneously expressed. Kerschmann et al., [17] found that (80%) of the KAs showed nuclear staining with anti-p53 antibody, distributed along the outermost layers of the aggregates of neoplastic cells, while (60%) of the SCCs were p53 positive. This overlapping expression patterns of p53 in KA and SCC supports the hypothesis that these tumors represent a possible biologic spectrum.

These contradictory results may be explained by differences in the number of cases or in the methods used in staining evaluation.

In the current study it was found that the nuclear expression of Ki-67 antigen was detected in 83.3% of SCC cases and 41.7% of KA cases. The distribution pattern of Ki-67 positive cells could be assessed in the same distribution pattern as p53-positive cells in different cases. This was a statistically significant correlation (p<0.05). In agreement with our results, Batinc et al., [7] reported that the positivity of Ki-67 proteins differed significantly among KA and SCC cases. Also, Lucia et al., [18] found that Expression of Ki-67 was decreased in the involutional stage of KA than in SCC. However, Chon et al., [1] reported that the mean Ki-67 labeling index was slightly higher for SCC group (KA-like SCC=30.72%, SCC=31.23%) than for KA (25.30%), but this difference was not statistically significant. Keratoacanthoma showed more pheirperal basal pattern (91%), whereas SCC group showed more diffuse pattern (77%).

The relatively low cell proliferation rate found in KA suggests its benign nature and cannot be solely responsible for its rapid growth phase. Results suggest that the balance between resistance and susceptibility to apoptosis could play a role in KA progression. In addition, it should be remem-

bered that there is a lower proliferation rate in regressing KA [7].

On the other hand, the distribution pattern of Ki-67 protein expression was similar to that of p53 protein expression, suggesting an association between cell proliferation and p53 expression.

CD 10 immunopositivity were detected in 16.7% of KA and 100% of SCC biopsies. CD10 labeled tumor stroma in 100% of SCC cases. CD 10 staining was present in peripheral tumoral cells in 11.1% of SCC cases, but negative in central tumoral cells. These results were in agreement with results reported by Hayam and Hayam [18], who found that in all the SCC cases, tumor cells failed to stain with CD10 in contrast to the stromal cells that showed CD10 expression in 81% of cases. Also, Takahara et al., [19] found that All SCCs showed weak to strong stromal CD 10 expression and weak CD10 expression was observed in only 2 of 15 samples of KA cases. Aslani et al., [11] found that 100% of the SCC cases had stromal CD 10 reactivity, with strong reactivity in 70% of the cases. In 10% of these cases, immunoreactivity was detected in the tumor cells at the center of the epithelial nests. Heidarpour et al., [14] reported that (96.2%) of SCC samples failed to stain with CD 10 in tumoral cells whereas CD 10 expression of stromal cells was identified in all SCC cases (100%). Sabeti et al., [20] reported that only 2/17 SCC cases exhibited weak focal positivity in tumoral cells. The peri-tumoral cells showed positive reaction in 71% of SCC cases.

Conversely, Wagoner et al., [10] found that CD 10 was negative in the tumor cells in 13 out of 13 invasive SCCs. CD10 expressed weakly in the surrounding stromal cells of 2 out of 13 SCCs. It can be postulated that the CD 10 functional expression might be confined only to the early evolution of SCC.

In their work, Wagoner et al., [10] concluded that the amount of expressed surface CD 10 appears related to the growth rate of the cells and is typically increased in conjunction with malignant neoplasms or regenerating tissues with an increased proliferative index. Their conclusion also explains the statistically significantly correlation of Ki-67 expression to the nuclear expression of p53 (p<0.01) and to the stromal expression of CD10 (p<0.05) which was detected in this study. High Proliferation index of the tumor is related to high anti-apoptotic activity and is detected at rapidly growing and invading areas of the tumor.
In conclusion: Our results suggest that Ki-67 and p53 have similar distribution patterns, but the former is more precise in differentiation between KA and SCC. The combined evaluation of the expression of Ki67 and CD10 may be helpful in resolving challenging differential diagnosis between KA and SCC, and thereby helps in directing appropriate treatment strategies.

References


