

Original Article

Evaluation of Diagnostic Values of EMA and Ber-Ep4 in Distinction between Basal Cell Carcinoma and Squamous Cell Carcinoma of the Skin

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Abstract

Background and Objective: Basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are two common tumors of the skin. In some cases, distinction between BCC and SCC can be difficult. This study aimed to clarify this uncertainty through immunohistochemical analysis. In this respect, epithelial membrane antigen (EMA) and Ber-Ep4 are the two immunohistochemical markers on which we focus in differentiating skin BCC from SCC.

Materials and Methods: Archived paraffin-embedded tissue samples of BCC (n = 40) and SCC (n=40) were stained immunohistochemically using Ber-Ep4 and EMA antibodies.

Results: It was found out that 37 (92.5%) out of the BCC samples stained positive for Ber-Ep4 and 2.5% of SCC samples showed positive staining. The majority of SCC group (37 out of 40) expressed EMA, while 5% of BCC samples showed positive staining.

Conclusion: Distinction of BCC and SCC of the skin can be readily achieved through Ber-Ep4 and EMA immunohistochemical markers. Regarding potential false positive and negative results through immunostaining techniques, we may recommend the use of these two antibodies together.

Key words: Basal Cell Carcinoma, Squamous Cell Carcinoma, Skin, Ber-Ep4, Epithelial Membrane Antigen

Introduction

The incidence of non-melanocytic skin cancers (NMSC) including squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) is increasing in the white-skinned population. Exposure to ultraviolet (UV) radiation is regarded as one of the major risk factor (1-3). In contrast to BCC with extremely rare rates of metastasis, invasive SCC is a potentially metastasizing tumor (1, 3). Accurate tumor typing has important implications to the patient since each of these

tumors has different modes of behavior and metastatic potential. In some cases, however, the distinction between BCC and SCC can be difficult (4).

Several studies have attempted to address some of these issues using immunohistochemistry (5-9), but this has not been fully resolved. In this regard, the obtained results have been variable and sometimes conflicting.

Therefore, we aimed to clarify this uncertainty, using antibodies that are widely available, and to establish a simple method to distinguish BCC and SCC through immunohistochemistry.

Received: 15 September 2006

Accepted: 10 December 2006

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Materials and Methods

Forty cases of BCC and 40 cases of SCC were analyzed through performing immunohistochemistry on paraffin-embedded sections using Ber-Ep4 and epithelial membrane antigen (EMA) antibodies. Paraffin-embedded blocks were retrieved from the histopathology archives in Alzahra Hospital. All cases were reviewed on hematoxylin and eosin (H&E) stained sections and categorized as BCC or SCC, using recognized criteria (4). The patients with BCC (26 males and 14 females) were between 49 and 82 years old (mean age = 62 years). Tumors were typed according to the classification outlined by Rippery (10). There were 26 nodular, 5 infiltrative, 5 adenoids, and 4 pigmented BCCs. The age of patients with SCC (28 males and 12 females) was between 40 and 98 years (mean age=74 years). All SCC samples were well or moderately differentiated.

Immunohistochemistry was performed through streptavidin-biotin method on 5 µm-thick tissue sections. These sections were deparaffinized with xylene for 15 minutes and then treated. For EMA antigen retrieval, the sections were treated in a microwave oven using a 0.01 mol/l citrate buffer (pH = 6.0) for 30 minutes. For Ber-Ep4 antigen retrieval, the sections were treated with proteinase for 5 minutes at room temperature. These sections were then incubated with mouse monoclonal antibodies (IgG) against EMA (clone E29) at a dilution rate of 1:50-1:100 and against Ber-Ep4 antigen (clone Ber-Ep4) at a dilution rate of 1:50.

Appropriate positive controls were considered for each staining run (meningioma for EMA and pancreatic tissue for Ber-Ep4). The normal tissues surrounding the tumor were considered as negative controls.

Results

All samples were successfully stained. Thirty-seven cases (92.5%) of BCC group showed strong positive staining with Ber-Ep4 with both membranous and cytoplasmic patterns (Figure 1) and three cases (7.5%) were negative. In contrast, only one (2.5%) of the SCC cases was positive for Ber-Ep4 (Tables 1 and 2). Thirty

seven out of 40 SCC cases (92.5%) were positive for EMA with membranous and variably cytoplasmic pattern (Figure 2). Meanwhile, only three SCC cases (7.5%) were negative for EMA. Two BCC samples (5%) demonstrated reaction for EMA, although the surrounding areas of pseudoepitheliomatous hyperplasia were highlighted (Tables 3 and 4). Sensitivity and specificity of Ber-Ep4 for BCC were calculated as 92.5% and 97.5% and were 92.5% and 95% for EMA regarding SCC respectively.

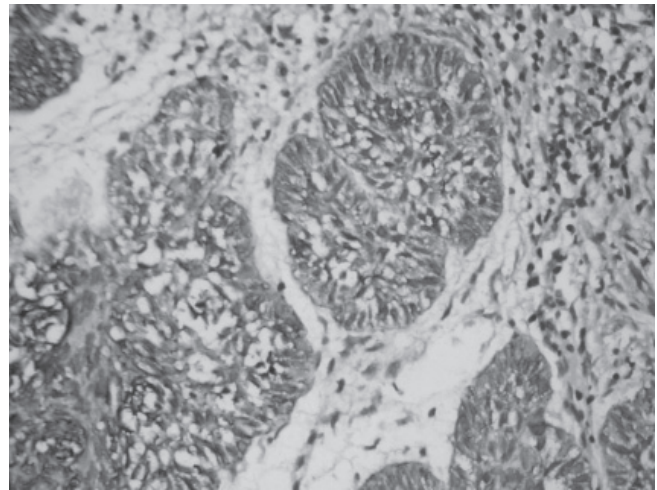


Figure 1. Diffuse Ber-Ep4 staining in a basal cell carcinoma

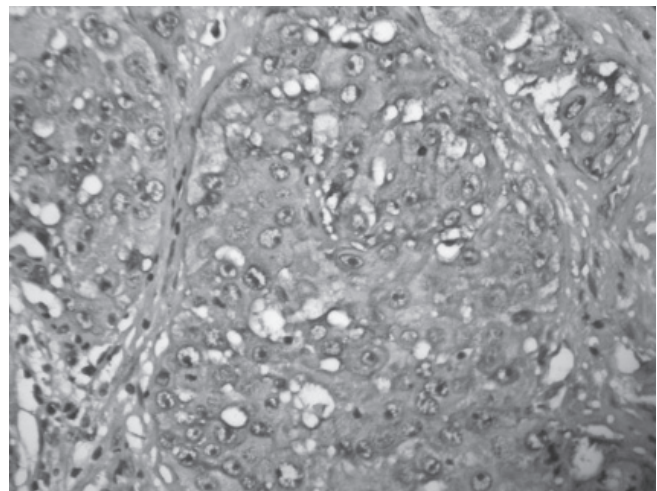


Figure 2. EMA staining in a squamous cell carcinoma

Table 1. Ber-Ep4 positive cases in various types of BCC

Histologic Type	Positive		Negative	
	Incidence	Percent	Incidence	Percent
Nodular BCC	25	62.5%	1	2.5%
Infiltrative BCC	4	10%	1	2.5%
Adenoid BCC	4	10%	1	2.5%
Pigmented BCC	4	10%	0	0%
Total cases of BCC	37	92.5%	3	7.5%

Table 2. Comparison of Ber-Ep4 expression in BCC and SCC

Histologic Type \ Staining Ber-Ep4	BCC	SCC
Ber-Ep4 Positive	37	1
Ber-Ep4 Negative	3	39
Total	40	40

Table 3. EMA expression in various types of SCC

EMA Staining \ Histologic Grade	Positive		Negative	
	Incidence	Percent	Incidence	Percent
Well Differentiated SCC	26	65%	2	5%
Moderately Differentiated SCC	1	27.5%	1	2.5%
Total Cases of SCC	37	92.5%	3	7.5%

Table 4. Comparison of EMA expression in BCC and SCC

Histologic Type \ Staining EMA	SCC	BCC
EMA Positive	37	2
EMA Negative	3	8
Total	40	40

Discussion

Cutaneous carcinomas are the most frequent tumors occurring in the white-skinned population and have a substantial impact on public health (11). Two major tumor types are distinguished as BCC and SCC that predominantly arise in sun-exposed sites. Distinction of these entities has great clinical relevance since BCC rarely metastasizes and may be treated with local radiotherapy, whereas SCC has a distinct risk of metastasis and radiotherapy may be inappropriate (12). In some tumors, categorization is difficult and probably highly subjective (4, 11). This is particularly the case when biopsies are small or the lesion is ulcerated. Confirmation of tumor type is important before embarking upon a Mohs' procedure and we have shown that the degree of diagnostic certainty can be enhanced using immunohistochemistry. It may also be possible to use rapid immunostaining during a Mohs' procedure to define the nature of tissue at the surgical margin. This may prevent tumor recurrences or unnecessary excision of normal tissue. BCC is sometimes associated

with pseudoepitheliomatous hyperplasia. Ber-Ep4 expression clearly delimited the reactive and neoplastic elements which may be difficult with conventional stains (4) and helps to assess the excision margins. Tellechea et al (5) have noted that Ber-Ep4 may be helpful in the distinction of BCC and SCC. However, performing a single immunostaining technique is not reliable, especially when there is poor Ber-Ep4 staining after more than 48 h of formalin fixation (13).

Previous studies have also shown that CAM 5.2, cyclooxygenase-2, p53, and CEA are not useful in distinguishing tumor type (14, 15, 16, 17). In another study, Jones et al stated that amongst Ber-Ep4, p53, and TGF- α , only Ber-Ep4 is helpful to perform the differential diagnosis of BCC and SCC (6). In addition, Swanson et al determined that bcl-2 and Ber-Ep4 markers were successful to indicate the distinction between SCC and BCC (18). In two separate studies, Morales and his colleagues depicted that among bcl-2, p53, and Ki-67, only bcl-2 is helpful in the distinction between SCC and BCC (19-20). In another study, Ber-Ep4 and EMA were highly successful in differentiation of SCC and BCC (7).

The results of our study showed that BCC and SCC can be readily distinguished using routine immunostaining for Ber-Ep4 and EMA. According to our results, EMA and Ber-Ep4 are highly sensitive and specific for SCC and BCC respectively. Because of the potential false positive and negative results with immunostaining techniques, we recommend the use of these two antibodies together. Other immunohistochemical markers also deserve evaluation and attention. In Connie's study, it was stated that Est-1 is not expressed in cutaneous BCC but is expressed in well-differentiated SCC (21). In another study by Muchemwa, it is stated that heat shock protein-105 (HSP-105) is over-expressed in SCC but not in BCC (22).

Conclusion

Est-1 and HSP-105 are important new prognostic and diagnostic markers in non-cutaneous cancers and are probably good substitutes for EMA and Ber-Ep4 in diagnosis of SCC and BCC. Further research is recommended on this issue.

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