DOI: 10.4274/gulhane.galenos.2020.859
Gulhane Med J 2020;62:63-71

Immunohistochemistry expression of EMA, CD10, CEA, and Bcl-2 in distinguishing cutaneous basal cell from squamous cell carcinoma: A systematic review

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Date submitted:
27.10.2019
Date accepted:
07.02.2020
Online publication date:
15.06.2020

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Keywords: Squamous cell carcinoma, basal cell carcinoma, immunohistochemistry, differential diagnosis

ABSTRACT

Cutaneous basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are the most popular neoplastic entities in cutaneous medicine. These two neoplasms are commonly well recognized on the basis of their clinical and histopathological features and the differentiation between the two mentioned carcinomas is clinically important as there is a significant difference in their rates of aggressiveness and metastatic potential. In case of difficulties in distinguishing BCC from SCC, in addition to the well-defined histological criteria, immunohistochemistry methods can be used and, in the literature, numerous studies underline their usefulness. Therefore, the present systematic review aimed to assess the expression of epithelial membrane antigen (EMA), cluster of differentiation 10 (CD10), carcinoembryonic antigen (CEA), and B-cell lymphoma-2 (Bcl-2) in distinguishing cutaneous BCC from SCC. A comprehensive search was done from 1983 to September 2017 in the PubMed/Medline, Web of Science, and Scopus databases without language restriction. The studies had a cross-sectional design on human tissue. The pooled staining of biomarkers showed that staining results of EMA and CEA in SCC tissues were significantly more positive than in BCC tissues ($p<0.00001$ and $p=0.008$, respectively), as well as CD10 and Bcl-2 in BCC tissues, were significantly more positive than in SCC tissues ($p<0.00001$ and $p<0.00001$, respectively). Findings demonstrate that the use of these markers will be very useful in mentioned cases in which routine microscopy is not able to distinguish between these two entities.

Introduction

Basal cell carcinoma (BCC) is the most frequent cutaneous neoplasm, accounting for around 70% of all skin cancers. It is regionally aggressive and its metastases are rare (1). The second most common malignancy in humans is cutaneous squamous cell carcinoma (SCC), with around double metastases compared to BCC (2). Therefore, BCC and SCC are the most commonly found tumoral entities in cutaneous medicine. They are commonly well-recognized on the basis of their clinical and histopathological features and differentiation between these two carcinomas is clinically important as there is a significant difference in their rates of aggressiveness and metastatic potential. In case of difficulties in the differential diagnosis between the two entities; in addition to the well-defined histological criteria, immunohistochemistry (IHC) methods can be of help and, in the literature, many studies have previously reported their role (3-5). The cluster of differentiation 10 (CD10) is an enzyme of the cell surface with metalloendopeptidase activity and reduces cellular response to peptide hormones by regulating local peptide hormone concentrations (4). CD10 is correlated with biological invasions in human malignancies, but this marker is more commonly used for diagnosis and prognosis with a more complexity (6). B-cell lymphoma-2 (Bcl-2) protein suppresses cell death and thus may be considered

to allow malignant cells for proliferation (7). In addition, Bcl-2 protein preserves cell against apoptosis caused by various death-inducing signals (8). Carcinoembryonic antigen (CEA) is a complex macromolecule with high glycosylation and is used as a marker in carcinomas worldwide (9). Epithelial membrane antigen (EMA) is another highly glycosylated protein with expression mainly in normal and tumor epithelium (10). The differences in biologic behavior mandate the application of more accurate diagnostic methods distinguishing between SCC and BCC. In the literature, there was just one study (11) that checked EMA, CD10, CEA, and Bcl-2 markers together and other studies used one or two markers for distinguishing between cutaneous BCC and SCC. Therefore, the present systematic review aimed to assess the expression of EMA, CD10, CEA, and Bcl-2 in distinguishing cutaneous BCC from SCC.

This systematic review was achieved based on the guidelines for the Preferred Reporting Items for Systematic Reviews and Meta-Analyses PRISMA (12).

Search strategies

A comprehensive search was conducted starting from 1983 to September 2017 using the search terms of "squamous cell carcinoma" (or "SCC") or basal cell carcinoma (or "BCC") and "EMA" (or "epithelial membrane antigen") or "CEA" (or "carcinoembryonic antigen") or "CD10" (or "cluster of differentiation 10") or "Bcl-2" (or "B-cell lymphoma 2") in the PubMed/Medline, Web of Science, and Scopus databases without language restriction. In addition, we manually checked the references of eligible articles to our subject for finding possible missed studies.

Study selection and eligibility criteria

One author (M.S) searched and selected the relevant studies. The second author (M.R) re-checked the studies. All articles in this study were examined for the evaluation of the expression of EMA, CD10, CEA, or Bcl-2 in distinguishing between cutaneous BCC and SCC. The studies included in the systematic review involved the following inclusion criteria: a) cross-sectional design; b) human tissue; and c) IHC staining of EMA, CD10, CEA, or Bcl-2. The exclusion criteria were as follows: a) duplication of a previous publication; b) review or case-series; c) conference paper; d) no full-text; and e) no relevant data.

Data extraction

Two authors (M.S & M.R) checked the studies involved in the systematic review and extracted the relevant data. The third author (E.Z) re-checked the data. We extracted the author's name, publication year, country, the number of BCC or SCC patients/tissues; the number of BCC or SCC tissues with positive IHC of each marker, used antibody and cut-off from each study were included in the systematic review.

Quality assessment

The quality of each study was evaluated by the Newcastle-Ottawa Scale (13). One author (M.R) checked the quality of the studies. The maximum total score was nine for cross-sectional studies. A high-quality study was considered as a study with ≥7 scores.

Statistical Analysis

The data were analyzed applying SPSS version 22 software (IBM Corp., Armonk, NY, USA) and the chi-square test. $P < 0.05$ (two-sided) was considered statistically significant.

Results

Study selection

Out of 250 studies retrieved in the databases, after excluding duplicates and not relevant studies, 38 full-text studies were assessed for eligibility (Figure 1). Then, seven studies were excluded for some reasons (one article was animal study, two articles were review studies, two articles reported mean score of markers, one article mixed BCC and SCC patients as one group, and one article duplicated with another study). At last, a total of 31 studies were entered and analyzed in the systematic review.

Characteristics of the studies

The characteristics of the 31 studies covered in the systematic review are presented in Table 1. The studies were published between 1983 and 2017. Eight studies (3,14-20) were from USA, four (4,5,11,21) from Iran, three (22-24) from UK, three (25-27) from Japan, two (28,29) from Turkey, two (30,31) from Egypt, and also Australia (32), Austria (33), Netherlands (34), Taiwan (35), China (36), Croatia (8), Romania (37), India (38), and Germany (39) each with one study. All studies in the systematic review included 694 BCC and 536 SCC patients/tissues. Fifteen studies reported Bcl-2 and included 339 BCC and 263 SCC patients; eight studies reported CD10 and included 257 BCC and 180 SCC patients; five studies reported CEA and included 111 BCC and 87 SCC patients; and ten studies reported EMA and included 177 BCC and 158 SCC patients. Other characteristics such as the number of patients/tissues with positive staining for each marker, used antibody and cut-off are shown in Table 1.

IHC staining

The pooled staining of biomarkers based on mentioned cut-offs in each study showed that staining results of EMA and CEA in SCC tissues were significantly more positive than BCC tissues ($p<0.00001$ and $p=0.008$, respectively), as well as CD10 and Bcl-2 in BCC tissues, were significantly more positive than SCC tissues ($p<0.00001$ and $p<0.00001$, respectively) (Table 2). Therefore, these markers can be useful biomarkers for distinguishing between both BCC and SCC.

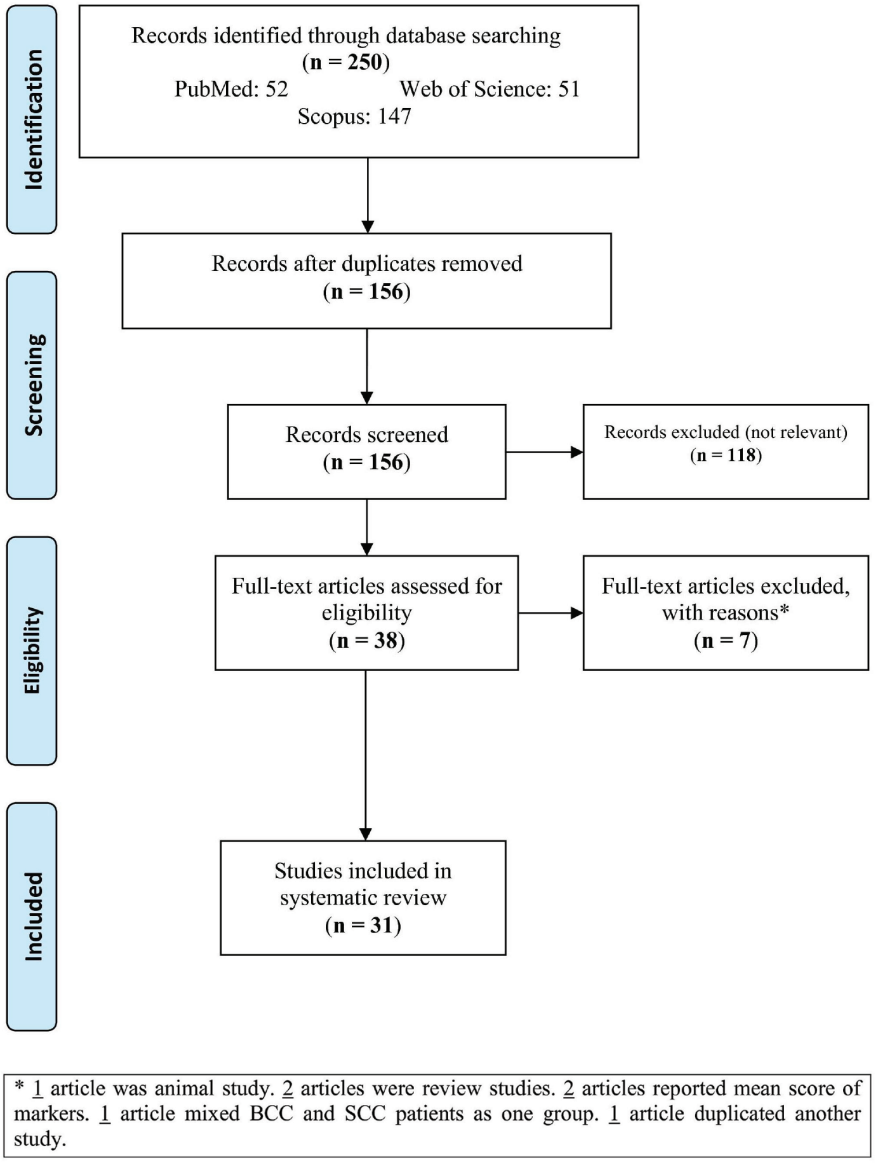

Figure 1. Flow-chart of the study

Quality assessment

The quality assessment of each study is shown in Table 3. A mean score of 6.7 was reported for all studies and twenty-six studies had high quality.

Discussion

It is critical to differentiate SCC from BCC clinicopathologically (21). In most cases, the differentiation of SCC and BCC is straightforward in routine H&E staining (4). The distinction of these neoplasms is clinically important because of the more aggressive behavior and metastatic potential of SCC, which mandates more radical treatment and closer follow-up (4,21). The SCC recurrence rate is about twice higher than that of BCC. So, more aggressive treatment is needed for SCC (21). Due to similarity in histopathology, differentiation between SCC and BCC is sometimes difficult (30). In fact, keratotic and metatypical BCCs may be indistinguishable from basaloid SCC (bSCC) in routine histopathology slides (3,4). Therefore, differentiation between BCC and SCC is mostly performed by routine histopathology, which may cause difficulty in superficial small biopsies. CD10 and Bcl-2 markers are of benefit in this condition (31). The present systematic review evaluated IHC staining of four biomarkers including EMA, CD10, CEA, and Bcl-2 in BCC compared to SCC tissues. BCC presentation is typically an ulcerated pearly papule/nodule with telangiectasia (40,41). SCC presentation is typically shallow crusted ulcer with raised margin accompanying actinic damage (40). Differentiation between SCC and BCC is very important in

Table 1. The characteristics of the included studies in the systematic review (n=31)

First author (year)	Country	Number of BCC patients or tissues	Number of SCC patients or tissues	Number of tissues with positive marker: (BCC/SCC)	Antibody manufacturer	Cut-off value
Scurry and de Boer (32)	Australia	10	10	CEA: (1/10)	DAKO & IMULOK	NR
Heyderman et al. (22)	UK	23	15	EMA: (8/15) CEA: (8/12)	Sigma	NR
Cerroni and Kerl (33)	Austria	20	20	Bcl-2: (20/0)	DAKO	NR
Nakagawa et al. (25)	Japan	15	4	Bcl-2: (15/4)	DAKO	5%
Morales-Ducret et al. (14)	USA	23	20	Bcl-2: (23/2)	DAKO	NR
Rodriguez-Villanueva et al. (15)	USA	17	11	Bcl-2: (17/0)	DAKO	NR
Wikonkal et al. (34)	Netherlands	17	22	Bcl-2: (13/6)	M0887 (DAKO A/S)	1%
Chang et al. (35)	Taiwan	10	8	Bcl-2: (10/0)	DAKO	NR
Swanson et al. (16)	USA	45	22	Bcl-2: (41/4)	Clone 124 (DAKO)	1%
Delehedde et al. (17)	USA	17	14	Bcl-2: (17/0)	Clone 124 (DAKO)	NR
Sinard (18)	USA	16	14	EMA: (1/11)	Anti–BCA-255 (BRST-1)	NR
Beer et al. (23)	UK	39	23	EMA: (0/22) CEA: (8/7)	EMA: Clone E29 (DAKO) & CEA: Clone 11-7 (DAKO)	5%
Niu et al. (36)	China	40	33	Bcl-2: (40/1)	NR	1%
Yada et al. (26)	Japan	51	9	CD10: (44/0)	DAKO	NR
Coflkun and Çobanolu (28)	Turkey	20	20	Bcl-2: (18/4)	NR	1%
Aiad and Hanout (30)	Egypt	21	16	CD10: (20/13)	Clone 56C6 (Zymed, Cat)	10%
Serarslan et al. (29)	Turkey	22	10	Bcl-2: (10/8)	Neomarkers-Biogen, mouse	10%
Wagoner et al. (3)	USA	16	13	CD10: (14/0)	NR	1%
Puizina-Ivić et al. (8)	Croatia	20	20	Bcl-2: (20/0)	M887 (DAKO)	1%
Sramek et al. (19)	USA	6	9	EMA: (0/6)	E29 (DAKO)	10%
Ansai et al. (27)	Japan	10	10	EMA: (0/9) CEA: (1/2)	EMA: E29 (DAKO) & CEA: Polyclonal (DAKO)	6%
Heidarpour et al. (21)	Iran	30	26	CD10: (26/1)	N-vision (DAKO)	10%
Abu Juba et al. (37)	Romania	14	10	Bcl-2: (12/5)	Clone 124 (DAKO)	1%
Sari Aslani et al. (5)	Iran	55	50	CD10: (52/0)	N-vision (K4061, DAKO)	10%
Mulay et al. (38)	India	18	25	EMA: (0/25)	Clone E29 (Cell Marque)	1%
Sabeti et al. (4)	Iran	27	17	CD10 (20/2)	RTU-CD10-270 (Novocastra)	10%

Table 1. Continued

First author (year)	Country	Number of BCC patients or tissues	Number of SCC patients or tissues	Number of tissues with positive marker: (BCC/SCC)	Antibody manufacturer	Cut-off value
Gaballah and Ahmed (31)	Egypt	30	20	Bcl-2: (24/0) CD10: (16/0)	CD10: 56C6 (DAKO) & Bcl-2: 100/D5 (Thermo Scientific)	10%
Plaza et al. (20)	USA	21	22	EMA: (0/16)	DAKO	1%
Mittal et al. (24)	UK	8	10	EMA: (0/5)	M0614 (DAKO)	1%
Ramezani et al. (11)	Iran	29	29	Bcl-2: (29/10) EMA: (0/4) CEA: (0/10) CD10: (22/0)	EMA: Clone E29,N1504 (DAKO) & CD10: M0727 (DAKO) & CEA: Clone II-7, N1586 (DAKO) & Bcl-2: Clone 124, N1587 (DAKO)	EMA: 1%, CD10:10%, CEA: 1%, Bcl-2: 5%
Schmitz et al. (39)	Germany	4	4	EMA: (0/4)	Clone E29. N0613 (DAKO)	NR

NR: Not reported, BCC: Basal cell carcinoma, SCC: Squamous cell carcinoma, EMA: Epithelial membrane antigen, CD10: Cluster of differentiation 10, Bcl-2: B-cell lymphoma 2, CEA: Carcinoembryonic antigen, IHC: Immunohistochemistry

Table 2. The comparison of biomarkers staining in tumor cells of basal cell carcinoma and squamous cell carcinoma tissues

Marker	BCC tissue, n (%)	SCC tissue, n (%)	p value
EMA: N (%)			
Positive	9 (5.1)	117 (74)	<0.001
Negative	168 (94.9)	41 (26)	
CD10: N (%)			
Positive	214 (83.2)	16 (8.9)	<0.001
Negative	43 (16.8)	164 (91.1)	
CEA: N (%)			
Positive	18 (16.2)	41 (47.1)	0.008
Negative	93 (83.8)	46 (52.9)	
Bcl-2: N (%)			
Positive	309 (91.1)	44 (16.7)	<0.001
Negative	30 (8.9)	219 (83.3)	

N: Number, BCC: Basal cell carcinoma, SCC: Squamous cell carcinoma, EMA: Epithelial membrane antigen, CD10: Cluster of differentiation 10, Bcl-2: B-cell lymphoma 2, CEA: Carcinoembryonic antigen

clinic and laboratory (21,30). Out of ten studies in systematic review to check EMA (11,18-20,22-24,27,28,39), five studies (22,23,27,38,39) showed EMA as positive in ≥90% SCC tissues and eight studies (12,20,21,25,28-31) did not show EMA as positive in BCC tissues (0%). In addition, out of five studies that checked CEA (11,22,23,27,32), two studies (22,32) showed CEA as positive in ≥80% SCC tissues and three studies showed ≤10% BCC tissues. Out of eight studies included in the systematic review that checked CD10 (3-5,21,26,30,31), six studies (3,5,21,26,30,31) reported CD10 as positive in more than 85% BCC tissues and five studies (3,5,11,26,31) did not show CD10 as positive in SCC tissues (0%). In addition, out of fifteen studies that checked Bcl-2 (8,11,14-17,25,28,29,31,33-37), thirteen studies (8,11,14,17,25,28,31,33,35-37) identified Bcl-2 as positive in ≥80% BCC tissues and eight studies (8,14,15,17,31,33,35,36) identified Bcl-2 as positive in ≤10% SCC tissues. Therefore, BCC and SCC can be readily distinguished using routine IHC for these markers. Based on the results of the systematic review, at least, if tumor cells were CD10 and BCl-2 positive, this would favor BCC over SCC and if tumor cells were EMA and CEA positive, this would favor SCC over BCC diagnosis.

In most cases, BCCs and SCCs are manifested on sun-damaged skin, suggesting a main role for ultraviolet (UV) radiation and their incidence is rising in Whites (37,40). UV-rays, for example, trigger new mechanisms (molecular changes in protein structure, the release of proinflammatory cytokines, and oxidative stress) overlapping those of the cutaneous carcinogenesis process (37).

Basosquamous carcinoma (bSCC) of the skin is an uncommon variant with histopathological aspects of BCC and SCC. Some authors consider it as a variant of BCC, while others as an aggressive entity (42). In the research of Beer et al. (23), a panel of antibodies was used. They found that all cases of BCCs were stained positively for the Ber EP4 antibody (Antibody to Ep-CAM/Epithelial Specific Antigen), with no staining of SCCs. bSCC demonstrated areas of

Table 3. Quality assessment based on the Newcastle-Ottawa Scale

First author, year	Selection (score)	Comparability (score)	Exposure/Outcome (score)	Total score
Scurry and de Boer (32)	4	1	3	8
Heyderman et al. (22)	0	1	3	4
Cerroni and Kerl (33)	3	1	3	7
Nakagawa et al. (25)	4	1	3	8
Morales-Ducret et al. (14)	3	1	3	7
Rodriguez-Villanueva et al. (15)	3	1	3	7
Wikonkal et al. (34)	3	1	3	7
Chang et al. (35)	0	1	3	4
Swanson et al. (16)	3	1	3	7
Delehedde et al. (17)	0	1	3	4
Sinard (18)	3	1	3	7
Beer et al. (23)	3	1	3	7
Niu et al. (36)	3	1	3	7
Yada et al. (26)	3	1	3	7
Coflkun and Çobanolu (28)	0	1	3	4
Aiad and Hanout (30)	3	1	3	7
Serarslan et al. (29)	3	1	3	7
Wagoner et al. (3)	3	1	3	7
Puizina-Ivić et al. (8)	4	1	3	8
Sramek et al. (19)	4	1	3	8
Ansai et al. (27)	3	1	3	7
Heidarpour et al. (21)	3	1	3	7
Abu Juba et al. (37)	2	1	3	6
Sari Aslani et al. (5)	3	1	3	7
Mulay et al. (38)	3	1	3	7
Sabeti et al. (4)	3	1	3	7
Gaballah and Ahmed (31)	3	1	3	7
Plaza et al. (20)	3	1	3	7
Mittal et al. (24)	3	1	3	7
Ramezani et al. (11)	3	1	3	7
Schmitz et al. (39)	3	1	3	7
Mean score				6.7

BerEp4 positivity. In this paper, BCCs did not stain with EMA, but most of the SCCs did. Only one bSCC showed a focal EMA positivity. The authors concluded that the distinction between BCCs and SCCs was possible by using BerEp4 and EMA, and that identification of bSCC could also be achieved with these antibodies.

Another challenging entity is bSCC, a quite rare type of SCC, which may resemble BCC with squamous metaplasia. In this context, BerEp4 is unreliable for differentiation between the two entities, and adding the staining for cytokeratin 14 (CK14) or CK17 is needed for differentiation (43). In this regard, Winters et al. (44) have reported the use of BerEp4 as a helpful diagnostic marker for bSCC as positive in 82% of their cases, but also in 68% of SCC cases. Positivity of BerEp4 was also found in 26.3% of cases in Bowen disease, a variant of SCC *in situ*, and caused difficulty in differentiation from BCC and other keratinocyte neoplasms (45). Stanoszek et

al. (46) reviewed the histologic mimics of BCC including non-neoplastic processes (i.e., follicular induction over dermatofibromas), benign adnexal tumors (mainly of follicular origin), and cutaneous carcinomas with basaloid appearance. Distinguishing required clinicopathological correlation and IHC. A panel including PHLDA1 (Pleckstrin Homology Like Domain Family A Member 1), CK20, androgen receptor, CD10, Bcl-2, CD34, Ber-EP4, CD200, Claudin 4, EMA, CK15, and CEA was successfully used for a wide range of diagnoses. The limitations of this study were as follows: 1) in most studies, there was no sensitivity and specificity of markers between SCC and BCC, 2) sensitivity and specificity of used antibodies were different among the studies and 3) in some studies, the cut-off of markers was different. The strengths of this study were as follows: 1) most of the studies had high quality, and 2) the used method in all studies was similar (IHC).

Conclusion

The findings of the systematic review presented a high efficiency of EMA, CD10, CEA, and Bcl-2 markers in differentiating between SCC and BCC. Moreover, the use of these markers will be useful in such cases that routine microscopy cannot differentiate between the two mentioned carcinomas. Further larger studies in various environmental areas are needed to reach more precise estimates of the sensitivity and specificity of these markers.

Acknowledgement

In addition, the authors would like to thank the Clinical Research Development Center of Imam Reza Hospital for Consulting Services.

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.R., E.Z., Design: M.S., Data Collection or Processing: M.R., M.S., Analysis or Interpretation: M.R., E.Z., Literature Search: M.S., Writing: M.S.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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