IMMUNOHISTOCHEMICAL CHARACTERISTICS OF BASAL CELL CARCINOMA AT NATIONAL HOSPITAL OF DERMATOLOGY AND VENEREOLOGY, VIETNAM

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ABSTRACT

Objectives: To investigate the immunohistochemical characteristics of basal cell carcinoma at the National Hospital of Dermatology and Venereology.

Methods: A descriptive, cross-sectional, retrospective, and prospective study was conducted on 312 patients diagnosed with basal cell carcinoma at the National Hospital of Dermatology and Venereology from January 2019 to September 2023. Histopathological and immunohistochemical analysis was performed using four immune markers: BerEP4, Bcl-2, p63, and EMA.

Results: BerEP4 immunostaining was positive in all 312 cases of basal cell carcinoma, representing various histological subtypes. Bcl-2 immunostaining was also positive in all 312 cases of basal cell carcinoma, with notably stronger expression in peripheral tumor cells compared to the central region. P63 immunostaining yielded positive results in 310 cases of basal cell carcinoma, accounting for 99.4% of the cases studied. EMA immunostaining, on the other hand, was negative in 308 cases of basal cell carcinoma, equivalent to 87.2%. The four cases of EMA-positive basal cell carcinoma were all classified as the histopathological subtype of squamous basal cell carcinoma (within the invasive category).

Conclusions: These results suggest that immunohistochemistry with the four immune markers, BerEP4, Bcl-2, p63, and EMA, can be used to support the definitive and differential diagnosis of basal cell carcinoma compared to other cutaneous carcinomas.

Keywords: *Basal cell carcinoma, immunohistochemistry, BerEP4, Bcl-2, p63, EMA.*

1. INTRODUCTION

Basal cell carcinoma (BCC) is increasingly prevalent, and many research studies have focused on its pathophysiology and risk factors. Biopsy of the lesion and histopathological examination are the gold standards for definitive diagnosis. Worldwide, numerous authors have concentrated

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on analyzing histopathology, utilizing immunohistochemical staining techniques to differentiate diagnoses, guide treatment, and prognosticate for patients. In various studies, authors have noted that the histopathological images of BCC can be misdiagnosed as hair follicle and sebaceous gland cysts, as well as poorly differentiated sweat gland tumors, without immunohistochemical staining for definitive diagnosis[1-4].

Different studies around the world have shown that BerEP4 exhibits high sensitivity and specificity in detecting BCC cells. The use of immunohistochemical BerEP4 for periodic screening of BCC cases is expected to enhance early diagnosis and prevent recurrence after surgery. Epithelial Membrane Antigen (EMA) is considered a useful marker to distinguish BCC (BerEP4+/EMA-) from squamous cell carcinoma (BerEP4 EMA+)[1,5]. In normal adult human skin, Bcl-2 is expressed in the basal layer of the epidermis, hair follicle outer root sheath cells, the dermal papilla, and the epithelial cells of eccrine sweat glands, while it is negative in squamous epithelial cells. Thus, Bcl-2 positivity in basal cell carcinoma and negativity in squamous cell carcinoma suggests that basal cell carcinoma is thought to originate from basal cells, while squamous cell carcinoma arises from squamous epithelial cells[6,7]. In normal skin, p63 is positive in the basal cell layer and in squamous cells just above the basal layer, but negative in late-stage squamous epithelial cells. The expression of p63 protein in basal cell carcinoma indicates that the tumor cells originate from progenitor basal cells[8].

In Vietnam, there have been no studies on this topic, particularly regarding the histopathological features and immunohistochemistry of basal cell carcinoma. Therefore, we conducted this study to describe the immunohistochemical characteristics of basal cell carcinoma and its correlation with various histopathological types.

2. SUBJECTS AND METHODS

2.1. Study subjects

The selection criteria included patients diagnosed with basal cell carcinoma who visited and received treatment at the National hospital of Dermatology and Venereology from January 2019 to September 2023. Patients must have complete medical records and preserved samples stained with hematoxylin and eosin (HE) and immunohistochemistry, including wax blocks. Exclusion criteria involved patients lacking complete information and clinical images in their medical records, as well as those who declined to participate in the study.

2.2. Study methods

Study design

This is a descriptive, cross-sectional, retrospective combined with prospective study. We collected retrospective and prospective samples until the end of the study period.

Procedures

We selected medical records of patients diagnosed with basal cell carcinoma with complete clinical, histopathological, and immunohistochemical information from January 2019 to September 2023. Specimens were reviewed, and all relevant histopathological and immunohistochemical information was recorded. We cut, stained, and re-examined histopathological and immunohistochemical samples that did not meet quality standards.

Statistical analysis

The collected data were entered, managed, and processed using SPSS version 20.0. Comparative analysis between two groups was performed using chi-square tests and t-tests. Comparisons were considered statistically significant at $p < 0.05$.

2.3. Ethics

Personal information of patients was kept confidential and used solely for the purpose of this study, in accordance with the Helsinki Declaration of 2013. This research was approved by the National hospital of Dermatology and Venereology.

3. RESULTS

From January 2019 to September 2023, a total of 312 medical records of patients diagnosed with basal cell carcinoma met the study criteria.

3.1. Expression of BerEP4 in Basal Cell Carcinoma Types

Table 1. Expression of BerEP4 in basal cell carcinoma (N = 312)

BerEP4 marker	Positive		Negative	
	n	%	n	%
Nodular type	245	100%	0	0
Superficial type	30	100%	0	0
Invasive type	37	100%	0	0
Total	312	100%	0	0

The BerEP4 marker was positive in all 312 cases of basal cell carcinoma across all different types. There was no statistically significant difference between the histopathological types of basal cell carcinoma ($p > 0.05$) (Table 1).

3.2. Expression of Bcl-2 in Basal Cell Carcinoma Types

Table 2. Expression of Bcl-2 in basal cell carcinoma (n = 312)

Bcl-2 marker	Positive		Negative	
	n	%	n	%
Nodular type	245	100%	0	0%
Superficial type	30	100%	0	0%
Invasive type	37	100%	0	0%
Total	312	100%	0	0%

The Bcl-2 marker was positive in all 312 cases of basal cell carcinoma across all types. Notably, the expression of Bcl-2 was stronger in the peripheral tumor cells compared to the central region. There was no statistically significant difference between the histopathological types of basal cell carcinoma ($p > 0.05$) (Table 2).

3.3. Expression of EMA in basal cell carcinoma

Table 3. Expression of ema in basal cell carcinoma (N = 312)

EMA marker	Positive		Negative	
	n	%	n	%
Nodular type	0	0%	245	100%
Superficial type	0	0%	30	100%
Invasive type	4	10.8%	33	89,2%
Total	4	12.8%	308	87,2%

The EMA marker was negative in 308 cases of basal cell carcinoma, corresponding to 87.2%. The 4 cases of basal cell carcinoma that were positive for EMA all belonged to the basal squamous type (a subtype of the invasive type). There was a statistically significant difference between the histopathological types of basal cell carcinoma and the invasive type ($p < 0.05$) (Table 3).

3.4. Expression of p63 in basal cell carcinoma

Table 4. Expression of p63 in basal cell carcinoma (N = 312)

p63 Marker	Positive		Negative	
	n	%	n	%
Nodular type	243	99.2%	2	0.8%
Superficial type	30	100%	0	0%
Invasive type	37	100%	0	0%
Total	310	99.4%	2	0.6

The p63 marker was positive in 310 cases of basal cell carcinoma, accounting for 99.4% of the study population (Table 4).

4. DISCUSSION

According to the study results, we found that the BerEP4 marker was expressed in all cases of basal cell carcinoma across all different types. The application of the BerEP4 marker in the diagnosis of basal cell carcinoma was first evaluated by Beer and colleagues. In their study, they found that all 39 samples of basal cell carcinoma were positive for BerEP4[1]. The three cases that were negative for the BerEP4 marker were classified as pseudoepitheliomatous hyperplasia.[1] Subsequent studies assessing the expression of BerEP4 also yielded similar results. In a review by Sunjaya et al. published in 2017, which included 285 cases of basal cell carcinoma gathered from 12 studies, it was shown that the BerEP4 marker was positive in all cases[2].

Notably, BerEP4 plays a crucial role in diagnosing squamous basal cell carcinoma, which is the most malignant type of basal cell carcinoma characterized by extensive infiltration of surrounding tissues[3]. Patients should be monitored long-term to detect local recurrence and distant metastasis. In our study, all 4 cases of squamous basal cell carcinoma were positive for BerEP4; however, unlike other cases of basal cell carcinoma, the expression of this marker was only positive in individual foci rather than diffuse. This result aligns with findings from Karahan (2006) [4].

Additionally, BerEP4 is used by pathologists to differentiate basal cell carcinoma from squamous cell carcinoma. Both types of cancer arise from the epidermis and are associated with sun-exposed skin, which often leads to similar clinical presentations. However, squamous cell carcinoma has a higher metastatic rate than basal cell carcinoma. Thus, accurately diagnosing these two pathologies is crucial for treatment and prognosis. Data from various studies compiled by Sellheyer (2013) showed that all 75 cases of squamous cell carcinoma stained with BerEP4 were negative[5]. Similar results were reported by Karahan (2006) and Dasgeb (2013)[4,6]. These findings are supported by research from Ozawa (2004), which indicated that the BerEP4 antigen was not found in normal keratinocytes and squamous cell carcinoma[8]. Mashhood et al. (2011) determined the sensitivity and specificity of BerEP4 in diagnosing basal cell carcinoma compared to squamous cell carcinoma to be 99.6% and 99.2%, respectively[7].

Histopathologically, basal cell carcinoma needs to be differentiated from other tumor types with a basal cell-like structure, such as sebaceoma. Fan's study (2007) comparing the expression of the BerEP4 marker in basal cell carcinoma and sebaceoma showed that among 25 tested sebaceoma samples, 24 were negative, while one sample exhibited weak positivity (< 10% of tumor cells). In contrast, all 51 samples of basal cell carcinoma tested were moderately or strongly positive, with positivity rates exceeding 20%[6].

In our study, all 312 basal cell carcinoma samples were positive for the Bcl-2 marker. This result is consistent with several previous studies. According to Neira (2008), all 20 samples of basal cell carcinoma in the study, including 5 nodular, 5 follicular, 5 trabecular, and 5 sclerotic types, were positive for Bcl-2. Bcl-2 expression was diffuse and uniform in the cytoplasm of all tumor cells but was stronger in the peripheral region of the lesions[2]. A more recent study by Raheem in 2014 also showed Bcl-2 expression in 20 cases of basal cell carcinoma with various types, including nodular, superficial, tubular, and invasive forms[1]. Bcl-2 protein has been demonstrated to prevent cell death and protect cells from apoptosis induced by various agents. The stronger expression of Bcl-2 in the peripheral regions of basal cell carcinoma may suggest that tumor cells at the edges are protected against programmed cell death, allowing the tumor to spread[6].

One application of Bcl-2 that has been studied is its role in differentiating basal cell carcinoma from squamous cell carcinoma. Numerous studies have yielded similar results, indicating that basal cell carcinoma is positive for Bcl-2 while squamous cell carcinoma is negative, as shown in research by Kim HC (1996), Zhenlong Zheng (2005), and Neira (2008)[1-3]. A recent study by Mohammad A Gaballah (2015) involving 30 cases of basal cell carcinoma and 20 cases of squamous cell carcinoma evaluated the sensitivity and specificity in differentiating these two types of carcinoma

at high levels of 100% and 80%, respectively.[7] In normal adult skin, Bcl-2 is expressed in basal epidermal cells, melanocytes, outer root sheath cells, the papillary dermis of hair follicles, and the epithelial cells of eccrine sweat glands, but it is negative in keratinocytes. Therefore, the positivity of Bcl-2 in basal cell carcinoma and negativity in squamous cell carcinoma can be attributed to the belief that basal cell carcinoma originates from basal cells, while squamous cell carcinoma arises from keratinocytes[6].

Bcl-2 is also a useful indicator in differentiating basal cell carcinoma from follicular epithelial tumors. Clinical diagnosis can be challenging for physicians in certain cases, necessitating biopsy. However, even in histopathology, since both basal cell carcinoma and follicular epithelial tumors share similar features with a basal cell-like structure, pathologists may need to conduct more in-depth immunohistochemical tests. Some studies indicate that Bcl-2 is diffusely positive in basal cell carcinoma[5,6], while it is only positive in the basal-like layer in follicular epithelial tumors[7,8]. A more recent study by Sayed (2014) showed that Bcl-2 was positive in both basal cell carcinoma and follicular epithelial tumors, but the key difference lies in the fact that Bcl-2 expression is diffuse throughout the tumor in basal cell carcinoma, while in follicular epithelial tumors, there is a distinctly stronger expression in the basal-like cell layer[7].

In our study of 312 cases of basal cell carcinoma, 308 cases were negative (89.2%) and 4 cases were positive (10.8%) for the EMA marker. All positive cases were of the squamous type of basal cell carcinoma with areas of keratinized differentiation. This result is supported by numerous previous studies[1,2]. Additionally, there are studies indicating that squamous cell carcinoma is positive for EMA[3,4]. In a comprehensive systematic review of many studies conducted from 1893 to 2017, Mazaher Ramezani summarized that five studies found EMA positivity in ≥ 90% of squamous cell carcinoma tissues, while eight studies found EMA negativity in all basal cell carcinoma tissues[5]. Therefore, EMA is considered a useful marker for differentiating squamous cell carcinoma from basal cell carcinoma.

In our study of 312 samples of basal cell carcinoma, the majority of cases (99.4%) were positive for p63. Previous studies by S. Bircan (2006), J.S. Reis-Filho (2002), Zheng (2005), and M.R. Hussein (2022)[1,2,6,7] also indicated diffuse positivity of p63 in the nuclei of basal cell carcinoma cells. This helps distinguish cases of pigmented basal cell carcinoma from melanoma. In normal skin, p63 is positive in the basal cell layer and in keratinocytes just above the basal layer but is negative in fully differentiated squamous epithelial cells. The expression of the p63 protein in basal cell carcinoma not only indicates that tumor cells are derived from basal cell precursors but also suggests its role in the tumorigenesis of this cancer. So far, the role of p63 in the development of basal cell carcinoma remains largely unknown. According to research by Barbieri (2006), it was concluded that endogenous p63 may inhibit genes related to tumor metastasis, which explains the association between the loss of p63 expression and poor prognosis in cancer. Supporting this, another study by Johnson reported the expression of p63 protein in primary basal cell carcinoma cells in the skin but negative in cancer cells when metastasizing to lymph nodes. This may explain why basal cell carcinoma rarely metastasizes[7].

5. CONCLUSIONS

The BerEP4, Bcl-2, and p63 markers exhibit high sensitivity in diagnosing basal cell carcinoma (> 99%). BerEP4 has high specificity for differentiating basal cell carcinoma from other carcinoma types. p63 is positive in both basal cell carcinoma and squamous cell carcinoma. Bcl-2 is primarily positive in the peripheral regions of the tumor. EMA is often negative in basal cell carcinoma. The results of this study suggest that immunohistochemistry with the four markers BerEP4, Bcl-2, p63, and EMA may be utilized to assist in confirming and differentiating basal cell carcinoma from other skin carcinomas.

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