Original Investigation
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Exploring the immunohistochemical profile of basosquamous carcinoma and its clinicopathological associations

Bazoskuamöz karsinomun immünohistokimyasal profili ve klinikopatolojik ilişkilerinin araştırılması

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Abstract

Background and Design: Basosquamous carcinoma (BSC) is widely accepted as a basal cell carcinoma variant. Despite an unknown molecular pathogenesis, specific mutations are associated with its squamatization. Immunohistochemical studies offer insights into the tumor's genetic background. However, extensive investigations into its immunophenotype are lacking, and existing data present conflicting results.
Materials and Methods: We analyzed the clinicopathological and immunohistochemical characteristics (Ber-Ep4, CK14, CK17, p53, p63, and Ki-67) of BSCs diagnosed between 1996 and 2017. Data were collected on patient demographics and tumor features, including location, ulceration rate, margin status, tissue invasion, mitotic activity, predominant cell type, and peritumoral lymphocytic infiltration. We explored the correlations among all parameters, including staining positivity rates and percentages, to understand their relationships better.
Results: One hundred and four BSCs (68 males, mean age 67.99±14.95) were included. The most common location was the nose. Ulceration rate and margin positivity were 70.2% and 22.1%, respectively. The cartilage, muscle, lymphovascular, and perineural invasion prevalences were 3.8%, 11.5%, 5.8%, and 5.8%, respectively. The mitotic activity was moderate-high in 68% of the tumors. The immunopositivity rates were; Ber-Ep4, 78.8%; CK14, 93.3%; CK17, 89.4%; p53, 80.8%; p63, 98.1%; Ki-67, 100%. Ulceration was associated with squamous cell predominance and CK14 positivity. The Ber-Ep4 intensity was higher in lesions with lymphovascular invasion. CK17, p53, and p63 expressions were higher on the scalp and face than in other sites. The p53 staining was associated with ulceration and peritumoral lymphocytic infiltration. The mitotic activity was correlated with the Ki-67 score.
Conclusion: This study sheds light on the relationship between the clinicopathological and immunophenotypic characteristics of BSCs through an investigation of a large cohort. It has a high proliferation and ulceration rate. High margin positivity favors wide-excision margins. Long-term follow-up studies will clarify the prognostic significance of immunohistochemical markers mentioned in the study.
Keywords: Basosquamous carcinoma, pathology, immunohistochemistry

Öz

Amaç: Basoskuamöz karsinom (BSK), bazal hücreli karsinomun bir varyantı olarak kabul edilmektedir. Moleküler patogenezi bilinmemekle beraber, belirli mutasyonlar skuamatizasyonla ilişkilendirilmiştir. İmmünohistokimyasal çalışmalar, tümörün genetik zeminine dair ipuçları sunmaktadır. Ancak, BSK'nin immünofenotipi üzerine kapsamlı çalışmalar yoktur ve mevcut veriler çelişkilidir.
Gereç ve Yöntem: Bu çalışmada 1996-2017 yılları arasında tanı konulan BSK'lerin klinikopatolojik ve immünohistokimyasal özellikleri (Ber-Ep4, CK14, CK17, p53, p63 ve Ki-67) analiz edildi. Hasta demografik özellikleri ve tümörün yeri, ülserasyon oranı, sınır pozitifliği, çevre dokulara invazyonu, mitotik aktivitesi, baskın hücre tipi ve peritümöral lenfositik infiltrasyon varlığı gibi tümör özelliklerine ilişkin veriler toplandı. İmmünohistokimya boyanma oranları ve yüzdeleri dahil tüm parametreler arasındaki ilişkiler incelenerek, bu parametreler arasındaki bağlantılar araştırıldı.
Bulgular: Çalışmaya 104 BSK (68 erkek, ortalama yaş 67,99±14,95) dahil edildi. En sık yerleşim yeri burundu. Ülserasyon oranı ve cerrahi sınır pozitifliği sırasıyla %70,2 ve %22,1 idi. Kıkırdak, kas, lenfovasküler ve perinöral invazyon prevalansları sırasıyla %3,8, %11,5, %5,8 ve %5,8 idi. Tümörlerin %68'inde mitotik aktivite orta-yüksek düzeydeydi. İmmünopozitiflik oranları şöyleydi: Ber-Ep4, %78,8; CK14, %93,3; CK17, %89,4;

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p53, %80,8; p63, %98,1; Ki-67, %100. Ülserasyon, skuamöz hücre baskınlığı ve CK14 pozitifliği ile ilişkili bulundu. Lenfovasküler invazyon olan lezyonlarda Ber-Ep4 yoğunluğu daha yüksekti. CK17, p53 ve p63 ekspresyonları, diğer bölgelere kıyasla saçlı deri ve yüzde daha yüksekti. P53 boyanması, ülserasyon ve peritümöral lenfositik infiltrasyon ile ilişkiliydi. Mitotik aktivite, Ki-67 skoru ile koreleydi.
Sonuç: Geniş bir kohortun incelendiği bu çalışma, BSK'lerin klinikopatolojik ve immünofenotipik özellikleri arasındaki ilişkiyi aydınlatmaktadır. BSK proliferasyon ve ülserasyon oranı yüksek bir tümördür. Cerrahi sınır pozitifliğinin sık olması geniş eksizyon önerilerini desteklemektedir. Çalışmada kullanılan immünohistokimyasal belirteçlerin prognostik önemi uzun süreli takip çalışmaları ile netleşecektir.
Anahtar Kelimeler: Bazoskuamöz karsinom, patoloji, immünohistokimya

Introduction

Basosquamous carcinoma (BSC) is a cutaneous malignancy exhibiting histopathological features of both basal cell carcinoma (BCC) and squamous cell carcinoma (SCC)[1]. Although often considered an aggressive BCC subtype, the molecular pathogenesis of BSC remains unclear[2]. Recently, BSC has been proposed as a distinct entity with clinical differences, including anatomical location, gender, and age distribution, compared to BCC and SCC[1]. Genomically, BSC is more similar to BCC than SCC, with subsequent mutations following BCC-related abnormalities linked to squamatization[2].

Immunohistochemical studies play a pivotal role in elucidating the phenotype of tumors arising from a genetic background, thereby aiding in their diagnosis[2]. The lack of large-scale studies on the immunophenotype of BSC hampers its proper characterization[3-8]. Additionally, BSC is often confused with other basaloid tumors and mislabeled as metatypical basal cell carcinoma (mBCC), contributing to the paucity of data on its morphology[9-11].

Ber-Ep4, a marker for basaloid tumors, aids in identifying BSC. While the basaloid component shows strong positivity, areas with squamous differentiation are negative for Ber-Ep4. The gradual loss of Ber-Ep4 staining in the transition zone can help distinguish BSC from collision tumors[1,9,10,12]; however, this zone is not always apparent[13], and staining may be lost due to technical errors[12]. Small series on BSC report Ber-Ep4 positivity rates of 90-100%[8,12,14]. Evidence suggests low diagnostic accuracy of Ber-Ep4 in BCCs with extensive squamous differentiation[14]. Linskey et al.[9] found 60% Ber-Ep4 reactivity in mBCC and recommended adding CK14 and CK17 stains to distinguish mBCC, particularly from basaloid squamous cell carcinoma (bSCC). The roles of CK14 and CK17 in identifying BSC remain underexplored.

Mutations in the *p53* gene, associated with p53 overexpression, are common in BSC[2]. While p53 expression is more frequent in aggressive BCC types, including BSC[6,15], reactivity rates in BSC vary significantly across studies[4,6,7]. Notably, a recent study found the lowest p53 expression in BSCs compared to other BCC variants[5].

Ki-67 expression indicates tumor cell growth characteristics. All BSCs expressed Ki-67 in a 1997 study[6], but a recent report found 13% positivity[5]. Due to conflicting data on BSC's biological behavior[10,16], solid evidence on the Ki-67 index could clarify its proliferative capacity.

The role of p63 in BCC pathogenesis remains unclear. In addition to suggesting a basaloid progenitor cell origin, it has been debated as a tumorigenesis factor in BCC[3,17]. No significant differences in p63 expression were found among BCC subtypes, though BSC was not specifically assessed[17]. A recent study reported 100% p63 positivity in eleven BSCs, but the small sample size requires validation through larger studies[3].

Accordingly, the lack of extensive search on the immunohistochemistry of BSC and conflicting results in currently available literature highlights the need for large-scale studies to better characterize this tumor[1,9]. In this study, we assessed the expression of Ber-Ep4, CK14, CK17, p53, and p63 in a large set of BSC patients and examined the involvement of proliferation pathways through the analysis of Ki-67. We also investigated the association between the clinicopathological characteristics and the immunohistochemical staining patterns, which, to the best of our knowledge, was not previously addressed.

Materials and Methods

Patient selection and data acquisition

The Başkent University Ethical Committee approved this study (approval number: KA15/188, date: 10.06.2015). The study was conducted in accordance with the Declaration of Helsinki. Patients diagnosed with BSC between 1996 and 2017 were included in the study. The clinical and epidemiological characteristics of patients, including gender, age at diagnosis, the localization of the tumor, and concomitant skin lesions (solar elastosis, actinic keratosis, seborrheic keratoses, sebaceous hyperplasia, BCC, SCC, and melanoma) were recorded by reviewing the institutional database.

Histopathological examination

Hemotoxylin and eosin-stained slides of patients were retrospectively retrieved from the histopathology archives and re-examined by the dermatopathologist (G.Ö.) experienced in skin cancer for reconfirmation of the diagnoses. All lesions were considered separate cases for patients with more than one BSC.

For the BSC diagnosis, widely accepted histopathological criteria were used as follows: a tumor containing infiltrative, mitotically active hyperchromatic basaloid cells with peripheral palisading and aggregates of cells with squamous differentiation (with large polygonal eosinophilic cytoplasm) in portions of the tumor with areas of transition between the two[1,12,13,18] (Figure 1a, b). Keratinization was not considered an essential feature[12,18]. The collision tumors were excluded from the study. In cases where the diagnosis is less clear, Ber-Ep4 staining is routinely performed in our clinic to facilitate the diagnosis. Histopathology specimens were evaluated for the parameters indicated in Table 2.

Immunohistochemical method

Specimens were then immunohistochemically stained for Ber-Ep4 (except for those that were stained previously) (Mouse monoclonal, dilution 1:100, DAKO, Denmark), CK14 (Mouse monoclonal, dilution 1:100, DAKO, Denmark), CK17 (clone E3, Mouse monoclonal, dilution 1:100, DAKO, Denmark), p63 (clone DAK, Mouse monoclonal, dilution 1:100, DAKO, Denmark), p53 (clone DO-7, Mouse monoclonal, dilution 1:100, DAKO, Denmark), and Ki-67 (clone MIB-1, Mouse monoclonal, dilution 1:100, DAKO, Denmark). Four-micrometer-thick serial sections

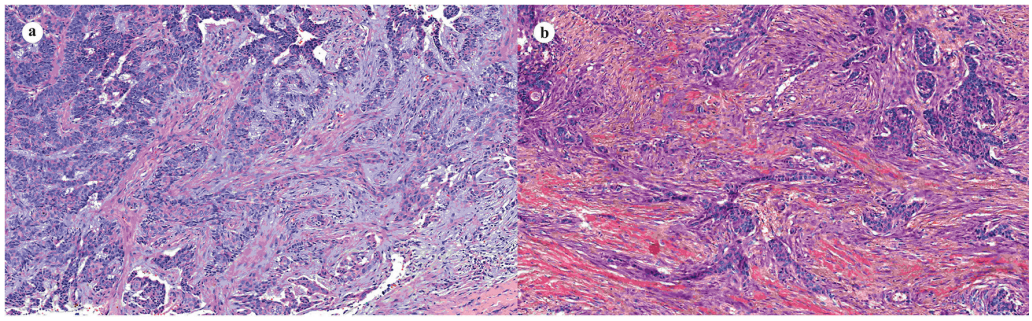

Figure 1. (a, b) Histopathological findings of basosquamous carcinoma show islands of infiltrative basaloid cells with peripheral palisading intermingled with cells with squamous differentiation and intermediate cells in the transition zones [**(a, b)** hematoxylin and eosin, x100]

were obtained from formalin-fixed, paraffin-embedded blocks. Immunohistochemical staining was performed on a DAKO OMNIS autostainer (DAKO, Denmark) using the standard immunohistochemical methodology, and a peroxidase/DAB detection system (DAKO) was used for visualization.

The positivity of staining (staining of less than 5% of tumor cells was considered negative) and the percentage of positive cells were recorded. The intensity of Ber-Ep4, CK14, CK17, and p63 staining was graded and expressed as "weak", "moderate", or "strong". The Ki-67 scores were expressed as the percentage of positively stained cells.

Statistical Analysis

Descriptive analyses were presented using medians (minimal-maximal values) and mean ± standard deviations. Chi-square or Fisher's exact tests were used for comparative analysis of categoric data. Correlation coefficients and their significance were calculated using the Spearman's test. Statistical analysis was performed using the statistical package for social sciences (SPSS) Software (SPSS version 24, Chicago, USA). P values less than 0.05 were considered significant.

Results

One hundred four patients were included in the study. The clinical characteristics and the histopathological features are given in Tables 1, 2, respectively. Accordingly, 65.4% of the patients were male. The most common location was the nose (34.6%), and 70.2% of the tumors were ulcerated. The immunohistochemical profiles of the tumors are given in Table 3 (Figure 2a-f). The rate of Ber-Ep4 positivity was 78.8%. After thoroughly examining the interrelationships between clinical findings, histopathological characteristics, and immunostaining patterns, we identified several noteworthy associations. The significant findings are as follows: The percentage of Ber-Ep4 staining was higher in women than in men (p=0.03) and was also higher in patients with lymphovascular invasion (LVI) compared to those without (p=0.04). CK14 staining was more prevalent in patients younger than 70 than in those older (p=0.01), in men than in women (p=0.02), and in lesions with ulceration compared to those without (p=0.03). CK17 reactivity was higher in lesions with squamous cell dominance than in those with basal cell dominance (p=0.03) and in lesions located on the scalp and face compared to other sites (p=0.04). The percentage of p53 staining was greater in face and scalp lesions than in trunk and extremity lesions (p=0.04), in lesions with ulceration than those without (p=0.04), and in those with a peritumoral inflammatory response than those without (p=0.006). P63 staining was significantly higher in facial and scalp lesions compared to other sites (p=0.02). There was also a significant positive correlation between mitotic activity and the Ki-67 score (r=0.226, p=0.02).

Table 1. Demographic and clinical data of the patients with basosquamous carcinoma (n=104)

Epidemiological and clinical parameters		
Age (mean ± SD)		67.99±14.95
Gender (n, %)	Female	36 (34.6)
	Male	68 (65.4)
Location (n, %)	Nose	36 (34.6)
	Ear	24 (23.1)
	Periorbital	13 (12.5)
	Cheeks	7 (6.7)
	Scalp	7 (6.7)
	Forehead	5 (4.8)
	Perineal and vulvar region	5 (4.8)
	Legs	3 (2.9)
	Neck	2 (1.9)
	Chest	2 (1.9)
Associated lesion (n, %)	Solar elastosis	60 (57.7)
	None	26 (25.0)
	BCC	7 (6.7)
	Seborrheic keratoses	5 (4.8)
	Actinic keratoses	3 (2.9)
	SCC	2 (1.9)
	Actinic keratoses + seborrheic keratoses	1 (1.0)

SD: Standard deviation, BCC: Basal cell carcinoma, SCC: Squamous cell carcinoma

Discussion

The current study clarifies the inconsistent results in the literature regarding the immunohistochemical profile of BSC and addresses the association between the clinicopathological and immunohistochemical features in a large cohort.

In accordance with the literature, we found that BSC is more common in men[1,10,11,16,18], and around the age of 70, albeit slightly younger than in previous studies[10,11,16,18]. Gualdi et al.[1] reported BSC was more

Table 2. The histopathological features of basosquamous carcinoma lesions (n=104)		
The presence of ulceration (n, %)	Yes	73 (70.2)
	No	31 (29.8)
The depth of invasion (mm) (mean ± SD)		2.89±2.19
Breslow thickness (mm) (mean ± SD)		2.84±2.16
Surgical margin positivity (n, %)	Yes	23 (22.1)
	No	81 (77.9)
Nearest surgical margin (n, %)	Lateral margin	47 (45.2)
	Deep margin	49 (47.1)
Distance to surgical margin (mm) (mean ± SD)		2.41±2.45
Distribution of tumors by surgical margin proximity (n, %)	<1 mm	13 (12.5)
	1-1.9 mm	26 (25.0)
	2-2.9 mm	18 (17.3)
	3-3.9 mm	8 (7.7)
	4-4.9 mm	4 (3.8)
	5-9.9 mm	9 (8.7)
	≥10	3 (2.9)
Mitosis (n, %)	≤1/1 HPFs	36 (34.6)
	2-9/1 HPFs	60 (5.7)
	≥10/1 HPFs	8 (7.7)
Cartilage invasion (n, %)	Yes	4 (3.8)
	No	100 (96.2)
Muscle invasion (n, %)	Yes	12 (11.5)
	No	92 (88.5)
LVI (n, %)	Yes	6 (5.8)
	No	98 (94.2)
PNI (n, %)	Yes	6 (5.8)
	No	98 (94.2)
Peritumoral inflammatory infiltrate (n, %)	Severe	20 (19.2)
	Moderate	15 (14.4)
	Mild	47 (45.2)
	None	22 (21.2)
The predominant cell type (n, %)	Basaloid	73 (70.2)
	Squamous	31 (2.8)

HPFs: High power fields, LVI: Lymphovascular invasion, PNI: Perineural invasion, SD: Standard deviation

common between the ages of 80 and 99. The most common location was the nose, confirming the previous studies[1,13,16,18]. The second most common location was the ear, in line with Wermker et al.[16] and Kececi et al.[13]. Truncal involvement has been reported between 10-20%[1,10,11]. It was 6.7% in the present study, which is similar to Ciążyńska et al.[18].

The incidence of BSC has been reported to be between 1.2-2.7% of all non-melanoma skin cancers (NMSCs)[13,19]. On the other hand, evidence suggests that the true incidence is hampered by its synonymous use with mBCC and the derivation of data from a few retrospective series[20,21]. It was recently found to be more common than previously reported, with an incidence of 4.8%[1]. Considering all skin cancers diagnosed in our institution for the last ten years, BSCs represented 5.5% of all NMSCs. Our higher-than-expected tumor prevalence can likely be attributed to the substantial population of solid organ transplant recipients within our institution.

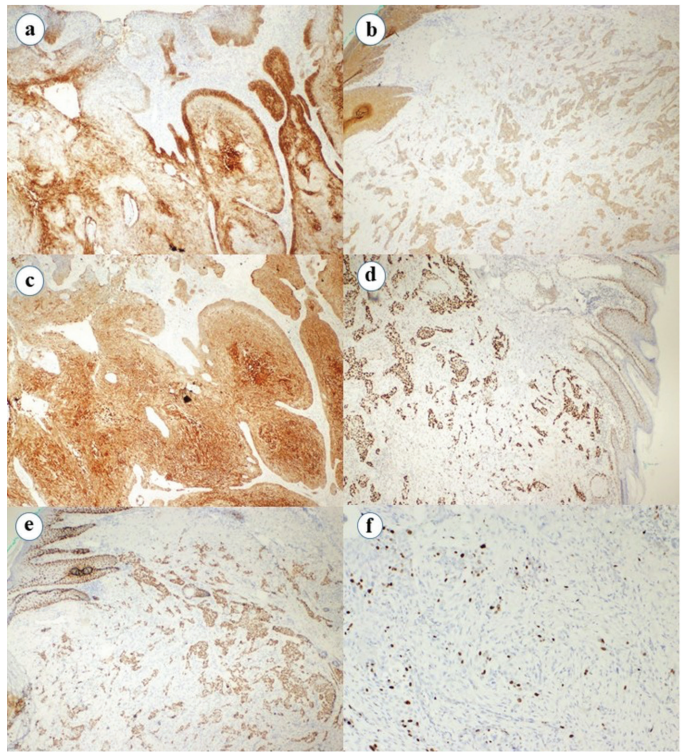

Figure 2. (a-f) Basosquamous carcinoma showing positivity for BerEp4 [**(a)**, x100], cytokeratin 14 [**(b)**, x100], cytokeratin 17 [**(c)**, x100], p53 [**(d)**, x100], p63 [**(e)**, x100] and Ki-67 [**(f)**, x400] immunohistochemical stains

The mean Breslow thickness was 2.84±2.16 mm in the current study, a histological feature associated with poor prognosis[16]. However, due to the lack of information on the mean depth of invasion in other BSC studies, we were unable to make comparisons.

The prevalence of adjacent tissue involvement varies considerably among studies. The LVI rate ranged between 1.3-16%[10,16,18], and PNI has been reported as 2.7-8%[10,18,22]. In this cohort, the rate of LVI and PNI was both 5.8%. Two previous reports found no case with PNI[13,21]. Muscle invasion was 11.5%, slightly lower than in Wermker et al.[16] (15%). The absence of data in recent literature limits comparative analysis on the cartilage invasion rate, which was 3.8% in this cohort.

The mean excision margin was 2.41±2.45 mm, narrower than previous studies (ranging between; 3.8±2.1 and 4.5±2.6)[10,18]. Margin positivity, which was 22% in our cohort, has been associated with poor prognosis[23]. The margin involvement has been found to be 13%[16] and 17%[19] in previous studies. In other reports, where resection margins of <1 mm were considered positive, it was 23%[10] and 24%[18]. Higher margin positivity (31.4%) was also reported[14]. Accordingly, wider excision margins have been recommended[13,19], and Mohs surgery, especially for wider tumors, has been advocated[19].

Ulceration has been associated with aggressive BCC variants rather than indolent subtypes[24]. The prevalence of ulceration has been reported as 10%[11], 46%[16], and 62.5%[21] in different studies, while it was 70.2% in the current report. Evidence for an association between ulceration and squamoid features has been demonstrated previously[12]. The present study supports this evidence by showing that ulceration was significantly higher in tumors with squamous cell dominance than

Table 3. The immunohistochemical profile of basosquamous carcinoma lesions (n=104)

	Stain	Ber-Ep4	CK14	CK17	p53	p63	Ki-67
The positivity rate (n, %)		82 (78.8)	97 (93.3)	93 (89.4)	84 (80.8)	102 (98.1)	104 (100)
The negativity rate (n, %)		22 (21.2)	7 (6.7)	11 (10.6)	20 (19.2)	2 (1.9)	0
The percentage of cells with positivity (mean ± SD)		58.89±39.78	74.51±34.38	72.40±35.54	39.83±38.91	67.40±34.64	28.67±23.8*
The intensity of expression (n, %)	Strong	54 (51.9)	42 (40.4)	58 (55.8)		56 (53.8)	
	Moderate	20 (19.2)	37 (35.6)	30 (28.8)		29 (27.9)	
	Weak	8 (7.7)	18 (17.3)	5 (4.8)		17 (16.3)	
	None	22 (21.2)	7 (6.7)	11 (10.6)		2 (1.9)	

CK: Cytokeratin, *Ki-67 score

in those with basal cell dominance.

Patients with BSC have been reported to have a higher rate of concomitant skin tumors than those with other NMSCs, up to 40-52%[18,25]. These rates indicate the presence of multiple skin cancers per patient over the entire study period. In the current study, 7% of patients had BCC, and 2% had SCC at the time of diagnosis.

The studies on the immunophenotype of BSC have been characterized by limited sample sizes[3-9]. Despite the suggested utility of Ber-Ep4 in the diagnosis of basaloid tumors, the reactivity may be low in BCCs with squamous differentiation, as the squamoid areas do not react[14]. Ber-Ep4 positivity has been reported to range between 80-100% in BCCs[8,12,26]. Considering subtypes with squamous differentiation, Ber-Ep4 stained either 60% (mBCC, n=43)[9] or 90% (BSC/mBCC, n=10)[14] of the lesions in different studies. Ber-Ep4 reactivity was 80% in our cohort, with a staining percentage of around 59%. This discrepancy in results may be explained by the different cut-off values used to define positive staining or by the predominance of squamous cells in our tumors. A finding that merits attention is that Ber-Ep4 staining intensity was associated with LVI. Although LVI has been linked to a worse prognosis in BSC[16,23], a prognostic role has not yet been reported for Ber-Ep4 intensity in BSC.

CK14 and CK17 expression points to the derivation of the tumor from the outer root sheath of the hair follicle, which is suggested to be the origin of BCC. Linskey et al.[9] have recommended the inclusion of CK14 and CK17 to differentiate BCCs with squamoid features from bSCCs, as all mBCC tumors (n=43) were positive for both cytokeratins with a distinct pattern from bSCC. Extensive studies are lacking on the cytokeratin expression of BSC. Regarding the CK17 profile, in two reports, all BSCs (n=10 and n=8, respectively) were stained positive with CK17[27,28]. In our cohort, CK14 and CK17 staining rates were 93% and 90%, respectively, with expression percentages of 75% and 72%. The association between CK14 positivity and ulceration may be of importance, and future studies may elucidate a prognostic role for CK14. Our finding of a higher percentage of CK17 staining in tumors on the scalp and face compared to other sites may be related to ultraviolet (UV)-induced alterations in BSCs. However, a recent study demonstrated that CK17 expression did not differ between BCCs in sun-exposed areas and non-exposed areas[29]. A potential link between CK17 expression and the squamous phenotype in BCC has been previously suggested[30]. In line with this, our cohort exhibited a higher percentage of CK17 staining in tumors predominantly composed of squamous cells. Further investigation is warranted to elucidate the role of CK17 in the process of squamatization in BSC.

Contrasting results are found considering the biological behavior of BSC[10,16], though it has been defined as a tumor with high mitotic activity[1]. The mitotic rate was moderate-to-high in 68% of the tumors in this cohort. Studies have yielded inconsistent findings on the Ki-67 index of BSC. Ki-67 positivity was 100% in two reports with a small number of cases (n=6 and 15, respectively)[6,15]. Conversely, it was 13.71% in eight BSC cases in a recent study[5]. While we observed 100% positivity for Ki-67, with a staining percentage of approximately 29%, it is noteworthy that previous literature, dating back to 1997, predominantly reported staining percentages ranging from 10% to 20%[6]. The significant positive correlation between mitotic activity and Ki-67 score suggests that Ki-67 may be a reliable indicator of mitotic activity in BSC.

While p53 positivity was found similar among BCCs with different infiltration patterns[7], a recent contrasting study demonstrated higher p53 reactivity in aggressive BCC types, although the expression rate in BSCs (n=2) was not specified[15]. The expression of p53 in BSC has been previously reported in the range of 33% to 75% based on small-scale studies from earlier years[4,6,7]. Recently, the lowest p53 expression rate (12.7%) was observed in BSCs (n=8) among different BCC variants[5]. In this study, p53 positivity was notably high at approximately 81%, with a staining percentage of around 40%, surpassing the rates reported in previous studies.

The significant association between p53 staining and peritumoral lymphocytic infiltration in the present study may support the relationship between p53 mutation and the immune response against the tumor, which has become a subject of interest in recent years. The p53 mutation has been shown to modify the immune system, causing the migration of immune suppressor cells and leading to tumor progression[31]. However, since we did not analyze the subtype of the infiltrating cells, they may also represent immune cells that play a protective role in tumor rejection. The identification of an association between p53 expression and ulceration raises the question of whether this relationship is linked to the aggressiveness of the tumor, thus warranting further investigation.

Overexpression of p63 in BCCs is thought to result from either the tumor's origin in undifferentiated basal cells or p63's role in maintaining basaloid cell proliferation[3,17]. The p63 mutation, associated with reduced/absent protein expression, is uncommon in BSCs[2]. In a previous study that did not include BSC, p63 hyperreactivity was found to be independent of the histopathological subtype of BCC[17]. Recently,

the p63 positivity in all BSCs (n=11) has suggested a stem cell trait for BSC[4]. In our cohort, p63 positivity was 98%, which confirms the previous findings[3,17]. We did not find an association between p63 expression and the predominant cell type. Further studies are needed to clarify whether p63 plays a role in differentiation or carcinogenesis in BSC.

Higher expression of p53 and p63 in the face and scalp locations compared to other sites may be related to genetic alterations induced by UV exposure. Supporting this, UV light-induced p53 mutations have been shown to alter cellular signaling pathways involving p63, thereby contributing to the progression of skin cancer[32].

Study Limitations

There are some limitations in the present study. Due to the retrospective design, only patients with established BSC diagnoses were included in the study. Since the differentiation of BSC, especially from mBCC, is challenging, there may still be false positives. However, the consistent results with previous reports regarding the clinicopathological features of BSCs data reassure the validity of the findings. Additionally, we could not compare the clinicopathological and immunohistochemical parameters of BSC with BCC and SCC within the same cohort, which would have enabled us to identify the distinct tumor characteristics. Finally, the lack of long-term follow-up limits the evaluation of the prognostic significance of immunohistochemical markers used in the study.

Conclusion

The current study contributes to the limited literature on the immunohistochemical profile of BSC (Ber-Ep4, CK14, CK17, p53, p63, and Ki-67) and provides a clearer understanding of the prevailing contradictory data through an analysis of a substantial cohort. Additionally, the study offers insights into the histopathological characteristics of the tumor, their interrelations, and their association with immunostaining patterns. Ulceration is a frequent finding in BSC and is associated with the squamous phenotype. Margin positivity in one-fifth of the tumors supports the recommendations for wider excision. Observing a moderate-to-high mitotic rate in 68% of the tumors, coupled with Ki-67 expression in all, likely indicates the aggressive nature of the tumor. The higher reactivity of CK17, p53, and p63 on the face and scalp compared to other sites may be related to sun exposure. The association between intense Ber-Ep4 staining and the poor prognostic indicator, LVI, warrants further investigation. CK14 and p53 expressions were associated with ulceration. Higher p53 positivity in lesions with peritumoral infiltration may be related to p53-induced immunosuppression, promoting tumor progression. Future long-term follow-up studies are needed to confirm the role of these markers in predicting outcomes and their potential implications in the risk stratification of BSCs.

Ethics

Ethics Committee Approval: The Başkent University Ethical Committee approved this study (approval number: KA15/188, date: 10.06.2015).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Concept: A.K., T.G., D.S., Design: C.T.A., A.K., T.G., D.S., D.Ö., Supervision: D.Ö., Materials: G.Ö., Ö.Ö., Data Collection or Processing: C.T.A., G.Ö., Ö.Ö., D.Ö., Analysis or Interpretation: G.Ö., Ö.Ö., Writing: C.T.A.

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References

1. Gualdi G, Soglia S, Fusano M, et al.: Characterization of basosquamous cell carcinoma: a distinct type of keratinizing tumour. Acta Derm Venereol. 2021;101:adv00353.
2. Chiang A, Tan CZ, Kuonen F, et al.: Genetic Mutations underlying phenotypic plasticity in basosquamous carcinoma. J Invest Dermatol. 2019;139:2263-71.e5.
3. Hussein MR, Ahmed AM: Expression profile of CD10, Bcl-2, p63, and EMA in the normal skin and basal cell carcinomas: an immunohistochemical reappraisal. Actas Dermosifiliog. 2022;113:848-55.
4. İlter N, Poyraz A, Dursun A, Akyol G, Gürer MA: Bcl-2 and p53 protein expression in non melanoma skin cancers. Turkiye Klinikleri J Dermatol. 2001;11:191-4.
5. Cojocaru A, Bîrjovanu C, Ciurea AM, et al.: Immunohistochemical expression of p53, Ki67, α-SMA, CD44 and CD31 in different histological subtypes of basal cell carcinoma. Rom J Morphol Embryol. 2022;63:383-93.
6. Barrett TL, Smith KJ, Hodge JJ, Butler R, Hall FW, Skelton HG: Immunohistochemical nuclear staining for p53, PCNA, and Ki-67 in different histologic variants of basal cell carcinoma. J Am Acad Dermatol. 1997;37:430-7.
7. Demirkan NC, Colakoglu N, Düzcan E: Value of p53 protein in biological behavior of basal cell carcinoma and in normal epithelia adjacent to carcinomas. Pathol Oncol Res. 2000;6:272-4.
8. Jones MS, Helm KF, Maloney ME: The immunohistochemical characteristics of the basosquamous cell carcinoma. Dermatol Surg. 1997;23:181-4.
9. Linskey KR, Gimbel DC, Zukerberg LR, Duncan LM, Sadow PM, Nazarian RM: BerEp4, cytokeratin 14, and cytokeratin 17 immunohistochemical staining aid in differentiation of basaloid squamous cell carcinoma from basal cell carcinoma with squamous metaplasia. Arch Pathol Lab Med. 2013;137:1591-8.
10. Oldbury JW, Wain RAJ, Abas S, Dobson CM, Iyer SS: Basosquamous carcinoma: a single centre clinicopathological evaluation and proposal of an evidence-based protocol. J Skin Cancer. 2018;2018:6061395.
11. Tarallo M, Cigna E, Frati R, et al.: Metatypical basal cell carcinoma: a clinical review. J Exp Clin Cancer Res. 2008;27:65.
12. Beer TW, Shepherd P, Theaker JM: Ber EP4 and epithelial membrane antigen aid distinction of basal cell, squamous cell and basosquamous carcinomas of the skin. Histopatholog. 2000;37:218-23.
13. Kececi Y, Argon A, Kebat T, Sir E, Gungor M, Vardar E: Basosquamous carcinoma: is it an aggressive tumor? J Plast Surg Hand Surg. 2015;49:107-11.
14. Gill P, Naugler C, Abi Daoud MS: Utility of Ber-EP4 and MOC-31 in Basaloid skin tumor detection. Appl Immunohistochem Mol Morphol. 2019;27:584-8.
15. Mendez-Flores RG, Martínez-Fernández DE, Vega-De la Torre DE, et al.: Role of Bcl-2, p53, and Ki-67 expression in basal cell carcinoma and their association with aggressive and non-aggressive histological phenotypes. Postepy Dermatol Alergol. 2022;39:517-23.
16. Wermker K, Roknic N, Goessling K, Klein M, Schulze HJ, Hallermann C: Basosquamous carcinoma of the head and neck: clinical and histologic

characteristics and their impact on disease progression. Neoplasia. 2015;17:301-5.

17. Bircan S, Candir O, Kapucoglu N, Baspinar S: The expression of p63 in basal cell carcinomas and association with histological differentiation. J Cutan Pathol. 2006;33:293-8.
18. Ciążyńska M, Sławińska M, Kamińska-Winciorek G, et al.: Clinical and epidemiological analysis of basosquamous carcinoma: results of the multicenter study. Sci Rep. 2020;10:18475.
19. Betti R, Crosti C, Ghiozzi S, Cerri A, Moneghini L, Menni S: Basosquamous cell carcinoma: a survey of 76 patients and a comparative analysis of basal cell carcinomas and squamous cell carcinomas. Eur J Dermatol. 2013;23:83-6.
20. Garcia C, Poletti E, Crowson AN: Basosquamous carcinoma. J Am Acad Dermatol. 2009;60:137-43.
21. Allen KJ, Cappel MA, Killian JM, Brewer JD: Basosquamous carcinoma and metatypical basal cell carcinoma: a review of treatment with Mohs micrographic surgery. Int J Dermatol. 2014;53:1395-403.
22. Leibovitch I, Huilgol SC, Selva D, Richards S, Paver R: Basosquamous carcinoma: treatment with Mohs micrographic surgery. Cancer. 2005;104:170-5.
23. Martin RC 2nd, Edwards MJ, Cawte TG, Sewell CL, McMasters KM: Basosquamous carcinoma: analysis of prognostic factors influencing recurrence. Cancer. 2000;88:1365-9.
24. Yalcin O, Sezer E, Kabukcuoglu F, et al.: Presence of ulceration, but not high risk zone location, correlates with unfavorable histopathological subtype in facial basal cell carcinoma. Int J Clin Exp Pathol. 2015;8:15448-53.
25. Bowman PH, Ratz JL, Knoepp TG, Barnes CJ, Finley EM: Basosquamous carcinoma. Dermatol Surg. 2003;29:830-2.
26. Ansai S, Takeichi H, Arase S, Kawana S, Kimura T: Sebaceous carcinoma: an immunohistochemical reappraisal. Am J Dermatopathol. 2011;33:579-87.
27. Kazantseva IA, Khlebnikova AN, Babaev VR: Immunohistochemical study of primary and recurrent basal cell and metatypical carcinomas of the skin. Am J Dermatopathol. 1996;18:35-42.
28. Costea CF, Turliuc MD, Sava A, et al.: Periocular basal cell carcinoma: demographic, clinical, histological and immunohistochemical evaluation of a series of 39 cases. Rom J Morphol Embryol. 2019;60:77-86.
29. García-de-la-Fuente MR, Santacana M, Valls J, et al.: Cytokeratin profile of basal cell carcinomas according to the degree of sun exposure and to the anatomical localization. Am J Dermatopathol. 2018;40:342-8.
30. Misago N, Satoh T, Narisawa Y: Basal cell carcinoma with tricholemmal (at the lower portion) differentiation within seborrheic keratosis. J Cutan Pathol. 2003;30:196-201.
31. Blagih J, Zani F, Chakravarty P, et al:. Cancer-specific loss of p53 leads to a modulation of myeloid and T cell responses. Cell Rep. 2020;30:481-96.e6.
32. Missero C, Antonini D: Crosstalk among p53 family members in cutaneous carcinoma. Exp Dermatol. 2014;23:143-6.