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Host immune responses and peritumoral stromal reactions in different basal cell carcinoma subtypes: histopathological comparison of basosquamous carcinoma and high-risk and low-risk basal cell carcinoma subtypes

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Background/aim: The literature does not include sufficient data on the associations between host immune responses and stromal reactions in different basal cell carcinoma (BCC) subtypes. The aim of the study was to compare host immune responses and stromal reactions between basosquamous carcinoma (BSC) and high-risk (HR) and low-risk (LR) BCC subtypes.

Materials and methods: The study included 35 BSC, 40 HR-BCC, and 40 LR-BCC patients. Age, sex, lesion location, density of peritumoral/adjacent perivascular inflammation, presence of lymphoid follicle formation, and stromal reaction type were compared between groups.

Results: In all 3 groups, age, sex distribution, and lesion location were similar. Overall, 70% of lesions in the LR-BCC group exhibited mild peritumoral inflammation, whereas in the BSC and HR-BCC groups dense inflammation was observed in 50% and 57.5% of lesions, respectively ($P < 0.001$). All lesions (100%) in the LR-BCC group had fibromyxoid stroma, whereas 61.8% and 80% of lesions in the BSC and HR-BCC groups, respectively, had desmoplastic stroma ($P < 0.001$).

Conclusion: The BSC and HR-BCC groups were similar in terms of host immune responses and stromal reactions. Furthermore, BSC and HR-BCC were associated with dense peritumoral inflammation, adjacent perivascular inflammation, and desmoplastic stroma.

Key words: Basal cell carcinoma, basosquamous carcinoma, inflammation, stroma

1.Introduction

Basosquamous carcinoma (BSC) is a rare variant of basal cell carcinoma (BCC) characterized by neoplastic cells, with both squamoid and basaloid differentiation. BSC is generally regarded as having an aggressive course and a high risk of recurrence and metastasis (1–3). Although it is widely known that BSC also has desmoplastic stroma, research on stromal and peritumoral inflammatory reactions in BSC is lacking (1). Furthermore, associations between host immune responses and stromal reactions in different BCC subtypes are not clearly known. As such, the aim of the present study was to evaluate peritumoral inflammation and stromal reaction patterns in BSC and compare them with those in low-risk (LR) and high-risk (HR) BCC subtypes.

2. Materials and methods

This retrospective study examined the clinical and histological features of 115 lesions in 115 patients diagnosed between January 2011 and February 2014. Lesions were obtained by searching the pathology department's computerized database using the terms 'diagnosed as BCC' and 'diagnosed as BSC'. All lesions identified via this search were then reexamined microscopically and conventional stainings (Alcian blue and Masson's trichrome) were performed on new 4-µm-thick cut sections. Patients with insufficient clinical data and/or histopathological specimens were excluded from the study. The clinical and histopathological data for 35 BSC, 40 LR-BCC, and 40 HR-BCC patients were included in the study. The regional ethics committee approved the study protocol.

The following clinical parameters obtained from pathological reports were evaluated: age, sex, and lesion location. Lesion location was grouped as follows: head and neck region (subgrouped as scalp, forehead, periorbital region, cheek, ear, nose, and neck), trunk, or extremity. BCC subtype, host immune responses, and peritumoral

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stromal reactions were evaluated as histopathological parameters. For evaluation of histopathological parameters formalin-fixed, paraffin-embedded hematoxylin and eosin (H&E)-stained sections were reexamined, and Alcian blue and Masson's trichrome stainings were performed on new cut sections to evaluate stromal reaction types. Lesions were histopathologically diagnosed as BSC if areas of both BCC and squamous cell carcinoma (SCC) were admixed together in a single tumor, with a transition zone between them. Collision tumors, keratotic BCC, and basaloid SCC were excluded. Collision tumors were defined as distinct BCC and SCC components with distinct and independent origin in the epidermis without a transition zone between them (1). Keratotic BCC was defined as BCC exhibiting central keratinization in some nodules, with uniform basal and squamoid components, without a transition zone or densely collagenized proplastic stroma (1,4). Furthermore, basaloid SCC was defined as a high grade variant of SCC composed of highly atypical basaloid cells, with abrupt foci of squamous differentiation in nests without a transition zone and frequently associated with comedo-type necrosis and epidermal squamous atypia or SCC in situ (5,6).

Subtypes of BCC were defined as previously described (7,8):

1. Nodular BCC: A tumor with rounded masses of peripherally palisading atypical basaloid cells with well-circumscribed outer margins in the dermis.
2. Micronodular subtype: A poorly circumscribed tumor composed of infiltrative small rounded nodules and nests that are ≤0.15 mm in diameter.
3. Superficial BCC: A tumor with nests of peripherally palisading basaloid cells protruding from and connected to the undersurface of the epidermis.
4. Infiltrative BCC: A poorly circumscribed tumor composed of infiltrative elongated strands and cords of basaloid cells.

Patients with superficial and nodular BCC constituted the LR-BCC group, and those with micronodular and infiltrative BCC constituted the HR-BCC group. If ≥2 subtypes were observed in the same lesion, the predominant subtype was accepted as the subtype.

As components of host immune response, the density of peritumoral inflammation, the presence of lymphoid follicle formation, and the density of perivascular inflammation in the adjacent dermis were evaluated. The density of peritumoral inflammation was scored according to Kaur et al. as follows (7):

- 0: No inflammation.
- 1: Mild inflammation, scattered clusters of inflammatory infiltrate surrounding <25% of the tumor nests.
- 2: Moderate inflammation, inflammatory infiltrate surrounding 25%–75% of the nests.
- 3: Dense inflammation, thick clusters or sheets of inflammatory infiltrate surrounding >75% of the nests.

Furthermore, the density of perivascular inflammation in the adjacent dermis was graded as follows:

0: No or occasional, inflammatory cells.
Mild: Few loosely arranged inflammatory cells.
Moderate: <3 concentric rows of inflammatory cells.
Dense: ≥3 concentric rows of inflammatory cells.

Peritumoral stromal reactions were evaluated based on cellularity (hypo/hypercellular), type of collagen bundles (thin/thick), and myxoid change according to Kaur et al. and were categorized as fibromyxoid or desmoplastic stroma (7).

Statistical analysis was performed using SPSS 18.0 for Windows (SPSS Inc., Chicago, IL, USA). Continuous variables are presented as mean ± SD or median (range), and categorical variables are presented as percentage. The normality of the distribution of numeric variables was evaluated using the Kolmogorov–Smirnov test. One-way analysis of variance was used to compare continuous variables between the BSC, LR-BCC, and HR-BCC groups. The chi-square test was used to identify associations between categorical variables. The level of statistical significance was set at $P < 0.05$.

3. Results

The study included 115 lesions in 115 patients (40 LR-BCC cases, 40 HR-BCC cases, and 35 BSC cases). The mean age of the patients in the LR-BCC, HR-BCC, and BSC groups was 65 ± 11.4 years, 69.4 ± 11.1 years, and 70.3 ± 11.1 years, respectively, and the female/male ratio in each group was 17:23, 25:15, and 17:18, respectively. Age and sex did not differ significantly between the 3 groups ($P = 0.184$). In all 3 groups the vast majority of the lesions were located in the head and neck region (HR-BCC group: 97.5%; BSC group: 94.3%; LR-BCC group: 92.5%). Although the difference was not significant, in the LR-BCC group cheek localization predominated (32.5%), whereas nasal localization predominated in the HR-BCC group (37.5 %) and BSC group (28.6%) ($P = 0.090$).

In the LR-BCC group, 4 (10%) of the lesions were superficial BCC and the remaining 36 (90%) were nodular BCC. In the HR-BCC group, 10 (25%) of the lesions were micronodular BCC and the remaining 30 were infiltrative BCC. In all 115 lesions examined (except for 1 nodular BCC lesion) peritumoral inflammation was observed. The density of inflammation in the 80 BCC lesions was mild in 30 (37.5%) (28 LR-BCC, 2 HR-BCC) lesions, moderate in 24 (30%) (9 LR-BCC, 15 HR-BCC) lesions, and dense in 25 (31%) (2 LR-BCC, 23 HR-BCC) lesions. Among the BCC lesions, 76% of the lesions with dense inflammation were infiltrative BCC, whereas 83.3% of the lesions with mild inflammation were nodular BCC. In the BSC group,

mild, moderate, and dense inflammation was observed in 4 (11.4%), 13 (37.1%), and 18 (51.4%) of the lesions, respectively. In the LR-BCC group, 28 (70%) lesions exhibited mild inflammation, whereas 18 (51.4%) and 23 (57.5%) of the lesions in the BSC and HR-BCC groups, respectively, exhibited dense inflammation ($P < 0.001$) (Figures 1–5).

Among the 115 lesions included in the study, lymphoid follicle formation was observed in only 8 (7%) lesions (7 in the HR-BCC group [5 infiltrative BCC and 2 micronodular BCC] and 1 in the LR-BCC group [nodular BCC]) ($P = 0.003$).

In all, 53 (46.1%) of the 115 lesions had desmoplastic stroma, versus fibromyxoid stroma in 62 (53.9%) of the lesions (Figures 6 and 7). In lesions with desmoplastic stroma, hypercellular stroma with thick collagen bundles was detected by Masson's trichrome stain, whereas in lesions with fibromyxoid stroma, hypocellular myxoid stroma was detected by Alcian blue stain (Figures 8 and 9). Desmoplastic stroma was most commonly observed in lesions with dense (56.6%) and moderate (39.6%) inflammation, whereas fibromyxoid stroma was most commonly observed in lesions with mild inflammation (51.6%).

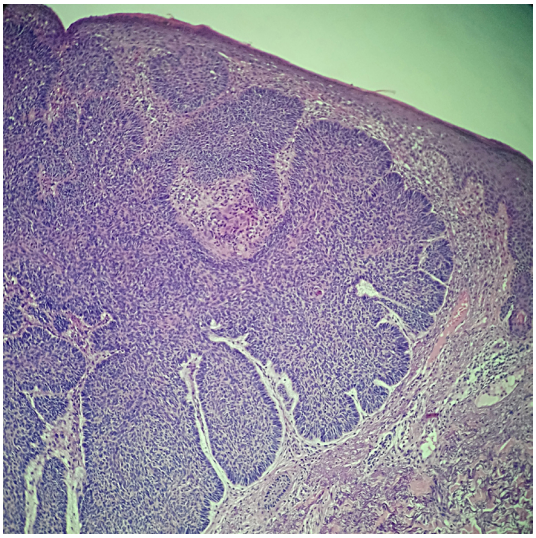

Figure 1. Nodular BCC with mild inflammation (H&E, 200×).

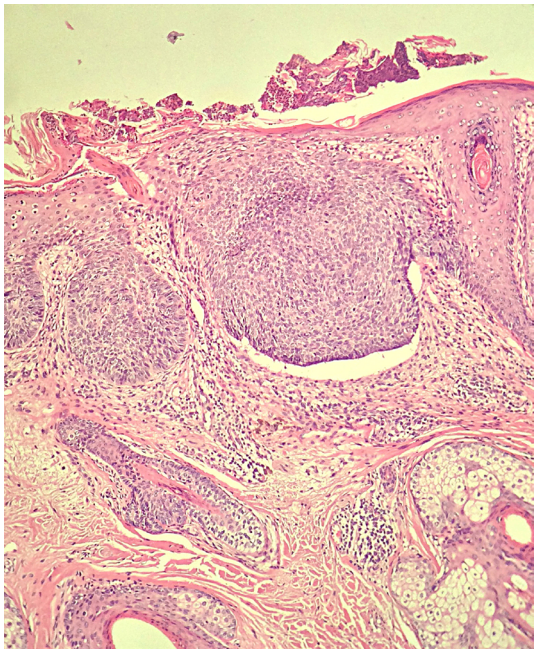

Figure 2. Superficial BCC with mild inflammation (H&E, 200×).

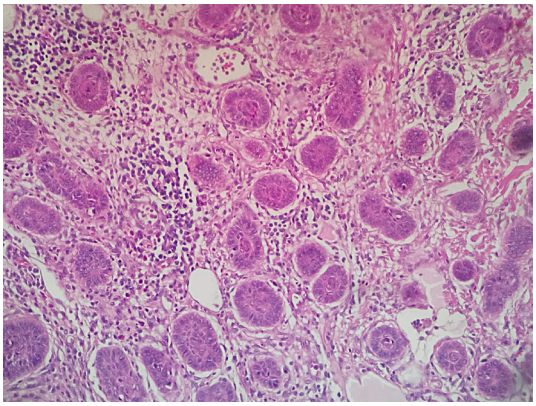

Figure 3. Micronodular BCC with moderate inflammation (H&E, 400×).

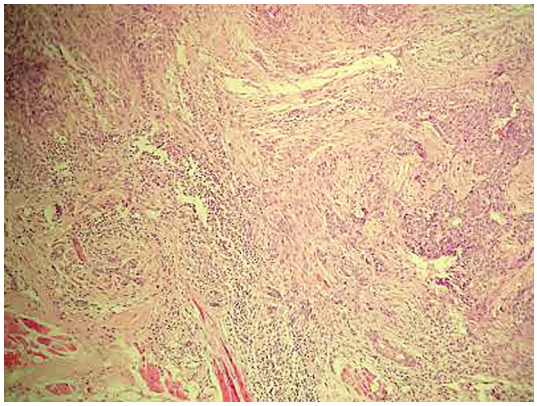

Figure 4. Infiltrative BCC with dense inflammation (H&E, 200×).

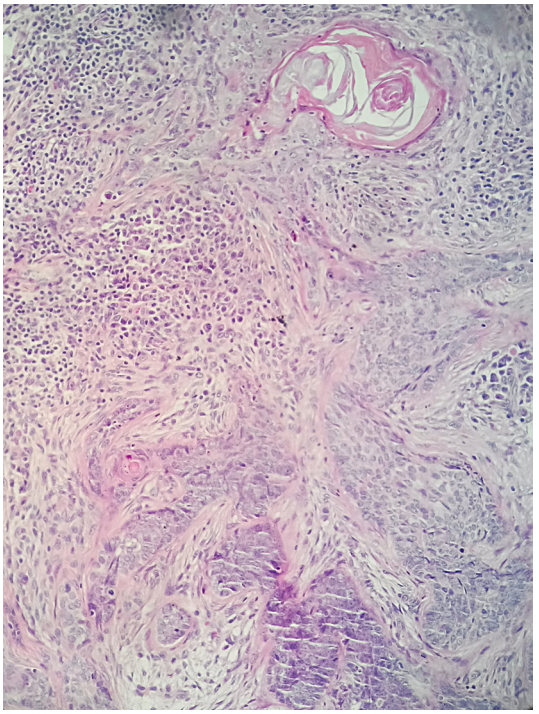

Figure 5. BSC with a transition zone with dense inflammation (H&E, 400×).

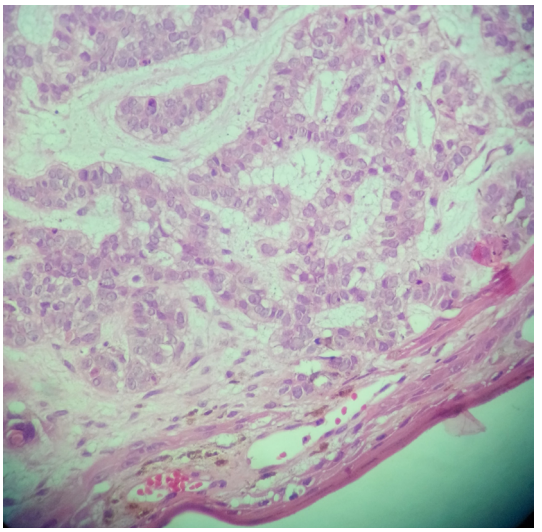

Figure 6. Tumor cells separated by myxoid stroma (H&E, 400×).

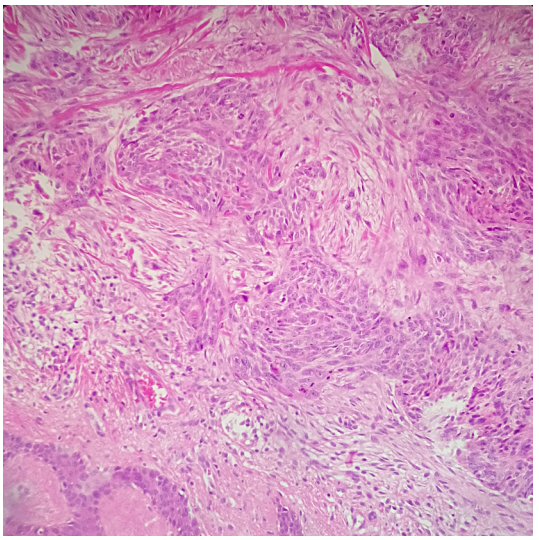

Figure 7. Tumor cells separated by desmoplastic stroma (H&E, 400×).

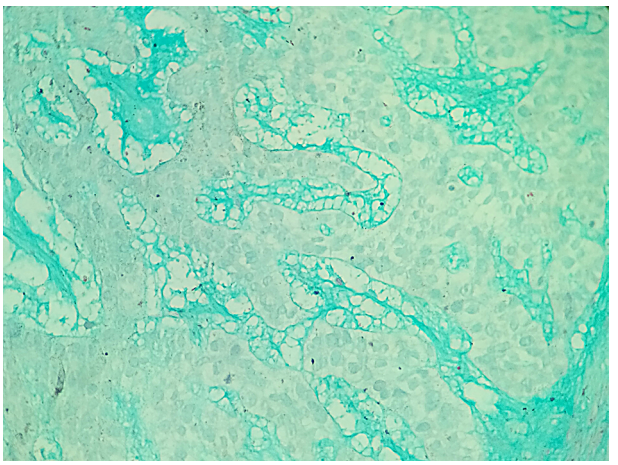

Figure 8. Histological section showing myxoid stroma reaction with Alcian blue (400×).

All lesions (100%) in the LR-BCC group had fibromyxoid stroma, whereas 21 (61.8%) and 32 (80%) of the lesions in the BSC group and HR-BCC group, respectively, had desmoplastic stroma ($P < 0.001$). In the HR-BCC group all of the infiltrative BCC cases had desmoplastic stroma, whereas 80% of the micronodular BCC cases had fibromyxoid stroma. Perivascular inflammation in adjacent dermis was present in 91.4%, 97.5%, and 55% of the lesions in the BSC, HR-BCC, and LR-BCC groups, respectively ($P < 0.001$). All of the lesions with dense perivascular inflammation (100%) were observed in the HR-BCC (65%) and BSC (35%) groups. Statistical analysis showed that there was no association between lesion location and sex, age, or peritumoral inflammation ($P = 0.375$, $P = 0.146$, and $P = 0.697$, respectively), or between stromal reactions and sex, age, or peritumoral inflammation ($P = 0.064$, $P = 0.351$, and $P = 0.351$, respectively). A detailed comparison of the

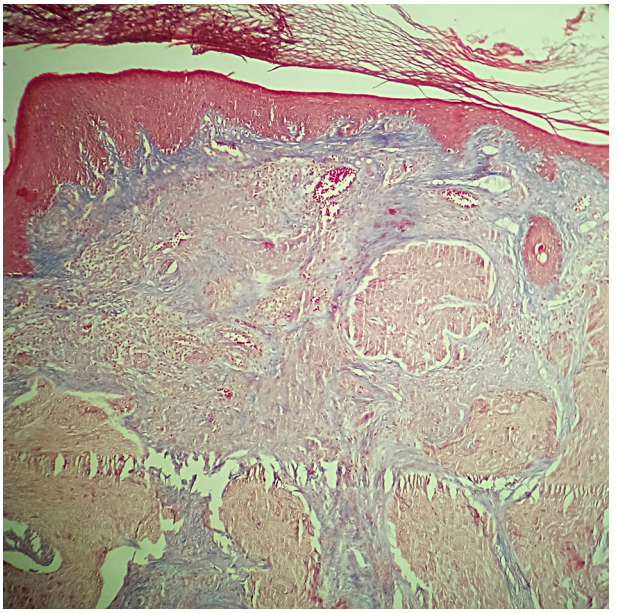

Figure 9. Histological section showing desmoplastic stroma reaction with Masson's trichrome (200×).

BSC, LR-BCC, and HR-BCC groups is shown in Table 1. A comparison of host immune responses and stromal reactions according to BCC subtype is shown in Table 2.

4. Discussion

The correlation between host immune responses and BCC subtypes is a contentious issue. Kaur et al. reported that significant stromal and inflammatory patterns correlate with BCC subtypes and tumor progression (7). They observed moderately dense peritumoral inflammation in superficial BCC with features of old regression, whereas little or no peritumoral inflammation was observed in micronodular BCC. Furthermore, they did not observe dense inflammation in any infiltrative BCC. Lesions with dense inflammation were more commonly noted in the LR-BCC (superficial/nodular) group than in the HR-BCC (infiltrative/micronodular/morphoeic) group in their study. In contrast, in the present study most of the infiltrative BCC lesions had dense inflammation and most of the micronodular BCC lesions had moderately dense inflammation. Furthermore, lesions with dense inflammation were most commonly observed in the present study's HR-BCC group. In addition, unlike Kaur et al. (7), we did not evaluate regression components in BCC lesions and, furthermore, the number of superficial BCC cases in the present study was limited, which makes it difficult to compare the superficial BCC findings. In Kaur et al.'s study, lymphoid follicle formation was infrequent (3%) and was associated with HR-BCC (7). Similarly, in the present study lymphoid follicle formation was observed in

Table 1. Comparison of the BSC, LR-BCC, and HR-BCC groups.

Clinicopathological feature	BSC (n = 35)	HR-BCC (n = 40)	LR-BCC (n = 40)	P
Mean age, years, mean ± SD	70.3 ± 11.1	69.4 ± 11.1	65 ± 11.4	0.098
Female/male	17/18	25/15	17/23	0.184
Location, n (%)				
• Head-neck	33 (94.3)	39 (97.5)	37 (92.5)	0.293
• Trunk	0 (0)	1 (2.5)	1 (2.5)	
• Extremity	2 (5.7)	0 (0)	0 (0)	
Peritumoral inflammation, n (%)				
• Absent	0 (0)	0 (0)	1 (2.5)	<0.001
• Mild	4 (11.4)	2 (5)	28 (70)	
• Moderate	13 (37.1)	15 (37.5)	9 (22.5)	
• Dense	18 (51.4)	23 (57.5)	2 (5)	
Adjacent perivascular inflammation, n (%)				
• Absent	3 (8.6)	1 (2.5	18 (45)	0.001
• Mild	12 (34.3)	6 (15)	16 (40)	
• Moderate	13 (37.1)	20 (50)	6 (15)	
• Dense	7 (20)	13 (32.5)	0 (0)	
Lymphoid follicle formation	0 (0)	1 (2.5)	8 (7)	0.003
Peritumoral stroma				
• Fibromyxoid	14 (40)	8 (20)	40 (100)	<0.001
• Desmoplastic	21 (60)	32 (80)	0 (0)	

Table 2. Comparison of host immune responses and stromal reactions between the LR-BCC and HR-BCC groups.

Feature	LR-BCC (n = 40)		HR-BCC (n = 40)		P
	Superficial BCC (n = 4)	Nodular BCC (n = 36)	Micronodular BCC (n = 10)	Infiltrative BCC (n = 30)	
Peritumoral inflammation, n (%)					
• Absent	0 (0)	1 (2.8)	0 (0)	0 (0)	
• Mild	3 (75)	25 (69.4)	1 (10)	1 (3.3)	
• Moderate	0 (0)	9 (25)	5 (50)	10 (33.3)	<0.001
• Dense	1 (25 %)	1 (2.8)	4 (40)	19 (63.3)	
Adjacent perivascular inflammation, n (%)					
• Absent	1 (25)	17 (47.2)	1 (10)	0 (0)	
• Mild	2 (50)	14 (38.9)	3 (30)	3 (10)	
• Moderate	1(25)	5 (13.9)	4 (40)	16 (53.3)	<0.001
• Dense	0 (0)	0 (0)	2 (20)	11 (36.7)	
Lymphoid follicle formation, n (%)	0 (0)	1 (2.8)	2 (20)	5 (16.7)	0.131
Peritumoral stroma, n (%)					
• Fibromyxoid	4 (100)	36 (100)	8 (80)	0 (0)	<0.001
• Desmoplastic	0 (0)	0 (0)	2 (20)	30 (100)	

only 7% of lesions and was also associated with HR-BCC. Moreover, in Kaur et al.'s study (7), sex- and site-specific differences were not observed, as in the present study. In contrast to Kaur et al.'s study (7), we also scored the density of perivascular inflammation in the adjacent dermis and noted that dense perivascular inflammation was associated with HR-BCC and BSC.

The functional role of peritumoral inflammation in BCC is not clear. In previous studies both antitumor and protumor effects of peritumoral inflammation were reported (9). Differences in findings between studies might be related to the different types of immune cells in peritumoral inflammation, i.e. an increase in the number of regulatory T cells suppresses antitumor response and is associated with a poor prognosis, whereas dendritic cells and tumor-associated macrophages play a role in tumor eradication (10–12).

The presence of denser inflammation in the HR-BCC and BSC groups in the present study might have been due to two factors: inflammatory cells could be recruited in response to some characteristics of the tumors, or inflammatory cells could secrete factors that influence the BCC phenotype. Delineation of the inflammatory cell types in peritumoral inflammation in such tumors is needed to further understand their functional roles.

The association between BCC subtypes and peritumoral stroma is also contentious. Lesack and Naugler evaluated the morphometric characteristics of tumor nests and their associated peritumoral stroma in 98 digitized BCC histology slides (including superficial, nodular, and infiltrative lesions), and they reported that the infiltrative subtype was associated with thicker stroma and a lower tumor/stroma ratio (13). Dixon et al. and Jacobs et al. observed that fibrous stroma was associated with less aggressive tumors, and that the incidence of hyalinization in stroma increased with BCC aggressiveness (14,15). Bartos et al. reported that there was marked dermal fibroplasia and chronic inflammatory infiltration of varying intensity in the majority of relapsing BCC lesions (16). Similarly, Pieh et al. showed that multiple recurrences were associated with sclerosing BCC (17). Kaur et al. also evaluated stromal reactions in various BCC subtypes and noted that infiltrative/morphoeic BCC was associated with desmoplastic tumor stroma (7). Similarly, in the present study all infiltrative BCC lesions had desmoplastic stroma. However, Kaur et al. reported loss of stromal tumor responses in micronodular BCC, whereas most micronodular BCC lesions in the present study had fibromyxoid stroma (7). Despite some differences between these studies, all the findings indicate that there are both quantitative and qualitative differences in peritumoral stroma in different BCC subtypes. However the functional role of peritumoral stroma in BCC remains unclear (13,18,19).

Peritumoral stroma is composed largely of activated fibroblasts and inflammatory cells in a complex interaction with tumor nests. Many paracrine factors, including extracellular matrix proteins and growth factors, are secreted by both stromal fibroblasts and inflammatory cells in stroma, and these are able to change the expression of many genes and affect tumor growth, angiogenesis, and metastasis (13,20). To date, identification of the precise

mechanisms underlying the complex interactions between stroma and tumor requires additional research.

The present findings show that desmoplastic stroma was associated with dense inflammation, which might have been due to two factors: inflammatory cells secreting factors that change stromal characteristics, or stroma-derived factors that may recruit additional inflammatory cells. A better understanding of the interactions between peritumoral inflammation and stromal characteristics would be possible if the types and functions of immune cells in peritumoral inflammation were identified.

In contrast to earlier studies, the present study also evaluated host immune responses and stromal reactions in BSC. Although the literature is lacking in studies on stromal reactions in BSC, they are suggested to be desmoplastic by some authors (1). In addition, the present findings show that although some BSC lesions had fibromyxoid stroma, most of the BSC lesions had desmoplastic stroma and dense inflammation, as in the HR-BCC group. The present findings also add support for considering BSC in the HR-BCC group.

In conclusion, BSC lesions in the present study were similar to HR-BCC lesions in terms of host immune responses and stromal reactions. Furthermore, dense peritumoral inflammation and desmoplastic stroma were associated with HR-BCC and BSC. In order to gain a better understanding of the functional role of host peritumoral inflammation and stroma in BCC subtypes, additional research is required.

References

1. Garcia C, Poletti E, Crowson AN. Basosquamous carcinoma. J Am Acad Dermatol 2009; 60: 137–143.
2. 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: 83–86.
3. Volkenstein S, Wohlschlaeger J, Liebau J, Arens A, Lehnerdt G, Jahnke K, Neumann A. Basosquamous carcinoma--a rare but aggressive skin malignancy. J Plast Reconstr Aesthet Surg 2010; 63: e304–e306.
4. Crowson AN. Basal cell carcinoma: biology, morphology and clinical implications. Mod Pathol 2006; 19: 127–147.
5. Bhanja KC, Mallick SK, Sharma S, Saha R. Cutaneous basaloid squamous of the face, a rare variant: a case report. IJCRI 201 2; 3: 20–23.
6. Tan SH, Chong AW, Nazarina AR, Prepageran N. Basaloid squamous cell carcinoma of the oropharynx: comparison of two cases and review of the literature. Otolaryngol Pol 2014; 68: 268–270.
7. Kaur P, Mulvaney M, Carlson JA. Basal cell carcinoma progression correlates with host immune response and stromal alterations: a histologic analysis. Am J Dermatopathol 2006; 28: 293–307.
8. Betti R, Menni S, Radaelli G, Bombonato C, Crosti C. Micronodular basal cell carcinoma: a distinct subtype? Relationship with nodular and infiltrative basal cell carcinomas. J Dermatol 2010; 37: 611–616.
9. Kaporis H, Del Rosso JQ. Peritumoral inflammation in basal cell carcinoma: fundamentals, clinical significance, and changes after topical imiquimod therapy. Cosmet Dermatol 2008; 21: 38–43.
10. Kaporis HG, Guttman-Yassky E, Lowes MA, Haider AS, Fuentes-Duculan J, Darabi K, Whynot-Ertelt J, Khatcherian A, Cardinale I, Novitskaya I et al. Human basal cell carcinoma is associated with Foxp3+ T cells in a Th2 dominant microenvironment. J Invest Dermatol 2007; 127: 2391–2398.
11. Zou W. Regulatory T cells, tumour immunity and immunotherapy. Nat Rev Immunol 2006; 6: 295–307.
12. Stary G, Bangert C, Tauber M, Strohal R, Kopp T, Stingl G. Tumoricidal activity of TLR7/8-activated inflammatory dendritic cells. J Exp Med 2007; 204: 1441–1451.
13. Lesack K, Naugler C. Morphometric characteristics of basal cell carcinoma peritumoral stroma varies among basal cell carcinoma subtypes. BMC Dermatol 2012; 12: 1.
14. Dixon AY, Lee SH, McGregor DH. Factors predictive of recurrence of basal cell carcinoma. Am J Dermatopathol 1989; 11: 222–232.
15. Jacobs GH, Rippey JJ, Altini M. Prediction of aggressive behavior in basal cell carcinoma. Cancer 1982; 49: 533–537.
16. Bartos V, Adamicova K, Kullova M, Pec M. Comparison of histological types of primary and subsequent relapsing basal cell carcinomas of the skin. Klin Onkol 2012; 25: 262–266.
17. Pieh S, Kuchar A, Novak P, Kunstfeld R, Nagel G, Steinkogler FJ. Long-term results after surgical basal cell carcinoma excision in the eyelid region. Br J Ophthalmol 1999; 83: 85–88.
18. Tlsty TD, Coussens LM. Tumor stroma and regulation of cancer development. Annu Rev Pathol 2006; 1: 119–150.
19. Bhowmick NA, Moses HL. Tumor-stroma interactions. Curr Opin Genet Dev 2005; 15: 97–101.
20. Reuter JA, Ortiz-Urda S, Kretz M, Garcia J, Scholl FA, Pasmooij AM, Cassarino D, Chang HY, Khavari PA. Modeling inducible human tissue neoplasia identifies an extracellular matrix interaction network involved in cancer progression. Cancer Cell 2009; 15: 477–488.