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Cluster Differentiation 133 Expression in Patients with Basal Cell Carcinoma: An Immunohistochemical Review

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Abstract
Basal cell carcinoma (BCC) is the most common skin cancer globally. The role of cancer stem cell (CSC) markers like *CD133* in BCC pathogenesis and diagnosis remains controversial, with limited data from the Indonesian population. This study aimed to determine the difference in *CD133* expression between BCC tissue and normal skin, assessing its potential as a diagnostic biomarker. An analytical observational study with a case-control design was conducted. Samples included 13 archived paraffin-embedded BCC tissues and 13 normal skin controls from Dr. Moewardi General Hospital (2021–2024). *CD133* expression was evaluated using immunohistochemical staining and assessed with the Allred scoring system by a single pathologist. Data were analyzed using the Chi-square test. The mean Allred score for *CD133* expression was high in both groups: 7.15 ± 0.56 in BCC tissue and 7.31 ± 0.48 in normal skin. Strongly positive expression was observed in 92.3% (12/13) of BCC samples and 100% (13/13) of normal skin controls. Statistical analysis revealed no significant difference in *CD133* expression between the two groups ($p = 0.308$). *CD133* expression is not significantly elevated in BCC compared to normal skin. These findings indicate that *CD133* lacks utility as a standalone diagnostic or prognostic marker for BCC in this setting. Future research should explore alternative or complementary CSC markers and consider fresh tissue samples to validate these results.
Keywords: Basal Cell Carcinoma; CD133; Cancer Stem Cell; Immunohistochemistry

INTRODUCTION

Basal cell carcinoma (BCC), previously known as basal cell epithelioma, is the most common type of cancer in humans. Although *BCC* exhibits slow growth and rarely metastasizes, it can lead to significant local tissue damage if left untreated or if treatment is delayed (McDaniel et al., 2024). Accounting for about 80% of all non-melanoma skin cancers, *BCC* arises from the basal layer of the epidermis and hair follicle sheaths. The risk factors include genetic, environmental, and immunological factors (Toha et al., 2019).

The incidence of *BCC* has steadily increased over the past five decades at an annual rate of 3–10%, demonstrating a higher prevalence in males, especially in elderly populations exposed to chronic ultraviolet radiation (Borzęcka-Sapko et al., 2020). In Indonesia, skin cancer is the third most common malignancy after cervical and breast cancer, with *BCC* representing 65.5% of total skin cancers, followed by squamous cell carcinoma (SCC) (23%) and malignant melanoma (7.9%) (Wilvestra et al., 2018). *BCC* typically appears as papules that are skin-colored or pink with ulceration or telangiectasia. *BCC* most commonly affects the head and neck regions. Surgical treatments, including excision, electrodesiccation and curettage, cryosurgery, and Mohs micrographic surgery, generally have more than a 95% cure rate for localized lesions (Dai et al., 2018; Giorgi et al., 2020).

In recent years, research has increasingly shown that tumor growth and development are controlled by a small group of cells called cancer stem cells (CSCs). *Cluster of Differentiation 133* (CD133) is emerging as a potential biomarker of interest in various malignancies, including skin cancers. CD133 is a glycoprotein with five transmembrane domains that has been identified in various human tissues (Sabet et al., 2014). It is associated with stem cell characteristics, proliferation, and differentiation potential (Liu et al., 2016). Studies show CD133 expression is significantly higher in *BCC* patients than in trichoblastoma (TB) patients. In addition, CD133 expression was significantly associated with mitotic activity in KSB patients (Bi et al., 2022). However, another study showed negative immunohistochemical staining for CD133 in both normal skin and cases of *BCC*, SCC, and TB (Youssif et al., 2022). Based on this controversy and the limited number of studies currently

available on the CD133 expression profile of *BCC* patients, especially in Indonesia, further studies are essential to establish its potential diagnostic and prognostic significance in *BCC* patients.

This study aimed to analyze the difference in CD133 expression in *basal cell carcinoma* tissue compared to normal skin. Specifically, this study was designed to evaluate whether CD133 can act as a diagnostic or prognostic marker in KSB patients in the Indonesian population. The benefits of this study include: (1) providing empirical evidence regarding the expression profile of CD133 in KSB patients in Indonesia; (2) serving as a reference for pathologists and clinicians in assessing the potential of CD133 as a biological marker of tumors; and (3) providing the basis for further research on the role of cancer stem cells in the pathogenesis of *basal cell carcinoma*.

RESEARCH METHOD

This analytical observational study with case control design was conducted at the Dr. Moewardi General Hospital in Surakarta from April to July 2025. The study samples included 13 BCC tissues and 13 normal skin controls taken from paraffin blocks from 2021-2024. Inclusion criteria of BCC cases were >50 years old patients who underwent skin biopsy with primary basal cell carcinoma diagnosis, with no previous history of other malignancies, infections, or autoimmune disease. Exclusion criteria included recurrent basal cell carcinoma cases and unidentifiable paraffin blocks. Normal skin controls had the same inclusion and exclusion criteria without basal cell carcinoma history. Ethical approval was obtained from the Dr. Moewardi General Hospital Ethics Committee (number: 2.487/X/HREC/2024).

Samples from paraffin blocks were immunohistochemically stained with primary antibodies based on the Novolink Min Polymer Detection system to measure CD133 expression. It involved sectioning paraffin-embedded tissue blocks at 4–5 μm, mounting on poly L-lysine slides, and incubating at 37 °C overnight. Sections were deparaffinized in xylol and graded alcohols then treated with 3% H_2O_2 in methanol to block endogenous peroxidase. Antigen retrieval was performed using Tris-EDTA pH 9 buffer in a microwave. The tissue is then incubated with a primary antibody, followed by biotin and streptavidin, and finally with a peroxidase enzyme substrate (DAB) to visualize positive staining. Slides are mounted and cover-slipped. CD133 expression density is evaluated under a light microscope by an anatomical pathologist, assessing at least four randomly selected fields of view. Allred score is calculated by summing percentage of positive tumor cells as proportion score and intensity score from staining strength of the positive tumor cells. Allred scores of 0 to 2 are considered negative, scores of 3 to 4 are considered weakly positive, scores 5 to 6 are considered moderately positive, while scores 7 to 8 are considered strongly positive.

Data analysis was conducted using SPSS Statistics version 26.0. The patient characteristics were described as univariat analysis. Bivariate analysis was done using Chi square test to compare mean Allred CD133 scores between patient groups.

RESULTS AND DISCUSSION

This study obtained a total of 26 samples consisting of 13 BCC patients and 13 normal skin subjects. Demographic analysis revealed that both groups were predominantly female, with 9 participants (69.2%) in each group, while males constituted 4 participants (30.8%) in each group. The age distribution showed that BCC subjects were most commonly found in those more than 55 years old (53.8%). While normal skin subjects demonstrated the highest frequency in the 51-55 years age range (53.8%) (Table 1).

Occupational characteristics revealed varying patterns between the two groups. The BCC subject predominantly consisted of individuals with high UV exposure occupations, including farmers, laborers, and sellers (92.1%). In contrast, the normal skin control group

showed different occupational distribution with sellers (46.0%), cleaning service (30.7%), and security guards (15.3%). UV exposure intensity analysis found that all BCC subjects (100%) experienced 3-6 hours of UV exposure daily. While normal skin subjects showed greater variability from less than 2 hours to more than 6 hours of daily UV exposure (Table 1).

Table 1. Demographic characteristic of the study subject

Characteristics	BCC		Normal Skin	
	Number (n=13)	Precentage (%)	Number (n=13)	Precentage (%)
Gender				
Female	9	69.2	9	69.2
Male	4	30.8	4	30.8
Age				
46-50 y.o	0	0	2	15.4
51-55 y.o	6	46.2	7	53.8
56-60 y.o	7	53.8	4	30.8
Occupation				
Laborer	4	30.7	1	0
Farmer	6	46.1	0	0
Seller	2	15.3	4	30.7
Cleaning service	0	0	4	30.7
Security	0	0	2	15.3
Housewife	0	0	1	7.6
Driver	0	0	1	7.6
Employee	1	7.6	0	0
UV Exposure Intensity				
≤ 2 hours/day	0	0	4	30.7
3-6 hours/day	13	100	8	61.5
> 6 hours/day	0	0	1	7.6

Source: Primary data, 2025

Clinical presentations of BCC subjects showed nodular type was the most prevalent subtype (61.5%), followed by morpheaform (23.1%) and pigmented type (15.4%). BCC subject in this study most commonly affected the H-zone, including orbital and nasal region (69.3%) **(Figure 1).**

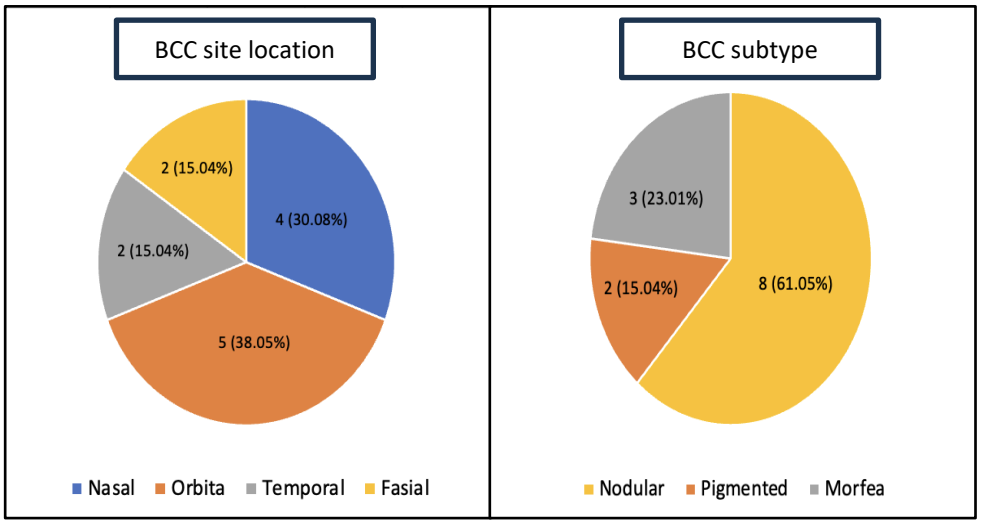

Figure 1. Characteristic of BCC subject based on anatomical lesion and histopathological subtype

Source: Researcher Documentation, 2025

Immunohistochemical examination of CD133 expression using the Allred scoring system demonstrated comparable results between both groups. The BCC group showed a mean Allred score of 7.15 ± 0.555, with scores ranging from 6 to 8. While the normal skin group showed a mean Allred score of 7.31 ± 0.480, with scores ranging from 7 to 8. Both groups had a median value of 7 **(Table 2) (Figure 2 & 3)**.

Table 2. Description of CD133 expression in BCC and normal skin subject

Group	CD133 expression (*Allred score*)				
	Minimum	Maximum	Median	Mean	Deviation standard
BCC	6	8	7	7.15	0.555
Normal Skin	7	8	7	7.31	0.480

Source: Primary data, 2025

All subtypes and anatomical locations of BCC subject demonstrated Allred scores within the 7-8 range, which categorize as strongly positive for CD133 expression. The greatest mean Allred score (7.33) was seen in morpheaform type, followed by nodular type (7.13), and pigmented type (7.00). Anatomical site analysis revealed the maximum mean Allred score was 7.50 in temporal region lesions, followed by orbital region with a value of 7.20, while the nasal and facial regions showed the same mean scores of 7.00 **(Table 3).**

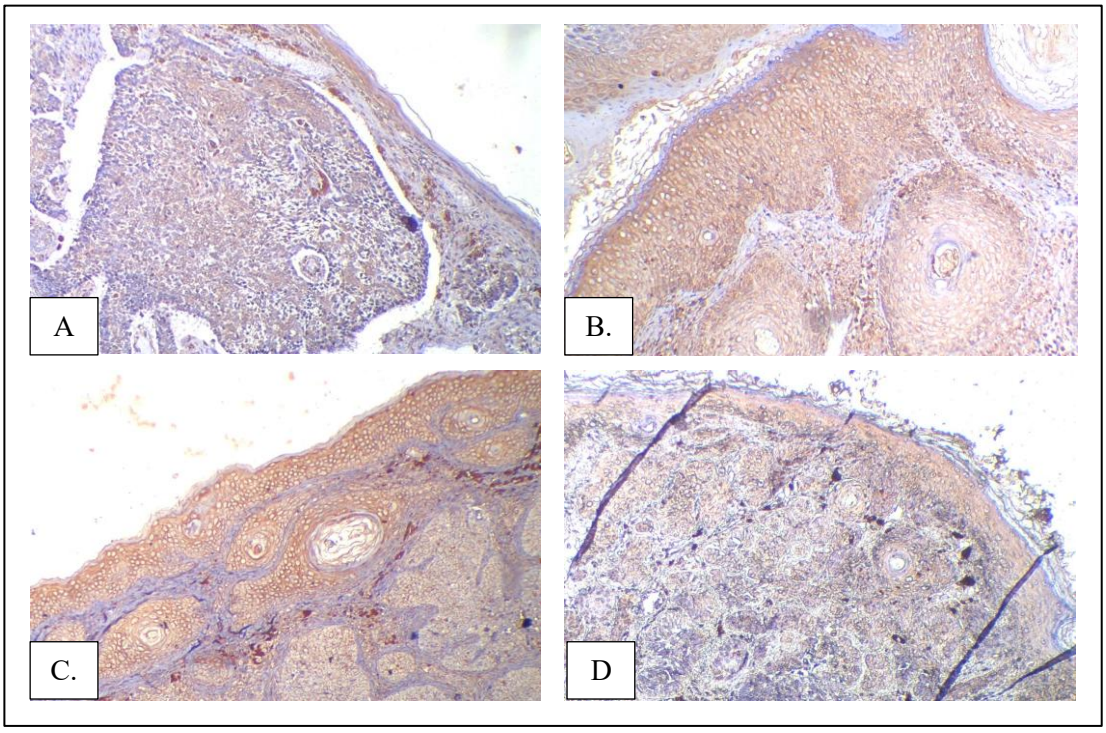

Figure 2. Immunohistochemical images of CD133 expression in the BCC group (100x magnification). (A-C) Immunohistochemical image of CD133 expression with Allred score 7. (D) Immunohistochemical image of CD133 expression with Allred score 8.

Source: Researcher Documentation, 2025

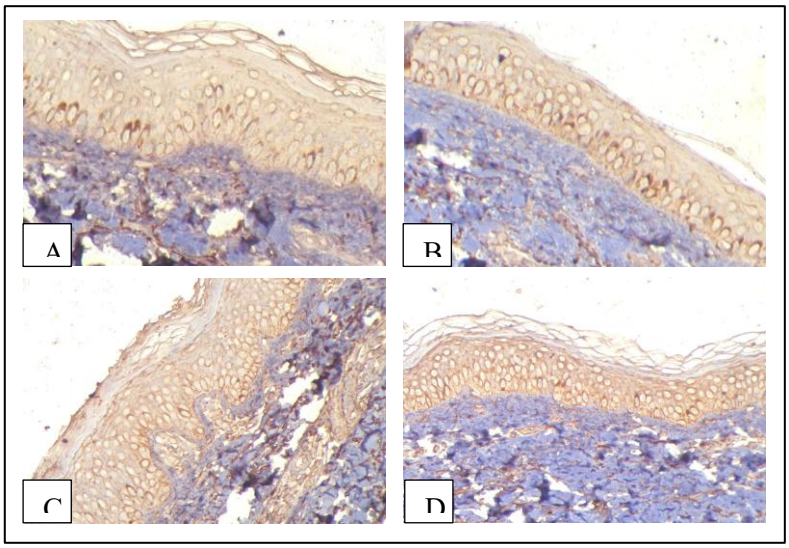

Figure 3. Immunohistochemical images of CD133 expression in the normal skin group (100x magnification). (A-C) Immunohistochemical image of CD133 expression with Allred score 7. (D) Immunohistochemical image of CD133 expression with Allred score

Source: Researcher Documentation, 2025

Table 3. Mean Allred score difference in BCC group based on histopathological subtype and anatomical site

		CD133 expression (Allred score)				
		Minimum	Maximum	Median	Mean	Number
BCC Subtype	Nodular	7	8	7	7.13	8
	Pigmen	7	7	7	7.00	2
	Morfea	6	8	8	7.33	3
BCC Anatomical Site	Nasal	7	7	7	7.00	4
	Orbita	6	8	7	7.20	5
	Temporal	7	8	7.5	7.50	2
	Fasial	7	7	7	7.00	2

Source: Primary data, 2025

Statistical analysis using the Shapiro-Wilk test showed abnormal data distribution ($p<0.05$). Chi-square analysis comparing CD133 expression in BCC and normal skin groups showed that 12 out of 13 BCC patients expressed strong positive CD133, while 1 patient expressed moderate positive expression. In contrast, all 13 normal skin samples showed strong positive CD133 expression (Figure 4). The chi-square test yielded a p-value of 0.308, indicating no statistically significant difference in CD133 expression between BCC lesions compared to normal skin tissue. These results contradicted initial hypothesis that BCC would express significantly higher CD133 levels compared to normal skin.

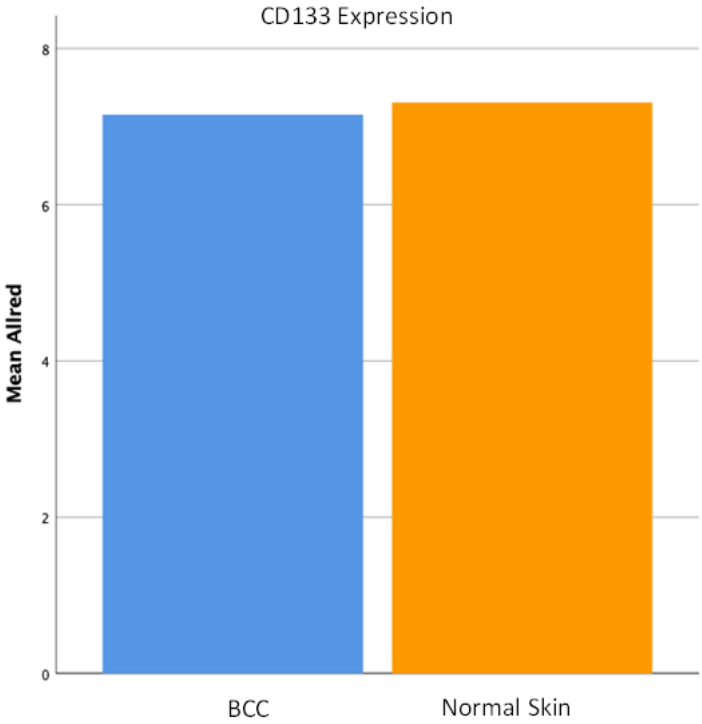

Figure 4. Graphic of mean Allred score between KSB and normal skin groups

Source: Researcher Documentation, 2025

The predominance of females (69.2%) subject in the BCC group is consistent with several previous studies showing variability in gender in BCC incidence depending on

population and geography (Bi et al., 2022). This finding is supported by studies in Indonesia over 20 years at Cipto Mangunkusumo Hospital showing BCC patients were dominated by women. Women's skin is structurally thinner with lower collagen density in the dermis compared to men. This leads to different skin responses to environmental stress including UV exposure (Arisanty et al., 2021). In addition, the greater attention to appearance means that BCC is more likely to be detected early in female than male (Gangan, 2022).

This study shows that most BCC patients are found in the 51 – 60 years age category (92.3%). It is consistent with global epidemiological data reporting increased BCC incidence with age, especially after the fifth decade of life. One of the major risk factors for skin cancer is prolonged sun exposure. Damage accumulation caused by sun exposure increases the risk of skin cancer related to age (Rulandani et al., 2025; Hardianto et al., 2025). In addition, multiple meta-analyses studies showed that outdoor occupations have a 1.43 times higher risk for developing BCC compared to indoor occupations (Bauer et al., 2011). In line with the study, occupations with a history of high UV exposure including farmers, laborers and salespeople were found in 92.1% of patients.

The results of this study indicate that all BCC subjects (100%) received sun exposure for 3–6 hours per day. These results are in line with a study conducted at Hasan Sadikin Hospital, which showed that most BCC patients experienced sun exposure for 8–40 hours per week (Rulandani et al., 2025). Intense and intermittent UV exposure is associated with BCC because the high accumulation of intense UV radiation received contributes to DNA damage and tumorigenesis in skin cells. This type of exposure overwhelms the skin's natural repair mechanisms, causing mutations that promote the development of BCC (Iannacone et al., 2012).

Prevalence of histopathological subtypes with nodular type predominance (61.5%) is consistent with Cochrane systematic reviews finding that identify nodular BCC as the most common subtype. Identification of morpheaform type (23.1%) has important clinical implication because this subtype forms part of high-risk BCC subgroup with higher recurrence potential (Thomson et al., 2020). The location distribution in orbital and nasal areas totaling 69.3% supports evidence from multiple RCTs showing BCC predilection in the H-zone of the face which has high UV exposure. This is consistent with BCC pathophysiology driven mainly by sun damage (Jansen et al., 2018).

CD133 expression based on Allred Score in the normal skin group showed strong positive results for all samples in this study. This is comparable and even higher than the BCC group with strong positive Allred Score in 12 cases and moderate positive in 1 case. CD133 expression in both normal and BCC skin groups had nearly identical values, indicating no variation for comparison. This could result in the chi-square test showing no significant results.

CD133 expression can physiologically appear in normal skin regions, including hair follicle bulges and basal layers of the epidermis, both of which are known to be reservoirs of skin stem cell. CD133 is found in these regenerative zones, indicating its role in maintaining epidermal homeostasis and promoting skin regeneration. CD133 expression in normal human skin is confined to the apical or apicolateral plasma membrane of cells in the glandular structures of eccrine glands, particularly within the ductal and secretory components. Keratinocytes and non-glandular epidermal cells do not have significant CD133 positivity. This location highlights the importance of CD133 in the secretory function essential for the preservation of epidermal stem cells (Nam-Cha et al., 2013). Additionally, CD133 is found in hepatic Hering canals, bile ducts, small interlobular bile ducts, endometrium, and cervix, indicating its role as a common apical or apicolateral epithelial membrane marker in glandular tissues.

High CD133 expression facilitates tissue signaling network formation at the plasma membrane by recruiting proteins involved in cytoskeletal reorganization, protein exchange, and signal transduction, which are essential for regulating membrane protrusion structure, ciliary

dynamics, and various cellular processes (Moreno-Londoño & Robles-Flores, 2024; Pleskač et al., 2024). Furthermore, elevated CD133 expression provides adaptive advantages by enhancing cell survival under stress conditions, with protein expression dynamically regulated by hypoxia through HIF-1α upregulation, enabling cells to better cope with oxygen-limited environments (Moreno-Londoño & Robles-Flores, 2024; Glumac & LeBeau, 2018).

CD133 expression can appear similar between normal skin and BCC skin through cell plasticity mechanisms known as "phenotype switching" phenomena. It occurs on the apical surface of glandular epithelial cells, such as eccrine and apocrine glands, which physiologically express CD133. This suggests that CD133 is a marker of glandular development, not a marker of stem cell (Nam-Cha et al., 2013). In addition, the neoplastic transformation process in BCC is frequently accompanied by tissue structural alterations and loss of differentiation, which significantly reduces the areas that physiologically produce CD133. Tumor cells will lose ductal structural and polarity, prioritizing proliferation above secretory function, resulting in diminished CD133 expression or its localization to specific regions. Thus, the elevated expression of CD133 as a stemness marker may be counterbalanced by the loss of differentiation in the majority of tumor cells (Nam-Cha et al., 2013; Korn et al., 2021). In colorectal cancer, CD133 is found on the luminal glandular surface of differentiated tumor cells, whereas undifferentiated tumor cells at the invasion front are CD133-negative. Studies demonstrate that CD133 is ubiquitously expressed in differentiated colonic epithelium in both adult mice and humans. CD133 positivity found in all EpCAM-positive epithelial cells indicating that CD133 represents a characteristic of normal intestinal epithelial differentiation rather than an exclusive stem cell marker. Overall, there is still debate over the reliability of CD133 as a CSC marker. Study results vary significantly based on several factors, including experimental design, culture conditions, cancer subtypes, study subjects, cell variability, and the microtumor environment (Glumac & LeBeau, 2018).

Future studies should prioritize the use of fresh tissue samples to minimize the confounding effects of paraffin block aging on biomarker expression. This is consistent with studies showing significantly reduced estrogen and progesterone receptor expression in samples older than a years (Chen et al., 2020). Additionally, it is important to fix the sample promptly to prevent time-dependent protein degradation. To address the inherent variability in CD133 expression and minimize inter-individual confounding factors such as genetic background, UV exposure history, immune system differences, and baseline tissue characteristics, future investigations should employ paired sampling approaches comparing intralesional with perilesional tissues from the same patients (Atkinson et al., 2013; Lupu et al., 2019). Given the controversial nature of CD133 as a cancer stem cell marker, subsequent studies should incorporate complementary immunohistochemical markers including BerEP4 as the gold standard for BCC diagnosis with 97-100% sensitivity and specificity. Furthermore, using CD31 for stromal assessment and CD44 for evaluating tumor aggressiveness patterns across different histopathological subtypes may be additional markers in future studies (Sunjaya et al., 2017; Cojocaru et al., 2022).

CONCLUSION

This study found no significant difference in CD133 expression between basal cell carcinoma (BCC) patients and those with normal skin, underscoring the ongoing controversy regarding CD133 as a diagnostic marker for BCC. Considering the study's limitations, future research should use fresh tissue samples to avoid antigen degradation, employ paired sampling of lesion and perilesional tissue to control for individual variability, and combine CD133 with other immunohistochemical markers such as BerEP4, CD44, and CD31 to better assess tumor characteristics. Additionally, exploring other stemness markers is necessary to gain a more comprehensive understanding of cancer stem cells' role in BCC pathogenesis.

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