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CD44 Expression in Malignant Epidermal Skin Tumors

Conflict of interest: nothing to declare.
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Abstract

Introduction. A transmembrane glycoprotein designated as CD44 is expressed in various cell types, including cancer stem cells. It also generally exhibits other variants that have been linked to the onset and progression of cancer.
Purpose. The aim of this study was to investigate how the CD44 molecule is expressed in epidermal skin tumors.
Materials and methods. In this study, we investigate the expression of CD44 in 63 formalin-fixed, paraffin-embedded tissue specimens. It includes 36 cases of basal cell carcinoma (BCC) and 27 cases of cutaneous squamous cell carcinoma (CSCC). By using immunohistochemistry (IHC), we examined the expression of CD44 in these cutaneous malignancies.
Results. Cutaneous squamous cell carcinomas (CSCC) had significant and strong CD44 expression in most of cases (77.8%), particularly at the invasive part; however, the majority of BCC typically showed no or focal reactivity (27.8%). The normal skin close to the lesion, demonstrated CD44 immunoreactivity. Chi-square test and effect size were calculated, and a strong link was found between CD44 positive expression and CSCC ($p<0.01$).
Conclusion. Findings suggest that CD44 expression in these skin malignancies may be related to tumor expansion and dissemination rather than just malignant transformation.
Keywords: CD44, squamous cell carcinomas, basal cell carcinomas, immunohistochemistry, skin cancer

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Экспрессия CD44 в злокачественных эпидермальных опухолях кожи

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Резюме

Введение. Трансмембранный гликопротеин, обозначаемый как CD44, экспрессируется в различных типах клеток, включая раковые стволовые клетки. Он также обычно имеет другие варианты, которые связаны с возникновением и прогрессированием рака.
Цель. Изучение экспрессии молекулы CD44 в эпидермальных опухолях кожи.
Материалы и методы. В данном исследовании мы изучили экспрессию CD44 в 63 образцах тканей, фиксированных формалином и заключенных в парафин. В исследование включены 36 пациентов с базальноклеточной карциномой кожи (БКК) и 27 – с плоскоклеточной карциномой кожи (ПКК). С помощью иммуногистохимии (ИГХ) мы исследовали экспрессию CD44 в материалах пациентов с данными злокачественными новообразованиями кожи.
Результаты. В большинстве случаев (77,8%) ПКК продемонстрировали значительную и сильную экспрессию CD44, особенно в инвазивной части; однако большинство случаев БКК обычно не проявляли реактивности или проявляли ее очагово (27,8%). В нормальной коже вблизи поражения наблюдалась иммунореактивность CD44. Были рассчитаны критерий хи-квадрат и величина эффекта, обнаружена сильная связь между положительной экспрессией CD44 и ПКК ($p<0,01$).
Заключение. Полученные данные свидетельствуют о том, что экспрессия CD44 в этих злокачественных новообразованиях кожи может быть связана с расширением и распространением опухоли, а не просто со злокачественной трансформацией.
Ключевые слова: CD44, плоскоклеточная карцинома, базальноклеточная карцинома, иммуногистохимия, рак кожи

■ INTRODUCTION

CD44 is a cell surface glycoprotein that occurs in many different molecular forms, such as the standard isoform and CD44 variant isoforms [1]. Human CD44 is encoded by 20 exons, 10 of which are constant for all isoforms. The standard isoform is encoded by ten constant exons and any pairing of the remaining variant exons are present in CD44 variant isoforms [2, 3]. CD44 participates in a number of biological functions, including cell adhesion, activation, proliferation, and differentiation [3]. Human cells that express CD44 include differentiated cells, embryonic stem cells and cancer cells [1, 2]. CD44 serves as a receptor for hyaluronan and various other extracellular matrix elements along with a cofactor for cytokines and growth factors [4]. Cancer progression and metastasis are significantly influenced by CD44 and hyaluronan interactions, which regulate cell proliferation, differentiation, survival and migration [5, 6]. CD44-binding with high molecular weight hyaluronan increases CXCL12-induced signaling and angiogenesis, their binding and consequent interaction to the neural Wiskott – Aldrich syndrome protein triggers polymerization of actin and EGFR activation [6–8]. It is typically expressed by keratinocytes, hair follicles, and sebaceous as well as eccrine epithelial cells in normal skin [9]. CD44 expression has been extensively studied in the context of various cancers, including skin tumors, where it plays essential role in tumor progression, spread, and resistance to treatment [3, 10]. CD44 is represented as a common marker for tumor stem cells and enhances epithelial to mesenchymal shift [1]. Different patterns of CD44 expression are seen in skin tumors, such as squamous cell carcinoma (SCC), basal cell carcinoma (BCC), and malignant melanoma (MM), reflecting the distinct molecular traits and pathophysiology of each tumor type [10].

Unregulated growth of squamous cells in the epidermis is known as cutaneous squamous cell carcinoma (CSCC) [11]. It contributes to 20% of all skin malignancies and is the second most frequently occurring malignant skin cancer after BCC [11, 12]. BCC is the most prevalent type of skin malignancy, typically slow-growing and locally invasive, and it originates in the basal layer of the epidermis and its appendages [13]. CD44 expression is frequently elevated in CSCC and is linked to the invasive capacity of the tumor; it is also associated with aggressive behavior and a bad prognosis indicating that it plays a role in promoting tumor cell adhesion and metastasis [10, 14]. However, its expression is less prominent in BCC that may contribute to tumor growth through changing in basal cells biology [15].

Understanding the diverse roles of CD44 in these skin tumor types offers insight into its potential as a therapeutic target, especially given its involvement in immune evasion, cancer cell interactions with the extracellular matrix and metastasis.

■ MATERIALS AND METHODS

Study design and setting

A retrospective study was conducted to assess the CD44 expression in skin tumors (CSCC and BCC).

Data collection

Data collection was carried out for a one-year duration during the period from 2023 to 2024 from the pathology archives of the Al-Sader Teaching Hospital and a private

lab. The study comprised 63 cases of skin malignancies. Selection was based on the availability of adequate clinical data and sufficient tissue material. Cases unsuitable for immunohistochemical analysis were excluded.

Staining

Formalin-fixed, paraffin-embedded tissue samples were obtained from the pathology archives of the Al-Sader Teaching Hospital and private lab from patients diagnosed with skin tumors, including 36 cases of BCC, and 27 cases of cutaneous squamous cell carcinoma (CSCC). Tissue blocks were cut into 4 μm-thick slices using a microtome and set on glass slides, then deparaffinized using xylene for 5 minutes and rehydrated through a succession of ethanol grades. The slides underwent hematoxylin staining (ready to use) for 8 minutes then rinsed in tap water. After that, counter stain with eosin (ready to use) was used for 1 minute then rinsed with water to remove excess stain. After staining, complete dehydration in absolute alcohol was done; and the slides were cleared and mounted. Then, the slides were left to cool at room temperature.

Immunohistochemistry (IHC) for CD44

After slide preparation, deparaffinization and rehydration of the sections was carried out by putting the slides in an oven with hot air set between 50–60 °C for 15 minutes, later moved to a xylene bath (two 5-minute xylene changes). Rehydration through two changes of fresh absolute ethanol (90–80%) 3 minutes for each. After being carefully rinsed in tap water for 30 seconds, the slides are placed in phosphate-buffered saline (0.01 M PBS, pH 7.4 PBS, Product No. P4417-tablet) for 30 min at room temperature for further rehydration. For antigen retrieval, primary antibodies CD44s monoclonal antibody (mouse anti-human CD44, clone DF1485, from Dako) have been applied for 45 minutes at room temperature and washed in PBS for 3 minutes. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide (ready to use) for 5 minutes then slides were immersed in PBS wash bath for 2 minutes. 100 μl of biotinylated secondary antibody (ready to use) conjugated with Sterptavidin-horseradish peroxidase (HRP) enzyme (ready to use) was applied to each slide, covering the tissue sections and incubated in a humidity chamber at room temperature for a minimum of 30 minutes. One drop (about 20μl) of DAB chromogen was used to visualize the enzyme-conjugated secondary antibody which combines with the enzyme to form a visible precipitate. Mayer's hematoxylin was used to counterstain the slides before they were mounted for microscopic examination.

Ethical approval

The institutional ethics committee offered its approval to all procedures, where essential, informed consent was acquired.

Statistical analysis

GraphPad Prism Edition 10 was used for data analysis. A chi-square test and effect size (phi equation) were done to assess if there is an association between CD44 expression and the type of tumor. P-values of 0.01 or less were considered a statistically significant association.

■ RESULTS

According to our study, which includes 63 cases (27 cases were diagnosed histopathologically as CSCC, as in figure 1, and 36 cases were diagnosed as BCC, as in figure 2). CD44 was highly expressed in squamous cell carcinoma (SCC) tissues compared to nearby normal epidermis. IHC analysis showed strong membranous staining for CD44 in the majority of tumor samples. Twenty-one of SCC cases (77.8%) had positive CD44 expression, with only 6 cases (22.2%) showing negative expression as in figures 3 A and B. The expression was more intense at the invasive part of the tumors, suggesting a possible role in tumor growth and epithelial–mesenchymal transition (EMT), figures 3 C and D.

In BCC, as compared to SCC, CD44 expression is usually more variable and less intense. CD44 expression was observed in only 10 out of 36 cases of BCC, figures 4 A and B. This positive corresponds to just 27.8% compared to 72.2% that had negative expression, figures 4 C and D. Unlike SCC, more differentiated, less aggressive tumors are generally linked with high CD44 expression in BCC.

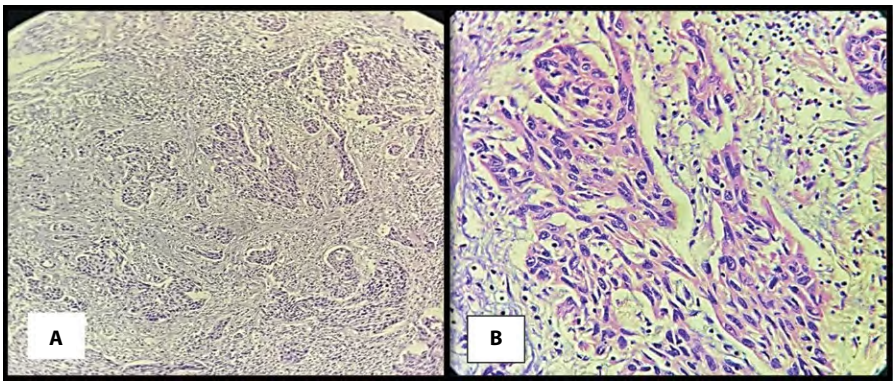

Fig. 1. Cutaneous SCC showing atypical keratinocytes with copious amounts of eosinophilic cytoplasm: A – H&E, 100X; B – H&E, 400X

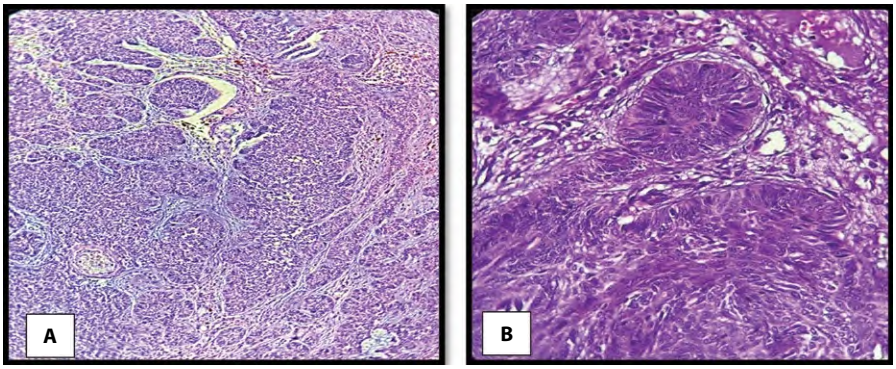

Fig. 2. BCC of the skin showing nests of basaloid cells with minimal cytoplasm, and peripheral palisading: A – H&E, 100X; B – H&E, 400X

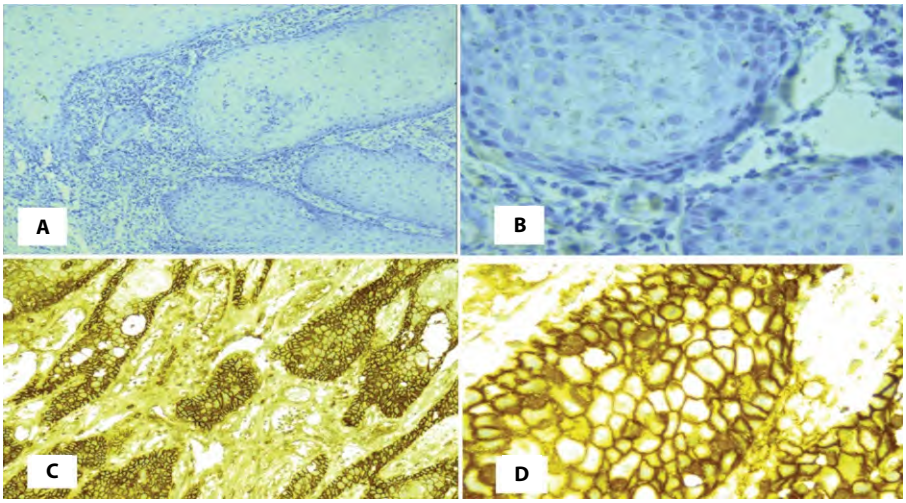

Fig. 3. A – Negative immunostaining of CD44 in CSCC (IHC, 100X); B – Negative immunostaining of CD44 in CSCC (IHC, 400X); C – Positive brownish membranous immunostaining of CD44 in CSCC (IHC, 100X); D – Positive brownish membranous immunostaining of CD44 in CSCC (IHC, 400X)

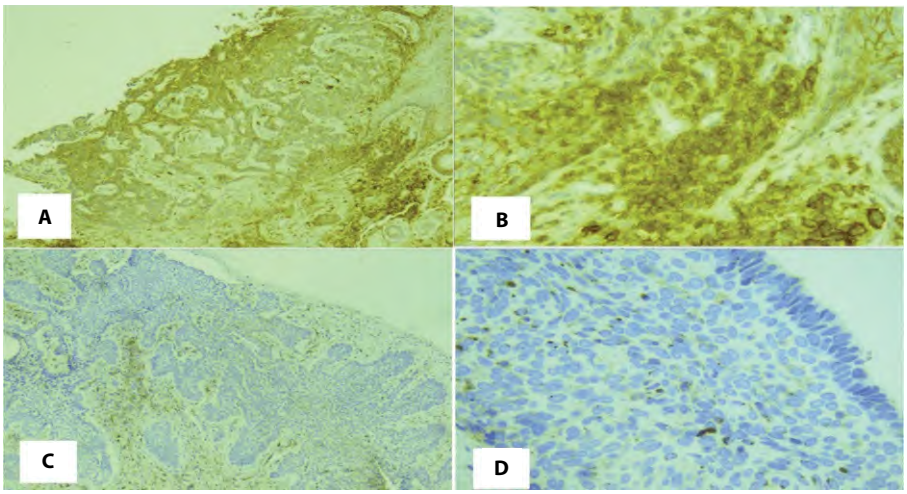

Fig. 4. A – Positive brownish membranous immunostaining of CD44 in BCC (IHC, 100X); B – Positive brownish membranous immunostaining of CD44 in BCC (IHC, 400X); C – Negative immunostaining of CD44 in BCC (IHC, 100X); D – Negative immunostaining of CD44 in BCC (IHC, 400X)

Association between tumor type and CD44 expression

Tumor type	CD44 positive	CD44 negative	Totals
CSCC	21	6	27
BCC	10	26	36
Totals	31	32	63

Notes: the chi-square statistic is 15.43; P<0.01.

Chi-square test was done, and a significant correlation ($P<0.01$) was found between CD44-positive expression and CSCC (table). Effect size was calculated using the Phi equation and was equal to 0.5 suggesting a strong association between CD44 expression and CSCC. These variations support the hypothesis that CD44 may be used as a diagnostic or prognostic marker to help differentiate between these two common skin cancers and determine their potential for progression.

■ DISCUSSION

CD44 is a cell surface molecule which is involved in a wide range of functions, like cell adhesion, migration, lymphocyte activation, angiogenesis, and cell-cell interactions [16]. It is a cell surface receptor for hyaluronic acid. Certain cells, such as epithelial cells, vascular endothelial cells, leukocytes, and cancer cells, possess cell surface adhesion receptors on their surfaces [17]. CD44 is primarily produced by basal keratinocytes in normal skin and helps in wound healing and epidermal homeostasis. However, in carcinogenesis, alterations in CD44 expression and its variants are linked to tumor growth, resistance to therapy, and raised invasive potential [9, 18].

CSCC is the second commonest epidermal skin cancer [19]. The unregulated growth of atypical epidermal keratinocytes is the initial trigger of CSCC, which is probably the consequence of an ongoing intraepidermal dysplasia process [20]. Although its clinical behavior is often benign, it can also exhibit local aggression and metastasis [21]. Prompt surveillance, timely detection, and rapid treatment are essential to reduce the risks of morbidity and mortality as incidence keeps on rising, posing considerable public health concerns [19, 20].

In the literature, only few studies have investigated CD44 expression in cutaneous epithelial neoplasms. Our results showed high expression of CD44 in cutaneous SCC that runs in alignment with other previous studies by Prieto VG et al who reported that all cases of SCC were immunolabelled for CD44 [9], also this finding is in agreement with what was reported in other studies [10, 22–24]. A study done by Karvinen S et al. in 2003 showed that moderate intensity and generally homogenous CD44 staining were observed in the well-differentiated invasive SCC tumors; however, decreased CD44 expression has been observed in the less differentiated SCC tumors [25].

Regarding BCC, it is the most frequent cancer and is typically a slow-growing tumor with little metastasis. It is rarely lethal, but when treatment is insufficient or delayed, it can cause significant damage and distortion to nearby tissues [26].

In contrast to SCC, BCC had a limited potential for metastasis, so most BCC does not express CD44. The expression of this particular protein may be correlated to some element of tumor progression, particularly the ability to metastasize [9]. In current study, CD44 showed focal or negative expression in most of cases (10 of 36 BCC cases) and this finding is in concordance with studies done by Prieto VG et al. [9], Simon J.C. et al. [10], Erfani E. et al. [22], Karvinen S et al. [25], but in contrast to other studies done by KP Dingemans et al. [15]. More study is required to completely evaluate CD44 role in SCC and BCC clinical behavior. Future further studies may be required to investigate CD roles in other cancer types like breast cancers [27–31], gastric cancer [32], CNS tumors [33], anal tumors [34], colorectal cancers [35–37], head and neck carcinoma [38–41], endometrium cancer [42], and ovarian tumor [43].

■ CONCLUSION

The different patterns of CD44 expression in CSCC and BCC could represent the unique origins of the tumors: SCC is a keratinocyte at an earlier stage in the differentiation cascade, and BCC is an undifferentiated keratinocyte. The variation in CD44 expressions between these malignancies may further contribute to the differences in their ability to metastasize.

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