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Loss of Correlation between Intensities of Desmoglein 2 and Desmoglein 3 Expression in Basal Cell Carcinomas*

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SUMMARY Desmosomal cadherins in humans involve three isoforms, each in two splicing variants, of desmocollin (DSC1-3) and four isoforms of desmoglein (DSG1-4). DSGs are transmembranous desmosomal adhesive glycoproteins, which are generally expressed in a differentiation-specific manner. The aim of this study was to measure intensities of expression of both DSG2 and DSG3 in basal cell carcinoma (BCC) and to compare them with those in normal epidermis. Using immunoperoxidase staining on frozen tissue with monoclonal antibodies against human DSG2 and DSG3, DSG2 and DSG3 expression was assessed in specimens from 26 BCC patients. There was a significant overexpression of DSG2 and a significantly lower expression of DSG3 in BCC tumor nests (BCCpos) compared to non-BCC-affected epidermis (BCCneg). There was no significant correlation between the intensities of DSG2 and DSG3 expression in BCCpos, but there was a significant correlation (r=+0.6092) between these markers in BCCneg. That loss of coordination of DSG2 and DSG3 expression in BCC, revealed with quantitative digital morphometry, might explain in part the BCC behavior as a locally invasive tumor. Our study further suggests that in human skin, DSG2-mediated adhesion appears to be more proliferation-associated, whereas DSG3-mediated adhesion seemingly is more differentiation-associated.

KEY WORDS: desmoglein 2, desmoglein 3, basal cell carcinoma

INTRODUCTION

Desmosomes form the intercellular junctional complex, essential for maintaining tissue integrity. They participate in a number of pathologic processes including tumor formation, invasion and metastasis (1). Desmosomal cadherins in humans involve three isoforms, each in two splicing variants, of desmocollin (DSC1-3) and four isoforms of desmoglein (DSG1-4). Each isoform arises from a different gene and has a characteristic expression pattern, which is dependent on tissue type and differentiation state (2,3). In some cases, the loss of DSGs in various types of carcinoma may be associated with an increased tumor cell capacity of migration (4).

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DSG1 and DSG3 are restricted to stratified epithelia (5). DSG1 is more intensively expressed as the cells differentiate toward stratum corneum, and is very little detectable in the basal layer, whereas DSG3 is localized to the basal and immediate suprabasal layers (6). In normal human epidermis, DSG2 is expressed at low levels, and has been reported to be restricted to the lowermost epidermis (5). DSG2 expression fades with keratinocyte differentiation, whereas DSG3 expression decreases somewhat gradually from the basal into the spinous cell layers (3). DSG4 expression is restricted to the highly differentiated upper cell layers (2). The distribution of DSGs in hair follicles has been described in some studies. Similar to epidermis, hair follicle is compartmentalized into a hierarchy of cell types based on the level of differentiation (6). DSG1 is expressed in the inner root sheath and the innermost layers of the outer root sheath. There is a report that DSG2 is highly expressed by the least differentiated cells of the cutaneous epithelium, including the hair follicle bulge of the fetus and adult, bulb matrix cells, and basal layer of the outer root sheath (6). Expression of DSG3 in hair follicle is correlated with different types of keratinization (all layers of the outer root sheath in the areas of trichilemmal keratinization and mainly basal layer in the areas of epidermal-like keratinization) (6). DSG4 is expressed specifically in the hair shaft cortex, lower hair cuticle, and upper inner root sheath cuticle (7). Probably this molecule is a key mediator of keratinocyte cell adhesion in hair follicle, where it coordinates transition from proliferation to differentiation (5).

Recent studies have suggested that bulge region might be a reservoir of stem cells of hair follicles (8,9). Hair follicle stem cells are multipotent and have a superior clonogenicity and proliferative capacity. They are capable of giving rise to all cell types of hair, epidermis and sebaceous gland (6,8,9). Bulge cells might be susceptible to genetic alteration and be a source of carcinogenic mutations (9). Some data suggest that several skin tumors including basal cell carcinoma (BCC) might be derived from hair follicle cells, particularly bulge cells (9).

Basal cell carcinoma is one of the most frequent types of cancer in humans. It is usually slow growing and rarely metastasizes; however, it can cause clinically significant local destruction and disfigurement when neglected or inadequately treated (10,11). The growth of BCC is usually localized to the area of its origin; nevertheless, disturbance of adhesion should be regarded as a feature of its pathogenesis (12). Clinical appearances and morphology are diverse (11). Basal cell carcinomas have many traditional histopathologic subtypes, depending on the predominating pattern, including the following (13): *solidum, adenoides, cysticum, keratoticum, pigmentosum, superficiale, cicatrisans, styloides,* and their combinations.

There is some evidence that BCC etiology is dependent on several signaling pathways, like hedgehog, Wnt signaling and MAPK pathway (11). Literature data suggest that overexpression of desmosomal marker, DSG2, can deregulate multiple signaling pathways associated with the development of skin tumors (14).

The aim of this study was to measure intensities of expression of both DSG2 (apparently not pemphigus autoantigen) and DSG3 (known pemphigus autoantigen) in BCC, and to compare them with those in normal epidermis in order to simultaneously assess the role of DSG2 and DSG3, both adhesive molecules, in differentiation and proliferation of keratinocytes in physiological and pathological (local malignancy) conditions in humans.

MATERIAL AND METHODS

The study included 26 patients (18 men and 8 women) with various histologic subtypes of BCC, diagnosed and treated at Department of Dermatology, Poznan University of Medical Sciences in Poland between November 2009 and July 2010. Expression of DSG2 and DSG3 was evaluated in BCC tumor nests (BCCpos) irrespectively of the BCC histologic subtype, and in non-BCC-affected epidermis (BCCneg) in patients with BCC. Conventional hematoxylin and eosin staining was performed in all cases to establish the diagnosis. The appropriate immunohistochemical procedure controls were performed. Frozen specimens had to be used for immunohistochemistry throughout the study because attempts with paraffin-embedded specimens for DSG3 visualization under various conditions proved futile.

The study of expression of DSG2 and DSG3 was performed on frozen tissues subjected to 4-µm cryosectioning and then mounted on poly-L-lysine coated glass slides. DSG2 and DSG3 were detected with the use of monoclonal murine antibodies (anti-DSG2 from AbD Serotec, UK) (anti-DSG3 from Invitrogen, USA, immunogen was human DSG3 extracellular domain). The slides were incubated with an appropriate dilution of antibodies at room temperature for 1 hour.

To visualize immunoreactivity, the sections were treated with LSAB+ system+ HRP visualization kit (Dako, Denmark). Then the slides were washed with PBS, counterstained with hematoxylin, coverslipped, and examined by light microscopy (BX40, Olympus, Japan) under magnification x400 and digitally photographed (Fig. 1A,B and Fig 2A,B).

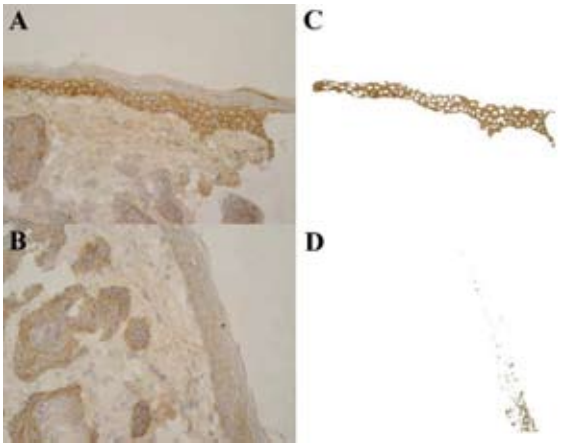

Figure 1. Intense DSG3 expression (A) compared to low DSG2 expression (B) in non-BCC-affected epidermis (BCCneg) in a patient with BCC (immunoperoxidase staining on frozen sections); DSG3 expression (C) and DSG2 expression (D) processed with digital microscopic image analysis in BCCneg. (original magnification x400)

We used the quantitative morphometric software (Amiga Analyser 4D, Poland) to evaluate quantitatively the positive immunostaining signals of DSG2 and DSG3 in BCCpos and in BCCneg serving as an internal control (Fig. 1C,D and Fig. 2C,D). Expression of these proteins in BCCpos and BCCneg on serial sections was measured in percentage of DSG2/DSG3 reaction (area of positive reaction divided by area studied and then multiplied by 100).

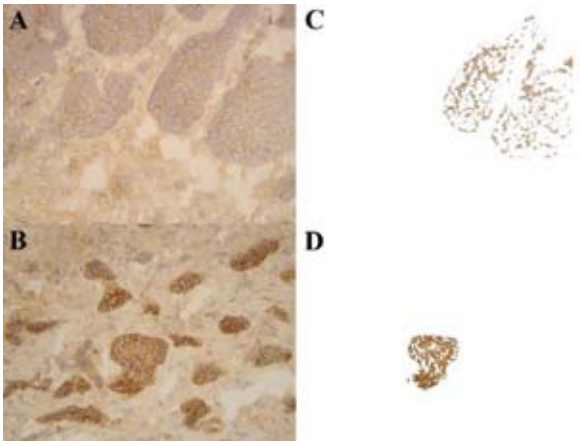

Figure 2. Decreased DSG3 expression (A) and overexpression of DSG2 (B) in BCC tumor nests (BCCpos) (immunoperoxidase staining on frozen sections); DSG3 expression (C) and DSG2 expression (D) processed with digital microscopic analysis in BCCpos. (original magnification x400)

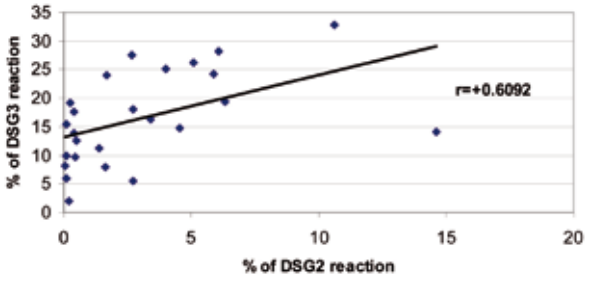

Figure 3. Significant correlation (r=+0.6092) between intensities of DSG2 and DSG3 expression (in percentage of DSG2/DSG3 reaction) in non-BCC-affected epidermis (BCCneg). Trend line is shown.

Statistical analysis was done using Wilcoxon test (DSG2 *versus* DSG3) and Mann-Whitney test (DSG2 in BCCpos *versus* DSG2 in BCCneg and DSG3 in BCCpos *versus* DSG3 in BCCneg). Correlation between DSG2 and DSG3 expression in BCCpos and BCCneg was calculated using Spearman's rank correlation coefficient. All analyses were performed using the Statistica PL 8.0 StatSoft statistical software.

RESULTS

Results are shown in Table 1 and Figures 1-4. On quantitative analysis of immunohistochemical examination, the expression of DSG2 and DSG3 showed differences between BCCpos and BCCneg. There was a significant overexpression of DSG2 and significantly lower expression of DSG3 in BCCpos compared to BCCneg (Table 1). In immunohistochemical study, most of the BCCneg areas were negative or weakly positive for DSG2 staining, but strongly positive for DSG3 staining (Fig. 1). Conversely, the BCCpos areas mostly were positive for DSG2 staining and negative/weakly positive for DSG3 staining (Fig. 2).

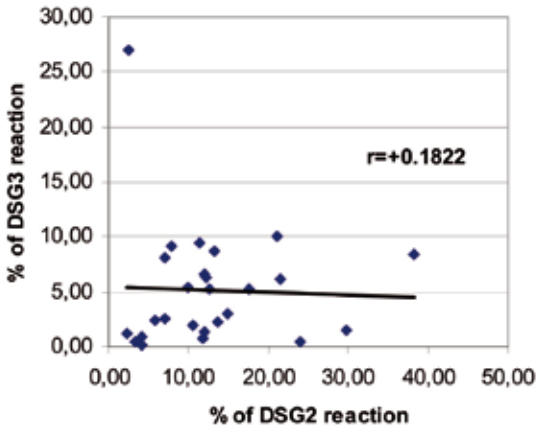

Figure 4. Lack of correlation (r=+0.1822) between intensities of DSG2 and DSG3 expression (in percentage of DSG2/DSG3 reaction) in BCC tumor nests (BCCpos). Trend line is shown.

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Table 1. Intensities of DSG2 and DSG3 expression (in percentage of DSG2/DSG3 reaction) in study groups

Study group	Number of cases (n)	DSG2 expression (% of DSG2 reaction) Mean ± SD	DSG3 expression (% of DSG3 reaction) Mean ± SD	DSG2 expression Statistical significance	DSG3 expression Statistical significance	DSG2 versus DSG3 expression Statistical significance
BCCpos	26	12.71±8.58	5.17±5.52	BCCpos versus BCCneg p=0.0001	BCCpos versus BCCneg p=0.0001	DSG2 versus DSG3 in BCCpos p=0.0002
BCCneg	25	3.04±3.60	-			DSG2 versus DSG3 in BCCneg p=0.0001
BCCneg	26	-	16.60±7.98			

There was a significant correlation (r=+0.6092) between the intensities of DSG2 and DSG3 expression in BCCneg (Fig. 3), but no such correlation between these markers in BCCpos (r=+0.1822) (Fig. 4).

DISCUSSION

Our results showed clear difference in DSG2 and DSG3 expression between BCCpos on the one hand and BCCneg on the other. These results corroborate those of our previous studies (3,15). Some results of this study are also compatible with data obtained by Brennan *et al*. (14), who demonstrated an increased expression of DSG2 in malignant skin tumors. Analysis of literature on molecular components of desmosomes and their association with tumorigenesis reveals that coordination of expression of these molecules in tumor cells is often altered. However, these data are still controversial.

Findings obtained by Chen *et al.* (16), who demonstrated the expression of DSG3 in normal oral keratinocytes to be very low in contrast to high levels of expression in head and neck cancer cells, are incompatible with this view. Moreover, they found that 61% of patients with head and neck cancer studied had DSG3 protein overexpression in tumor tissues compared to paired grossly normal counterparts. Similarly, Chi-Che *et al.* (1) report on overexpression of DSG3 in inverted papilloma (IP) and IP with squamous cell carcinoma (SCC). This group of researchers has also demonstrated that this overexpression correlated with malignant transformation of IP. Moreover, at the transcriptional level of DSG3, overexpression was observed in pulmonary SCCs, but in adenocarcinomas the expression was very limited (17). Also, their results do not corroborate the finding by Shirakata *et al*. (18), who demonstrated DSG3 to be the predominant desmoglein isoform in both keratinizing and non-keratinizing oral epithelia. Therefore, it might be advantageous to analyze DSG3 expression more thoroughly, as its levels apparently do show significant variations in different types of carcinoma. To resolve this issue, we suggest using a wider scope of investigated sample materials in respect to both the type of carcinoma and the cells assessed (oral keratinocytes *versus* skin keratinocytes), as well as different clones of the monoclonal antibodies to DSG3 which would be capable of recognizing various epitopes of a protein undergoing posttranslational modifications.

During the last decade, the expression of cadherins and desmosomal proteins during tumor progression has been subjected to extensive studies, the majority of which have been focused on the role of E-cadherin. The loss of E-cadherin expression and/or function has been observed during tumor progression of most carcinomas (19). Kurzen *et al.* (20) found no qualitative correlation of desmoplakin or plakoglobin expression with the risk of metastasis in SCC of the skin. On the other hand, many literature reports have documented alterations in the expression of desmosomal cadherins during the process of tumorigenesis (21). Recent studies have shown the loss of DSG2 in gastric cancer (22,23), DSC2 in colorectal cancer (24) and DSC3 in breast cancer (25). In contrast to gastric cancer, DSG2 was overexpressed in both SCC of the skin (18) and in BCC (26). Tada *et al*. (27) semiquantitatively analyzed immunofluorescent images suggesting that the expression of DSG1 and plakoglobin is markedly reduced or absent in both SCC and BCC tumor cells (27). Goyal *et al*. have recently reported no statistically significant difference in the expression of E-cadherin and catenins (α-, β-, γ-catenin) in the paired normal background and tumor tissues (human breast cancer) (28). Some studies (29)

demonstrated that down-regulated expression of E-cadherin, catenins and p120 occurs frequently in hepatocellular carcinomas. Future research may show whether the expression levels of certain desmosomal protein coding genes, or perhaps an expression level network of many genes might serve as a new biomarker of tumorigenesis.

Furthermore, hair follicles, which physiologically express DSGs, apparently are involved in both pemphigus pathogenesis and BCC pathogenesis (15). Particularly, bulge cells are in the area of research interest because of their possible role in tumorigenesis (9), including that of BCC. Several research groups have noted that some BCCs express K-15 that is usually expressed in human follicle bulge cells (9). Interestingly, it has been found that overexpression of *Shh* gene (sonic hedgehog), a gene essential for hair follicle morphogenesis, resulted in the formation of BCC-like tumors (9). However, Youssef *et al.* (30) have very recently revealed that BCC does not originate from bulge stem cells, as previously thought. With the use of clonal analysis in a mouse model of the disease, they found that BCC arised from long-term resident progenitor cells of the interfollicular epidermis and upper infundibulum (30). Still, DSGs can be important in BCC pathogenesis regardless of the issue of BCC cellular origin, as they are expressed in both bulge region and upper infundibulum of the hair follicle and interfollicular epidermis. Furthermore, analyzing DSGs expression patterns in relation to various histologic subtypes of BCC might help clarify the issue of differences in the biological behavior of these BCC subtypes.

CONCLUSION

Thus, our results seem to indicate that both DSG2 and DSG3 might be involved in BCC pathogenesis. Seemingly, the expression of DSG2 and DSG3, adhesion molecules that plausibly play different roles in proliferation and differentiation of epidermis, is coordinated in BCCneg, but this apparent coordination is lost in BCCpos. That loss of coordination of DSG2 and DSG3 expression, revealed with quantitative digital morphometry, in BCC might in part explain BCC behavior as a locally invasive tumor.

There is still a very interesting issue of what role desmosomal adhesion and desmosomal components (particularly DSGs) play in carcinogenesis. The disturbance of desmosomal adhesion can result in tissue integrity damage and possibly induction of tumor cell migration and proliferation. Our BCC study, in which both physiological and pathological expression of both DSG2 and DSG3 was statistically compared using quantitative digital morphometry further suggests that in human skin DSG2-mediated adhesion appears to be more proliferation-associated, whereas DSG3-mediated adhesion seemingly is more differentiation-associated.

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