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Desmosomal defects in acantholytic squamous cell carcinomas

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Abstract

Background—Acantholytic squamous cell carcinoma (Acantholytic SCC) are epithelial tumors characterized by a loss of cell adhesion between neoplastic keratinocytes. The mechanism underlying loss of cell-cell adhesion in these tumors is not understood.

Methods—A retrospective analysis of acantholytic SCC (n=17) and conventional SCC (n=16, controls not showing acantholysis) was conducted using a set of desmosomal and adherens junction protein antibodies. Immunofluorescence microscopy was used to identify tumors with loss of adhesion protein expression.

Results—The vast majority of acantholytic SCC (89%) showed focal loss of at least one desmosomal cell adhesion protein. Most interestingly, 65% of these tumors lost expression of two or more desmosomal proteins.

Conclusions—Loss of cell adhesion in acantholytic SCC is most likely linked to the focal loss of desmosomal protein expression, thus providing potential mechanistic insight into the patho-mechanism underlying this malignancy.

Keywords

Acantholytic squamous cell carcinomas; Desmosomes; Desmocollin; Desmoplakin; Desmoglein

INTRODUCTION

Acantholytic squamous cell carcinoma (acantholytic SCC) is a histopathologic subtype of squamous cell carcinoma (SCC) characterized by focal loss of cell-cell adhesion. These tumors are typically well-differentiated. Further, acantholysis may be so prominent in focal areas that it produces a pseudoglandular pattern. In addition, dyskeratotic cells may be prominent (e.g. (1–4)). Approximately 2–4% of all SCC are classified as acantholytic SCC (5).

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Acantholytic SCC most commonly occur in men on the sun-exposed skin of the face, the neck and the hands of older patients (3). Further, acantholysis has been postulated to be a risk factor for metastasis, specifically in patients with larger lesions (3, 4, 6). The mechanism underlying loss of cell-cell adhesion in these tumors is not understood; however, acantholysis is observed in patients and animals with impaired desmosome function (7, 8), suggesting the possibility that desmosomal defects might occur in acantholytic SCC.

Desmosomes are cell adhesion structures (junctions) that are abundant in epidermal cells. Structurally, these junctions can be divided into a transmembrane core, which is crucial for connecting the plasma membranes of neighboring cells, and a plaque that connects the transmembrane core to the keratin filaments of keratinocytes (see references in (7, 9)). The core is composed of transmembrane proteins that are sequence-related to cadherins, and which have been termed desmosomal cadherins (10). Two families of transmembrane proteins are found in desmosomes, desmocollins (DSC) and desmogleins (DSG). Both groups contain several proteins which are encoded by different genes (DSC1-3, DSG1-4). These proteins are differentially expressed in desmosome-forming tissues. It is assumed that the different desmosomal cadherins vary in their biological properties. For example, the complement of desmosomal cadherins expressed in a given cell might affect adhesive strengths between cells and the ability to migrate. The desmosomal plaque contains plakins; desmoplakin (DSP), plakophilin(s) (PKP) and junctional plakoglobin (JUP) (9). DSP plays a crucial role in connecting the desmosome to the keratin intermediate filament network (11). JUP is unique in that it integrates into two different junctions; desmosomes and adherens junctions (AJ). All of the proteins listed above have been shown or postulated to be crucial for cell-cell adhesion (7, 8, 11–13).

We assessed the expression of constitutive desmosomal proteins as well as the expression of two marker proteins of adherens junctions (E-cadherin and β -catenin) in acantholytic SCC and conventional SCC. Our results indicate that focal loss of desmosomal adhesion protein occurs in the majority (89%) of acantholytic SCC analyzed, providing a possible mechanistic explanation for the development of acantholysis in this tumor type.

MATERIAL AND METHODS

A searchable electronic database was used to retrieve cases of cutaneous acantholytic SCC or conventional SCC from 2010 to 2013 from the University of Colorado Dermatopathology Consultant archives. The cases were identified by typing in either “acantholytic squamous cell carcinoma” or “squamous cell carcinoma” in the search menu of the database. The archived slides were retrieved and re-examined by a dermatopathologist (JEF). Cases that were excluded from the study included those specimens that were superficial, lacked enough residual tissue on to the block to study or did not demonstrate acantholysis in at least 25% of the invasive portion of the tumor. Each specimen was assigned a database-generated identification number for patient confidentiality before being released for laboratory study.

A total of 17 acantholytic SCC and 18 SCC were identified that met the criteria required for inclusion in the study. All acantholytic SCC's were classified as being moderate (2 cases) or well-differentiated (15 cases). Fourteen of the 17 cases were from males and three of 17

were from females. The mean age was 68.4 years (range was 53 to 89 years). This is consistent with previously published data which has reported that acantholytic SCC's are more common on older male patients (3). The conventional SCC without acantholysis were classified as being poorly-differentiated (1 case), moderately-differentiated (2 cases) or well-differentiated (16 cases). Nine tumors were from males and nine tumors were from females. The mean age was 71.5 years (range was 53 to 89 years).

Antibody Staining

Formalin-fixed and paraffin-embedded tumor sections were stained for immunofluorescence microscopy with the following antibodies using standard protocols: DSC3 (U114; Cat #61093, Progen; Heidelberg, Germany), DSG1/2 (DG3.10; Cat #61002; Progen; Heidelberg, Germany), DSG3 (5G11; Cat #MCA2273T; AbD Serotec; Oxford, UK), DSP (2.15, 2.17; Cat #10R-D108A; Fitzgerald; Acton, MA), JUP (PG 5.1; Cat #10RP128A, Fitzgerald; Acton, MA), β -catenin (Cat #610153, BD Transduction Laboratories; San Jose, CA), E-Cadherin (24E10; Cat #3195, Cell Signaling; Danvers, MA), Keratin 5 (KRT5, Cat #PRB-160P, Covance; Emeryville, CA). For a description of the desmosomal antibodies used refer to the references (14, 15). Fluorescent dye-conjugated secondary antibodies were purchased from Invitrogen (Eugene, OR). Desmosomal and adherens junction protein antibodies were used in combination with KRT5 antibodies (double staining) to identify protein expression in epithelial tissues. Antibody dilutions were determined empirically using human skin as a substrate. Antigen retrieval was done as recommended by the manufacturer of the antibodies. Fluorescence staining was documented with a Nikon Eclipse 90I microscope equipped with a Coolsnap HQ2 and a DS-Fi1 camera. Image processing was done with the NIS Elements 3.10 imaging software (Nikon). All pictures were taken with the same lens (100X magnification) and each antibody was imaged with the same exposure time. Loss of fluorescent staining was confirmed by two independent observers.

RESULTS

Using acantholytic SCC (n=17) and conventional SCC (n=18, controls) tumor sections obtained from a clinical dermatopathology laboratory, we performed a retrospective study to determine whether junctional proteins are deregulated in these carcinomas.

Previous studies have not systematically analyzed the expression of constitutive desmosomal proteins in acantholytic SCC. The vast majority of acantholytic SCC (89%) in our study showed loss of at least one desmosomal protein (Table II). One acantholytic SCC showed normal expression of these proteins as judged by immunofluorescence microscopy (data not shown) while a second acantholytic SCC showed expression of all five markers, albeit with reduced expression levels of three desmosomal proteins (Table I and data not shown). Nevertheless, given that reduced expression is difficult to quantitate reliably by immunofluorescence microscopy, we restrict our discussion to samples in which proteins were apparently absent (see examples in Fig 1). Note that in most cases, loss occurred focally and did not extend throughout the entire tumor tissue, thus providing an internal control for our staining experiments.

The results listed in Table I and III show that in acantholytic SCC, DSG1/2 (note that the antibody used cross-reacts with two DSG isoforms, DSG1 and DSG2) and DSP were the proteins most likely to be lost, followed by DSG3 and DSC3. Further, our analysis showed that many acantholytic SCC showed loss of multiple desmosomal proteins (Table II). Acantholytic SCC9 showed loss of all desmosomal proteins assessed while maintaining expression of the adherens junction markers. Acantholytic SCC11 showed loss of all adhesion proteins tested, including the adherens junction markers. Strikingly, our conventional SCC controls showed far less loss of desmosomal protein expression than acantholytic SCC. Only 28% of these tumors lost at least one desmosomal marker. Further, from the 18 SCC assessed, none showed loss of more than 2 desmosomal markers (Table II). None of the AJ markers were lost in the SCC samples analyzed (Table II).

DISCUSSION

The patho-mechanism underlying acantholysis in SCC is not understood. Several studies have attempted to link defects in cell-cell adhesion in these neoplasias to the loss of specific proteins. Several groups identified E-Cadherin and syndecan-1 as proteins lost or functionally inactive in acantholytic SCC (1, 5, 16, 17). However, at least with respect to syndecan-1, it appears that its role in the disease remains unclear since one study did not confirm de-regulation of this protein in acantholytic SCC (16). Lastly, over-expression of IKK α has also been claimed to induce acantholytic SCC-like tumors in a mouse model (18). This is somewhat surprising given that IKK α plays an important role in the normal differentiation of epidermal keratinocytes. Nevertheless, it is possible that IKK α serves a different function in transformed cells when compared to normal keratinocytes. It remains to be seen whether IKK α de-regulation is a common occurrence in acantholytic SCC.

In the present study, we determined whether acantholytic SCC lose expression of adhesion proteins. Note that we define the term “lost” as the absence of antibody staining in immunofluorescence microscopy. Loss of an immunofluorescence signal is indicative of a significant reduction in protein synthesis or stability. We are aware that this finding is not identical to a null mutation, which completely abolishes protein synthesis. Nevertheless, the absence of protein detection in our system is likely to indicate severe defects in desmosome function.

Two of the desmosomal cadherins tested, DSG3 and DSC3, have previously been linked to acantholysis in the epidermis and the oral mucosa. We have demonstrated that loss-of-function mutations in the *Dsg3* gene cause acantholysis in stratified epithelia of mice (19, 20). Similarly, using mouse models, we showed that epidermis-specific loss of *Dcs3* leads to acantholysis and skin blistering (21), a phenomenon that appears to be replicated in patients with *DSC3* gene mutations (7, 8). Nevertheless, DSG3 and DSC3 were not the primary targets of protein loss in acantholytic SCC. Further, our analysis failed to demonstrate statistical significance for our finding of reduced DSG3 and DSC3 expression (Table III). Instead, loss of DSG1/2 and DSP appeared to be statistically significant in our cohort of acantholytic SCC (Table III). Note that the antibody used to detect Dsg1/2 recognizes two desmogleins, DSG1 and DSG2, proteins with different expression patterns in the epidermis. DSG2 is only weakly expressed in the interfollicular epidermis (22). Further, this protein is

down-regulated during keratinocyte differentiation while DSG1 is up-regulated, explaining the basal DSG2 and the suprabasal DSG1 expression in the epidermis (e.g. (22)). The biological consequences of DSG2 loss in the epidermis are not known since neither animal models nor human patients have been described with loss-of-function mutations in this gene. Given the low DSG2 expression levels in interfollicular epidermis, it is questionable whether this protein plays a major role in cell adhesion between epidermal keratinocytes. Loss of DSG1, on the other hand, has been linked to epidermal pathology (8). Recently, Samuelov and colleagues (12) described patients with a homozygous *DSG1* mutation which is predicted to result in a loss-of-function phenotype. These patients develop spinous and granular acantholysis with split desmosomes in the epidermis.

The second most frequently lost desmosomal protein in our tumor group was DSP. Mouse studies have demonstrated that loss of DSP in the epidermis can lead to stress induced acantholysis (11). Interestingly, mice with a conditional DSP null mutation in the skin also formed less adherens junctions. In our acantholytic SCC group, only two tumors showed loss of both adherens junction markers examined (E-cadherin and β -catenin). In these tumors, DSP expression was lost as well.

Tanaka and colleagues recently reported patients with a genodermatosis caused by a DSP loss-of-function mutations which resulted in skin blistering at sites of exposure to mechanical stress, confirming that DSP is required for cell-cell adhesion in human skin as well (13).

The findings described above clearly demonstrate that all of the desmosomal proteins frequently lost in acantholytic SCC are essential to maintain tissue cohesion in the epidermis. Surprisingly, many acantholytic SCC tumors showed loss of several desmosomal proteins, with almost 12% of the tumors losing all five proteins examined (Table II). It is tempting to speculate that loss of multiple desmosomal proteins might facilitate acantholysis and potentially tumor progression and metastasis. Unfortunately, due to a lack of data regarding clinical outcome in our acantholytic SCC group, we currently cannot address this possibility. In our control SCC group, very few desmosomal proteins were lost. This further supports the hypothesis that loss of cell adhesion in acantholytic SCC is primarily due to impaired desmosome function, not a defect in AJ.

In summary, we predict that loss of cell-cell adhesion in acantholytic SCC is primarily a desmosomal defect with most tumors (82% in our study) showing loss of either DSG1/2, DSP or both proteins.

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Abbreviations

DSG	Desmoglein
DSC	Desmocollin
DSP	Desmoplakin
JUP	Junctional Plakoglobin
SCC	Squamous Cell Carcinoma

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Fig 1A

Fig 1B

Fig 1C

Fig. 1.

Three acantholytic SCC (A–C) were stained with the antibodies indicated. In each of the three tumors shown, two desmosomal proteins show overlapping loss of synthesis in the same areas. Arrowheads point to areas in which the marker proteins are normally expressed. Arrows indicate focal loss of desmosomal protein expression. The open arrowhead in Fig 1A demarcates an area in which DSG3 staining appears to be cytoplasmic, suggesting functional inactivation of this protein. Desmosomal cadherins require cell membrane localization to function as cell adhesion proteins. (Final magnification, 100X)

Table 1

Desmosomal protein expression in acantholytic SCC and conventional SCC (FL, Focal Loss)

Tumor	DSG1/2	DSP	DSG3	DSC3	JUP	E-Cadherin	β-Catenin
Acantholytic SCC 1							
Acantholytic SCC 2	FL					FL	
Acantholytic SCC 3	FL					FL	
Acantholytic SCC 4	FL						
Acantholytic SCC 5	FL	FL			FL		
Acantholytic SCC 6							
Acantholytic SCC 7	FL	FL	FL		FL		
Acantholytic SCC 8	FL	FL					
Acantholytic SCC 9	FL	FL	FL	FL	FL		
Acantholytic SCC 10	FL	FL					
Acantholytic SCC 11	FL	FL	FL	FL	FL	FL	FL
Acantholytic SCC 12	FL		FL				
Acantholytic SCC 13		FL					
Acantholytic SCC 14		FL	FL				
Acantholytic SCC 15	FL	FL		FL		FL	FL
Acantholytic SCC 16			FL	FL			
Acantholytic SCC 17	FL	FL	FL	FL			
Conventional SCC 1							
Conventional SCC 2s							
Conventional SCC 3	FL						
Conventional SCC 4							
Conventional SCC 5		FL					
Conventional SCC 6							
Conventional SCC 7							
Conventional SCC 8			FL				
Conventional SCC 9							
Conventional SCC 10	FL				FL		

Tumor	DSG1/2	DSP	DSG3	DSC3	JUP	E-Cadherin	β -Catenin
Conventional SCC 11							
Conventional SCC 12							
Conventional SCC 13							
Conventional SCC 14							
Conventional SCC 15			FL	FL			
Conventional SCC 16							
Conventional SCC 17							
Conventional SCC 18							

Table II

Loss of multiple junctional protein expression as determined by immunofluorescence microscopy

	Acantholytic SCC (n=17)	Conventional SCC (n=18)
1 Desmosomal protein lost	4/17 (24%)	3/18 (17%)
2 Desmosomal proteins lost	5/17 (29%)	2/18 (11%)
3 Desmosomal proteins lost	2/17 (12%)	0/18 (0%)
4 Desmosomal proteins lost	2/17 (12%)	0/18 (0%)
5 Desmosomal proteins lost	2/17 (12%)	0/18 (0%)
1 AJ protein lost	2/17 (12%)	0/18 (0%)
2 AJ proteins lost	2/17 (12%)	0/18 (0%)

Table III

Loss of junctional protein expression as determined by immunofluorescence microscopy. P values were determined using Fisher's Exact Test. Note that $p < 0.05$ is considered statistically significant.

	Acantholytic SCC (n=17)	Conventional SCC (n=18)	P-Values
DSG1/2	12/17 (71%)	2/18 (11%)	0.000491
DSG3	7/17 (41%)	2/18 (11%)	0.059908
DSC3	5/17 (29%)	1/18 (6%)	0.087683
DSP	10/17 (59%)	1/18 (6%)	0.000945
JUP	4/17 (24%)	1/18 (6%)	0.177419
E-Cadherin	4/17 (24%)	0/18 (0%)	0.045455
β -Catenin	1/17 (12%)	0/18 (0%)	0.48517
1 Desmosomal protein lost	15/17 (89%)	5/18 (28%)	0.000491
1 AJ protein lost	4/17 (24%)	0/18 (0%)	0.045455