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Differential Expression of Desmosomal Glycoproteins in Keratoacanthoma and Squamous Cell Carcinoma of the Skin: An Immunohistochemical Aid to Diagnosis

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The distinction between keratoacanthoma and squamous cell carcinoma is a common dermatopathological dilemma. Although the mainstay of the diagnosis is still clinico-pathological correlation, many dermatopathologists now include keratoacanthomas in the spectrum of squamous cell carcinomas. Recent reports, however, have pointed out that keratoacanthoma is "deficient squamous cell carcinoma" since it loses the expression of bcl-2 antigen, consistent with initiation of apoptosis, i.e. its involution. Electron microscope studies performed in keratoacanthomas and squamous cell carcinomas also revealed significantly reduced desmosomes in squamous cell carcinoma, but not in keratoacanthoma.

A series of 38 keratoacanthomas and 62 squamous cell carcinomas of the skin (28 well-differentiated, 21 moderately differentiated and 13 poorly differentiated) were stained immunohistochemically with the monoclonal antibody 32–2B to desmosomal glycoproteins desmoglein 1 and desmoglein 3. Thirty-five keratoacanthomas showed extensive pericellular desmoglein expression. Three keratoacanthomas and 20 squamous cell carcinomas (19 well-differentiated, 1 moderately differentiated) showed focal staining, and in 11 squamous cell carcinomas (2 moderately differentiated, 9 poorly differentiated) the staining was negative. The remaining 31 squamous cell carcinomas (9 well differentiated, 18 moderately differentiated, 4 poorly differentiated) showed juxtanuclear staining. None of the squamous cell carcinomas exhibited the extensive pericellular pattern found in keratoacanthomas. Assessment of staining intensity, by 3 independent examiners, revealed a strong negative correlation between desmoglein expression and degree of dysplasia in the squamous cell carcinomas ($p < 0.01$).

This antibody therefore clearly distinguishes these tumours and may be of value in the differential diagnosis of keratoacanthoma and squamous cell carcinomas in routine histopathology.

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The distinction between solitary keratoacanthoma (KA) and squamous cell carcinoma of the skin (SCC) is a common dermatopathological dilemma (1). Rapid onset of the lesion as well as a firm dome-shaped aspect with a keratin-filled crater are highly indicative of KA, but cannot exclude well-differentiated SCC. Conversely, cell atypia and deep stromal or intravascular invasion characterize SCC but may also occur in the early phase of KA (1–3). Some immunohistochemical studies (distribution of H blood group antigen (4), peanut lectin receptor and carcinoembryonic antigen (5), major histocompatibility complex antigen expression (6), intercellular substance staining pattern (7), nucleolar organizer region enumeration (8)) can be adjuncts in differential diagnosis of these tumours. Others (involucrin (9), filaggrin (10), keratin (9), β-2 microglobulin (11) and p53 oncoprotein expression (12)) have shown conflicting results. For these reasons, in the last decade, some authorities in dermatopathology have begun to consider KA and SCC as variants of a single entity (13). Confusingly, it has been considered as a pseudoneoplastic growth (14), a benign epidermal lesion (15), a preneoplastic lesion (16), an abortive malignancy that rarely progresses to invasive SCC (1), or as low-grade SCC (13). Clearly it is of the utmost importance to distinguish between benign lesions and those that may or will metastasize.

Electron microscope studies performed on KA and SCC (2, 17) showed significantly reduced expression of structures relating to the cohesiveness of epithelial cells (numerical and surface density of desmosomes, volume density of intercellular space) in SCC but not KA, so these characteristics may be useful in differential diagnosis. Only small areas of tumour cells can be scanned ultrastructurally, but results are also compatible with studies of intercellular substance (ICS) staining patterns (7, 18), which were completely preserved in KA but expressed focally, decreased or absent in SCC. The marker for ICS was pemphigus vulgaris serum with a high titre of anti-ICS antibody, applied to the frozen sections of both tumours, and visualized by indirect immunofluorescence.

The major pemphigus foliaceus (19) and pemphigus vulgaris (20) antigens have recently been characterized as desmoglein 1 (Dsg 1) and desmoglein 3 (Dsg 3). They have been shown to be reduced in invasive SCC (21). We have examined the expression of these desmosomal glycoproteins using a monoclonal antibody 32–2B developed by Vilela et al. (22). This antibody, an IgG2a class mouse immunoglobulin, recognizes the cytoplasmic domains of human Dsg 1 and Dsg 3 (23, 24), and can be used on sections from paraffin-embedded material. The study was designed to determine the pattern of desmoglein staining in KA and SCC, their relationship with prognosis and their potential value in routine histopathology.

MATERIAL AND METHODS

Strict clinical (1) and histological (2, 3) criteria were used to differentiate between KA and SCC (Table I). Tissue specimens were retrieved from dermatopathology files (Dermatopathology sections of the Department of Dermatology, University of Belgrade School of Medicine and St. John's Institute for skin diseases, London, UK), dated January 1991 to June 1994, and initial haematoxylin-eosin (HE) glass slides were reviewed by one author (AK). Small tissue samples,

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Table I. *Clinical and histological criteria used for the differential diagnosis of KA and SCC*

	KA	SCC
Clinical criteria		
Macroscopic appearance	pink-coloured, crateriform nodule with central keratin plug, regular circular border, with distinct margin	indurated, scaly plaque, indistinct margin, irregular border, sometimes eroded or ulcerated
Duration	less than 3 months	more than 6 months
Biological behaviour	rapid growth phase, then static or decreasing in size	continuous slow growth
Histological criteria		
Low power	invaginated, keratin-filled crater with epidermal collarette	multiple keratin-filled nests
High power	proliferation of the keratinocytes at the base and sides of the lesion, few mitoses, little anaplasia; large, eosinophilic, "ground glass" keratinocytes; intraepithelial abscesses; mixed cell infiltrate at the dermal side of the tumour;	disordered proliferation of the keratinocytes, multiple or atypical mitoses, pronounced anaplasia

specimens in which the lesion comprised only a minority of the tissue, were excluded, as well as cases with incomplete or absent clinical data from the patients' charts. From the remaining 100 paraffin blocks (38 KA and 62 SCC) 5-μm thick sections for HE were obtained, as well as 4-μm thick sections for immunochemical evaluation.

Immunocytochemical procedures

Immunocytochemistry was performed using the avidin-biotin complex immunoperoxidase method (ABC) as described by Hsu et al. (25), with the modifications outlined below. After the sections had been cut from paraffin blocks, they were deparaffinized in xylol, rehydrated through graded alcohols to water and treated with 1% H_2O_2 in methanol to block endogenous peroxidase activity. The sections were then trypsinized (0.1% trypsin in 0.1% $CaCl_2$, pH 7.8 at 37°C for 10 min) and washed first in water and then in Tris-buffered saline (TBS; 0.5M Tris/HCl, pH 7.8, diluted 1:10 with 0.15M NaCl). Each incubation lasted 30 min and was followed by three 10-min washes in TBS. The sections were then incubated with 1% bovine serum albumin (BSA) in Dulbecco's Minimum Essential Medium (DMEM) plus 20% fetal calf serum (FCS) for at least 30 min to eliminate non-specific binding. Excess BSA solution was then blotted from the slides and the diluted (1:20) monoclonal antibody applied. The sections were then treated with biotinylated rabbit anti-mouse IgG for 30 min, followed by the ABC (ABC kit, DAKO) for 60 min. Incubation was followed by three 5-min washes with TBS. Bound peroxidase-labelled antibody was revealed by the application of 3.3′ diaminobenzidine (DAB). Sections were then counterstained with Harris's haematoxylin. As a negative control normal mouse serum was used in place of the primary antibody, while regions of uninvolved epidermis served as a positive control when tumour staining was absent.

Classification and scoring of histological sections

HE sections were examined by a histopathologist (NPS and OC) and the SCC were further classified according to the degree of differentiation by the method of Broders (26), with the modifications summarized by Wick (27):

1) well-differentiated (WD) (more than 75% of keratinized cells – Broders I)
2) moderately differentiated (MD) (25% to 75% keratinized cells – Broders II/III)
3) poorly differentiated (PD) (less than 25% of keratinized cells – Broders IV)

The sections stained with the antidesmoglein antibody were examined independently by 3 of the authors (AK, DRG, GSO) without knowledge of the histological assessment or subsequent clinical course of the patient. The degree of desmoglein staining was graded as follows:

+++ extensive pericellular staining up to the tumour-host border
++ focal pericellular staining
+ juxtanuclear staining pattern
0 absent staining.

Statistical analysis was performed by the chi-square test.

Fig. 1. Widespread intense pericellular staining of keratoacanthoma with classical crateriform architecture with monoclonal antibody 32–2B. Staining extends to all non-keratinized epithelial layers. The keratinized crater which is not stained is indicated with an *arrowhead*. *D* = dermis. (×43).

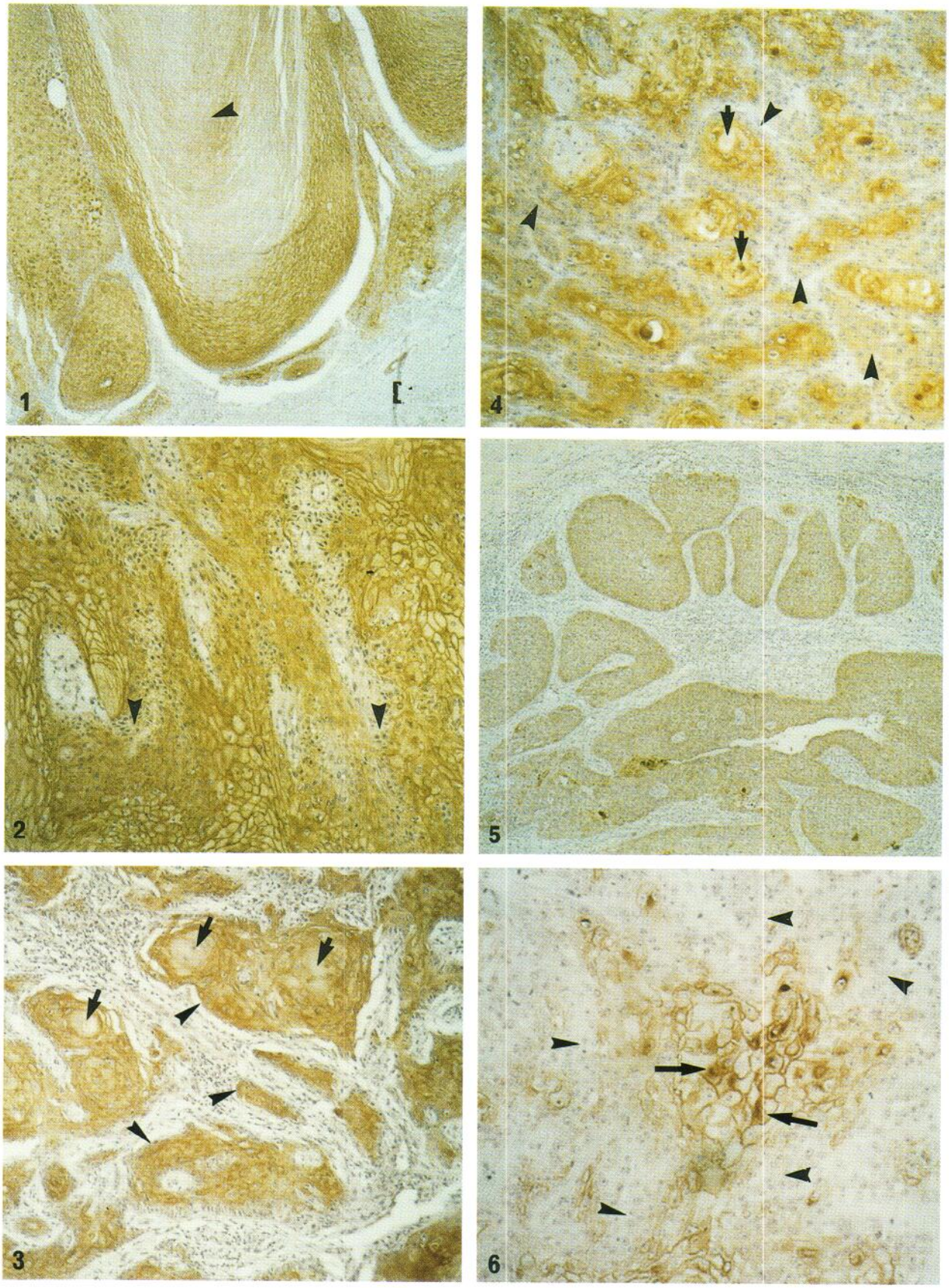
Fig. 2. Keratoacanthoma showing extensive pericellular staining with monoclonal antibody 32–2B (×86). Virtually all tumour cells show intense staining with only a few showing reduced staining (*arrowheads*).

Fig. 3. Isolated islands of keratoacanthoma showing complete retention of pericellular staining with 32–2B except in keratinized areas (*arrows*). *Arrowheads* indicate the boundaries of tumour islands where they abut on the extracellular matrix. Note that intense staining persists right up to this boundary. (×86).

Fig. 4. Well-differentiated squamous cell carcinoma showing patchy staining with 32–2B. Staining surrounds the unstained keratinized areas (*arrows*). However, each tumour island is surrounded "basally" by at least one layer of unstained cells (*arrowheads*) in stark contrast to the appearance of the KA shown in Fig. 3. (×86).

Fig. 5. Poorly differentiated squamous cell carcinoma showing complete absence of staining with 32–2B. (×43).

Fig. 6. Moderately differentiated squamous cell carcinoma showing juxtanuclear staining with 32–2B in localised areas (*arrows*) but absence of staining elsewhere (*arrowheads*). (×86).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RESULTS

All 38 specimens of KA exhibited significant expression of desmoglein. Thirty-five KA (92.1%) showed extensive pericellular staining (+ + +) throughout the non-cornified layers of the abnormal epidermis with very few or no cells remaining unstained (Figs. 1 and 2). There was no staining in the keratinized crater or at the contacts between the basal cells and the basement membrane (Figs. 1 and 2). This staining pattern is therefore entirely similar in distribution to that found in normal epidermis with this and other desmosomal antibodies (32). The remaining 3 KA (7.9%) showed extensive desmoglein expression in a focal pericellular pattern (+ +) (Fig. 3). Here again, the staining was detectable at the cell-cell boundaries of all non-keratinized epithelial cells, including those immediately adjacent to the extracellular matrix (arrowheads in Fig. 3).

Among 62 SCC examined, 20 SCC (32.3%–19 WD and 1 MD) exhibited focal pericellular expression of desmoglein (+ +) (Fig. 4), especially concentrated around regions of keratinization (horn pearls). However, in none of these cases did the staining extend to all epithelial layers: many or all of the cells adjacent to the matrix were negative (arrowheads in Fig. 4). Eleven SCC (17.7%–2 MD and 9 PD) were completely negative (Fig. 5). The remaining 31 (50%–9WD, 18 MD and 4 PD) specimens of SCC showed predominant juxtanuclear expression (+) of desmoglein (Fig. 6). None of the SCC studied showed pericellular staining of desmoglein throughout all epithelial cells, in marked contrast to KA.

Desmoglein expression was strong in well-differentiated tumours, but was absent in poorly differentiated ones. Moderately differentiated SCC mostly demonstrated juxtanuclear desmoglein expression. This relation was statistically highly significant ($p < 0.01$). Strong and extensive pericellular expression of desmoglein was noted only in KA (Table II).

DISCUSSION

In this study we found that KA retains desmoglein antigen throughout the whole body of the tumour, except in areas where the cells show advanced keratinization. This strong pericellular pattern of desmoglein expression is similar to the labelling characteristics of normal epidermis, previously reported (22). These results are compatible with the complete retention of pemphigus antigen in KA shown in several indirect immunofluorescence studies (7, 18). By contrast, we found that desmoglein staining in SCC was always either partially reduced or completely absent. These results appear consistent with an electron microscope analysis, where number and surface density of desmosomes were found to be significantly lower in well-differentiated SCC than in KA without any overlap (17). This ultrastructural study also found an intermediate number and size of desmosomes in KA between normal epidermis and well-differentiated SCC and suggested that KA presents an intermediate lesion in this spectrum. The focal absence of pericellular desmoglein expression in the central areas of 3 KA examined is probably consistent with advanced keratinization and keratinocyte-corneocyte transformation, with subsequent loss of organized desmosomal structures on the cell membranes, as shown previously in normal skin (22).

Table II. *The relationship between desmoglein expression and differentiation of the tumours examined*

The degree of desmoglein staining was assessed in the following way: + + + = diffuse pericellular expression; + + = focal pericellular expression; + = juxtanuclear expression; 0 = negative staining

Tumour type	Degree of desmoglein staining + + +	+ +	+	0	Total
Keratoacanthoma	35	3	0	0	38
Well-differentiated squamous cell carcinoma	0	91	9	0	28
Moderately differentiated squamous cell carcinoma	0	1	18	2	21
Poorly differentiated squamous cell carcinoma	0	0	4	9	13
Total	35	23	31	11	100

Focal absence of desmoglein expression in well-differentiated SCC may provide an early index of impaired differentiation, leading to loss of desmosomes and reduced intercellular adhesion. The weak expression of desmogleins 1 and 3 or their complete absence may suggest potentially invasive behaviour, since tumour cells without well-developed desmosomes may be easily detached from the primary site, thus initiating metastases (28). It has been shown previously that reduction in staining for desmoglein correlates with invasion in transitional cell carcinoma (29) and with metastatic spread in SCC of the head and neck (28). Juxtanuclear expression of desmoglein, as found here in some SCCs, probably represents internalized desmosomes (22) and may also indicate reduced intercellular adhesion. This event characterizes malignant behaviour and has been reported in several electron microscope studies of many different types of carcinoma (30). However, juxtanuclear pattern of desmoglein expression was not detected in normal epidermis as shown by Vilela et al. (22) and was also consistently absent in all KAs examined in our study.

Retention of desmosomes in KA shows that this keratinocyte neoplasm is well-differentiated and suggests that this may explain the difference in clinical behaviour when compared to SCC. The ability to metastasize, while not an absolute criterion, is generally accepted as an indicator of malignancy. The suggestions from some authorities that KA is a variety of SCC (13) or that classical KA may metastasize (13, 31) are not entirely convincing. It is likely that in the majority of cases so-called metastasizing KA represents wrongly diagnosed SCC. Recent studies indicate that KAs lose bcl-2 expression in the process of maturation, as pointed out by Sleater et al. (32). Since bcl-2 protooncogene is responsible for the inhibition of apoptosis, its loss is compatible with the involution of KA. In his study, proliferative KAs are bcl-2-positive only at the basal cell area, with subsequent loss of the staining in regressive KAs. Basal cell pattern of bcl-2 staining has also been detected in normal epidermis and adnexal structures (33). This may be concordant with controlled and predetermined evolution of KA through the phases of proliferation, maturation and involution. Conversely all examined SCC in Sleater's study exhibited diffuse presence of bcl-2 consistent with uninhibited, uncontrolled proliferation present in these tumours. Emphasizing the striking difference in the staining patterns, Sleater at al. (32) also questioned if KAs were "deficient squamous cell carcinomas".

An alternative explanation for "metastasizing KAs" could be that the initial lesion represented a combined SCC and KA

or that SCC may have developed from a preexisting KA. It is possible that KA and SCC represent part of a biologic spectrum with KA at one end and poorly differentiated SCC at the other. However, we believe that the results of our immunohistochemical study with 32–2B antibody indicate that desmosomal glycoproteins present important markers that may be helpful in the distinction of these two neoplasms.

In the authors' opinion it is most likely that KA does not metastasize and classical SCC does not undergo spontaneous involution. One possible exception to this is the condition known as familial self-healing carcinoma of Ferguson-Smith, which histologically may be indistinguishable from a well-differentiated SCC. Use of immunological markers already mentioned (4–8, 18) as well as the present study of desmosomal glycoproteins should help to distinguish KA from SCC more clearly.

In conclusion, immunohistochemical investigation with 32–2B antibody is a valuable technique which may help in the differential diagnosis of KA and SCC and, since it reacts with paraffin-embedded tissue, is of potential value in routine histopathology. It has a clear advantage over electron microscope evaluation since it is easily performed and not time-consuming, and larger areas of the lesion can be examined at the light microscopic level. It also has an advantage over many previously reported immunohistochemical studies for KA and SCC (4–8, 18), because it reacts with fixed, paraffin-embedded tissues and so is suitable for retrospective studies.

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