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Abnormal Desmoglein Expression by Squamous Cell Carcinoma Cells

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Abnormal expression of cell adhesion molecules and related proteins has been observed in various carcinoma cells. We compared expression patterns of desmosomal cadherins, E-cadherin, and cytoplasmic plaque proteins of four different human squamous cell carcinoma cell lines and in vivo squamous cell carcinoma cells with those of normal human keratinocytes. Unlike normal human keratinocytes, the squamous cell carcinoma cells, both in culture and in vivo, exhibited diminished or unusual expression of desmoglein 3 and desmoglein 1, which bear pemphigus vulgaris and pemphigus foliaceus antigens, respectively. Abnormal expression of E-cadherin and cytoplasmic plaque proteins such as desmoplakin and plakoglobin was also observed. Western blotting study demonstrated that three squamous cell carcinoma cell lines expressed two desmogleins with a predominant 150 kDa molecule, and a minor 130 kDa one. Although these molecular sizes were similar to those of cultured normal human keratinocytes, the 130 kDa desmoglein, which usually carries pemphigus antigenic epitopes, was weakly or negatively reactive with pemphigus vulgaris serum. One squamous cell carcinoma cell line showed a doublet of 140 and 145 kDa bands in addition to the 130 kDa band. All the carcinoma cell lines constantly expressed desmoglein 2 and desmoglein 3 mRNA, whereas cultured normal human keratinocytes always expressed desmoglein 1 and desmoglein 3 mRNA, with or without desmoglein 2 mRNA. These findings indicate that the squamous cell carcinoma cells revealed abnormal expression of desmoglein isoforms, which may be related to tumour cell kinetics such as cell invasion and metastasis. ***Key words: desmosomes; cadherin; cell adhesion; keratinocyte; acantholysis.***

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Acantholysis, a histologic hallmark of pemphigus, is often seen in squamous cell carcinoma (SCC) and solar keratosis despite the absence of pemphigus autoantibodies. Previous electron microscopic studies of SCC cells showed decreased numbers of desmosomes with altered structures, suggesting the impairment of cell-cell cohesion (1). Biologically, cell adhesion by desmosomes is mediated by two major desmosomal cadherins, desmogleins and desmocollins (2). Recent molecular studies disclosed that desmogleins include 130/140 kDa desmoglein bearing pemphigus vulgaris antigens (Dsg3 or PVA), 150/160 kDa desmoglein bearing pemphigus foliaceus antigens (Dsg1 or DGI), and colon type desmoglein (Dsg2 or HDGC) (3).

In the adherent junction of the epidermis, cell-cell adherence is induced by homophilic binding of E-cadherins (4). It has been reported that expression of E-cadherin is diminished in epithelial carcinomas, which induces cell detachment from the primary tumour cells, with subsequent cell invasion or distant metastasis (5). Because desmogleins and pemphigus antigens are a group of adhesion molecules specific for epidermal desmosomes, we studied their expression patterns by SCC cell lines. We found abnormal expression patterns of desmogleins and their isoform mRNA in the SCC cells.

MATERIALS AND METHODS

Cells and culture study

We used keratinocytes of different origins: normal human keratinocytes from foreskin (EpiPack, Clonetics Co., San Diego, CA, U.S.A.), three biopsy specimens from normal skin, and four human SCC cell lines, termed HSC-1, 5, 6, and KOM-1, respectively. The HSC-1, 5, and 6 cell lines were generous gifts from Drs. S. Kondo and Y. Katagata (Yamagata University, Yamagata, Japan). The KOM-1 cell line was established in our laboratory, from a biopsy specimen obtained from a patient with SCC.

Normal human keratinocytes were cultured in serum-free medium (Gibco Laboratories, NY, U.S.A. or Clonetics Co., CA, U.S.A.) until 60–70% confluency was gained. In order to form cell-cell contact, the medium was changed to minimum essential medium containing 10% foetal calf serum (MEM/FCS) and the culture continued for 48 h. The SCC cell lines were cultured in MEM/FCS throughout.

Three biopsy specimens were obtained from 3 patients with SCC, and stored at −80°C until use.

Serum samples

Pemphigus sera were obtained from 5 patients with pemphigus vulgaris and 4 with pemphigus foliaceus. The sera contained IgG pemphigus antibodies at titres ranging from 1:10 to 1:640, as determined by immunofluorescence using normal human skin as substrate.

Antibodies and immunofluorescence study

Monoclonal antibodies to human E-cadherin (clone HECD-1), desmoglein (DG3.10), desmoplakin (DP2.15), and plakoglobin (PG5.1) (Progen, Heidelberg, Germany) were used at a dilution of 1:200 for Western blotting. The monoclonal anti-desmoglein antibody (DG3.10) reacted with an epitope of cytoplasmic domain of desmogleins (6), and gave 150 and 130 kDa bands in Western blotting study using extracts of cultured normal human keratinocytes (7). Fluorescein isothiocyanate-labelled goat anti-mouse IgG or goat anti-rabbit IgG was used for a second antibody.

Cryostat sections of the biopsy specimens from 3 patients with SCC or cultured keratinocytes on Chamber Slides (Lab-Tek, Naperville, U.S.A.) were incubated with diluted sera or the primary antibodies at room temperature for 60 min and then rinsed in phosphate-buffered saline (PBS). The subsequent reaction with the conjugated antibodies was carried out for 30 min each. After washing in PBS and mounting with 50% glycerin in PBS, immunoreaction was examined with a fluorescence microscope.

Western blotting

Approximately 7.5×10^5 cells of the cultured keratinocytes were homogenized in 100 µl of extraction buffer containing 50 mM Tris-HCl, 0.5% sodium dodecyl sulphate (SDS), 1 mM phenylmethylsulphonylfluoride, 5 mM N-methylmaleimide, 3 mM calcium chloride. One-fourth volume of the extract was separated by SDS-polyacrylamide gel electrophoresis in 6% slab gel, followed by transblotting to

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nitrocellulose paper. The blotted paper strips were first blocked with Block Ace (Dainippon Pharmaceutical Co. Osaka, Japan) and incubated with pemphigus sera or monoclonal anti-desmoglein antibody. The strips were reacted with biotin-labelled anti-human or anti-mouse IgG, and subsequently incubated with horseradish peroxidase-labelled streptavidin. Between each incubation, the strips were washed three times with Tris-HCl buffer containing 0.5% Tween 20. The peroxidase activity was visualized by incubation with 4-chloro-1-naphthol.

Reverse transcriptase-polymerase chain reaction

Total RNA was isolated from approximately 1×10^5 cells of the cultured keratinocytes, and poly(A)+ RNA was separated using oligo(dT)-cellulose. cDNA was synthesized by means of hexanucleotide random primers in the presence of reverse transcriptase. The RNA extraction and cDNA synthesis were performed by Micro-Fast Track® of mRNA isolation kit and cDNA Cycle Kit® (Invitrogen, CA, U.S.A.), respectively. One-tenth volume of the cDNA was processed for PCR amplification using the following sequence specific primers: Dsg1 (DGI): sense, 5′-TCAAGTTCGCAGCAGCCTGT-3′ and antisense, 5′-TGTTCGGTTCATCTGCGTCA-3′ (expected size of amplified products; 411 bp), Dsg2 (HDGC): sense (JA156), 5′-CAGTAGCTTCCCAGTTCC-3′ and antisense (JA157), 5′-CTGTAAGCTTCATGAAAAATCAG-3′ (products; 803 bp) (8), Dsg3 (PVA): sense, 5′-ACCGAATCTCTGGAGTGGGAA-3′, and antisense 5′-GCACCACTCACAACCAGACGAT-3′ (products; 467 bp). Primers specific for β-actin were purchased from Stratagene Cloning Systems, U.S.A. (products; 661 bp).

PCR was carried out in 50 µl of buffer containing 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 100 µg/ml gelatin, 200 mM deoxynucleotide triphosphate (dNTP), 1.25 U of Taq polymerase, and 20 pmol of the respective primers (9). The reaction consisted of 40 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 2 min, and extension at 72°C for 2 min. The amplified DNA was analyzed on 2% agarose gel and the fragment size was determined by comparison with control DNA fragments.

RESULTS

Expression of desmosomal antigens by SCC cells

Cultured keratinocytes demonstrated differences in the expression of desmosomal antigens between normal human keratinocytes and SCC cell line cells. The cell-cell contact areas of the cultured normal human keratinocytes were constantly positive for pemphigus vulgaris antigens, desmoglein (DG3.10), desmoplakin (DP2.15), plakoglobin (PG5.1), and E-cadherin (HECD-1) (Fig. 1). As previously reported (7), pemphigus foliaceus antigens were detected in the intercellular

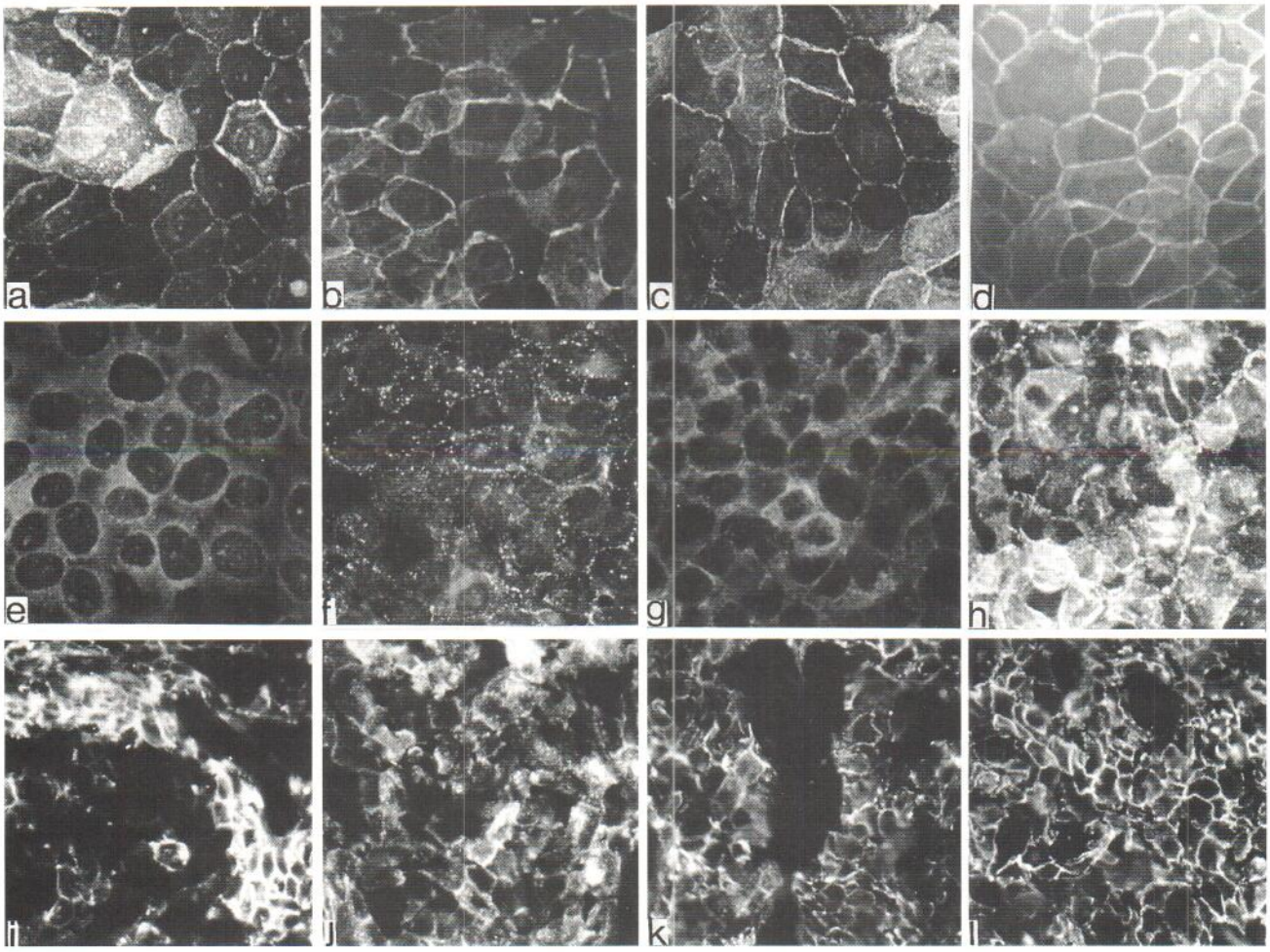

Fig. 1. Expression patterns of pemphigus vulgaris antigens (Dsg 3), desmoglein 1 (Dsg 1), plakoglobin (PG), and E-cadherins by cultured normal human keratinocytes, HSC-1 cells, and SCC cells in the biopsy specimens. Top (*a–d*); cultured normal human keratinocytes, middle (*e–h*); HSC-1 cells, bottom (*i–l*); SCC biopsy specimen. Pemphigus vulgaris antigens (Dsg 3); *a*, *e* and *i*, Dsg 1; *b*, *f*, and *j*, PG; *c*, *g*, and *k*, and E-cadherin; *d*, *h*, and *l*.

spaces of the stratified keratinocytes, but not in the monolayers of cultured normal human keratinocytes.

The expression patterns of the desmosomal antigens by the SCC cell line cells were different from those by the normal human keratinocytes. Most cell line cells exhibited diminished or lacked expression of desmosomal antigens, including pemphigus vulgaris, pemphigus foliaceus, desmoglein, desmoplakin, and plakoglobin antigens. E-cadherin was detected on the cell surfaces as well as cytoplasm of the cell line cells in a dot-like or discontinuous fashion (Fig. 1).

The intercellular spaces of normal human epidermis were positively stained with pemphigus vulgaris sera, pemphigus foliaceus sera and antibodies to desmoglein, desmoplakin, plakoglobin, and E-cadherin, as observed in the cultured normal human keratinocytes (data not shown). In three biopsy specimens obtained from patients with SCC, some tumour cells expressed those antigens in the intercellular spaces, but other cells in the different portions did not (Fig. 1). The differences of expression patterns were not clear between superficial and deep areas, or between cell dense and sparse areas.

Western blotting study

Cultured normal human keratinocytes constantly expressed 150 and 130 kDa bands positively stained with monoclonal anti-desmoglein antibody (DG3.10) (Fig. 2A). The minor 130 kDa band was stained with pemphigus vulgaris sera (Fig. 2B). Among the SCC cell line cells, the HSC-5, 6, and KOM-1 cells expressed both 150 and 130 kDa bands similar to the cultured normal human keratinocytes. The HSC-1 cells, however, expressed a doublet of 140 and 145 kDa bands in addition to the 130 kDa band.

The 130 kDa molecules expressed by the SCC cell line cells were weakly stained with pemphigus vulgaris sera in the HSC-6 and KOM-1, and almost negative in the HSC-1 and 5 (Fig. 2B).

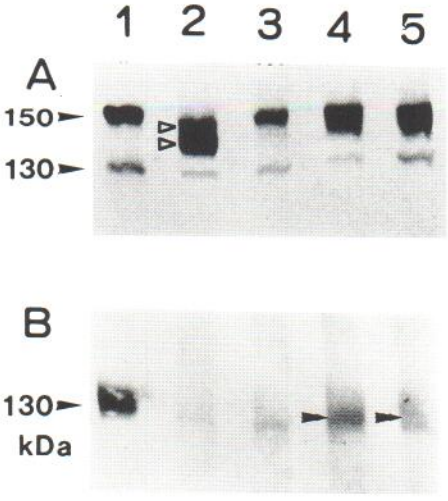

Fig. 2. Western blotting study using extracts of cultured normal human keratinocytes (*lane 1*), HSC-1 (*lane 2*), HSC-5 (*lane 3*), HSC-6 (*lane 4*), and KOM-1 (*lane 5*) cells, stained with monoclonal anti-desmoglein antibody (DG3.10) (A) and a pemphigus vulgaris serum (B). *Open triangles*; unusual sizes of desmogleins with 145 and 140 kDa.

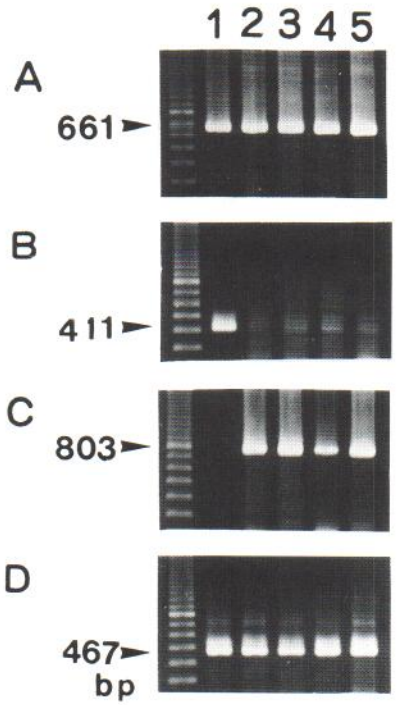

Fig. 3. Expression of desmoglein isoform mRNA by cultured normal human keratinocytes (*lane 1*), HSC-1 (*lane 2*), HSC-5 (*lane 3*), HSC-6 (*lane 4*), and KOM-1 (*lane 5*) cells. A; β-actin, B; Dsg 1 (DGI), C; Dsg 2 (HDGC), D; Dsg 3 (PVA).

Expression of desmoglein isoform mRNA by SCC cells

Both Dsg1 (DGI) and Dsg3 (PVA) mRNA was constantly detected in normal human keratinocytes cultured in the MEM/FCS medium for 0–72 h (Fig. 3). The expression of Dsg2 (HDGC) mRNA, however, was variable by skin samples and experimental conditions. Dsg2 (HDGC) mRNA was positive in one out of three primary cultures of the normal human keratinocytes, and was occasionally detected by repeated PCR experiments with the same cDNA samples which had once given a negative result. In contrast, all the four SCC cell lines always expressed both Dsg2 (HDGC) and Dsg3 mRNA intensely but did not produce Dsg1 (DGI) mRNA (Fig. 3).

DISCUSSION

Structural and quantitative abnormalities of desmosomes have previously been shown in SCC cells (1). The present study demonstrated that SCC cells, both in vivo and in culture, exhibited diminished or unusual expression patterns of desmosomal antigens including desmogleins, desmoplakins, and plakoglobins. In addition to the abnormal expression of the desmosomal antigens, E-cadherin expression was also irregular in the SCC cell lines as well as the SCC lesions. Although it has to be taken into account that the properties of cell line cells might have changed from those of original tumour cells during the long-term cultivation, the immunostain with tumour specimens supports the abnormal findings observed in the cell line cells. The results of RT-PCR amplification showed that cultured normal human keratinocytes mainly expressed Dsg1 (DGI) and Dsg3 (PVA) mRNA. Dsg2 (HDGC) mRNA

expression could be detected in some samples but not in all. These findings suggest that Dsg2 (HDGC) is not a constitutive molecule in normal epidermis, and its mRNA expression might be variable. Alternatively, a very small amount of Dsg2 (HDGC) might be expressed by certain types of keratinocytes in the epidermis.

In contrast, all the four SCC cell lines constantly expressed two desmoglein isoforms; Dsg2 (HDGC) and Dsg3 (PVA) mRNA. The cell lines lacked or diminished expression of Dsg1 (DGI) mRNA, which is a major desmoglein isoform in normal epidermis. Furthermore, the results of Western blotting study demonstrated that one of the SCC cell lines expressed abnormal sizes of desmoglein isoforms with 140 and 145 kDa. Therefore, the desmosomal components of the SCC cell lines were biologically different from those of normal human keratinocytes. Since Dsg1 (DGI) is predominantly observed in differentiated normal keratinocytes (7,8), the lack of this molecule in the SCC cells might be related to dedifferentiation of keratinocytes. We speculate that the lack of differentiation is an initial event of carcinoma cells, which leads to the various changes of biological properties of SCC, such as abnormal expression of cell adhesion molecules.

Because blocking the action of E-cadherin by monoclonal antibodies causes dispersion of compact cell colonies (10), this molecule is thought to be important in cell-cell adhesion. It has also been reported that the selective loss of E-cadherin expression can generate dedifferentiation and invasiveness of human carcinoma cells (5). Furthermore, the impaired expression of α-catenin, which is directly bound to the cytoplasmic domain of cadherins, is closely related to tumour cell dedifferentiation, infiltrative growth, and lymph node metastasis (11). Therefore, it became apparent that components of adherent junctions play an essential role for cell adhesion.

Recent studies, however, have demonstrated that acantholysis, a loss of cohesion of keratinocytes, is induced by blocking the function of Dsg1 (DGI) and Dsg3 (PVA) with pemphigus foliaceus and pemphigus vulgaris sera, respectively (12). Therefore, in addition to the impaired expression of E-cadherin, abnormal usage of desmoglein isoforms and abnormal glycosylation of the molecules might be responsible for the disruption of cell-cell adhesion. Although further studies are necessary to determine the dysfunction of desmogleins expressed by SCC cells, our results suggest that the abnormal expression of desmoglein isoforms might be related to the cell detachment from the primary tumours, with subsequent invasion of the cells.

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