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Aberrant expression, processing and degradation of dystroglycan in squamous cell carcinomas

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Abstract

The α- and β- dystroglycan (DG) proteins are involved in epithelial cell development, formation of the basement membrane and maintenance of tissue integrity. Recently, specific changes in the expression patterns of DGs have been described in some cancers. We studied the expression and localisation of α- and β-DG using Western blotting, immunohistochemistry and reverse transcriptase–polymerase chain reaction analyses in samples of normal oral mucosa, oral squamous cell carcinoma (SCC) and cancer cell lines. The α- and β-DG were localised in the basal layers of normal oral mucosa.However, β-DG expression in cancer tissues showed evidence of aberrant expression, processing and degradation. α-DG was altered in all oral cancer samples and cell lines, despite the persistent presence of DG mRNA in cancer cells. Using matrix metalloproteinase (MMP) inhibitors, we determined that β-DG degradation in carcinoma cell lines can be mediated by MMPs but this process is highly variable, even in cells from the same cancer type. Considering the multifaceted role of DG in epithelial development, it appears that the role of DG degradation in cancer growth and spread, although currently poorly understood, may be important.

Keywords: Squamous cell carcinoma; α- and β-Dystroglycan; Matrix metalloproteinases

1. Introduction

Oral squamous cell carcinoma (SCC) is the sixth most common malignancy worldwide. The tongue and the floor of mouth are the most common sites of origin in the Western world. Furthermore, the incidence of oral cancer has increased over the same time period in both the United Kingdom (UK) and United States of America (USA), particularly in the under 50-year old age group [3,16]. In UK alone, there are ≈3500 new cases of oral cancer diagnosed per year, and 1600 deaths. Both the disease and its treatment are associated with a high rate of morbidity, and despite improvements in the diagnosis and management of oral SCC, the crude 5-year mortality rate of ≈45% has changed little in the last 30 years. Moreover, metastasis to the cervical lymph nodes reduces the chance of cure by 50% [33]. A greater understanding of the disease processes is needed in order to improve patients outcome and develop novel treatment modalities.

The cell–cell and cell-extracellular matrix (ECM) interactions have a pivotal function in the development and maintenance of the cytoarchitecture in normal tissues and play an important role in the development and progression of many types of cancer. In fact, abnormalities in the interactions between tumour cells and ECM proteins are often implicated in the aberrant behaviour of carcinoma cells and defects in the structure and function of basement membranes are hallmarks of metastatic disease [19].

The roles of the different adhesion molecules and their binding ligands are not fully understood. It is clear

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that these proteins are multi-functional – they not only connect cells to the basal lamina, but also contribute to the exchange of information with the extracellular environment, connect and organise the cytoskeleton, and participate in signal transduction processes [19]. As such, adhesion molecules are critically important for proper tissue growth, differentiation and maintenance of cytoarchitecture. Until recently, studies on cell adhesion molecules involved in cancer have mainly concentrated on the integrin family [4,19]. However, there is a growing body of evidence that other adhesion molecules may play an equally important role.

We have focused our attention on the function of the dystroglycan (DG) complex. DG is a cellular receptor expressed in a variety of tissues and it interacts with extracellular proteins like laminin, perlecan and agrin [41] and also membrane proteins – e.g., neurexins [36].

The biosynthesis and structure of DG are complex [41]. DG is a product of a single gene, but the primary peptide is post-translationally cleaved, resulting in two protein subunits (α and β), which interact to form a functional non-covalent complex [18]. The β-DG is a 43 kDa transmembrane protein. The predicted 72 kDa α-DG peptide undergoes further post-translational modifications resulting in a heavily glycosylated extracellular protein. Its molecular mass can vary in different tissues (120–180 kDa), depending on the degree of glycosylation.

In muscle tissue, α/β-DG functions as part of a large dystrophin-associated protein (DAP) complex, which is an array of transmembrane, cytoplasmic and extracellular proteins that are crucial for proper muscle function. Mutations disrupting this complex result in muscular dystrophy. However, DG also has a role in non-muscle tissues. The DG gene knockout in mice results in embryonic death *in utero*. This early lethality is caused by disruption of the Reichert's membrane – an early basement membrane [39]. Later in foetal development, this protein has been shown to have a crucial role in kidney and salivary gland epithelial morphogenesis [8]. Moreover, in the adult mouse, DG is present in epithelial cells in several other non-muscle organs (including squamous epithelia) [7]. It is particularly enriched at the basal interfaces of cells directly apposing basement membranes [14]. Finally, disruption of brain DG results in neuronal migration errors in the developing brain [27,28]. This evidence points to DG having a role in epithelial cell development and the formation of the basement membrane and indicates its importance in the formation and maintenance of tissue integrity.

Recently, specific changes in DG expression in human breast, colon and prostate cancers have been described [15,25,32].

Herein, we analysed DG expression in samples of normal human oral epithelia, primary and metastatic SCCs and a range of tumorigenic SCC lines to study its involvement in oral cancer.

2. Materials and methods

2.1. Primary antibodies

Mouse monoclonal anti-β-DG NCL-b-DG (Novocastra, UK) recognising 15 amino acids at the extreme C-terminus of human DG; mouse monoclonal (clone 56, BD Biosciences, UK) generated against an epitope encompassing amino acids 655–767 in the middle of human β-DG and two mouse monoclonal antibodies anti-α-DG: VIA4-1 (Upstate Lab, USA) and anti-α-DG (IgM, US Biological, USA), both generated using rabbit skeletal muscle membrane preparation as an immunogen were used. Only VIA4-1 is suitable for immunolocalisation.

2.2. Cell lines

SCC-4, SCC-9, SCC-15 and SCC-25 (human tongue SCCs) and HT-29 (human colon adenocarcinoma grade II) were obtained from the American Tissue Culture Collection (LGC Promochem, Middlesex, UK). Cells were grown in Dulbecco's modified Eagle's medium supplemented with nutrient mixture F-12 Ham's (containing 15 mM HEPES, NaHCO, pyridoxine and L-glutamine), 10% foetal bovine serum, penicillin, streptomycin and 400 ng/ml hydrocortisone (Sigma–Aldrich, Poole, UK). Cultures were maintained at 37 °C in a humidified atmosphere with 5% CO_2.

For the matrix metalloproteinase (MMP) inhibition studies, cells were grown to confluence as described above, and the growth media substituted with a fresh one of the same composition but containing 25 μM of the GM6001 inhibitor (Ilomastat) or 2–20 μM of MMP2/MMP9 inhibitor IV (SB-3CT). Both inhibitors were obtained from Chemicon International and used according to the manufacturer's instruction. The inhibitor stocks were prepared in dimethyl sulfoxide (DMSO). Ilomastat was added directly to the culture medium, while SB-3CT was diluted in buffer R (50 mM HEPES [pH 7.5], 150 mM NaCl, 5 mM $CaCl_2$, 0.01% Brij-35 and 50% DMSO) prior to addition.

After incubation for 48 h in the presence of either inhibitor, cells were washed three times with phosphate buffered saline (PBS) and used for protein extraction as described below.

2.3. Tumour samples

Surgically resected primary tumours, metastatic lymph nodes and control samples of squamous epithelia were obtained and used in accordance with the institutional guidelines and the ethical permissions granted. All studies were performed using fresh-frozen specimens.

2.4. Immunolocalisation

Cryosections (10 μm) were cut, mounted on poly-L-lysine-coated glass slides and fixed with 4% (w/v) paraformaldehyde in PBS for 15 min on ice. Following blocking of endogenous peroxidases, the sections were pre-incubated for 30 min in PBS containing 10% (v/v) normal horse serum, and further treated with Vector Blocking kit (Vector Laboratories, UK) to block endogenous biotin activity. After blocking, the sections were incubated overnight at 4 °C in PBS containing 10% (v/v) normal horse serum and mouse monoclonal β-DG antibody NCL-b-DG at a 1:100 dilution. Following incubation with the primary antibody, the sections were washed, incubated for 30 min with horse anti-mouse biotinylated secondary antibody (1:200, Vector Laboratories, UK) diluted in PBS with 2% normal horse serum and the signal was visualised with Vectastain ABC reagent and Vector VIP substrate kit. The sections were counter-stained with methyl green (Vector Laboratories, UK).

For the immunolocalisation of α-DG with the mouse monoclonal antibody VIA4-1 the protocol was as described above, but with the following modifications. Blocking was in PBS with 8% bovine albumin and the primary antibody was diluted 1:500 in PBS with 1% bovine albumin.

2.5. Western blotting

2.5.1. Protein extraction

Tissues (tumour and control human muscle samples) were homogenised in ice-cold buffer containing 10 mM 2-[4-(2-Hydroxyethyl)-1-piperazine]ethane Sulfonic acid (pH 7.5), 0.5% Triton X-100, 5 mM EGTA, 5 mM ethylene (oxyethylene nitrilo)tetraacetic acid (EDTA), 2 mM sodium *ortho*vanadate, 50 mM sodium fluoride, 100 mM sodium chloride and protease inhibitors (Complete™, Roche) using polytron. After incubation on ice for 25 min on a shaking platform, the samples were centrifuged at 200*g* for 5 min at 4 °C and the supernatants were centrifuged again at 16 000*g* for 25 min at 4 °C. For immunoblotting, the pellets were solubilised for 4 min at 100 °C in solubilisation buffer (50 mM Tris–HCl; pH 7.5, 2% (w/v) sodium dodecyl sulphate (SDS), and 1% (w/v) dithiothreitol).

Proteins from the tumour cell lines were extracted as described above or using a simplified protocol: in this case, cells were scraped off the dish in PBS, pelleted and solubilised in the Triton lysis buffer (1% Triton X-100, 150 mM NaCl, 10 mM Tris pH 7.4, 1 mM EDTA, 1 mM EGTA, 0.5% Tergitol NP-40 with Complete™ protease inhibitors, Roche). Following 20 min incubation on ice with shaking and brief centrifugation, the supernatant containing the total protein extract was used for the SDS–polyacrylamide gel electrophoresis (PAGE) analysis. Both methods were run concurrently and produced equivalent results.

2.5.2. SDS–PAGE

Solubilised proteins (30–50 μg) were mixed with Laemmli sample buffer (Bio RAD, UK) and 0.05% mercaptoethanol, heated to 95 °C for 4 min, separated on 6–10% SDS–polyacrylamide gels and electroblotted onto Hybond ECL membranes (Amersham Pharmacia Biotech, Bucks, UK). The blots were incubated overnight with a blocking solution containing 5% non-fat milk powder in PBST (PBS/0.05% Tween 20) at 4 °C and then incubated with NCL-b-DG, clone 56, VIA4-1 or US Bio antibodies at 1:50, 1:500, 1:100 and 1:1000 dilutions, respectively, in the same blocking solution for 2 h at room temperature. Following three washes with PBST, membranes were incubated with horseradish peroxidase-conjugated secondary anti-mouse IgG (1:10 000, Amersham Pharmacia, Biotech UK) or, in the case of US Bio, anti-mouse IgM (Vector Laboratories, UK), at room temperature for 30 min and the signal was then visualised on films using the enhanced chemiluminescence (ECL) plus kit (Amersham Pharmacia Biotech, UK). As a negative control, the primary antibody was omitted from the incubation mixture and the rest of the protocol was performed as described above. Rat brain and human skeletal muscle were used as positive controls (detecting specific bands of 130 and 156 kDa, respectively).

2.6. Reverse transcriptase–polymerase chain reaction analysis

Total cellular RNA was extracted from SCC cells using the total RNA isolation system (Promega, Southampton UK) according to the manufacturer's instructions. RNA samples (3 μg) were digested with 3 U of amplification grade deoxyribonuclease I (Life Technologies, Paisley, UK) for 20 min at room temperature to eliminate the contaminating DNA. Half of the resulting RNA sample was reverse-transcribed at 42 °C using 200 ng of random primers, 400 U of Superscript II reverse transcriptase (Life Technologies, Paisley, UK) and 40 U of RNasin (Promega, Southampton UK) in a final volume of 50 μl. The remaining RNA was used to prepare a control sample in which the reverse transcriptase was omitted. One to two microlitre aliquots of each sample were used for reverse transcriptase–polymerase chain reaction (RT–PCR) analysis in a 50 μl reaction volume containing 200–500 nM primers, 3 mM $MgCl_2$, 200 μM of each deoxynucleoside triphosphate (dNTP) and 2.5 U of *Taq* polymerase (Qiagen, Crawley, UK or Life Technologies, Paisley, UK).

Two primer sets were designed and used as described before [12].

Set 1: Gly–1Rv: 5′-CGCGGGTGATGTTCTG-CAGGGTGA-3′ and Gly–2Fvd: 5′-ACCCA ACCAG-CGCCCAGAGCTCAAG-3′.

Set 2: Gly–1Rv: as above and Gly–3Fvd: 5′-AT-GGCTCCTCCAGTCAGGGATCCTG-3′.

Cycling conditions were: 94 °C for 4 min, followed by 35 cycles of 94 °C for 60 s, 55 °C for 60 s, 72 °C for 60 s with a final extension step of 72 °C for 7 min.

PCR products were resolved by electrophoresis in 1–2% agarose gels and visualised by ethidium bromide staining.

3. Results

We initially analysed the localisation of α- and β-DG in normal human squamous epithelium (tongue). Both α- and β-DG were found to co-localise in the basal cell layers of the squamous epithelium (Figs. 2(a) and 1(a), respectively). Staining with β-DG was concentrated almost exclusively on the basolateral surfaces of the basal cell layer, while weak staining with α-DG was also present in the upper layers. The specificity of staining with both antibodies was confirmed by an internal tissue

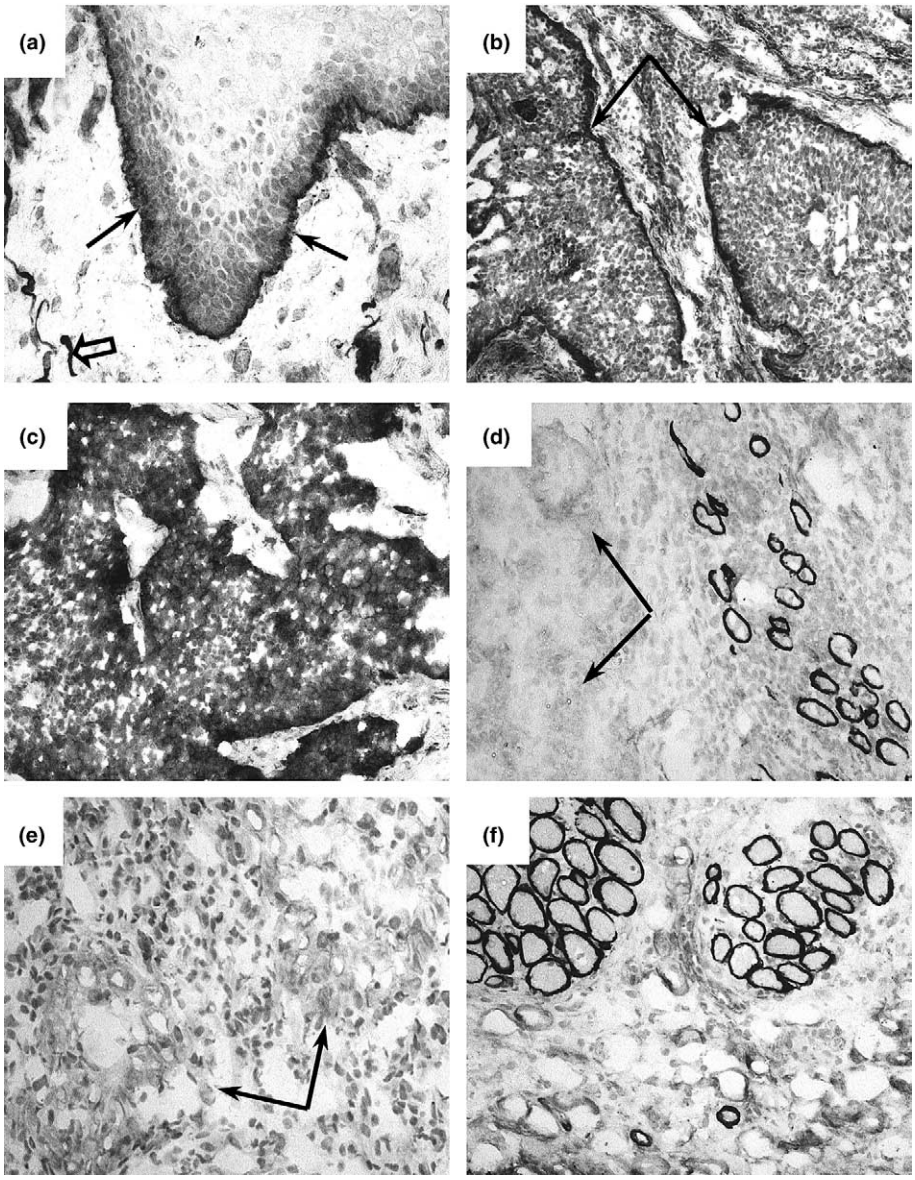

Fig. 1. Immunolocalisation of β-dystroglycan (β-DG) in normal oral epithelium and oral cancer. Mouse monoclonal anti-β-DG antibody: NCL-b-DG (Novocastra) was used in this study. (a) Normal oral epithelium. Note the strong staining in the basal cell layer (arrows) and in blood vessels in the stroma (open arrow). (b)–(f) Oral cancers (b). Soft palate squamous cell carcinoma (SCC), moderately/poorly differentiated. Strong expression in cancer cells in areas directly in contact with the stroma (arrows). (c) Soft palate SCC, moderately differentiated. Note the strong expression in cancer cell membranes in the entire tumour mass. (d) and (f) Tongue SCC, poorly differentiated. Note the complete lack of β-DG expression in the tumour mass (arrows) with strong expression in the muscle fibre membranes (e). SCC metastasis in a neck lymph node (arrows). The primary tumour from which this metastasis developed is shown in (b). Magnifications: (a), (e) and (f) 400×; (b) 100×; (c) and (d) 200×.

control as there was strong expression in the sarcolemma of the adjacent skeletal muscle fibres (Figs. 1(d) and (f) and 2(a)). There was also clear staining with β-DG antibody present in the blood vessels within the tissue (Fig. 1(a)).

In contrast, this pattern of expression was completely altered in the samples from 11 cases of primary oral SCCs (tongue, 8; soft palate/pharyngeal, 3). Unlike the specific expression pattern in normal epithelium, the β-DG staining in cancer tissue was highly variable. In three cases, the cancer cell membranes in areas directly in contact with the stroma showed strong staining, resulting in clearly delineated clusters of cancer cells (Fig. 1(b)). This resembled the pattern of staining found in the normal epithelium where the basal cell layer in contact with basal lamina was strongly positive. In two other oral cancer samples, the β-DG staining was significantly increased. The expression was no longer localised to the margin of the tumour mass, but most cancer cells were immunopositive, with both membrane and cytoplasmic staining being clearly noticeable (Fig. 1(c)). Finally, in three other cases the expression of β-DG was completely absent from the cancer cells (Fig. 1(d) and (f)). In the same samples, the membranes of skeletal muscle found between the infiltrating cancer cells were strongly positive (Fig. 1(d)). Apart from the blood vessels, all the stromal cells were negative for β-DG.

In contrast to β-DG, the α-DG staining was entirely absent from all the oral SCC samples (Fig. 2(b)–(d)). The skeletal muscle membranes between the infiltrating cancer cells were strongly positive (Fig. 2(b)), confirming staining specificity.

The same β- and α-DG antibodies failed to detect any staining in metastatic cancer in lymph nodes of the neck (Fig. 1(e)). These nodes were dissected from the two cases from which the primary carcinomas were analysed and found to be positive for β-DG.

To confirm these immunolocalisation findings, we analysed protein extracts from rat skin and primary SCC samples, which were used in the immunohistochemical studies (Fig. 3(a)). In skin and skeletal muscle samples used as positive controls, we found one band of the expected size (43 kDa). In all tumour samples, there were different proportions of two bands (43 kDa and a weaker ≈30 kDa band), the higher molecular mass band corresponded to the expected size for β-DG. In one sample (oral carcinoma), we found a band of ≈80 kDa, with little or no bands of 43 and ≈30 kDa. This large band was detected with two β-DG antibodies (against

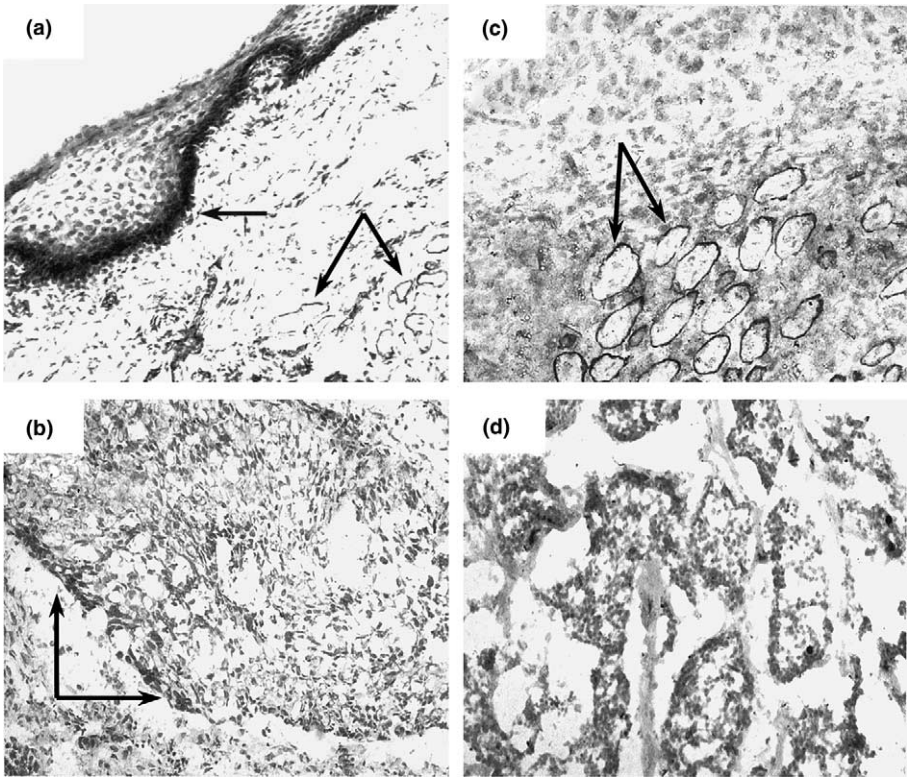

Fig. 2. Immunolocalisation of α-dystroglycan (α-DG) in normal oral epithelium and in oral cancer. (a) Normal oral epithelium. The strong staining located in the basal cell layer (arrow), in muscle sarcolemma and blood vessels in the stroma (arrows). (b)–(d) Oral cancer; note a total lack of α-DG staining in the tumour mass, while the skeletal muscle membranes are clearly stained. (b) Tongue squamous cell carcinoma (SCC), poorly differentiated. (c) Neck lymph node metastasis originating from a soft palate tumour. (d) Soft palate SCC, moderately differentiated. Magnifications: (a) 100×; (b) 400×; (c) and (d) 200×.

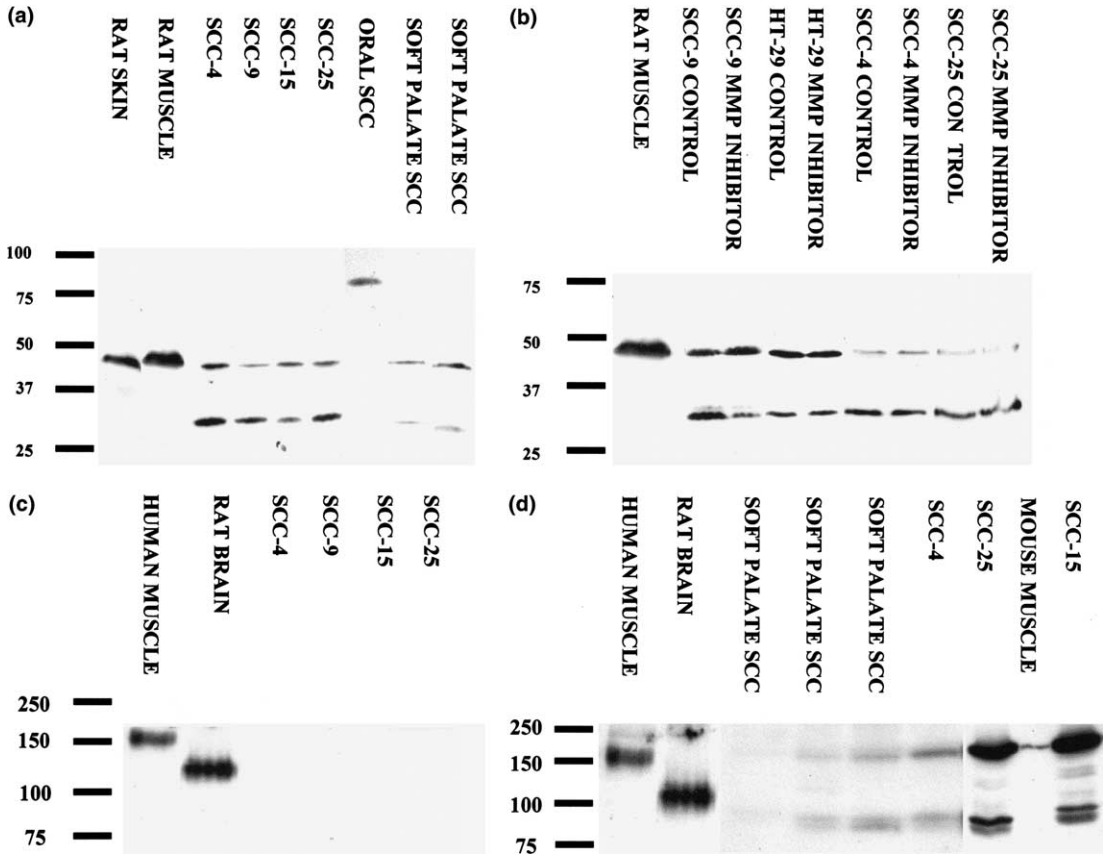

Fig. 3. Western blotting analysis of α- and β-dystroglycan (α- and β-DG) in oral cancer. (a) Protein samples from oral cancers and squamous cell carcinoma (SCC) lines (SCC-4, 9, 15 and 25) were processed for Western blotting and probed with β-DG antibody. (b) Effects of matrix metalloproteinase (MMP) inhibition on β-DG degradation. (c) Immunoblotting analysis of α-DG with VIA4-1 antibody. (d) Immunoblotting analysis of α-DG with US Bio antibody.

the N- and C-terminal epitopes), and corresponds to an improperly processed DG protein.

The same blots probed with the α-DG antibody used in the immunolocalisation analysis (VIA4-1) were negative in all tumour samples (data not shown). Different protein extraction procedures, use of varying sets of protease inhibitors and different amounts of protein all gave identical results. However, when another α-DG antibody was used (US Bio), two weak bands were detected, one corresponded to the expected size of α-DG and a smaller one. This smaller band was fuzzy and sometimes appeared as a doublet (Fig. 3(d)). In the control muscle samples, only a single, expected size band was observed.

Western blotting was carried out to analyse proteins extracted from several human SCC cell lines. In all of the cell lines, the same β-DG antibody detected a common 43 kDa band, identical to that in control skeletal muscle extracts and corresponding to the expected size for β-DG. In addition to this band, there was again a band of reduced molecular mass (about 30 kDa) in all of the cell lines (Fig. 3(a)). In three cell lines (SCC-25, SSC-4 and SSC-9), this band was more abundant than the 43 kDa band. When we used an antibody (BD Bioscience) recognising an epitope located in the middle of the β-DG molecule (as opposed to the C-terminal epitope targeted by the first antibody), only the 43 kDa band was detected (data not shown).

In some normal tissues [42] and cancer cell lines [25,32], β-DG can be degraded by the MMPs. To study whether the ≈30 kDa protein was identical with this product of β-DG proteolysis, we studied the effects of inhibition of MMP activity on the β-DG protein in SCC lines. We found that in SCC-9 cells cultured in the presence of the pan-MMP inhibitor GM6001 (Ilomastat), there was a drastic decrease in the level of the lower molecular size band and a concomitant increase in the intensity of the normal β-DG protein (Fig. 3(b)). At the concentration used (25 μM), GM6001 inhibits a range of MMPs including 1, 2, 3, 8, 9, 12 and 14. However, the same treatment of SCC-25, SCC-4 and HT-29 cells (colorectal cancer cell line used as a positive control for β-DG degradation) showed no significant effects (Fig. 3(b)). Moreover, use of the specific MMP2/MMP9 inhibitor IV had no effect in any cell line studied (data not shown).

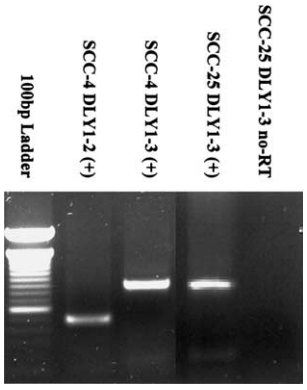

Fig. 4. Reverse transcriptase–polymerase chain reaction (RT–PCR) analysis of dystroglycan (DG) mRNA expression in squamous cell carcinoma (SCC) cells. The figure shows representative examples of agarose gel analysis of amplification products obtained with two specific primer sets. Lane 1: 100 bp ladder (Gibco-BRL). No-RT – negative control used to exclude genomic DNA contamination.

Interestingly, as in the primary tumour samples, in all SCC lines the α-DG antibody (VIA4-1) used for Western blotting gave negative results (Fig. 3(c)). However, another α-DG antibody (US Bio.) produced two bands, one of the expected size and another, smaller one (Fig. 3(d)). The smaller band often appeared as a doublet and in SCC cells further weak bands of variable size were often visible.

To analyse whether the lack of α-DG expression and aberrant β-DG expression could be the result of mRNA processing and to confirm the specificity of results obtained, we used two DG-specific primer sets in a RT–PCR analysis to amplify the pan-DG transcript. In all of the cell lines, the α/β-DG mRNA was clearly detectable and no major rearrangements within the region tested were found (Fig. 4), thus confirming the presence of DGs in SCC cells.

4. Discussion

DG α/β is a transmembrane/extracellular protein linking the extracellular basement membrane, cell membrane and intracellular cytoskeleton, providing structural integrity and interacting with a complex of proteins with putative signalling roles. To our knowledge, this is the first study demonstrating aberrant expression, processing and degradation of DG in oral SCCs.

We found that β-DG expression varies substantially among oral SCCs. In general, reduced expression of this subunit was more frequently associated with poorly differentiated tumours and increased expression was found in moderately/well-differentiated cancers. Moreover, the pattern of tumour staining was distinctive. In well-differentiated samples, the β-DG localised primarily on the tumor margin, resembling the staining pattern found in normal squamous epithelium. Moreover, we found a loss of β-DG expression in lymph nodes metastases, despite the presence of β-DG in the corresponding primary tumours. Unlike Hosokawa and colleagues [20], who found increased expression of β-DG in vascular endothelial cells within malignant tumours, we did not observe such an increase in oral cancer metastases. Our data is consistent with the recent results by Sgambato and colleagues [32]. This large series of breast and colon cancers showed a heterogeneous DG expression and low expression correlated with a higher tumour stage, high proliferation index and lower overall survival.

In addition to the variability in β-DG expression, we found no evidence of α-DG expression in the cancer tissues and SCC lines we investigated in this study with the VIA4-1 antibody. However, when another α-DG antibody was used, several immunoreactive bands were detected. Unfortunately, this antibody is not suitable for immunolocalisation. Nevertheless, this result indicates that the difficulty in detecting α-DG using the first antibody might be due to an altered post-translational processing, especially glycosylation of this protein in oral cancer. This would be in agreement with the detection of normal DG transcripts in our RT–PCR analyses. Abnormal glycosylation of DG has been found to be responsible for a number of human diseases [29] and abnormalities in glycosylation may play a role in cancer [24].

The significance of such a reduction/aberrance in DG expression in oral SCCs is currently unclear. DG is known to be involved in epithelial differentiation [6,8,21,30], epithelial cell migration/repair after injury [38] and interactions with basement membrane components [41]. Invasive growth of cancer cells is a complex process involving specific interactions between tumour cells and the orderly, integrated complexes of the ECM. Maintained integrity or disruption of the basement membranes has been proposed to play a critical role in regulating cancer invasion and metastasis. Components of the basement membrane and its overall structure are altered during tumour invasion [40].

We have shown here that the reduced expression of β-DG in oral cancer tissues and SCC lines *in vitro* was accompanied by increased levels of a ≈30 kDa product. This band was only detected by an antibody recognising the C-terminal epitope. This indicated that this smaller band was a N-terminally truncated form of β-DG. This observation is consistent with other data where this truncated form has been observed in other cancers [25,32].

An important group of proteolytic enzymes that are capable of degrading the basement membrane, as well as certain cell membrane proteins, are the MMPs [22]. We found (in one cell line) that the proteolysis of the β-DG subunit may be inhibited by a pan-matrix MMP

inhibitor. This agrees with observations made by Yamada and coworkers [42] in some normal tissues. However, we also found that the mechanism of β-DG degradation can vary dramatically in specific SCC, despite their common origin (all SCC lines used in this study were derived from tongue cancers). In three out of four cell lines, MMP inhibitors were ineffective. At the concentrations used in this study, GM6001 should inhibit MMP 1, 2, 3, 8, 9, 12 and 14, while SB3-CT should block MMP 2 and 9 function. We are currently analysing the proteinases expressed in SCC cell lines. There is evidence linking the activity of MMPs with both tumour invasion and metastasis in many tumours, including oral SCC [9,23,37,44] and the high activity of proteinases in oral carcinomas appears to be responsible for the highly invasive nature of these tumours [1,9,23].

Importantly, the data described here shows that, in addition to basement membrane proteins, β-DG may be yet another target for proteolytic degradation. Yamada and colleagues [42] showed that such processing disintegrates the DG complex and disrupts the link between the cells and the ECM.

Adhesion molecules, including DG, have multiple functions: they are critically important for proper tissue growth, differentiation and maintenance of cytoarchitecture. DG regulates the distribution of caveolin-3, which, in turn, affects α-integrin 7 receptor expression [5,35]. Therefore, dysregulation of DG may directly alter its binding to the ECM and indirectly affect adhesion *via* integrin pathways.

Moreover, degradation of β-DG may also affect cell signalling. β-DG itself is implicated in intracellular signal transduction *via* its interactions with Grb-2, an adaptor protein involved in several signalling pathways [31,34,43]. Furthermore, DG is part of the larger complex of dystrophin/utrophin-associated proteins (DAP) anchoring specific signalling molecules e.g., nitric oxide synthase [2], protein kinases [13,17,26] and specific ion channels [11]. Finally, hAG-2 and hAG-3, two unique secreted proteins involved in differentiation, interact with α-DG in a subset of breast cancers [10].

It is therefore, clear that the disruption of the DG complex may have far-reaching consequences other than just a loss of attachment to the basal lamina.

It has been shown that a lack of or altered glycosylation of brain DG in mouse knockouts results in disruption of the whole DAP complex [27,28]. It is conceivable that in cancer cells degradation of β-DG and aberrant processing/glycosylation of α-DG may have the same effect on DAP and result in changes in the properties of this complex.

In conclusion, our study indicates that abnormalities in DG expression may be a common feature in carcinogenesis and that the role of DG in cancer development and spread, although currently unclear, may be important. Considering the multifaceted role of DG in epithelial development, further studies into the clinical implications of these processes and into abnormal DG expression and processing in oral SCCs and in cancers in general are warranted.

Conflict of Interest Statement

The authors declare they have no conflict of interest.

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