

Expression of desmosomal proteins in acantholytic squamous cell carcinoma of the skin

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Summary. Acantholytic (adenoid) squamous cell carcinoma (ASCC) is a subtype of squamous cell carcinoma (SCC) in which neoplastic tumour cells form gland-like structures. Little is known about the pathogenetic mechanisms of ASCC. We hypothesised that they may be related to the composition of desmosomes. We analysed the immunohistochemical expression of desmosomal proteins in 5 cases of ASCC of the skin, in comparison to 5 cases of conventional SCC of the skin. The most consistent findings were loss of desmoglein 1 (DSG 1), desmoglein 3 (DSG3), desmocollin 1 (DSC1), desmocollin 2 (DSC2), desmocollin 3 (DSC 3), and plakophilin 1 (PKP 1), and decreased expression of desmoplakin 1 (DP 1) and plakoglobin (PG). In conventional well to moderately differentiated SCC, the expression of desmosomal proteins was decreased, but membranous staining was mostly preserved with patterns similar to normal epidermis.

Our results suggest that loss of desmosomal cadherins and decreased expression of desmosomal plaque proteins might be responsible for the formation of gland-like structures in ASCC. It seems that desmosomal cadherins, which correspond to the transmembrane core of desmosomes, are predominantly affected in ASCC, while DP 1 and PG, which correspond to cytoplasmic plaque of desmosomes, probably play a lesser role in maintenance of tumour cell cohesion. Our results also

indicate that, in addition to previously described verrucous and spindle cell carcinoma, ASCC is another subtype of SCC with a characteristic expression pattern of desmosomal proteins.

Key words: Squamous cell carcinoma, Skin, Acantholytic, Desmosomal proteins

Introduction

Acantholytic (adenoid) squamous cell carcinoma (ASCC) is a subtype of squamous cell carcinoma (SCC) in which neoplastic tumour cells form gland-like structures with variably sized, discohesive, acantholytic cells (Lever, 1947). Some authors believe that it is more aggressive than conventional SCC (Bennet, 1988; Toyama et al., 1995), while others did not find a higher potential for metastasis than in conventional SCC (Johnson and Helwig, 1966; Carpeto and Garcia-Perez 1972; Peter and Haustein, 1998). Prognosis of ASCC appears to be more dependent on the characteristics of the host, and the location, size and grade of the tumour (Johnson and Helwig, 1966; Petter and Haustein, 2000; Rinker et al., 2001; Cassarino et al., 2006; Garcia and Crowson, 2011).

The pathogenetic mechanisms responsible for the development of ASCC are not completely understood. There is evidence to suggest that they might be related to the composition of cell-cell contacts, particularly desmosomes. Desmosomes connect the intermediate filaments of the neighbouring cells and are abundant in tissues that are subjected to severe mechanical stress,

such as squamous epithelium and the heart. They are composed of at least three protein families: one is the desmosomal cadherin family, with four isoforms of desmogleins (DSGs) and three isoforms of desmocollins (DSCs). Another is the so called armadillo-family, with plakoglobin (PG) and three isoforms of plakophilins (PKPs); the last protein family is the plakin family, with desmoplakin (DP) as a constituent of desmosomes. Desmosomal cadherins interact extracellularly with desmosomal cadherins of neighbouring cells. Intracellularly, they bind PG and PKPs, which in turn bind to DP, and DP mediates connection to the intermediate filaments.

Desmosomal proteins show tissue specific patterns of expression (Schmidt et al., 1994, 1999; Hatzfeld and Nachtshelm, 1996). In stratified epithelia, the desmosomal constituents, together with the keratin filaments, are among the most frequent proteins. The stratum-specific molecular ensembles, specifically the DSG and DSC glycoproteins, are also of marked importance with respect to the pathogenesis of autoimmune skin diseases of the pemphigus type, which show a more or less direct correlation with the specific desmosomal glycoprotein complement of the affected layer (Amagai, 2003; Godsel et al., 2004; Payne et al., 2004; Schmidt and Koch, 2008; Waschke, 2008).

Desmosomes not only function in cell to cell adhesion and maintenance of tissue integrity but also participate in processes such as cell proliferation, differentiation and tissue morphogenesis, probably through cell signalling. There is mounting evidence that desmosomal proteins are also involved in several aspects of carcinogenesis. Previous studies have shown down-regulation of various desmosomal components in several types of human cancer, which was associated with tumour differentiation and behaviour. It seems that alterations of desmosomal components contribute to the invasive and metastatic ability of the tumour cells by reducing cell adhesion and/or changes in cell signalisation (Shinohara et al., 1998; Depondt et al., 1999; Bankfalvi et al., 2002; Kurzen et al., 2003; Papagerakis et al., 2004; Chidgey and Dawson, 2007; Wang et al., 2007; Wong et al., 2008; Brennan and Mahoney, 2009; Odar et al., 2012).

Little is known about intercellular adhesion protein expression in ASCC. Previous studies have shown distinct expression patterns of desmosomal components and other intercellular adhesion proteins in SCC and its variants, with various effects on clinical behaviour and

prognosis. For example, a decreased expression of E-cadherin, a major constitutional glycoprotein of adherent junctions, has been found in both oral (Zidar et al., 2006) and cutaneous ASCC (Bayer-Garner and Smoller, 2001; Griffin et al., 2013). Loss of E-cadherin has been associated with carcinoma progression and poor prognosis in various human and mouse tumours (Birchmeier and Behrens, 1994). Moreno-Maldonado and colleagues (2008), however, found an increased cytoplasmic expression of E-cadherin in ASCC compared with SCC. Contradictory results are evident also concerning the expression of another adhesion molecule syndecan-1 (Bayer-Garner and Smoller, 2001; Griffin et al., 2013). It is important to stress that the term "expression" is frequently but incorrectly used to describe the presence or the absence of immunostaining. Different results may also be a consequence of different protocols and antibody clones used for immunohistochemistry (e.g. frozen or paraffin sections, immunofluorescence or light microscopy, cytoplasmic or extracellular molecular domain detection of E-cadherin). Furthermore, staining may be membranous or cytoplasmic (cytosolic), and loss of membranous staining may be associated with an increase in cytoplasmic staining. The finding of rare acantholytic cells showing strong cytoplasmic staining was reported for E-cadherin and syndecan (Bayer-Garner and Smoller, 2001; Griffin et al., 2013), and for DSG3 (Griffin et al., 2013). Experimentally, E-cadherin complexes are normally internalized to endosomes and thereafter recycled back to the cell surface or degraded in lysosome (Pecce and Gutkind, 2002), but this process is more prominent in tumour cells (Le et al., 1999).

Expression of desmosomal proteins in ASCC has not been comprehensively studied. We therefore analysed the intensity and patterns of immunostaining for different desmosomal proteins in ASCC of the skin in comparison with conventional SCC, in order to better understand the mechanisms of ASCC development. We hypothesized that ASCC is a subtype of SCC with a decreased expression of some desmosomal proteins resulting in acantholysis.

Material and methods

Five patients with ASCC of the skin were included in the study. Their characteristics are summarized in Table 1. In all patients, the tumours were excised and the margins were free of tumour. Perineural invasion was

Table 1. Clinical and pathologic characteristics of patients with acantholytic squamous cell carcinoma of the skin.

Patient No	Gender, age	Location of tumour	Tumour diameter/thickness	Lymphovascular and perineural invasion	TNM	Follow-up (in years)
1	M, 76	Temporal region	14 mm/5 mm	No	T1 N0 M0	3.5
2	M, 76	Forearm	11 mm/4 mm	No	T1 N0 M0	4
3	M, 68	Cheek	10 mm/5 mm	No	T1 N0 M0	6
4	M, 75	Auricule	21 mm/8 mm	Yes, perineural	T2 N0 M0	4
5	M, 74	Cheek	8 mm/4 mm	No	T1 N0 M0	6

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found in one patient but lymphovascular invasion was not found in any of them. TNM stage was assessed according to the WHO TNM system (Le Boit et al., 2006). No additional treatment was needed. The patients had been followed for 3.5 to 6 years and all had a favourable course, with no recurrence or metastases.

Five patients with conventional, well to moderately differentiated SCC, grade 2, matched for gender, age, localization and TNM, were used for comparison. Their characteristics are summarized in Table 2. As in patients with ASCC, all tumours were excised with margins free of tumour and none of the patients needed additional treatment. There was perineural invasion in one patient. All patients except one, who died due to ischaemic cerebrovascular infarction, were alive after 4 to 6 years of follow-up, with no recurrence or metastases.

For immunohistochemistry, all tumour blocks were selected to include at least 4 mm thick tumours with at least 2 mm of normal epidermis as positive control. We noticed that hyperplastic epidermis adjacent to tumours is not appropriate because of variable intensity of immunostaining. Sections were cut at 4-5 μ m from paraffin blocks and deparaffinization was carried out according to standard procedures. Antigen retrieval methods were optimized for each primary antibody. Briefly, we used microwave oven heating for 10 min at 750 W in sodium citrate buffer (Dako TRS, pH 6.0, Dako Glostrup, Denmark) or for 25 min at 800 W in ethylenediaminetetraacetic acid (EDTA) buffer (1 mM, pH 9). In some cases, additional enzymatic pretreatment with proteinase 2 (0.1 unit of enzyme activity, Ventana,

Tuscon, Arizona, USA) was performed. Immunohistochemical staining was carried out in an automatic immunostainer (Benchmark, Ventana), using commercially available primary antibodies against desmosomal proteins, purchased from Atlas Antibodies (Stockholm, Sweden), Invitrogen (Camarillo, California, USA), Novocastra Laboratories (Newcastle, UK) or Progen Biotechnik (Heidelberg, Germany). An overview of the source and clone, dilution of the primary antibodies and antigen retrieval methods used in this study is given in Table 3. After incubation with primary antibodies, sections were treated with biotinylated secondary antibodies (Ventana) and incubated with peroxidase-conjugated streptavidin. Immunoreactivity was visualised with 3,3'-diaminobenzidine. Sections were counterstained with haematoxylin.

Expression of desmosomal proteins in ASCC and SCC was assessed semiquantitatively, in comparison to morphologically normal epidermis. An image analysis system (Cell and Tissue Analysis, Leica, Wetzlar, Germany) was used. The following scoring system was adopted. In score 4, protein expression was increased compared with normal epidermis; in score 3, it was equivalent to the epidermis; in score 2, it was decreased and in score 1 it was barely visible. Protein expression was absent in score 0.

Statistical analysis was performed with SPSS 19.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Exact Mann-Whitney test was used to analyse the differences between the intensity of desmosomal protein immunostaining of ASCC and SCC. P value less than

Table 2. Clinical and pathologic characteristics of patients with conventional squamous cell carcinoma of the skin.

Patient No	Gender, age	Location of tumour	Tumour diameter/ thickness	Lymphovascular and perineural invasion	TNM	Follow-up (in years)
1	M, 75	Hand	15 mm/7 mm	No	T1 N0 M0	4
2	M, 78	Infraorbital region	19 mm/12 mm	No	T1 N0 M0	5
3	M, 69	Cheek	17 mm/6 mm	No	T1 N0 M0	6
4	M, 74	Forearm	20 mm/21 mm	Yes, perineural	T2 N0 M0	5
5	M, 76	Nose	18 mm/7 mm	No	T1 N0 M0	4

Table 3. Overview of the source and clone of the primary antibodies and pretreatment used for immunohistochemistry.

Antigen	Antibody clone	Pretreatment	Source
Plakophilin 1 (PKP 1)	5C2	citrate buffer + proteinase 2	Progen Biotechnik
Plakophilin 2 (PKP 2)	Polyclonal	EDTA	Atlas Antibodies
Desmoglein 1 (DSG 1)	Dsg1-P124	EDTA	Progen Biotechnik
Desmoglein 2 (DSG 2)	10G11	citrate buffer + proteinase 2	Progen Biotechnik
Desmoglein 3 (DSG 3)	5G11	citrate buffer	Invitrogen
Desmocollin 1 (DSC 1)	DCS1-U100	citrate buffer + proteinase 2	Progen Biotechnik
Desmocollin 2 (DSC 2)	polyclonal	citrate buffer + proteinase 2	Progen Biotechnik
Desmocollin 3 (DSC 3)	Dsc3-U114	EDTA	Progen Biotechnik
Desmoplakin 1 (DP 1)	DP-2.17	EDTA	Progen Biotechnik
Plakoglobin (γ -catenin)	11B6	EDTA	Novocastra

EDTA; ethylenediaminetetraacetic acid buffer.

0.05 was considered statistically significant.

Results

Expression of desmosomal proteins in the epidermis

There were 3 basic expression patterns in the epidermis: predominantly basal/parabasal expression (DSG 2), predominantly suprabasal expression (DSG 1, PKP 1), and both basal and suprabasal expression, with or without a changing staining intensity towards the surface (DSG 3, DSC 2, DSC 3, DP 1, PG) (Figs. 1A, 2A, 3A, 4A). Basal expression of DSC 3 was weak. PKP 2 was strongly expressed by epithelial cells of sweat glands and their excretory ducts and weakly by epithelial cells of sebaceous glands. Basal expression of PKP 2 in the epidermis was weak and focal, limited to the part adjacent to excretory ducts of sweat glands.

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The expression of desmosomal proteins was

markedly altered in comparison to normal epidermis and conventional SCC (Tables 4, 5). There was almost a complete loss of immunostaining for DSG 1 (Fig. 1C), DSG 3 (Fig. 2C), DSC 1, DSC 2, DSC 3 (Fig. 3C) and PKP 1 (median score 1), and a partial loss of immunostaining for DP 1 (Fig. 4C) and PG (median score 2). The reduction or loss of staining was most pronounced in areas with marked acantholysis, but rare acantholytic cells showed strong cytoplasmic staining for DSG 1, DSG 3, DSC 1, DSC 2, DSC 3 and PG. The staining intensity for DSG 1 and DSC 3 was significantly lower in ASCC than in conventional SCC ($p < 0.05$), while the decrease of DSG3 was of borderline significance ($p = 0.07$).

The immunoreactivity of DSG 2 was increased in comparison to normal epidermis (median score 4), with the exception of small areas of complete acantholysis, in which immunoreactivity was decreased. There was no immunoreactivity for PKP 2 in tumour cells.

The intensity and pattern of expression of desmosomal proteins in nonacantholytic areas of ASCCs was similar to conventional SCCs, with diminished but preserved membranous staining (see below). There was,

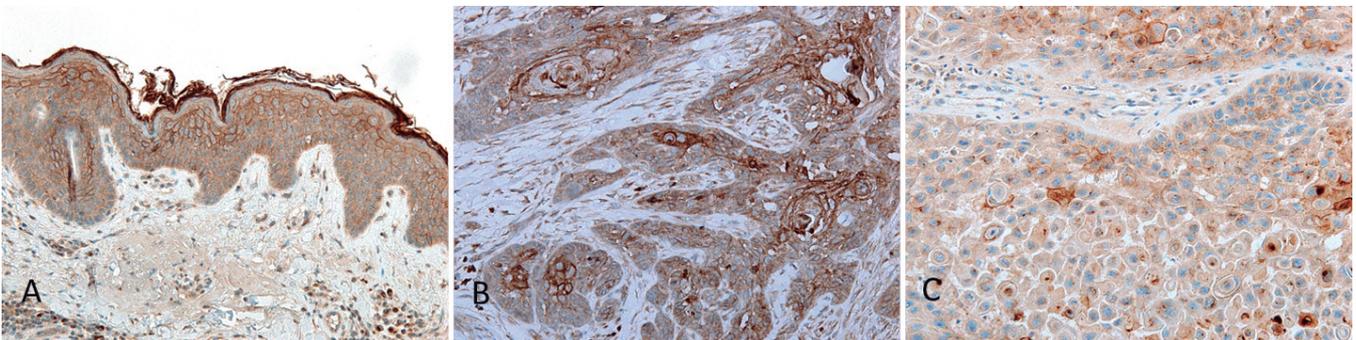


Fig. 1. Immunohistochemical staining pattern of desmoglein 1. In normal skin (A), staining is evident primarily in the upper part of the spinous layer and stratum corneum. In conventional, well-differentiated squamous cell carcinoma (B), the intensity of staining is decreased but the pattern is similar to normal epidermis. In acantholytic squamous cell carcinoma (C) there is almost no membranous staining but some dot-like perinuclear staining is present. Original magnification, x 200

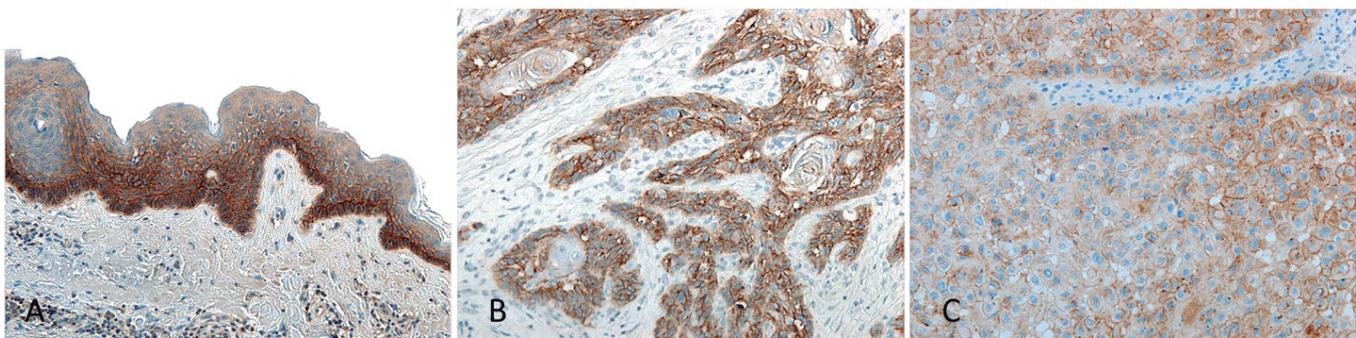


Fig. 2. Immunohistochemical staining pattern of desmoglein 3. In normal skin (A), staining is evident primarily in the basal layer and the lower part of the spinous layer. In conventional, well-differentiated squamous cell carcinoma (B), the intensity of staining is decreased but the pattern is similar to normal epidermis. In acantholytic squamous cell carcinoma (C), only some focal membranous staining is preserved. Original magnification, x 200.

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Table 4. Immunohistochemical expression of desmosomal proteins in 5 patients with acantholytic squamous cell carcinoma (SCC) and in 5 patients with conventional squamous cell carcinoma of the skin, as determined with semiquantitative immunohistochemistry.

Desmosomal protein	Acantholytic SCC*		Conventional SCC*	
	Median expression	(range) score	Median expression	(range) score
Desmoglein 1 (DSG 1)	1**	(1)	2**	(1-2)
Desmoglein 2 (DSG 2)	4	(4)	4	(4)
Desmoglein 3 (DSG 3)	1	(1)	2	(1-2)
Desmocollin 1 (DSC 1)	1	(1-2)	2	(1-2)
Desmocollin 2 (DSC 2)	1	(1-2)	2	(1-2)
Desmocollin 3 (DSC 3)	1**	(1-2)	2**	(2-3)
Desmoplakin 1 (DP 1)	2	(2)	2	(2-3)
Plakophilin 1 (PKP 1)	1	(1-2)	2	(1-2)
Plakophilin 2 (PKP 2)	0	(0)	0	(0)
Plakoglobin (γ -catenin)	2	(2-3)	2	(2-3)

* squamous cell carcinoma, **p<0.05

however, no keratinization in a form of keratin pearls as seen in conventional SCCs.

Expression of desmosomal proteins in conventional squamous cell carcinoma (SCC)

In conventional SCC, the expression of desmosomal

Table 5. Immunohistochemical expression of desmosomal proteins in 5 patients with acantholytic squamous cell carcinoma, as determined with semiquantitative immunohistochemistry.

Desmosomal protein	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Desmoglein 1 (DSG 1)	1	1	1	1	1
Desmoglein 2 (DSG 2)	4	4	4	4	4
Desmoglein 3 (DSG 3)	1	1	1	1	1
Desmocollin 1 (DSC 1)	1	1	2	1	1
Desmocollin 2 (DSC 2)	1	2	1	1	1
Desmocollin 3 (DSC 3)	1	1	2	1	1
Desmoplakin 1 (DP 1)	2	2	2	2	2
Plakophilin 1 (PKP 1)	1	1	2	2	1
Plakophilin 2 (PKP 2)	0	0	0	0	0
Plakoglobin (γ -catenin)	2	2	3	2	2

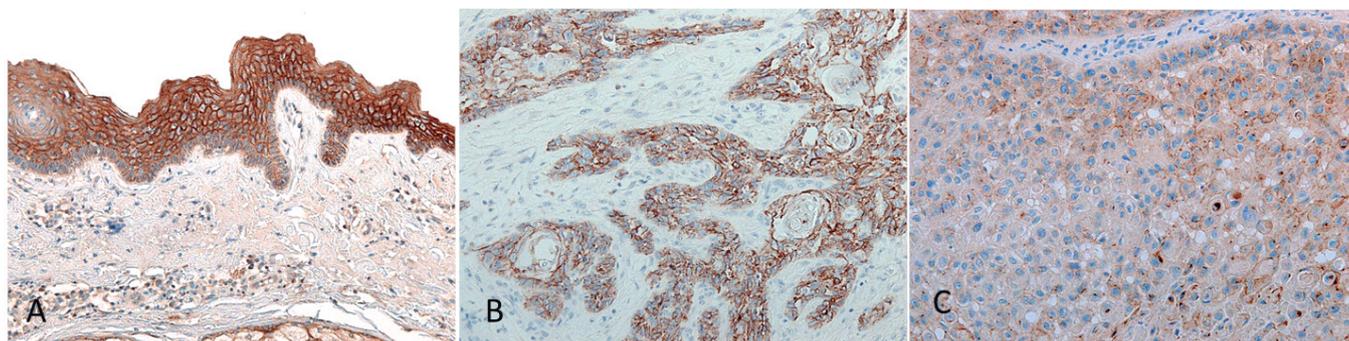


Fig. 3. Immunohistochemical staining pattern of desmocollin 3. In normal skin (A), staining is present in all layers with the exception of the stratum corneum. The basal layer is weakly stained. In conventional, well-differentiated squamous cell carcinoma (B), the intensity of staining is decreased but the pattern is similar to normal epidermis. In acantholytic squamous cell carcinoma (C), there is almost no membranous staining in the areas of acantholysis. As for desmoglein 1, focal dot-like perinuclear staining is present. Original magnification, x 200.

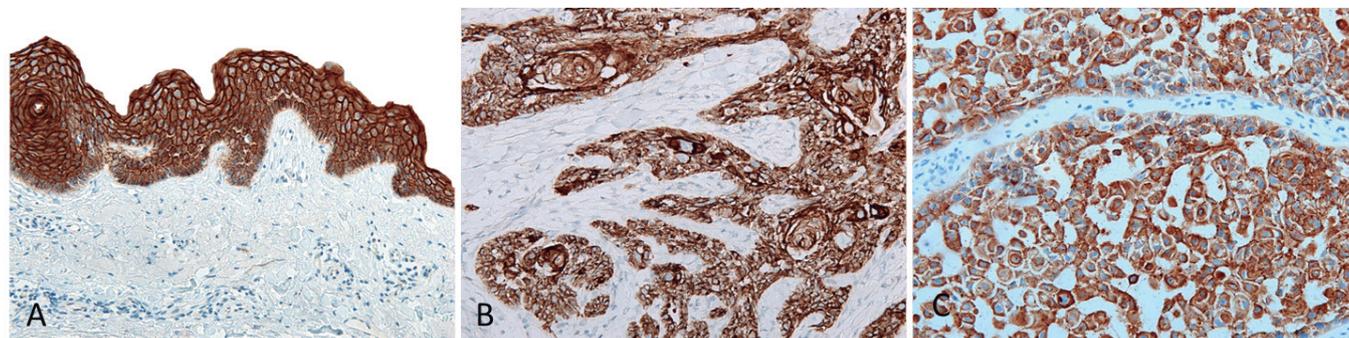


Fig. 4. Immunohistochemical staining pattern of desmoplakin 1. In normal skin (A), staining is present in all layers, including the stratum corneum. In conventional, well-differentiated squamous cell carcinoma (B), the pattern of staining is similar to normal epidermis but the intensity of staining is slightly decreased. In acantholytic squamous cell carcinoma (C), membranous staining is irregular but still preserved in the majority of cells. Original magnification, x 200.

proteins was altered in comparison to normal epidermis, but to a lesser extent than in ASCC (Table 4), since membranous staining was preserved and the pattern of staining was similar to normal epidermis (Figs. 1-4). The expression of all desmosomal proteins was moderately decreased (median score 2), with the exception of PKP 2, which was completely negative, and DSG 2, which was increased in comparison to normal epidermis (median score 4).

Discussion

We analysed the expression of desmosomal proteins in ASCC of the skin in comparison to conventional SCC. In ASCC, we found an almost complete loss of several desmosomal proteins, such as DSG 1, DSG 3, DSC 1, DSC 2, DSC 3 and PKP 1, and a decreased expression of DP 1 and PG. It seems that desmosomal cadherins are the predominantly affected desmosomal proteins in ASCC, which may be responsible for the formation of the acantholytic (adenoid) features in ASCC. Armadillo protein PG and plakin member DP 1 probably play a lesser role in maintenance of tumour cell cohesion than do desmosomal cadherins. The mechanisms responsible for altered expression of desmosomal proteins in ASCC are currently not known but it may result from reduced gene expression, the production of an altered molecule, or post-translational modifications (Schmidt and Koch, 2008). Our findings in relation to DSG 3 are in agreement with those reported by Griffin and co-workers (2013), who demonstrated a decrease in DSG 3 in ASCC in comparison to conventional SCC. Expression of other desmosomal proteins in ASCC, however, has not so far been studied.

In contrast to the aforementioned desmosomal proteins, DSG 2 showed increased expression in tumour cells in both ASCC and conventional SCC. This finding is consistent with some previous studies describing strong expression of DSG 2 in human SCC (Harada et al., 1996; Kurzen et al., 2003; Brennan and Mahoney, 2009; Odar et al., 2012). The significance of increased expression of DSG 2 in SCC is unknown. It might suggest an immature phenotype of neoplastic cells, because DSG 2 is normally found only in the basal layers. According to experimental studies, overexpression of DSG 2 may play an important role in cancer development: transgenic mice overexpressing DSG 2 in the epidermis developed spontaneous epithelial tumours and were more susceptible to chemical carcinogen-induced tumourigenesis (Brennan et al., 2007).

Desmosomal proteins normally exhibit a membranous pattern of expression. The patterns of expression in the normal epidermis were consistent with previously published studies (Kurzen et al., 2003; Donetti et al., 2005; Mahoney et al., 2006; Green and Simpson, 2007; Odar et al., 2012). Altered expression in ASCC manifested as a complete loss or as a decreased intensity of membranous staining. In addition, we also

observed aberrant expression patterns: e.g., cytoplasmic staining and perinuclear localization of desmosomal cadherins and PG, but only in rare cells. This phenomenon has been described in both ASCC and conventional SCC of the skin (Kurzen et al., 2003; Griffin et al., 2013) but its significance is unknown. Aberrant cytoplasmic (Bosch et al., 2005; Gao et al., 2005; Moreno-Maldonado et al., 2008) and nuclear (Bosch et al., 2005; Gao et al., 2005) expression of E-cadherin was found to correlate with a poor prognosis of SCC (Bosch et al., 2005; Gao et al., 2005). An experimental study showed that endocytosis of E-cadherin is more prominent in the absence of stable cell-cell contacts, which may be the reason for increased cytosolic expression (Le et al., 1999). Desmosomal proteins are, however, not disassembled and recycled during or after internalisation but instead are transported to the centrosomal region, where they are degraded (McHarg et al., 2014).

E-cadherin reappears in metastases during mesenchymal-epithelial transition, allowing tumour cells to regain epithelial properties and integrate into distant organs (Yang and Weinberg, 2008). It is tempting to assume the same may also be true for desmosomal proteins.

It may be speculated that aberrant expression patterns of desmosomal proteins in a prone individual may induce autoimmune skin diseases of the pemphigus type. Inaoki and co-workers (2001) described a patient with cutaneous SCC that expressed DSG 1. The tumour metastasized to the regional lymph nodes and the patient subsequently developed pemphigus foliaceus. Another patient, reported by Maumi and co-workers (2013), had SCC of the soft palate associated with autoantibodies to DSG 1 and DSG 3 but without clinical signs of pemphigus.

Little is known about desmosomal protein expression in SCC and its subtypes but there is mounting evidence that some subtypes of SCC exhibit distinct patterns of desmosomal protein expression. For example, spindle cell carcinoma of the head and neck is characterised by loss of desmosomal cadherins (Zidar et al., 2006). In verrucous carcinoma, a less aggressive subtype of SCC, the expression patterns of desmosomal proteins were found to be similar to normal epithelium and epidermis but differed significantly from those observed in conventional SCC (Odar et al., 2012). It seems that in conventional SCC, the expression patterns of desmosomal proteins depend on differentiation of the tumour, being similar to normal epithelium/epidermis in well differentiated SCC and markedly altered in poorly differentiated SCC (Kurzen et al., 2003; Bosch et al., 2005). Our results indicate that ASCC is another subtype of SCC, with a characteristic expression pattern of desmosomal proteins.

Clinically, ASCC is most often seen in sun-exposed areas of the head and neck of elderly patients, mostly males, which correlates with our results. There have been reports, however, of this tumour occurring in sun-

protected areas, such as the dorsum of the foot (Toyama et al., 1995). Like conventional SCC, ASCCs arising in burn scars are of high risk (Ikegawa et al., 1989).

None of our patients with ASCC or conventional SCC developed metastases. When the frequency of metastasis in SCC is evaluated, the size of the tumour at the time of initial excision must be considered. The metastasis rate increases threefold (from 9.1% to 30.3%) when the tumour diameter is larger than 20 mm (Peter and Hausteine, 2000). The median tumour diameter in our patients was 11 mm for ASCCs and 17 mm for conventional SCCs. With the exception of one tumour in each group, both measuring 21 mm, none was larger than 20 mm. In the skin, tumours up to 20 mm in diameter (T1), in both ASCC and common SCC, are far more common than tumours larger than 20 mm (Roozeboom et al., 2013). They are usually grade 2 SCC, which is the commonest grade of conventional cutaneous SCC (Roozeboom et al., 2013). The prognosis of skin SCC is generally more favourable than SCC in other locations, due to early diagnosis and excision of skin SCC, and it seems also to be the case for skin ASCC.

Several groups of authors concluded that published evidence does not support the assumption that skin ASCC is a more aggressive tumour than conventional SCC (Johnson and Helwig 1966; Carpeto and Garcia-Perez, 1972; Petter and Hausteine, 1998, 2000; Rinker et al., 2001; Garcia and Crowson, 2011). According to their opinion, prognosis of cutaneous ASCC appears to be more dependent on the characteristics of the host, and the location, size, and grade of the tumour (Petter and Hausteine, 2000; Rinker et al., 2001; Cassarino et al., 2006; Garcia and Crowson, 2011). In a review of 155 patients with 213 lesions (Johnson and Helwig, 1966), only three patients died of metastatic disease and two patients died of local invasion. One of the deaths due to local invasion involved a patient who refused treatment. In a review of 20 patients (Carpeto and Garcia-Perez, 1972), no patients were noted to have lymph node metastases, but three patients died of local intracranial extension of tumor. In a review of 36 patients (Nappi et al., 1989), 11 patients had local recurrence, five had visceral metastases, and two died of local intracranial extension of tumor. Another review of the same authors (Nappi et al., 1992) reported on three out of six patients with ASCC who died of lymph node metastasis, but two of these were immunocompromised. In a review of 18 patients (Peter and Hausteine, 1998), only one patient developed a local recurrence.

Squamous cell carcinoma grading system introduced by Broders in 1920 (well, moderately and poorly differentiated, according to the percentage of undifferentiated cells (Broders, 1920)) can hardly be applied to ASCC due to lack of appropriate criteria concerning the differentiation of ASCC (e.g. which cells are differentiated and which undifferentiated, since typical keratinization with keratin pearls as seen in conventional SCC is not present in ASCC).

Contrary to the poorly differentiated SCC and spindle cell SCCs which were negative or barely positive for desmosomal proteins (Odar et al., 2012) and E-cadherin (Andrews et al., 1997; Depondt et al., 1999; Bankfalvi et al., 2002; Bosch et al., 2005), we found that membranous expression of desmosomal proteins was at least focally present in ASCC. It seems also to be the case for DSG3, E-cadherin and syndecan (Bayer-Garner and Smoller 2001; Griffin et al., 2013). Interestingly, ASCC-like tumours in mice and human ASCC overexpressed IKK α , which plays an important role in epidermal keratinocyte differentiation (Moreno-Maldonado et al., 2008). At the same time, high molecular keratin cytokeratin 1 (K1), expressed by differentiated epidermal keratinocytes, was unexpectedly negative. In conventional SCC, K1 is positive in areas of keratinization. It seems that ASCC in addition to acantholysis also exhibits aberrant terminal differentiation, which may be a consequence of desmosome disassembly and loss of cytokeratin filaments.

In conclusion, we found altered expression of desmosomal proteins in ASCC in comparison to conventional SCC and normal epidermis. The most consistent findings were loss of DSG 1, DSG 3, DSC 1, DSC 2, DSC 3 and PKP 1 and a decreased expression of DP 1 and PG. This loss of desmosomal proteins is probably responsible for acantholysis in ASCC. Despite markedly altered expression of desmosomal proteins, ASCC does not seem to be more aggressive than conventional SCC. However, this must be confirmed in a larger series of patients.

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