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Expression of the α6β4 Integrin by Squamous Cell Carcinomas and Basal Cell Carcinomas: Possible Relation to Invasive Potential?

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We have studied the expression of α6β4 integrin, a carcinoma laminin receptor in ten squamous cell carcinomas (SCCs) and ten basal cell carcinomas (BCCs) of the skin in order to examine whether changes in α6β4 integrin expression may be related to invasive and metastatic potential. Monoclonal antibodies specific for each subunit were applied on cryosections, using a three step indirect peroxidase technique. In normal epidermis the basal cells expressed both the α6 and the β4 subunits, and the expression was polarized against the basement membrane. In SCCs the expression of the α6 and the β4 subunits paralleled each other, showing an increased intensity and loss of polarity. The BCCs, however, showed consistently decreased expression of both the α6 and the β4 subunits. The results of our study, as well as those of other studies, support the assumption that the increase and depolarization of α6β4 integrin that occurs in SCCs might be related to the invasive properties of this tumour type. ***Key words: Invasion; Immunohistochemistry.***

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Tumour invasion and metastasis depend, in part, on the ability of tumour cells to alter their adhesion to each other and to the extracellular matrix. Many studies on invasion and metastasis have highlighted the importance of tumour cell adhesion to laminin (1, 2). In general, it appears that invasive tumour cells exhibit enhanced adhesion and migration on laminin compared to less aggressive tumour cells. For this reason, studies aimed at understanding changes in the expression and function of specific laminin receptors during tumour progression are essential for understanding tumour behaviour.

Cell adhesion to laminin, as well as other extracellular matrix proteins, is mediated primarily by integrins and non-integrins. Integrins are a family of adhesion receptors comprised of two non-covalently linked subunits termed α and β (3–6). To date, six different integrins (α1β1, α2β1, α3β1, α6β1, α7β1, α6β4) have been implicated as laminin receptors (7–10). However, the α6β4 integrin has received considerable attention from cancer biologists because it is expressed predominantly on epithelial and carcinoma cells (3, 4, 7, 11–19). In normal epithelium, the expression of α6β4 is concentrated along the basal cell surface, which is in contact with the basement membrane (8–10, 20–23). One of the hallmarks of carcinoma cells is loss of polarity of α6β4 expression. In these cells, a diffuse distribution of this integrin over the entire cell surface is often observed.

A key issue that has not been resolved is whether the expression of α6β4 increases in metastatic cells compared to either normal epithelial cells or cells of primary tumours. The β4 integrin was initially characterized in mice as a metastasis-associated antigen (24), because its expression in carcinoma cells correlated with metastatic potential. However, studies on human tumours have not yielded such clear-cut results. For example, conflicting results on α6β4 expression in colon carcinoma have been reported (11, 12). In one study (11), it was suggested that the expression of this integrin did not change significantly in carcinomas compared to normal colon, while others report a marked decrease in α6β4 expression in primary as well as metastatic tumours (12). Immunohistochemical examination of α6β4 in pancreatic carcinomas revealed no change in expression compared to normal glandular epithelium, but studies on pancreatic cell lines indicated a direct correlation between loss of α6β4 expression and loss of differentiation (13). Breast carcinomas have been reported to be negative for α6β4 expression (14). Adenocarcinomas and squamous cell carcinomas of the lung are reported to have preserved expression of α6β4, while small cell carcinomas are negative (15). Squamous cell carcinomas of the head and neck exhibit increased expression of the A9 antigen (16, 17), recently demonstrated to represent a common epitope on the β4 subunit (18). Basal cell carcinomas of the skin have been shown to have strong α6β4 expression in the superficial portion of the tumour and less expression in the deeper portion of the tumour (19).

The complex picture that has emerged from the various studies on α6β4 expression in different cancers prompted us to initiate a careful analysis of expression of α6β4 in squamous cell carcinomas (SCCs) and basal cell carcinomas (BCCs) of the skin. In particular, we sought to determine whether differences in expression were related to the invasive and metastatic potential of these tumours.

MATERIAL AND METHODS

Source of tissue

Fresh tissue samples representing SCCs and BCCs were received from the Department of Plastic Surgery, Rigshospitalet, Denmark. After routine sampling for diagnostic purposes, rest tissue representing ten SCCs and ten BCCs, all of which included normal epidermis, was sampled and snap-frozen in liquid nitrogen. Five-μm cryostat sections were fixed in acetone for 10 min at room temperature.

Immunohistochemistry

A three-step indirect peroxidase technique was used (25). The primary antibodies were rat IgG2b monoclonal anti-human α6 (cat. no. MAB1972, lot 280CCB3, Chemicon, Temecula, Canada) (8) and mouse IgG1 monoclonal anti-human β4 (cat. no. MAB1964, lot 253TPC3, Chemicon, Temecula, Canada) (26). Sections were incubated with primary antibodies for 30 min. Primary antibodies were diluted in phosphate-buffered saline (100 mM sodiumphosphate, 154 mM NaCl,

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pH 7.4) containing 0.1 % bovine serum albumin. Secondary antibodies were horseradish-peroxidase conjugated rabbit anti-rat immunoglobulin (cat. no. P162, Dakopatts, Glostrup, Denmark) and horseradish-peroxidase conjugated rabbit anti-mouse immunoglobulin (cat. no. P260, Dakopatts, Glostrup, Denmark), both incubated for 30 min. Tertiary antibodies were horseradish-peroxidase conjugated swine anti-rabbit immunoglobulin (cat. no. P217, Dakopatts, Glostrup, Denmark), incubated for 30 min. All incubations were performed at room temperature and sections were rinsed with Tris-buffered saline (5 mM Tris-HCl, 147 mM NaCl, pH 7.6) between each step. Finally, sections were incubated for 10 min with freshly prepared substrate solution consisting of 0.04% ethyl-carbazole and 0.02% hydrogen peroxide in a sodium-acetate buffer (15 mM acetic acid, 35 mM sodium-acetate, pH 5.0), washed in distilled water, counterstained with Mayer's hematoxylin, and mounted in Kayser's glycerol-gelatine. Each immunohistochemical staining was performed three times to ensure reproducibility.

Controls included 1) replacing the primary antibody with antibodies of the same isotype as the primary ones, rat IgG2b (cat. no. 1118 129, lot 12539722-02, Boehringer Mannheim, Mannheim, Germany) and mouse IgG1 (cat. no. M724, lot 050, Dakopatts, Glostrup, Denmark), respectively; 2) deletion of primary antibodies; 3) deletion of primary and secondary antibodies; and 4) deletion of all antibodies.

RESULTS

In all sections both normal epidermis and tumour tissue were represented. The immunohistochemical expression of α6 and β4 in tumour cells was compared to the expression in normal epidermis.

The α6 subunit

In normal epidermis and in adnex structures only the basal cells expressed α6 (Fig. 1A). The expression was associated to the cell membrane, with a linear accentuation along the epithelial-stromal interface. A less constant and weaker expression was seen at the lateral and apical surface of the basal keratinocytes. Perineural and endothelial cells were positive, with the endothelial cells showing strongest immunoreactivity in small blood vessels.

All ten cases of SCCs showed an increased expression of α6. The immunoreactivity was strongest in the cells at the periphery of the tumour islands and equally strong all around the cells (Fig. 2A). Thus, both increased intensity and change of expression pattern with depolarization of α6 expression were observed. Some of the squamous cell carcinomas showed an additional slight cytoplasmic positivity of the tumour cells. In one SCC, serial sectioning revealed tumour cell invasion into a small blood vessel (Fig. 3). These invasive keratinocytes exhibited a strong α6 immunoreactivity. In two cases of SCCs scattered positive lymphocytes were seen.

The tumour cells of the ten BCCs consistently showed a weak and clearly reduced intensity of α6 immunoreactivity as compared to normal epidermis (Fig. 2B). The staining pattern of the BCCs was generally similar to that of normal epidermis, with a polarization of the positivity along the epithelial-stromal interface. When BCCs were ulcerated an apparently stronger reaction could be seen in tumour cells adjacent to the ulcerated surface. However, when the surface was not ulcerated, the down-regulation of α6 expression was uniform in all portions of the tumours. All controls were negative.

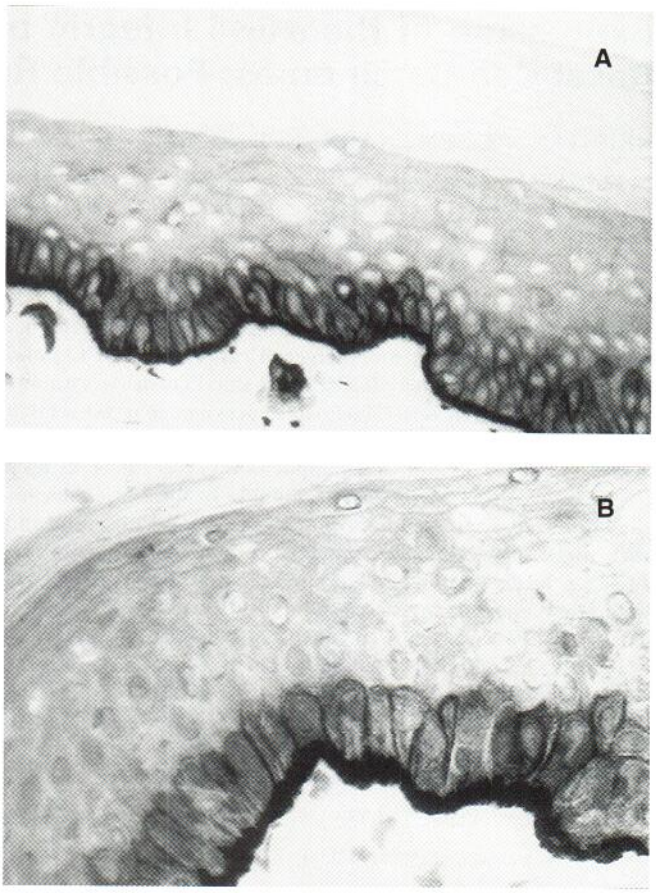

Fig. 1. Immunostaining of normal epidermis with monoclonal antibodies to α6 and β4. (A) α6 is expressed by basal cells and its expression is concentrated along the epithelial-stromal interface. A weaker and less constant expression is seen along the lateral and apical surface of the basal cells (laser scan microscope ×310). (B) β4 expression is similar to that of α6. A strong expression along the epithelial-stromal interface is seen and occasionally a weak expression along the lateral and apical surface of the basal cells (laser scan microscope ×500).

The β4 subunit

The staining pattern of β4 closely resembled the pattern of α6. In the normal epithelium a reaction was seen in the basal cells (Fig. 1B). The reaction was strongest at the epithelial-stromal interface. Also a weak expression along the lateral and apical surface of the basal keratinocytes was occasionally seen. Perineural and endothelial cells were positive, paralleling the pattern of the α6 staining. The α6-positive lymphocytes in two cases of SCCs were negative for β4.

The β4 staining of SCCs and BCCs closely paralleled the staining with α6 (Fig. 2C, D). Thus, SCCs showed an increased intensity and loss of polarization of β4, while BCCs showed a decreased expression. In cases of ulceration, the β4 expression pattern was similar to that of α6. However, as with the α6 staining, when the surface was not ulcerated the down-regulation of β4 expression was uniform in all portions of the tumours (Figs. 2D and 4). All controls were negative.

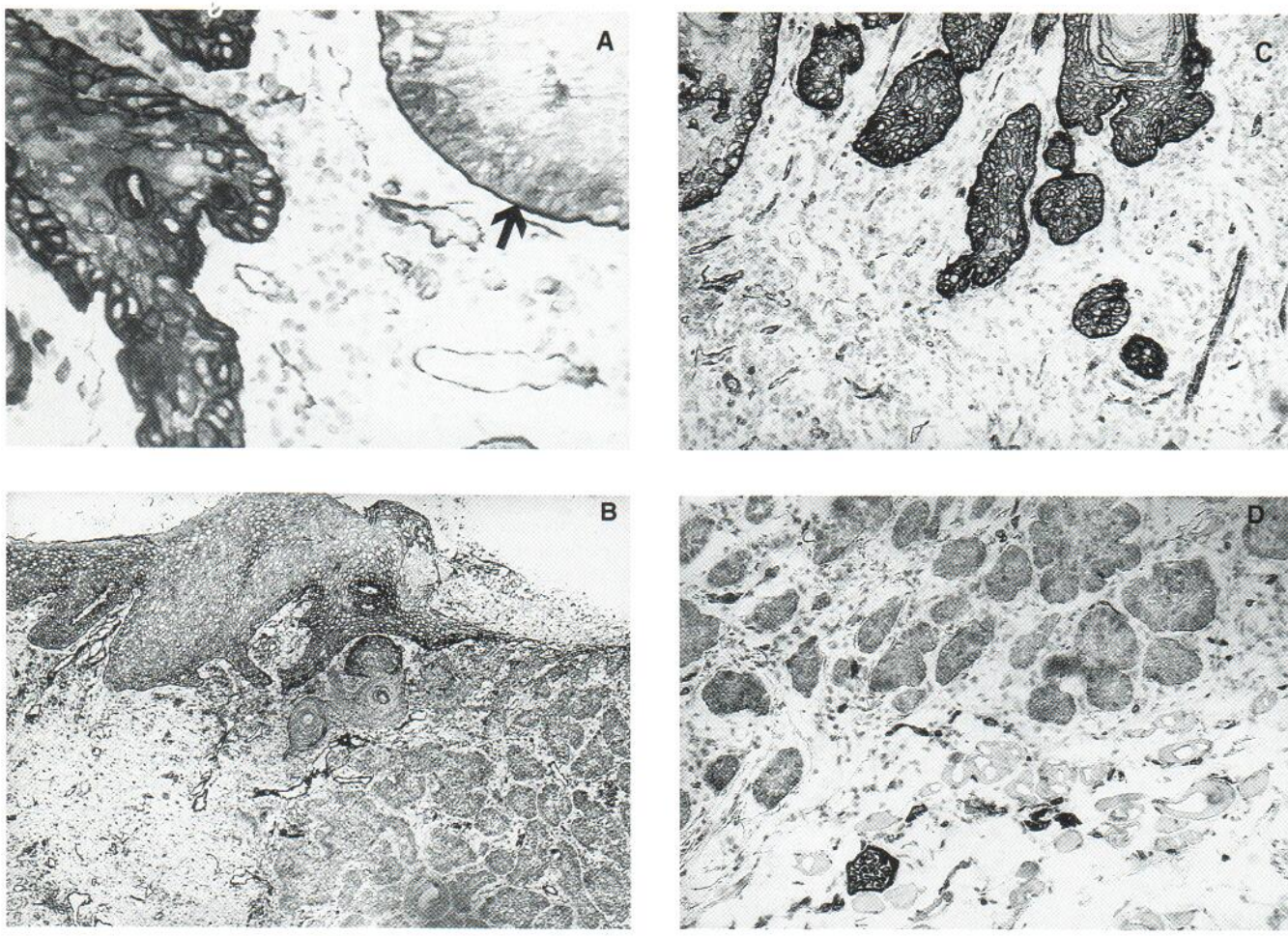

Fig. 2. Immunostaining of SCC and BCC with monoclonal antibodies to α6 and β4. (A) α6 expression in SCC cells is increased and its localization is depolarized compared to normal epidermis (*arrow*) (laser scan microscope ×270). (B) α6 expression in BCC cells is significantly reduced (×70). (C) β4 expression in SCC cells of the invasive front. The expression of β4 by the peripheral tumour cells is increased and its localization is depolarized (×170). (D) β4 expression in BCC cells of the invasive front. Its expression is significantly reduced (×170).

DISCUSSION

In the epidermis, the α6 integrin subunit associates primarily and probably exclusively with the β4 integrin subunit (20–23). This pattern of integrin association is probably retained in tumours derived from epidermal cells. Thus, immunohistochemical studies using monoclonal antibodies to either the α6 or the β4 subunit reflect expression of the α6β4 integrin heterodimer. The immunohistochemical results obtained in this study confirm this observation, because the staining patterns obtained with α6 and β4 specific antibodies were identical. More importantly, the results obtained indicate a clear distinction between BCCs and SCCs in their expression of the α6β4 integrin. The ten BCCs examined exhibited a significant decrease in their expression of this integrin as compared to normal epidermis. In contrast, a marked increase in the intensity of α6 and β4 staining was observed in the SCCs compared to normal epidermis. Also, SCCs were characterized by a depolarization of α6β4 expression compared to the basal surface polarization seen in normal epidermis. Because SCCs are, in general, more invasive and metastatic than BCCs, the possibility exists that increased α6β4 expression and loss of its basal polarity are related to tumour invasion and metastasis (Fig. 3).

The expression of the α6β4 integrin has been shown to increase during the malignant conversion of mouse epidermal keratinocytes (27). In humans, β4 expression has been shown to increase during the neoplastic progression of intraepithelial neoplasias of the cervix uteri (28). In human SCCs (16, 17), expression of the α6β4 integrin has been examined with the UM-A9 monoclonal antibody which recognizes the β4 integrin subunit (18). These studies, as well as our results, show that SCCs exhibit alterations in the polarity and intensity of the α6β4 expression. Moreover, A9 antigen expression has been used to predict the clinical outcome of SCCs of the head and neck. Tumours that most strongly expressed this antigen had a higher incidence of recurrence (17).

In a study by Sollberg et al. (19), which included six BCCs, it was shown that superficial portions of BCCs had a strong expression of β4 while the deeper portions were less positive. Furthermore, it was found that the lateral surface of the peripheral tumour cells in the BCCs was occasionally positive, a phenomenon that was not seen in normal epidermis. These

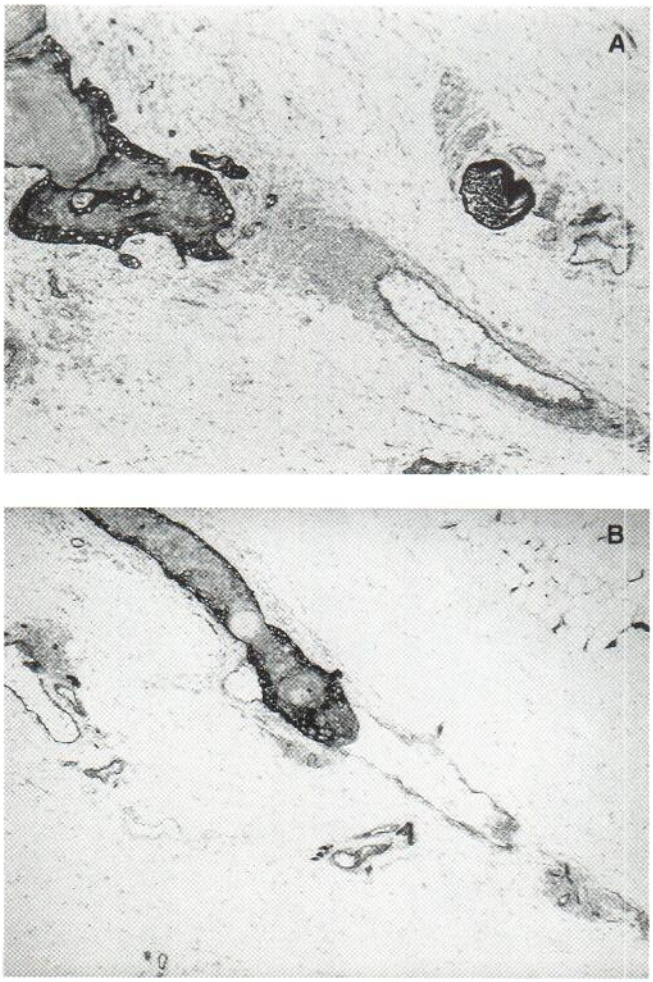

Fig. 3. Immunostaining of SCC with monoclonal antibodies to α6, serial sections (A&B). (B) Tumour cell invasion of a small blood vessel is observed. The invading cells exhibit a strong expression and depolarized localization of α6 (A: ×150, B: ×130).

results differ somewhat from ours. First, we did not find a significant difference of α6β4 expression within different portions of the BCCs (Figs 2D and 4). Only when the surface of the BCCs was ulcerated could a somewhat stronger expression be seen in the superficial portion. The phenomenon of an increased expression in these cases might represent some kind of reaction to the ulceration. Thus we do not find it possible to draw conclusions about invasive properties of superficial versus profound tumour cells in BCCs based on these observations. Second, a weak β4 expression along the lateral and apical surface of normal basal keratinocytes was occasionally seen in our preparations (Fig. 1). In other studies of β4 expression in normal squamous epithelium this detail has not always been clarified. Some results indicate that β4 is only expressed along the epithelial-stromal interface (29), while others support our results (30).

In an in vitro model of wound healing Kurpakus et al. (31) have shown that in keratinocytes migrating from the edges of a wound over bare connective tissue α6β4 appears along the entire cell surface. Only at a later stage, as cells become positive for cytosolic hemidesmosome compounds, the α6β4 is concentrated along the basal pole of the cell. These results indicate that basal keratinocytes are able to regulate the polarization of α6β4 integrin expression. The same events that determine the depolarization of α6β4 integrin in migrating keratinocytes of wound healing could very well be responsible for the depolarization of α6β4 expression in SCCs. This hypothesis raises a question for future investigation: is depolarization of α6β4 integrin on SCCs an event that contributes to the invasive properties of the SCC cells or is it a "normal physiological response" to the cell-stromal interactions of the invasive growing SCC cells?

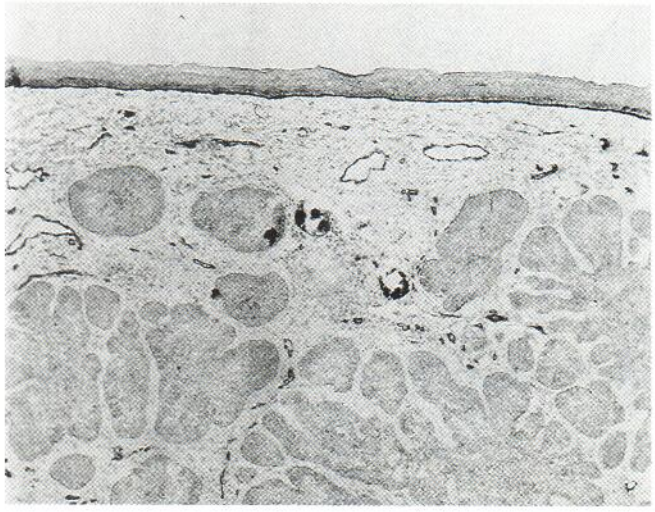

Fig. 4. Immunostaining of BCC with monoclonal antibodies to β4. The superficial non-ulcerated area of BCC exhibits the same staining pattern as the deep, invasive portion of the same tumour (Fig. 2D) (×170).

It is tempting to interpret the striking difference in expression of α6β4 integrin by SCCs and BCCs as being related to the difference in invasive and metastatic behaviour of the two tumour types. Yet, it must be remembered that even though SCCs and BCCs originate from the same epithelium the phenotypes of the two tumour types are very different, a circumstance that makes conclusions drawn from such a comparison uncertain. Even so, our results in combination with other studies suggest that in SCCs the increase in expression of α6β4 integrin may be related to invasion. Furthermore it is possible that the depolarization of α6β4 in SCCs may promote migrative/invasive properties. However, we do not find that this assumption necessarily implies that the loss of α6β4 expression seen in BCCs is functionally related to the low metastatic potential of this tumour.

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