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Integrin Molecules: A Clue to the Non-metastasizing Behaviour of Basal Cell Carcinomas?

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Does the integrin profile of basal cell carcinomas explain their non-metastasizing behaviour? Immunohistochemical investigation of nodular ($n=31$) and superficial ($n=17$) tumours yielded a strong expression of α 2, α 3, α 6, β 1, and β 3 subunits and a weak expression of α 4 subunits by the epithelial tumour component. α 5 subunits were focally detected in superficial basal cell carcinomas but not in the nodular type. Tumour cells were nearly devoid of α v subunits.

The integrin profile of basal cell carcinomas does not differ essentially from that of metastasizing tumour varieties and cannot be regarded as a major reason for the non-metastasizing phenotype of basal cell carcinomas. ***Key words: skin; tumour; adhesion; invasion.***

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One of the most fascinating biologic aspects of basal cell carcinomas (BCCs) is their non-metastasizing behaviour despite an overt invasive growth pattern. Hitherto, no satisfactory explanation of this paradox has been rendered. Following the metastatic cascade, tumour cells have to pass a sequence of steps which all require adhesion to and detachment from tumour cells, interstitial matrix proteins, connective tissue cells, and vessels. It is obvious that adhesion molecules play a key role during the metastatic process. The question, however, arises whether the profile of adhesion molecules is sufficient to explain the metastatic or non-metastatic phenotype of a tumour variety. Adding to former reports on the expression of integrins by basal cell carcinomas (1–7), we performed an immunohistochemical study on nodular and superficial BCCs ($n=48$) looking for the expression of α 2, α 3, α 4, α 5, α 6, α v, β 1, and β 3 subunits. The question to be answered was: Does the integrin profile of BCCs account for their non-metastasizing behaviour?

MATERIAL AND METHODS

Tissue samples

Slices (2 mm wide) of freshly excised nodular ($n=31$) and superficial ($n=17$) BCCs were snap-frozen in liquid nitrogen. Cryostat sections (5 µm) were mounted on poly-L-lysine (Sigma P 1399, Deisenhofen, Germany) coated slides and air-dried (2 h, room temperature [RT]) followed by fixation in acetone (10 min, 4 °C), air drying (a few minutes) and rinsing in 0.19 M Tris buffered saline (TBS), pH 7.4 (5 min, RT).

Immunohistochemical labelling

Background staining was blocked with heat-inactivated normal rabbit serum (DAKO X 902, Hamburg, Germany) or normal goat serum (DAKO X 907) diluted 1:1 in 0.19 M TBS, pH 7.4 (30 min, RT). A panel of monoclonal antibodies was used, directed against the following integrin subunits:

α 2 subunit (mouse mAb, clone Gi9; dianova 0717, Hamburg, Germany); α 3 subunit (mouse mAb, cloneM-KID2; dianova 1308); α 4 subunit (mouse mAb, clone HP2.1; dianova 0764); α 5 subunit (mouse mAb, clone SAM1; dianova 0771); α 6 subunit (rat mAb, clone GoH3; dianova 0769); α v subunit (mouse mAb, clone AMF7; dianova 0770); β 1 subunit (mouse mAb, clone K20; dianova 1151); β 3 subunit (mouse mAb, clone SZ21; dianova 0540).

The primary antibodies were diluted 1:100 in 0.19 M TBS, pH 7.4, with 1% BSA (Sigma A 9647) added, and were applied to the sections for 30 min at RT. Monoclonal mouse antibodies were detected with biotinylated rabbit-anti-mouse bridge antibody 1:400 (DAKO M 413), 30 min, RT, followed by streptavidin-peroxidase 1:400 (DAKO P 397), 30 min, RT. For detection of the rat mAb biotinylated goat-anti-rat bridge antibody 1:400 (dianova 112-066-062) was applied. AEC (Sigma A 5754) was used to visualize the peroxidase reaction. For negative controls, the primary antibodies were left out. No background staining was observed.

RESULTS

BCCs of the nodular and superficial type displayed a distinct and differential profile of integrins. Strong expression of the α 2 and α 3 subunits was found in a pericellular distribution on the tumour cells. The labelling was most intense at the periphery of the tumour strands and declined towards their central parts. Groups of BCC cells that failed to express α 2 or α 3 subunits were found within the palisaded zone of the tumour islands (Fig. 1). The α 4 subunit was expressed very faintly and only focally by BCC cells. Tumour cell nests of nodular BCCs were devoid of α 5 subunits in contrast to superficial BCC aggregates (Figs. 2 and 3). In the latter variety, a focal intercellular expression of α 5 was detected in addition to a discontinous labelling pattern of the BCC basement membrane (Fig. 3). α 5 subunits were richly

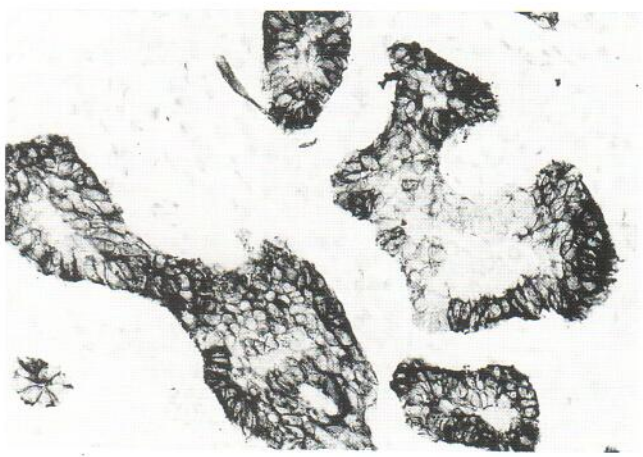

Fig. 1. Nodular BCC. α 2 subunit of VLA-integrin (streptavidin-biotin-Pox, ×220).

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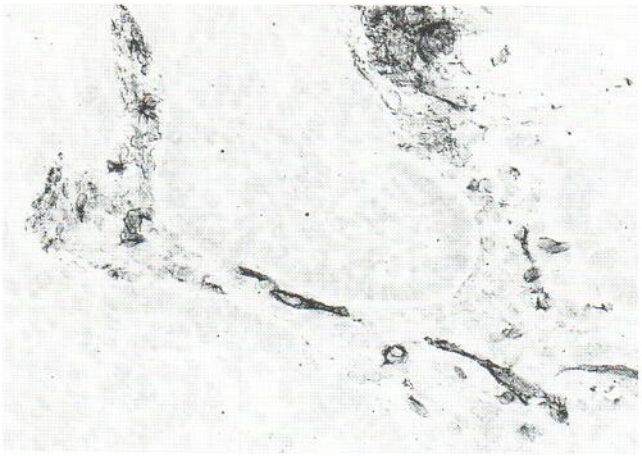

Fig. 2. Nodular BCC. α 5 subunit of VLA-integrin (streptavidin-biotin-Pox, ×220).

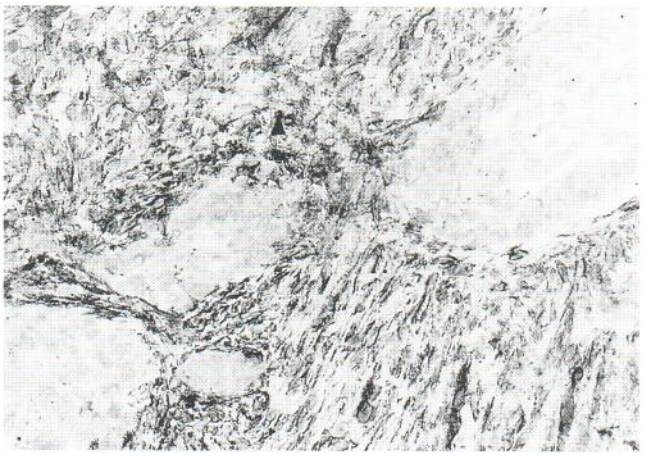

Fig. 5. Nodular BCC. α v subunit of the vitronectin receptor (streptavidin-biotin-Pox, ×220).

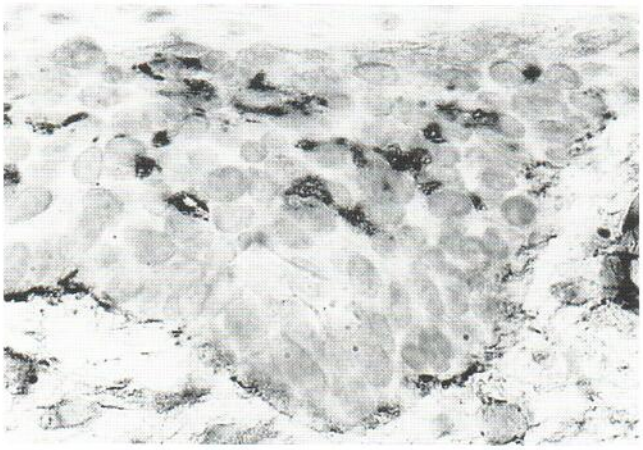

Fig. 3. Superficial BCC. α 5 subunit of VLA-integrin (streptavidin-biotin-Pox, ×550).

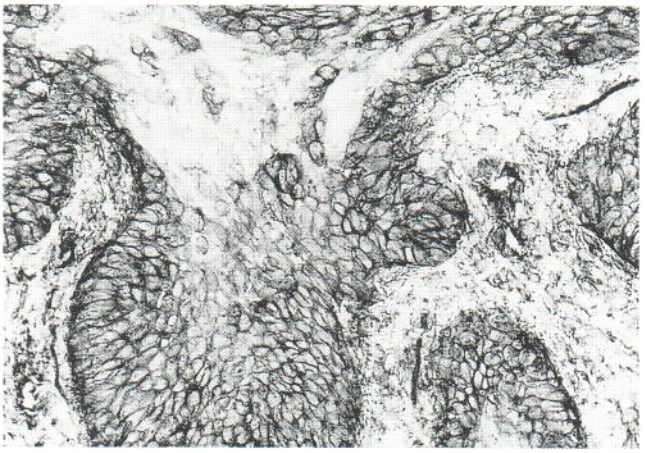

Fig. 6. Nodular BCC. β 1 subunit of VLA-integrin (streptavidin-biotin-Pox, ×220).

expressed by stromal cells and blood capillaries. As to the α 6 subunit, a staining pattern similar to α 2 and α 3 was found, i.e. a strong pericellular expression of α 6 by the peripheral tumour cells and a decreased labelling intensity of the centrally located cells (Fig. 4). The stromal capillaries exhibited a strong staining reaction for α 6. The α v subunit of the vitronectin receptor was hardly detectable in the epithelial component of BCCs. Instead, there was an intense labelling of stromal elements (fibroblasts and blood vessels) (Fig. 5). The β 1 and β 3 integrin subunits were distributed in a pericellular fashion on the surfaces of most of the BCC cells. There was a focal decrease or lack of immunoreactivity in central parts of the tumour strands (Fig. 6). The fibroblasts and the blood vessels of the BCC stroma displayed an intense staining reaction for both the β 1 and β 3 subunits. The staining patterns of the integrin subunits were uniform in all parts of the same tumour specimen. As to our observation, the expression of α 2, α 3, α 4, α 6, α v, β 1, and β 3 subunits did not correlate with the growth pattern or eventual inflammatory infiltrates of BCCs. In contrast, the α 5 subunit was restricted to superficial BCCs, where it could focally be detected between tumour cells and along the BCC basement membrane.

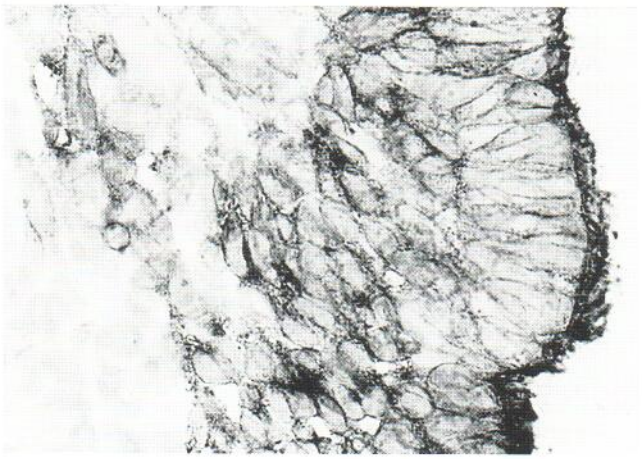

Fig. 4. Nodular BCC, detail of the palisaded cells. α 6 subunit of VLA-integrin (streptavidin-biotin-Pox, ×550).

DISCUSSION

Our investigation was prompted by the question whether the integrin profile of BBCs could give an explanation of their non-metastasizing behaviour. In accordance with former studies (1–4, 6, 7) we found a strong expression of α 2, α 3, β 1,

and β 3 subunits. As a rule, the peripheral cell rows of the tumour strands displayed a more intense labelling than the inner parts. Occasional cell groups devoid of any α or β subunits were mentioned by Stamp & Pignatelli (2). We confirm this observation as a consistent finding in nodular BCCs and explain it by a focal keratotic differentiation. In contrast, the fading of integrin subunits from the central parts of BCC strands might be due to a reduced functional performance of these tumour cells. As to the α 5 subunit, our results indicate that BCCs of the superficial type synthesize this molecule, which is not expressed in BCCs of the nodular variety or in epidermal keratinocytes. Savoia et al. (6) did not detect the α 5 subunit in BCCs but Peltonen et al. (1) and Tuominen et al. (7) reported a weak and focal expression of α 5 subunits by the tumour cells. Savoia et al. (6) and we ourselves had used the same mAb (clone SAM-1), whereas Peltonen et al. (1) and Tuominen et al. (7) had applied different α 5 antibodies (mAb rat IgG2a (1), and mAb mouse P1D6 (7)). The difference between our findings and those of Peltonen et al. (1) and Tuominen et al. (7) as to the α 5 expression could also be due to the tissue preparation (no fixation of the cryostat sections in (1, 7) vs. acetone fixation in our investigation). Savoia et al. (6), however, using unfixed sections like (1, 7) found the same staining pattern as we did. That is the reason why we estimate the source of the α 5 antibody to be the salient point, explaining the different α 5 immunoreactivity. The α 4 subunit was found to be negative (6) or positive (7) in BCCs. We detected a faint and heterogeneous immunoreactivity for α 4 subunits. The epithelial component of BCCs proved to be almost negative for the α v subunit but the tumour stroma abounded with α v immunoreactivity. Tuominen et al. (7) reported α v expression by BCC cells. Our result of a strong α 6 labelling of tumour cells stands out against the findings of Korman & Hrabovsky (4), Savoia et al. (6), and Tuominen et al. (7). These investigators state that BCC cells are devoid of α 6 or express it only focally in minor amounts. We applied an anti-α 6 mAb (rat IgG2a) derived from the same clone (GoH3) as used by Savoia et al. (5) and Tuominen et al. (7). A second series of immunolabelling experiments at our laboratory using a different lot of anti-α 6 mAb (clone GoH3) yielded the same staining pattern as before. The distinct and reproducible staining result cannot be regarded as an artefact. By that, we estimate the question of α 6 expression by BCCs not yet to be settled. Except for the α 4 β 1 integrin, which binds to VCAM-1 expressed on activated endothelial cells, all the other α and β subunits studied are components of integrins binding to extracellular matrix (ECM) proteins (collagen type I and type IV, fibronectin, vitronectin, laminin, fibrinogen) (8). By their differentiated profile of integrins, BCCs may form multiple contacts to stromal elements. The functional repertoire of integrins, however, includes intercellular adhesion (9, 10) and receptor functions initiating intracellular phosphorylation processes (11) or synthesis of collagenase (12). The activity of an integrin molecule itself is dependent on the state of phosphorylation, cytoskeletal association, or clustering within the cell membrane (8, 11). The expression of VLA-integrins and components of the vitronectin receptor on BCC cells not in contact with ECM proteins argues against the simple hypothesis that these membrane compounds merely mediate epithelial-stromal interactions. The ligand specificity and the intracellular processes subsequent to ligand binding strongly depend on the type of tumour cell and its environmental conditions (10, 13).

Does the pattern of integrins expressed by BCCs explain their non-metastasizing behaviour? No, it does not. BCCs exhibit principally the same adhesion molecules as metastasizing tumour varieties like malignant melanomas (13–15). The very low expression of α 4 subunits in comparison with melanoma cells is an unfavourable precondition for the adherence of BCC cells to activated endothelial cells via VCAM-1. It is, however, not proven that non-lymphoid tumour cells make use of this adhesion mechanism for intra- or extravasation. Taraboletti et al. (16) suggest that the formation of metastases may depend much more upon tumour cell adhesion to subendothelial basement membrane components than to the endothelium itself.

Savoia et al. (5) speculate that the simultaneous absence of a hemidesmosomal integrin (α 6 β 4) and its basement membrane ligand (BM-600/nicein) might play a role for the benign course of BCCs. It is clear that the quantity of integrin receptors differentially regulates the performance of cells. Up to now, however, it has not been demonstrated that quantitative differences of integrin profiles determine a metastatic vs. non-metastatic phenotype of tumour cells (15).

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REFERENCES

1. Peltonen J, Larjava H, Jaakkola S, Gralnick H, Akiyama SK, Yamada SS, et al. Localization of integrin receptors for fibronectin, collagen, and laminin in human skin. J Clin Invest 1989; 84: 1916–1923.
2. Stamp GWH, Pignatelli M. Distribution of β 1, α 1, α 2 and α 3 integrin chains in basal cell carcinomas. J Pathol 1991; 163: 307–313.
3. Sollberg S, Peltonen J, Uitto J. Differential expression of laminin isoforms and beta-4 integrin epitopes in the basement membrane zone of normal human skin and basal cell carcinomas. J Invest Dermatol 1992; 98: 864–870.
4. Korman NJ, Hrabovsky SL. Basal cell carcinomas display extensive abnormalities in the hemidesmosome anchoring fibril complex. Exp Dermatol 1993; 2: 139–144.
5. Savoia P, Trusolino L, Pepino E, Cremona O, Marchisio PC. Expression and topography of integrins and basement membrane proteins in epidermal carcinomas: basal but not squamous cell carcinomas display loss of α 6 β 4 and BM-600/nicein. J Invest Dermatol 1993; 101: 352–358.
6. Savoia P, Cremona O, Trusolino L, Pepino E, Marchisio PC. Integrins and basement membrane proteins in skin carcinomas. Path Res Pract 1994; 190: 950–954.
7. Tuominen H, Junttila T, Karvonen J, Kallioinen M. Cell-type related and spatial variation in the expression of integrins in cutaneous tumors. J Cutan Pathol 1994; 21: 500–506.
8. Albelda SM, Buck CA. Integrins and other cell adhesion molecules. FASEB J 1990; 4: 2868–2880.
9. Carter WG, Wayner EA, Bouchard TS, Kaur P. The role of integrins α 2 β1 and α 3 β 1 in cell-cell and cell-substrate adhesion of human epidermal cells. J Cell Biol 1990; 110: 1387–1404.
10. De Luca M, Tamura RN, Kajiji S, Bondanza S, Rossino P, Cancedda R, *et al.* Polarized integrin mediates human keratinocyte

adhesion to basal lamina. Proc Natl Acad Sci USA 1990; 87: 6888–6892.
11. Juliano R. Signal transduction by integrins and its role in the regulation of tumor growth. Cancer Metast Rev 1994; 13: 25–30.
12. Seftor REB, Seftor EA, Gehlsen KR, Stetler-Stevenson WG, Brown PD, Ruoslahti E, *et al.* Role of the α v β 3 integrin in human melanoma cell invasion. Proc Natl Acad Sci USA 1992; 89: 1557–1561.
13. Hart IR, Birch M, Marshall JF. Cell adhesion receptor expression during melanoma progression and metastasis. Cancer Metastasis Rev 1991; 10: 115–128.
14. Cheresh DA. Structure, function and biological properties of integrin α v β 3 on human melanoma cells. Cancer Metast Rev 1991; 10: 3–10.
15. Kramer RH, Vu M, Cheng YF, Ramos DM. Integrin expression in malignant melanoma. Cancer Metast Rev 1991; 10: 49–59.
16. Taraboletti G, Belotti D, Giavazzi R, Sobel ME, Castronovo V. Enhancement of metastatic potential of murine and human melanoma cells by laminin receptor peptide G: attachment of cancer cells to subendothelial matrix as a pathway for hematogenous metastasis. J Natl Cancer Inst 1993; 85: 235–240.