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Differential expression of α1 (IV) and α5 (IV) collagen chains in basal-cell carcinoma

Background: Basement membrane alterations are common in malignancies, and they may indicate tumoral aggressiveness. Distinct patterns of tumoral coverage by collagen IV were reported in nodular and aggressive basal-cell carcinomas (BCCs). Differential expressions of α (IV) collagen chains were also shown on frozen sections. The aim of our work was to document the immunohistochemical expression of α1, α3, and α5 (IV) collagen chains in BCC after routine fixation and processing.
Methods: The patterns of distribution of α1 (IV), α3 (IV), and α5 (IV) collagen chains were studied in 20 formalin-fixed and paraffin-embedded BCCs showing different infiltrative patterns. One trichoblastoma was used as control.
Results: In nodular BCCs, the expression of α5 (IV) collagen chain was downregulated and uneven. By contrast, α1 (IV) collagen chain expression was preserved around these tumors similar to the surrounding skin. However, the α1 (IV) collagen chain expression was discontinuous or absent in BCC areas showing an infiltrative pattern of extension. The α3 chain was absent both underneath all BCCs and non-neoplastic skin.
Conclusions: The basement membrane alterations around nodular BCCs involved more precisely the α5 (IV) collagen chains. Defects in α1 (IV) collagen chain expression seemed to be associated with a tumoral invasive and infiltrative pattern. The biological significance of these findings is unclear.

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Basement membranes (BMs) present at the interface between epithelial tissues and their underlying stroma act as a filtration barrier. They also play a decisive role in morphogenesis, tissue differentiation, and regeneration. They are also important in cancer progression by influencing tumoral invasion and dissemination. Neoplastic BMs may be derived from the tumor cells, stromal cells, or both.[1,2] They are often abnormal in their composition and in their capabilities for cellular attachment.[3]

Type IV collagen and laminin are two major BM components in every region of the body. In normal skin, these macromolecules are located at the dermoepidermal junction and around hair follicles, sebaceous glands, sweat glands, blood vessels, smooth muscles, and nerves. Collagen IV is a glycoprotein composed of three α-chains organized in a helicoidal structure. These three α-chains may be of the same type or may be different. Six distinct α-chains coded by six different genes have been identified. The α1 (IV) and α2 (IV) collagen chains are found in every BM. The α3 (IV) and α5 (IV) collagen chains are restricted to specialized BM such as those of the eye, ear, and kidney glomerula. The α5 (IV) and α6 (IV) collagen chains are found at the dermoepidermal junction and along kidney

collector tubes. The α5 (IV) collagen chain is typically absent in the glomerular and skin BM in Alport's syndrome.[4–7]

Basal-cell carcinoma (BCC) is the most frequent malignant cutaneous neoplasm. It is a locally invasive tumor but with very low propensity to metastasize. It is thought that the neoplasm may originate from an undifferentiated multipotential epithelial germ cell of the basal layer of the epidermis and hair follicles. Some cases are more aggressive and show progression into the hypodermis, muscle, and bone. Tumoral cell clusters of BCC are often lined by a continuous BM even in inflammatory areas.[8–12] However, focal absence of immunohistochemical positivity for collagen IV was reported inside BCC.[8,10]

As seen by immunofluorescence on unfixed specimens, differential expressions of the α1 (IV), α2 (IV), α5 (IV), and α6 (IV) collagen chains were reported to be linked with the potential aggressiveness of BCCs.[13] In superficial multifocal BCC, α1 (IV) and α5 (IV) collagen chains exhibited a linear continuous peritumoral pattern. In nodular BCCs, the network α1 (IV) collagen chains remained present in a continuous linear pattern, whereas the α5 (IV) collagen chains exhibited a discontinuous distribution or were absent. By contrast, the morphea-like BCC subtype was characterized by a discontinuous α1 (IV) collagen chain pattern, and α5 (IV) collagen chains were consistently absent.

The aim of the present work was to document the immunohistochemical expression of α1 (IV), α3 (IV), and α5 (IV) collagen chains in BCCs after routine fixation and processing.

Materials and methods

Fourteen nodular BCCs, three morphea-like BCCs, one plexiform BCC, two superficial BCCs, and one trichoblastoma, were fixed in neutral buffered formalin and embedded in paraffin. Six-micron thick sections were stained with hematoxylin and eosin. Other sections were immunostained using antibodies directed to α1 (IV), α3 (IV), and α5 (IV) collagen chains (Wieslab, Lund, Sweden). The immunostaining was performed using the avidin–biotin–peroxidase method after protease XXIV (Sigma P8038) digestion. The 3-amino-9-ethylcarbazole was used as chromogen. Semiquantitative assessments were performed at the dermoepidermal interface, at the junction between tumoral aggregates and stroma, as well as inside the tumoral collections and the peritumoral stroma. The staining intensity was rated as strong (++), moderate (+), or absent (0). The pattern was considered as continuous or discontinuous. Negative controls and internal positive controls were also considered.

Results

Data are presented in Table 1. The dermoepidermal interface outside BCCs showed a continuous linear staining for both α1 (IV) and α5 (IV) collagen chains. By contrast, the α3 (IV) collagen chain, used as negative control, was absent at the dermoepidermal junction, in the peritumoral stroma and around tumoral aggregates.

In nodular BCCs, the α1 (IV) chain immunoreactivity was strongly expressed in a thick continuous linear pattern at the junction between the tumoral cell clusters and the connective tissue (Fig. 1). Some peritumoral stromal positivity was also present in a granular pattern. In addition, peritumoral stromal cells showed a granular cytoplasmic staining with the anti-α1 (IV) collagen antibody. Inside tumoral collections, α1 (IV) collagen chains were focally present in globular, linear, and intracellular distributions. The density in these structures gradually decreased from the center of the tumoral cell clusters to their periphery.

Five BCCs focally exhibited infiltrative sclerodermiform or adenoid extensions. The pattern of immunoreactivity in these areas was different from the rest of the neoplasm. Indeed, the α1 (IV) collagen chain staining was discontinuous around adenoid BCC structures and was absent around infiltrative cords. The infiltrative BCCs showed a discontinuous staining pattern around tumoral cords for α1 (IV) collagen chain. In morphea-like BCCs, no staining was observed around infiltrative cords. In superficial BCCs, the interface between tumoral nests and the dermis was strongly positive.

With the anti-α5 (IV) collagen chain antibody, three nodular BCCs were weakly positive in a discontinuous linear pattern around tumoral collections but without any stromal staining. The 11 other BCCs were not covered by a BM-containing α5 (IV) collagen (Fig. 2). In these cases, the intratumoral α5 (IV) collagen chain immunostaining was moderately stronger than the stromal staining, and it remained essentially located inside the tumoral cells (Fig. 3). There was also a decrease in staining intensity from the center to the periphery of the tumoral masses.

The three morphea-like BCCs, the two superficial BCCs and one plexiform BCC, showed no staining around tumoral cords with the anti-α5 (IV) collagen antibody. The cellular nests of trichoblastoma were lined by a positive staining with the anti-α1 (IV) collagen antibody, but α5 (IV) collagen was absent.

Discussion

BCC is the most frequent cutaneous malignant neoplasm. Most BCCs share a good prognosis, but some cases are invasive exhibiting local aggressiveness and

Table 1. Immunohistochemical patterns for chains of α collagen IV

Case	Histology	α1 Peritumoral	α1 Intratumoral	α3 Peritumoral	α5 Peritumoral	α5 Intratumoral
1	Nodular	Interface ++ Stroma ++	+	–	–	–
2	Nodular	Interface ++ Stroma +	+	–	–	–
3	Nodular	Interface ++ Stroma ++	+	–	Interface dc Stroma –	IC
4	Nodular	Interface ++ Stroma ++	+	–	Interface dc Stroma –	IC
5	Nodular with infiltrative areas	Interface dc Stroma ++	–	–	–	–
6	Nodular	Interface ++ Stroma ++	+	–	Interface dc Stroma –	IC
7	Nodular	Interface ++ Stroma ++	+	–	–	–
8	Nodular	Interface ++ Stroma ++	+	–	–	–
9	Nodular with adenoid areas	Interface dc Stroma ++	+	–	–	IC
10	Nodular	Interface ++ Stroma +	+	–	–	–
11	Nodular	Interface ++ Stroma ++	+	–	–	IC
12	Nodular with infiltrative areas	Interface dc Stroma ++	+	–	–	–
13	Nodular with infiltrative areas	Interface dc Stroma ++	+	–	–	–
14	Nodular with infiltrative areas	Interface dc Stroma + +	+	–	–	–
15	Infiltrative	Interface dc Stroma +	–	–	–	–
16	Superficial	Interface ++	–	–	–	–
17	Superficial	Interface ++	–	–	–	–
18	Morphea like	–	–	–	–	–
19	Morphea like	–	–	–	–	–
20	Morphea like	–	–	–	–	–
21–25	Solid	Interface +	+	NS	dc	–
26	Morphea like	dc	–	NS	–	–
27	Morphea like	dc	–	NS	–	–
28	Morphea like	dc	–	NS	–	–
29	Morphea like	dc	–	NS	–	–
30	Superficial	++	+	NS	+	+
31	Superficial	++				

1–20, personal data; 21–31, from Tanaka et al.;[14] dc, discontinuous staining; NS, non-studied.

tissue destruction. Thus, the recurrence rate is variable, ranging from 0.5% to 14% according to the BCC type and the treatment modality. Most, if not all, recurrent cases are located on the face.[11] Rare cases showed metastases after multiple recurrences. These more aggressive forms were characterized on histological examination by thin rows of tumoral cells infiltrating a desmoplastic stroma. The tumoral strands were usually thinner and more irregularly shaped than those of the nodular variant. Peripheral palisading was often less prominent, and nuclei were more pleiomorphic.[12] These criteria are subjective, and other prognostic factors are welcome in order to predict the biological behavior of BCCs. Invasive tumors often show alteration of BM such as discontinuity or complete loss.[10,11,14,15] Discontinuity and breaches of the BM were previously shown in aggressive BCC subtypes.[8–10]

Collagen IV expression in BCCs can be categorized into three patterns, namely (a) linear deposits along the interface between the tumor and its stroma, (b) linear deposits inside the tumor sometimes connected to the peritumoral deposits, and (c) intratumoral granular and patchy distribution with gradient density toward the center of the tumoral clusters.[8] The linear staining inside the tumor was interpreted as irregular infoldings of the peritumoral BM. The focal intratumoral deposits may reflect the fact that tumor cells were still able to synthesize collagen IV or that there were not enough possibilities to degrade it. Our findings confirm the staining patterns with linear staining around tumoral cell aggregates and linear and granular intratumoral deposits. However, this was observed only for the α1 (IV) collagen chain. Rare cases showed intratumoral staining for α5 (IV) collagen chain. This was limited to the granular pattern that was more intense toward the center where the staining seemed to be intracellular. Hence, the deposition of these two collagen IV chains appears to be different.

The mechanism underlying these modifications is not yet elucidated; it may be a destructive one by

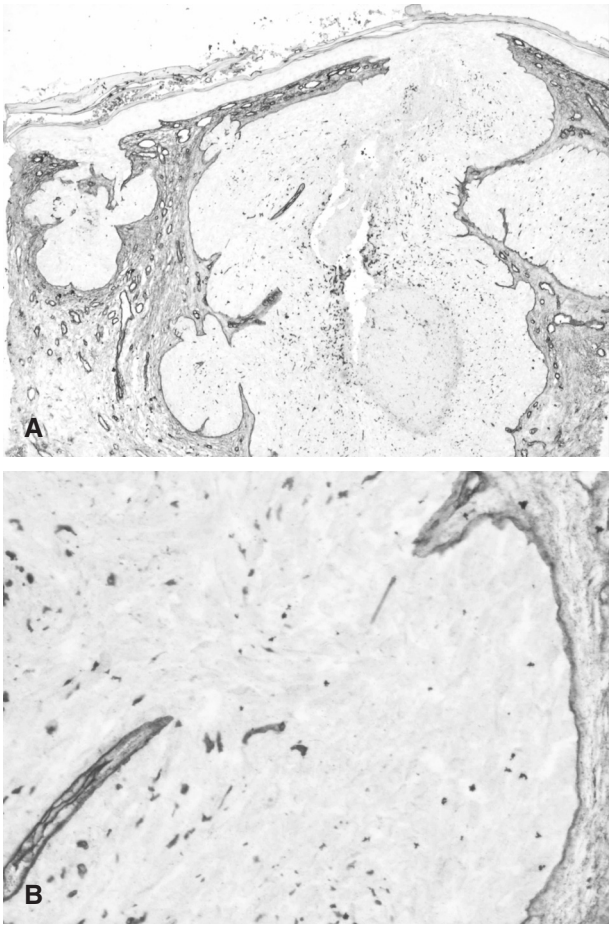

Fig. 1. Intratumoral, linear peritumoral, and stromal α1 (IV) collagen chain immunostaining. A) ×40; B) ×200.

enzymatic degradation or it may be the consequence of any defect in the production of BM components. Upregulation of matrix metalloproteinases (MMPs) is operative in BCCs.[16] However, no significant difference was yielded between nodular and aggressive BCCs. Moreover, psoriasis, some other dermatoses,

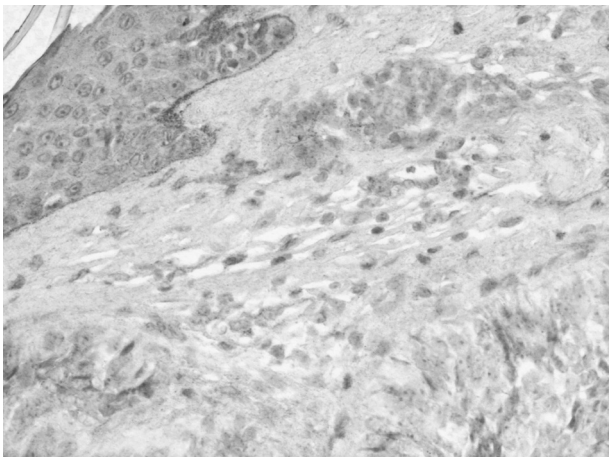

Fig. 2. Absence of peritumoral α5 (IV) collagen chain with positivity at the dermoepidermal junction (×200).

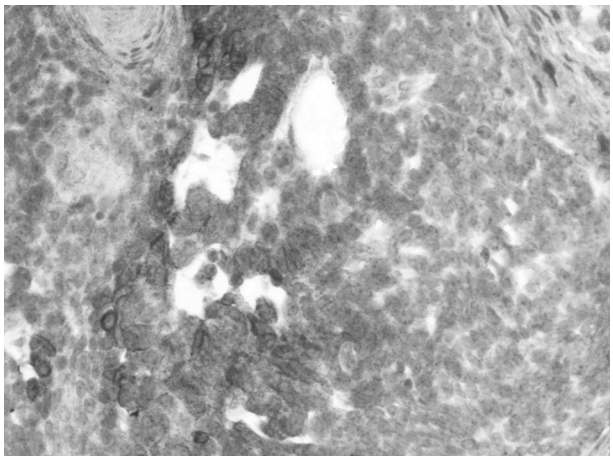

Fig. 3. Intratumoral α5 (IV) collagen chain immunostaining (×200).

and sun-damaged skin are also characterized by this enhanced MMP production. Hence, this mechanism does not appear to be solely responsible for the different behavior of these BCCs. Some alternative hypotheses may be offered. Other enzymes than those studied may be implicated in tumoral invasiveness. Indeed, a significant difference exists between collagen types IV and VII expressions around BCC, suggesting that human skin collagenases were able to degrade collagen VII but not collagen IV. The same hypothesis may be offered to explain the differential expression of α1 (IV) and α5 (IV) collagen chains.

The deregulation may also be the result of impaired synthesis and deposition of BM components. This situation occurs in numerous non-skin tumors.[1] Defective synthesis of α5 (IV) collagen chains in BCCs may be argued by the correlation with little intratumoral staining.

Aggressive behavior may also be due to the altered expression of adhesion molecules involved in tumor invasion. For instance, galectin-3 is one of the molecules that play a role in cell aggregation, cell adhesion, cancer invasion, and metastasis. Decreased expression of galectin-3 was reported in BCCs, colonic, breast, ovarian, and endometrial adenocarcinomas.[17] Decreased galectin-3 expression suggested decreased cellular adhesion and decreased homotypic cellular aggregation leading to small clusters of tumoral cells or to isolated cells favoring crossing throughout BM and local invasion. The aggressiveness of some tumors is also host-dependent rather than a tumor-dependent characteristic.[18]

Moreover, the absence of expression of α5 (IV) collagen chain in BCCs may perhaps be related to the follicular differentiation of these tumors. Indeed, the cell at the origin of BCCs is still discussed: undifferentiated multipotential epithelial germ cell, basal cell of the lower most layers of the epidermis, or outer root sheath of the pilosebaceous unit.[19–21] The

absence of α5 (IV) collagen chain in both BCCs and some areas of follicular outer root sheath could be offered as an argument for the follicular origin of these BCCs. The same pattern observed in trichoblastoma also favors this hypothesis. This merits further studies.[13]

Our findings confirm a previous study[13] showing preserved expression of α1 (IV) collagen chain, but altered expression of α5 (IV) collagen chain in nodular BCCs. A discontinuous lining by α1 (IV) collagen chain was described in association with the absence of α5 (IV) collagen chain in the infiltrative BCC variants.[13] In contrast with the same study,[13] no staining was observed for α5 (IV) collagen chain in the present cases of superficial BCCs. The biological significance of the differential expression between α1 (IV) and α5 (IV) collagen chains is unknown, and there is no evidence for clinical implication. However, focal areas in five of the present cases were more infiltrative, with morphea-like subtype aspect. In these areas, α1 (IV) collagen chain staining was less intense and discontinuous. In sum, we presently report that the immunoreactive patterns to the collagen IV chains can be assessed on formalin-fixed and paraffin-embedded tissue. Hence, these patterns of immunolabeling are wise to be scrutinized after routine processing when assessing the aggressiveness and risk of recurrence of BCCs.

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