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Immunohistochemical Localization of Syndecan in Mouse Skin Tumors Induced by UV Irradiation

Loss of Expression Associated with Malignant Transformation

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Immunoreactivity for syndecan, a cell surface proteoglycan, which binds extracellular matrix molecules and growth factors, was studied in hairless (hr/hr) mice exposed to UV-A and UV-B irradiation. Positive staining was observed at the surface of normal epidermal cells as well as in the dermal abortive hair follicle cysts characteristic to this mouse strain. Early reaction to UV-irradiation showing hyperplastic epidermis with slight cellular atypia showed also positive, although reduced, staining of epidermal cell surfaces. Specimens with severe dysplasia showed weak staining in the granular cell layer, whereas the basal cell layer was negative. In papillomas and keratoacanthomas, immunoreactivity for syndecan was observed in the benign hyperplastic epidermal cells as well as in the proliferating epidermal cells of the horn cysts. Malignant transformation of epithelium, expressed as the formation of early invasive and anaplastic squamous cell carcinomas, was uniformly associated with loss of syndecan staining. These results are consistent with the previous findings of reduced expression of syndecan associated with malignant transformation of cultured epithelial cells, but also suggest an important role for syndecan in the maintenance of normal tissue architecture and differentiation pattern of the skin. (Am J Pathol 1991, 139:1333–1340)

Extracellular matrix (ECM) participates in the regulation of cell shape, proliferation, migration, and differentiation.[1] Matrix receptors, cell surface molecules that bind ECM-molecules, are thus likely to have an important role in development and maintenance of normal tissue architecture. In addition, they also may be involved in the formation of benign and malignant tumors. The latter is supported by the observed changes in the matrix receptor expression during malignant transformation, especially among the members of the integrin family of matrix receptors.[2–4]

Syndecan is a cell surface proteoglycan that binds to various ECM molecules[5] and associates with cytoskeleton through its cytoplasmic domain[6]; therefore it is suggested to act as a matrix receptor. During the formation of tissues, syndecan expression follows morphogenetic rather than histologic boundaries and correlates with the reciprocal epithelial–mesenchymal interactions.[7–9] In the cell culture, syndecan becomes localized solely at basolateral cell surfaces on confluency.[6] If induced to change shape, cells loose syndecan by cleaving its extracellular domain from the membrane-associated domain.[10] Thus syndecan expression is altered in a fashion consistent with its role as a matrix receptor. Recent molecular cloning of mouse and human syndecan shows that syndecan actually represents a family of cell surface proteoglycans that share a common cytoplasmic and transmembrane domains, but differ in their extracellular domains.[11,12] In adult mouse tissues, syndecan expression is restricted to epithelial cells, with the exception of plasma cells and Leydig cells, as demonstrated by immunohistochemistry and Northern analysis.[11,13] Stratified squamous epithelial and transitional epithelial cells stain over their entire surfaces, whereas staining in simple cuboidal and columnar epithelial cells is restricted to lateral surfaces of the basal cells. In stratified squamous and transitional epithelia, the most superficial and differentiated cells fail to stain.[13]

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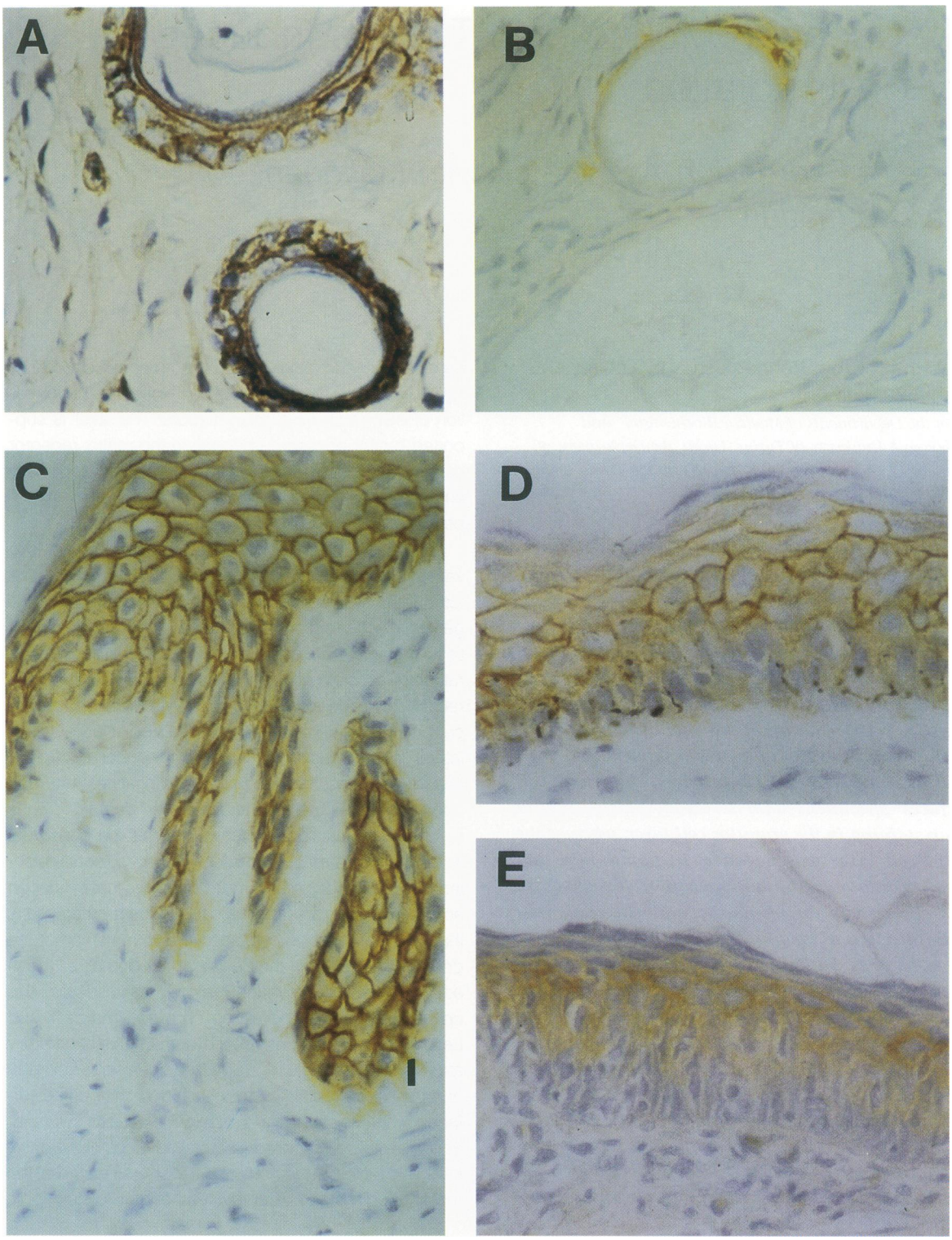


Figure 1. *Paraffin tissue sections from adult hr/hr hairless mice stained for syndecan by the avidin-biotin method.* A: *Positive staining for syndecan is noted in the epidermal cells as well as in the abortive hair follicle cysts in the dermis of untreated skin.* B: *Section showing dermal hair follicle remnants with variable staining.* C: *Hyperplastic UV exposed epidermis showing elongated rete ridges and distinct positive staining.* D: *Moderate epidermal dysplasia induced by UV-B with varying in cell size and shape, with positive, irregular staining.* E: *Severe dysplasia showing positive staining in the granular cell layer, the basal cell layer lacking staining. All magnifications ×400.*

Table 1. *Intensity and Regularity of Syndecan Staining in Normal and UV-irradiated Skin of Hairless Mice*

Skin component/lesion	Syndecan expression		Number of lesions
	Intensity	Type	
Epidermis			
Horn layer	0		
Granular layer	3	r, c	
Basal layer	1	r, dc	
Dermis	0		
Skin appendages			
Superficial-hair follicle cyst	3	r, c	
Deep-hair follicle cyst	0–1	r, c	
Epidermal hyperplasia	3	r, c	
Mild epidermal dysplasia	3	r, dc	11
Moderate epidermal dysplasia	2–3	ir, dc	6
Severe epidermal dysplasia	1–2	ir, dc	4
Papilloma	3	r, c	83
Keratoacanthoma	3	r, c	2
Squamous cell carcinoma	0		4
Fibrosarcoma	0		11

Staining intensity: 0 = none, 1 = slight, 2 = moderate, 3 = distinct.
Distribution: r = regular, ir = irregular, c = continuous, dc = discontinuous.

Exposing animal skin to an agent with known carcinogenic potential, like UV in this study, provides a model for studying the behavior of cells in an intact environment with preserved intercellular interaction and the regulatory function of surrounding cells. UV-B is a potent tumor-inducer with known progression of events leading to the formation of neoplasms similar to those in humans, accounting for a large proportion of clinically observable neoplasms. Previous experimental and clinical studies on the effects of UV exposure as well as chemical carcinogens in skin have shown the emergence of epidermal dysplasia as well as benign and malignant neoplasms from extended UV irradiation.[14] Ultrastructural studies have shown disturbed intercellular structures, a decrease in number of desmosomes, and disturbances in development of surface extensions in the intercellular space in close connection with morphologic signs of malignancy. Also considerable extracellular matrix remodeling happens on formation of a neoplasm, including proteolytic degradation of ECM components and changes in the composition of the ECM and the basement membrane.[15] In this article we report immunohistologic localization of syndecan in murine benign and malignant neoplasms, which were induced by subjecting hairless mice to various doses of UV-A and UV-B irradiation,[16] resulting in a total of 11 sarcomas, 4 squamocellular carcinomas, 2 keratoacanthomas, and 83 papillomas, together with epidermal dysplasia and numerous dermal and epidermal reactive lesions. The results of this paper indicate that development of both severe dysplasia as well as carcinomas results in the loss of syndecan, suggesting that, at least in part, loss of syndecan expression could contribute to the altered behavior of transformed epithelial cells responsible for tumor formation.

Materials and Methods

Tumor Production

Tumors were produced in 48 lightly pigmented hairless male mice of the hr/hr C3H/Tif strain (Bomholdgaard, Denmark) by subjecting the animals to UV-A and UV-B irradiation as described by Talve et al.[16] During the observation period of 12 months, a total of 83 papillomas, 2 keratoacanthomas, 4 squamocellular carcinomas, 1 combined carcinosarcoma, and 11 sarcomas occurred, with increased tumor formation associating with high UV-A (582 J/cm2) plus high UV-B (erythemally effective = EE) (1.0 J/cm2) as compared with low UV-A (71 J/cm2) plus high UV-B (EE) (0.8 J/cm2) (78 *versus* 28 tumors). In addition, mild epidermal dysplasia was observed in 11, moderate dysplasia in 6, and severe dysplasia in 4 animals.

Tissue Samples

Samples were taken from all grossly observable tumors as well as normal-looking UV-exposed skin. Some of the specimens were fixed in 10% buffered formalin, embedded in paraffin, sectioned, and routinely stained with hematoxylin and eosin (H&E). The rest of the examples were frozen in nitrogen and sectioned. The lesions were classified according the standard histopathologic criteria.[17] Periodic acid-Schiff staining (PAS) was also used in some cases for visualization of the basement membranes as well as Herovic, Weigert's, Masson's trichrome, and Gomori's stains for collagen types as well as other stains and electron microscopic analysis when needed.

Immunostaining

For immunohistochemical localization of syndecan, a rat monoclonal antibody 281–2 was used. This antibody is specific for the core protein of mouse syndecan ectodomain.[18] The avidin-biotin-immunoperoxidase technique was used to detect immobilized 281–2 as described by Hsu et al.[19] After deparaffinization and rehydration of the tissue sections, the endogenous peroxidase activity was blocked by incubating the slides in methanol containing 3% hydrogen peroxide for 30 minutes. The sections were then incubated with 2% normal goat serum (Vector Laboratories Inc, Burlingame, CA) in TRIS-buffered saline, pH 7.4 (TBS) for 30 minutes at room temperature (RT) to minimize nonspecific staining. The sections were covered with the primary antibody 281–2 at a protein concentration of 2 μg/ml in 1% (wt/vol) bovine serum albumin (BSA)-TBS and incubated overnight at 4°C. The slides then were incubated with biotinylated goat anti-rat IgG (Jackson's Immunoresearch Laboratories Inc., West Baltimore, PA) at 1:1000 dilution in 1% BSA-TBS for 30 minutes at RT, and finally with avidin-biotin-peroxidase complex (Vectastain kit, Vector Laboratories, Burlingame, CA) for 30 minutes at RT. After washes, the peroxidase activity was demonstrated by incubating the slides with 0.5 mg/ml 3,3′-diaminobenzidine tetrahydrochloride (DAB, Polysciences Inc., Northampton, England) in TBS containing 0.68 mg/ml imidazole and 0.01% hydrogen peroxide for 5 minutes in the dark. The slides counterstained lightly with Mayer's hematoxylin and and mounted Depex Mounting medium (BDH Limited Pool, England). Between all steps, the slides were washed three times with TBS. For control sections, normal rat IgG (Sigma, St. Louis, MO) was used at 2 μg/ml. A few frozen sections for each tumor type were also stained, resulting in an identical immunoreactivity as in the paraffin-embedded tissue sections. Changes in primary antibody concentration and incubation times did not alter the staining pattern significantly.

Results

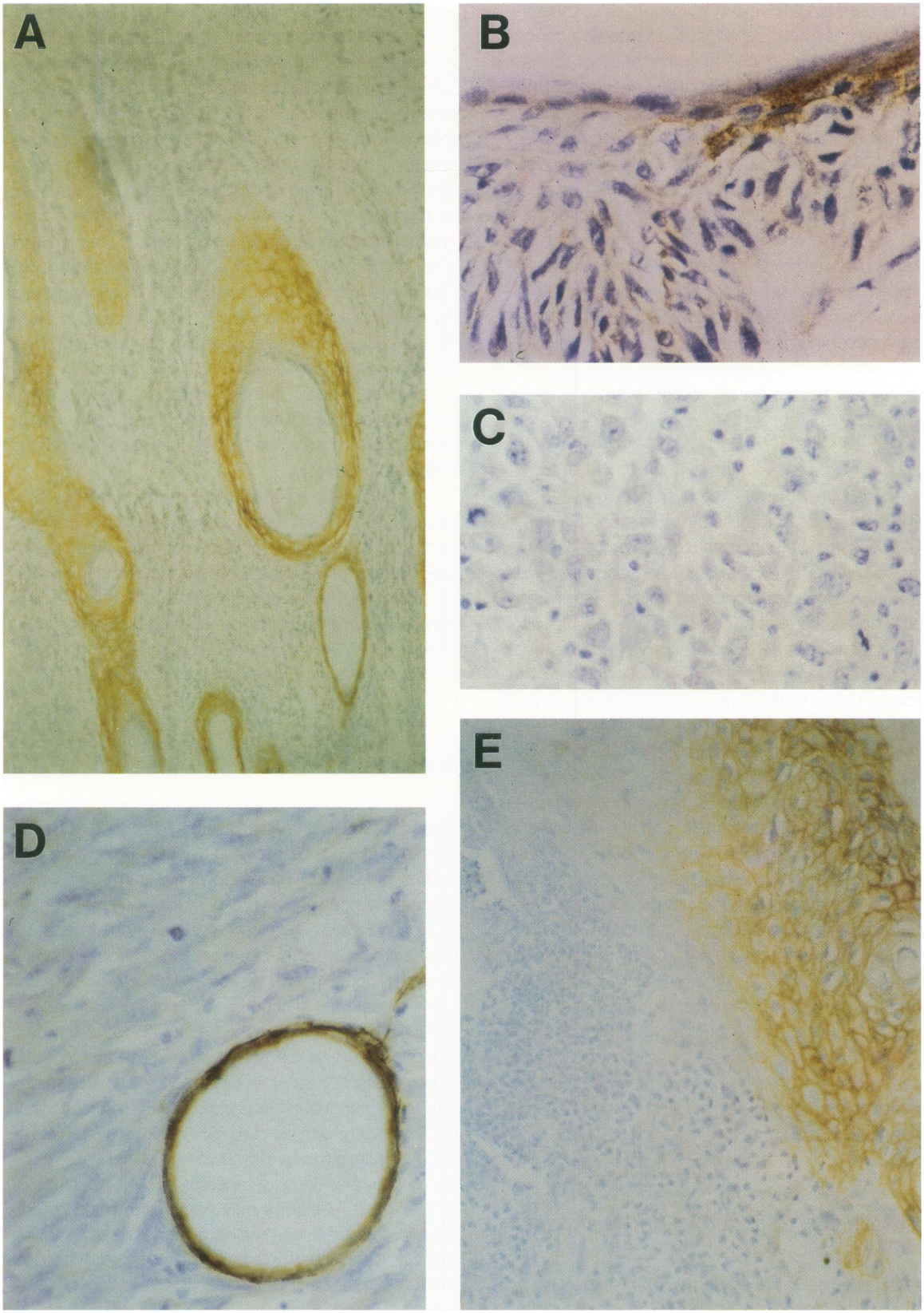
Normal Skin and Premalignant Epidermal Lesions

The skin of hairy mice consists of syndecan-negative dermis as well as the hair follicle apparatus and epidermis, with varying distribution of syndecan as shown earlier by Hayashi and co-workers[13] (Figure 1A). These staining patterns were duplicated also by hairless mouse skin and they are summarized in Table 1. The abortive dermal hair follicle cysts characteristic to this mouse strain were usually also positive for syndecan (Figure 1A). The cells in the walls of the superficial hair follicle remnants were all strongly syndecan positive, but the activity decreased in intensity when approaching the muscular layer, the deepest-lying cysts being completely negative (Figure 1B). These cysts did not participate in the neoplastic process; the decrease in syndecan was related to the degree of development of the cyst wall. Cyst wall cells similar to the surrounding cells were positive, whereas flattened or abortive, deeper located cells were negative. Similar staining as in unexposed skin was also observed in mice that received only UV-A irradiation and showed no histopathologic changes, as well as in normal skin from other mice strains. Control stainings with nonspecific rat IgG showed no staining (not shown).

Extended exposure to UV irradiation produced after several weeks epidermal hyperplasia and structural irregularities. Increased number of cells was an early occurrence and seen in animals exposed to predominantly UV-B as well as in animals given predominantly UV-A. These cells showed a regular even distribution of syndecan at the intercellular space, also when involving the superior part of the hair follicle remnants (Figure 1C). The basal cell layer was mostly unchanged and weakly positive for syndecan. Continued exposure to predominantly UV-B caused first the appearance of mild dysplasia, a solar-keratosislike condition but with limited cytologic disorganization. The epidermal cells varied somewhat in size and shape, the nuclei increased in size, and the nucleo/cytoplasmic ratio increased. These cells exhibited a distinct syndecan-positive border and the staining was slightly discontinuous and irregular.

Extended exposure to mainly UV-B caused moderate dysplasia, increase in cytologic irregularities, and a decrease in syndecan distribution (Figure 1D). The epidermal cells were polymorphic, angular, or irregular, with syndecan staining borders varying in thickness and staining intensity (Figure 1D). The superficial horny layer increased in thickness, being mostly syndecan negative. The basal cell layer, also varying slightly in size and shape, was still only one cell layer thick and displayed a very weak syndecan staining activity.

Figure 2. *Immunoreactivity for syndecan in epidermal neoplasms of hairless mice skin exposed to UV-B and UV-A irradiation.* **A:** *Keratoacanthoma showing distinct positive staining in the proliferating benign epidermal cells of the horn cysts, magnification ×260.* **B:** *Early invasive carcinoma with slight staining for syndecan in the granular cell layer, magnification ×400.* **C:** *Poorly differentiated squamous cell carcinoma cells lacking immunoreactivity for syndecan, magnification ×400.* **C & D:** *Syndecan-negative sarcoma and remnants of hair follicle cysts with syndecan-positive cells, magnification ×400.* **D:** *Section showing syndecan-negative sarcoma invading the epidermis, which appears hyperplastic and syndecan-positive, magnification ×260.*


A
B
C
D
E

At the end of the study, a number of animals, but only those exposed to UV-B, exhibited severe dysplasia, distinct cytologic disorganization, and disordered arrangements of cells, comparable to solar keratosis in man. These cells showed pleomorphism and anaplasia of their nuclei, which appeared large, irregular, and hyperchromatic. Epidermolytic hyperkeratosis, however, was uncommon. The atypic epidermal cells exhibited a weak although still distinctly observable syndecan staining (Figure 1E). The basal cell layer, continuous and distinct, was mostly syndecan negative. The remnants of the hair follicles did not participate in this process, and their staining properties remained unchanged, with superficially located ones being syndecan positive, deeper-laying ones being syndecan negative.

Benign Neoplasms

Another type of neoplastic progression observed in this study consisted of an increase in epidermal thickness, gradually assuming a papillomatous character. This type of alteration, in which a large number of papillomas were found, was observed predominantly in the group receiving large doses of both UV-A and UV-B. The major portion of the tumor cells were positive for syndecan, except for the basal layer and the superficial horn layer. The papillomas varied in structure with a different extent of connective tissue participation; however, invariable negative for syndecan, and varying extent of superficial keratin formation, also syndecan negative. Regardless of whether the neoplasms were verrucous, or acanthomalike, onionlike or fingerlike, the major part of the neoplastic cells were syndecan positive. Only in hyperplastic solar keratosislike conditions, the was the decrease in syndecan similar to that described in simple dysplasia. Neoplasia with acanthosis and irregular rete ridges extending into the dermis, with areas showing signs of early invasion also being negative for syndecan in these locations.

The other type of benign neoplasms were keratoacanthomas, composed of proliferating epidermis on a cupshaped base. The cells participating in this type of neoplastic proliferation were positive for syndecan, also in areas of epidermal cells with irregular borders, deep in the dermis (Figure 2A).

Squamous Cell Carcinomas and Sarcomas

Ultimately a large number of malignant tumors were found in animals exposed to UV-B. These were squamous cell carcinomas and fibrosarcomas. In an early invasive carcinoma, syndecan expression was absent except in a few cells in the granular cell layer at the edge of the carcinoma (Figure 2B). Other squamous cell carcinomas consisted of atypical squamous cells that showed considerable variation in cell size and shape, together with numerous mitoses and with a low degree of differentiation. The extent of keratin formation was minimal, the horn pearls being remnants of pre-existing structures not participating in the formation of tumors. In these neoplasms, the malignant cells were constantly negative for syndecan (Figure 2C). The syndecan distribution in these neoplasms was compared with that of similar neoplasms studied by electron microscopy, and indicated a close relation between the development of intercellular bridges and intercellular surface structures and syndecan activity, all being absent in these malignant invading neoplasms. Well-differentiated areas in these highly malignant neoplasms were sparse, and they were also devoid of syndecan activity.

The other type of neoplastic progression, distinct in this study, consisted of stromal proliferation of fibroblasts, leading to the formation of fibrosarcomas. These malignant neoplasms were keratin negative and also syndecan negative. The syndecan-positive cells in these neoplasms were remnants of pre-existing squamous cells (Figure 2D, E). All staining results with regard to their intensity and regularity are summarized in Table 1.

Discussion

Altered extracellular matrix assembly, reduced adhesion of cells to ECM, and change in cell morphology are processes associated with malignant transformation, which indicate changes also in matrix receptor-mediated cell–ECM interactions. Therefore, all studies that analyze altered expression of matrix receptors during malignant transformation will aid to understand the altered behavior of malignant cells. Although analysis of the expression of integrin molecules exist in the literature,[2–4] this study is a first paper to describe altered expression of syndecan during malignant transformation *in vivo*. Besides matrix molecules, syndecan binds also growth factors, eg, basic fibroblast growth factor (bFGF[20]). Recently heparin or heparan sulfate has been shown to regulate bFGF binding to its signal-inducing receptor,[21,22] suggesting that syndecanlike molecules may participate in the growth factor promotion during normal development.[23] With this respect, it has been interesting to observe enhanced syndecan expression by keratinocytes of regenerating wound.[24]

Irregular Cell Shape and Malignant Transformation Is Accompanied with Loss of Syndecan Expression in UV-irradiated Skin

The results in this study showed that syndecan is not exclusively an indicator of malignancy, but is also related to cellular proliferation and differentiation as a part of the normal process of cell turnover. Basal cells in the epidermis and parts of the hair follicle associated with cell growth were weakly syndecan positive. Similarly terminally differentiated cells and their products in the hair and epidermal cell surface were negative for syndecan. The horn cysts, aberrant remnants of hair follicles in the mouse strain hr/hr used in this study, were also positive when having features reminiscent of normal structures. The more aberrant hair follicle cysts situated deep in the dermis were completely syndecan negative, thus indicating that aberrant differentiation, although not associated with malignant progression, was associated with decreased expression of syndecan. The finding of reduced expression in epidermal dysplasia, together with the loss of expression in the basal epidermal cell layer and the aberrant, large hair follicle cysts, suggest that syndecan is important for the regulation of cell growth and maintenance of normal tissue architecture.

Syndecan as a Prognostic Marker of Malignant Transformation

This study shows a close association between malignant phenotype and absence of syndecan expression, a result consistent with the previous findings of reduced syndecan expression associated with malignant transformation of cells in culture.[25] The results indicate the applicability for syndecan analysis for determining the capacity of malignant progression of clinically observed lesions as well as lesions with doubtful clinical significance. The progression of solar keratosis is related to the degree of dysplasia, characterized by cytologic irregularities and structural disorganization, closely associated with a decrease of syndecan expression. Scar formation and cellular necrosis and proliferation are occasionally histologically similar, syndecan expression being distinctly dissimilar in these processes. The precise clinical usefulness of syndecan analysis in clinical settings requires, however, more studies using clinical material. This awaits the availability of good immunoprobes for human syndecan.

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References

1. Buck CA, Horwitz AF: Cell surface receptors for extracellular matrix molecules. Annu Rev Cell Biol 1987, 3:179–205
2. Plantefaber JC, Hynes RO: Changes in integrin receptors on oncogenically transformed cells. Cell 1989, 56:281–290
3. Cheresh DA, Smith JW, Cooper HM, Quaranta V: A novel vitronectin receptor integrin is responsible for distinct adhesive properties of carcinoma cells. Cell 1989, 57:59–69
4. Ruoslahti E, Giancotti FG: Integrins and tumor cell dissemination. Cancer Cells 1989, 1:119–126
5. Elenius K, Salmivirta M, Inki P, Mali M, Jalkanen M: Binding of human syndecan to extracellular matrix proteins. J Biol Chem 1990, 29:17837–17843
6. Rapraeger A, Jalkanen M, Bernfield M: Cell surface proteoglycan associates with the cytoskeleton at the basolateral cell surface of mouse mammary epithelial cells. J Cell Biol 1986, 103:2683–2696
7. Thesleff I, Jalkanen M, Vainio S, Bernfield M: Cell surface proteoglycan expression correlates with epithelial-mesenchymal interaction during tooth morphogenesis. Dev Biol 1988, 189:565–572
8. Vainio S, Lehtonen E, Jalkanen M, Bernfield M, Saxén L: Epithelial-mesenchymal interactions regulate the stage-specific expression of a cell surface proteoglycan, syndecan, in the developing kidney. Dev Biol 1989, 134:382–391
9. Solursh M, Reiter RS, Jensen KL, Kato M, Bernfield M: Transient expression of a cell surface heparan sulfate proteoglycan (syndecan) during limb development. Dev Biol 1990, 140:83–92
10. Jalkanen M, Rapraeger A, Saunders S, Bernfield M: Cell surface proteoglycan of mouse mammary epithelial cells is shed by cleavage of its matrix-binding domain from its membrane-associated domain. J Cell Biol 1987, 106:953–962
11. Saunders S, Jalkanen M, O'Farrel S, Bernfield M: Molecular cloning of syndecan, an integral membrane proteoglycan. J Cell Biol 1989, 108:1547–1556
12. Mali M, Jaakkola P, Arvilommi A-M, Jalkanen M: Sequence on human syndecan indicates a novel gene family of integral membrane proteoglycans. J Biol Chem 1990, 265:6884–6889
13. Hayashi K, Hayashi M, Jalkanen M, Firestone JH, Trestad RL, Bernfield M: Immunocytochemistry of cell surface heparan sulphate proteoglycan in mouse tissues. A light and electron microscopic study. J Histochem Cytochem 1987, 35:1079–1088
14. Stenbäck F: Life history and histopathology of ultraviolet light-induced skin tumors. Natl Cancer Inst Monogr 1978, 50:57–70
15. Stenbäck F, Wasenius V-M, Kallioinen M: Basement membranes in experimentally induced skin tumors. J Invest Dermatol 1986, 87:185–189
16. Talve L, Stenbäck F, Jansen CT: UV-A-irradiation increases the incidence of epithelial tumors in UV-B-irradiated hairless mice. Photodermatol Photoimmunol Photomed 1990, 7:109–115

17. Lever F, Scaumburg-Lever G: Histopathology of the skin. 7th edition. Philadelphia, JB Lippincott, 1990, pp 1–848
18. Jalkanen M, Nguyen H, Rapraeger A, Kurn N, Bernfield M: Heparan sulphate proteoglycans form mouse mammary epithelial cells: Localization on the cell surface with a monoclonal antibody. J Cell Biol 1985, 101:976
19. Hsu S, Raine L, Fanger H: Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981, 29:577–580
20. Kiefer MC, Stephans JC, Crawford K, Okino K, Barr PJ: Ligand-affinity cloning and structure of a cell surface heparan sulfate proteoglycan that binds basic fibroblast growth factor. Proc Natl Acad Sci 1990, 87:6985–6989
21. Yayon A, Klagsbrun M, Esko JD, Leder P, Ornitz DM: Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor. Cell 1991, 64:841–848
22. Rapraeger AC, Krufka A, Olwin BB: Requirement of heparan sulfate for bFGF-mediated fibroblast growth and myoblast differentiation. Science 1991, 252:1705–1708
23. Ruoslahti E, Yamaguchi Y: Proteoglycans as modulators of growth factor activities. Cell 1991, 64:867–869
24. Elenius K, Vainio S, Laato M, Salmivirta M, Thesleff I, Jalkanen M: Induced expression of syndecan in healing wounds. J Cell Biol 1991, 114:585–595
25. Leppä S, Härkönen P, Jalkanen M: Steroid-induced epithelial-fibroblastic conversion associated with syndecan suppression in S115 mouse mammary tumor cells. Cell Regul 1991, 2:1–11