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Tenascin expression in normal human adult skin and skin appendage tumours

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Abstract The expression and distribution of tenascin, an extracellular matrix glycoprotein, was investigated immunohistochemically using an anti-human tenascin monoclonal antibody (RCB 1) in formalin-fixed paraffin-embedded tissues obtained from 79 patients with skin appendage tumours, and compared with adjacent normal skin. Tissue specimens were pretreated with actinase and processed by the labelled streptavidin-biotin method. In normal skin, tenascin immunoreactivity was consistently found around the ductal portion of the sweat glands, around the lower part of the hair follicle and hair bulbs, and around or within blood vessels. Immunoreactivity was also observed variably around secretory coils of the sweat glands, and below the epidermis. No immunoreactivity was seen around the sebaceous glands. Tumours originating from sweat glands and hair follicles expressed tenascin around the tumour cells nests, while sebaceous gland tumours were immunonegative. Thus, tenascin expression in skin appendage tumours generally resembled that in corresponding normal tissue.

Key words Tenascin · Skin appendage tumour Immunohistochemistry

Introduction

Skin appendage tumours are relatively uncommon and often show heterogeneity in their features. Classification of these tumours is based on their resemblance to the normal adnexa and their degree of differentiation and although morphological features remain the bases of classification, functional aspects should also be added to this system [19]. Various markers have been introduced for this purpose [15, 16, 17, 18], with partial success.

Tenascin, an extracellular matrix glycoprotein was originally described as a myotendinous antigen. It is identical to cytotactin, glioma mesenchymal extracellular matrix protein, hexabrachion and J1, is associated with epithelial mesenchymal interfaces and is abundant in embryonic tissue, while its expression is restricted in adult tissue [11]. Prominent tenascin expression has been demonstrated in the extracellular matrix region in developing skin [2, 3, 4] and its presence in adult skin has also been reported [1, 8, 12]. Several monoclonal antibodies have been raised against tenascin [10] and an immunoperoxidase method using an anti-human monoclonal antibody (RCB 1) yielded satisfactory staining in formalin-fixed paraffin-embedded tissues with actinase treatment [13]. Little is known about the precise expression and distribution patterns of tenascin in normal human adult skin, and there have been no prior reports of tenascin immunostaining in skin appendage tumours. Accordingly, we used the labelled streptavidin-biotin (LSAB) method with a series of routinely processed surgically obtained material to investigate the expression of this antigen in various benign and malignant skin appendage tumours, and its distribution in these tumours is discussed in relation to their tissue of origin.

Materials and methods

Seventy-nine skin appendage tumours from the Osaka Red Cross Hospital, Matsushita Memorial Hospital and Higashiosaka Central Hospital were examined. All specimens were fixed in formalin and subjected to routine paraffin processing. Sections, 4 μ m thick, were carefully heated (60 °C, 5 min) to facilitate adherence to silane-coated slides, dewaxed and used for immunohistochemical investigation. All cases were classified into eccrine and apocrine sweat gland, sebaceous gland, or hair follicle types. All tumours used were histologically typical, and 34 cases of epidermis and skin appendages adjacent but not involved in the tumour were also evaluated.

For immunohistochemistry, after dewaxing, sections were incubated in 3% hydrogen peroxide in ethanol for 10 min, followed

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by actinase digestion. In brief, sections were treated with 0.01% actinase (Kaken Pharmaceutical Company, Tokyo) in 0.05 M phosphate buffered saline (pH 7.2) at 37 °C for 15 min to enhance antigenic exposure. The sections were then incubated in 0.5% bovine serum albumin for 5 min, and further incubated for 1 h at room temperature with a rat anti-human tenascin monoclonal antibody (RCB 1) at a dilution of 1: 500. They were then treated with a LSAB staining kit (Dako, Carpenteria, Calif.) according to the manufacturer's instructions. Reaction products were visualized with freshly prepared 0.02% diaminobenzidine (Wako Pure Chemical, Osaka) containing 0.003% hydrogen peroxide, and the sections were counterstained with Gill's haematoxylin. RCB 1 affinity purified against tenascin from human fibroblasts, has been described in detail previously [10]. For immunohistochemistry, for-

Table 1 Tenascin distribution in normal skin and skin appendages

Site	Tenascin localization
Epidermal-dermal junction	Variable at subepithelial layer
Eccrine and apocrine sweat glands	Consistent at periductal matrix, and variable at periacinar layer
Hair follicle	Consistent around the lower portion of hair follicle and hair bulb
Sebaceous gland	Negative
Vessels	Consistent at subendothelial matrix in small vessels and within the smooth muscle in large vessels

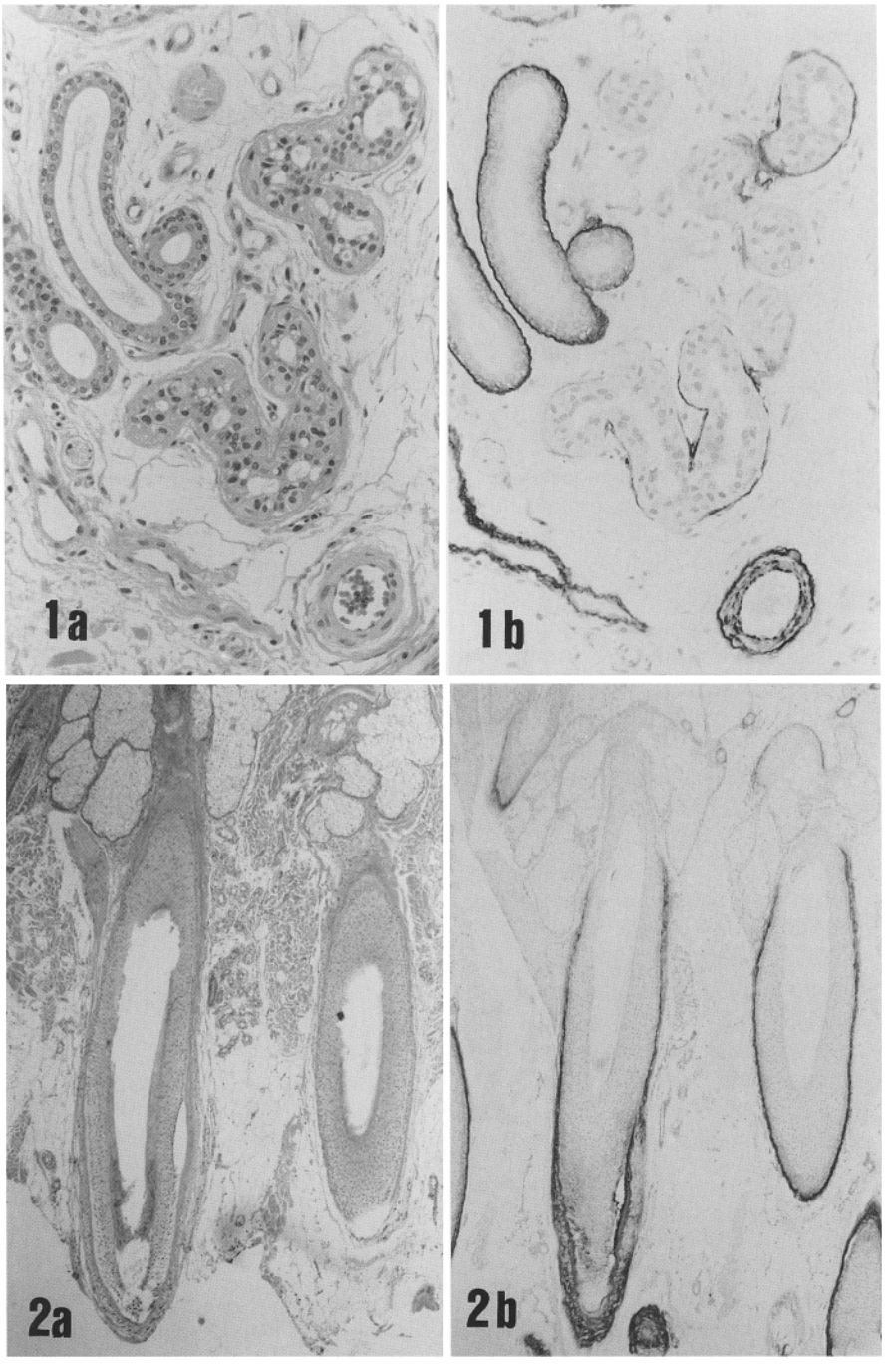

Fig. 1a, b Normal eccrine sweat gland. **a** Haematoxylin and eosin (H&E), × 200. **b** Tenascin immunoreactivity completely surrounded the eccrine duct, and variable weak staining was seen in the secretory coil. Immunostaining within the smooth muscle of the large vessel and subendothelial staining of the small vessel was also observed, × 200

Fig. 2a, b Normal pilosebaceous apparatus. **a** H&E, × 200, **b.** Intense perifollicular staining was seen around the lower portion of the hair follicle and around the hair bulb. There was no tenascin immunostaining around the sebaceous gland, × 40

malin fixation destroys most of the tenascin reactivity. However, pretreatment with actinase enhances tenascin staining [13]. As a negative control, serial sections were incubated with non-immune rat serum instead of the primary antibody at a comparable dilution, and the ubiquity of the blood vessel staining served as an internal positive control.

Results

As shown in Table 1, in 34 instances where epidermis and skin appendages were adjacent but not involved in the tumour, tenascin was variably distributed below the epidermis adjacent to the basal lamina. The expression differed among cases examined, in which some were negative. In the 20 positive cases, the staining was more pronounced within the papillary tip and was frequently sparse at the base of the rete ridge. In sweat glands (Fig. 1a), staining of the eccrine and apocrine sweat ducts was strong in all cases (Fig. 1b), completely surrounding the duct. Variable weak staining was observed in 12 cases at the secretory portion, but the intraepidermal portion was totally immunonegative. In pilosebaceous apparatus (Fig. 2a), hair follicles showed intense perifollicular staining at the lower portion and around the hair bulbs in all cases, whereas no staining was seen around the sebaceous glands (Fig. 2b). The anti-tenascin antibody stained blood vessels consistently, including small arterioles and veins and muscular arteries and large veins. In the smaller vessels, the immunoreaction products appeared in the subendothelial matrix, while in the large vessels staining appeared within the smooth muscle of the vessel wall and was not limited to the basement membrane (Fig. 1b). Erector, muscle was invariably immunonegative with this antiserum.

As shown in Table 2, all the eccrine and apocrine sweat gland tumours and those of the hair follicle showed tenascin immunoreactivity in the connective tissue surrounding tumour cell nests. Characteristic staining patterns observed were as follows; in syringomas and in eccrine hidrocystomas (Figs. 3a and 4a), an intense thick layer of staining was observed in the collagenous matrix near the proliferating cells (Figs. 3b and 4b). Other sweat gland tumours showed a thin layer of tenascin expression around tumour cell nests. In chondroid syringomas (Fig. 5a), immunostaining completely surrounded the cells but did not extend into the lumen, and immunoreactivity was also seen in areas of chondroid metaplasia (Fig. 5b). In hair follicle tumours, tenascin was observed in tumour stroma around basaloid islands of trichoepitheliomas (Fig. 6a, b), while trichofolliculomas showed positive staining around the rudimentary bulbs of the secondary follicle. Immunostaining for tenascin was positive in granulomatous stroma in pilomatrixomas. In contrast, no definite staining was observed with this antibody in either benign or malignant sebaceous gland tumours.

Discussion

Tenascin, a 320 kDa glycoprotein, is an important molecule in the chain of epithelial-mesenchymal interactions

Table 2 Tenascin immunoreactivity in skin appendage tumours (– Negative; + thin layer of positive staining; ++ thick layer of intense staining)

Type of tumour	Tenascin immunoreactivity (number of positive cases/number of cases examined)	Localization	Intensity and extent
Eccrine sweat gland tumour			
Eccrine poroma	8/8	Around tumour cell nests	+
Syringoma	4/4	Around tumour cell nests	++
Eccrine hidrocystoma	3/3	Around tumour cell nests	++
Eccrine acrospiroma	7/7	Around tumour cell nests	+
Eccrine spiradenoma	9/9	Around tumour cell nests	+
Chondroid syringoma	11/11	Around tumour cells and in chondroid matrix	++
Apocrine sweat gland tumour			
Apocrine cystadenoma	2/2	Around tumour cell nests	+
Papillary hidradenoma	1/1	Around tumour cell nests	+
Papillary syringocystadenoma	2/2	Around tumour cell nests	+
Hair follicle tumour			
Inverted follicular keratosis	2/2	Around tumour cell nests	+
Trichoepithelioma	4/4	Around tumour cell nests	++
Trichofolliculoma	3/3	Around rudimentary bulbs of the secondary follicle	+
Trichilemmal cyst	6/6	Around tumour cell nests	+
Steatocystoma	2/2	Around tumour cell nests	+
Pilomatrixoma	6/6	Granulomatous stroma	+
Sebaceous gland tumour			
Sebaceous nevus	0/4	–	–
Sebaceous epithelioma	0/1	–	–
Sebaceous adenoma	0/1	–	–
Sebaceous carcinoma	0/3	–	–

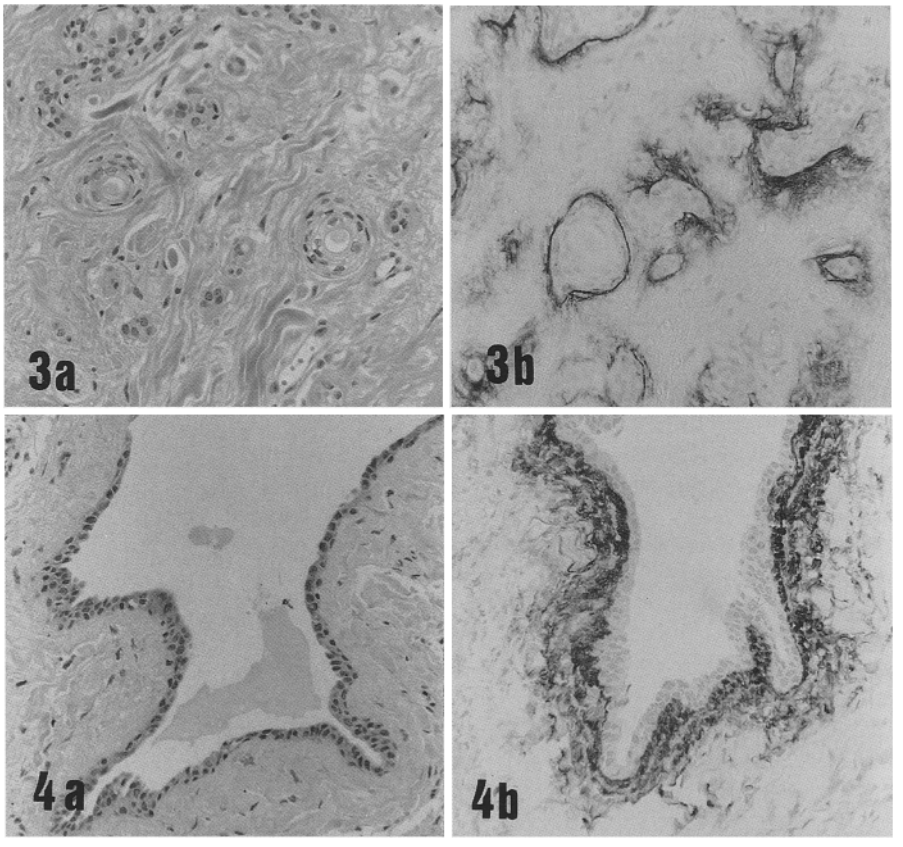

Fig. 3a, b Syringoma. **a** H&E, × 200. **b.** A thick layer of tenascin immunostaining was seen in the collagenous matrix adjacent to the proliferating small ducts, × 200

Fig. 4a, b Eccrine hidrocystoma. **a.** H&E, × 200. **b.** Thick staining was seen around the dilated eccrine dermal duct, × 200

during development, morphogenesis and neoplasia, and is also known to be expressed in certain normal adult tissues. The present immunohistochemical study clearly demonstrated the characteristic tenascin expression in normal adult skin, and in a variety of skin appendage tumours. Tenascin distribution in normal skin was generally consistent with that reported previously [1, 8, 12] but showed minor discrepancies. A possible explanation may lie in the different tissue-processing techniques used in the present and these previous studies. The use of routinely processed specimens may not only reduce the intensity but affect the results. However, in agreement with our previous report [13], pretreatment with 0.01% actinase was adequate, and the tenascin immunoreactivity was comparable to that observed in frozen sections or more reliable methacarn-fixed tissues [1, 8, 12]. Actinase digestion is greatly advantageous for immunohistochemical analysis of tenascin using routinely processed surgical materials. In the present study, tenascin staining around blood vessels was always consistent, and this served as a built-in positive control.

Tenascin is a structural component of adult dermis with a highly specific and unique distribution pattern. In normal adult human skin, tenascin is expressed in the papillary dermis [1, 12]. The existence of this protein near the basal lamina may suggest a possible role in maintenance of dermal-epidermal junction. The marked intraepidermal staining of the ductal portion, but virtual abscence in the intraepidermal portion of sweat glands and weak staining at the secretory coil is suggestive of a significant role of tenascin in the sweat glands. In the normal adult mammary gland, a modified skin adnexal gland, tenascin is distributed around the duct and ductule but not around the acini [13]. Tenascin immunoreactivity was detected around the lower hair follicle and around the hair bulb, both sites of intense morphogenic activity even in adults [14]. Tenascin is a large complex molecule in which multiple signals coexist, and this may lead to difficulty in interpretation of the role of tenascin in normal skin. Tenascin has several features in common with fibronectin and laminin [11]. However, the tenascin immunostaining pattern observed in the present study differed completely from that of laminin reported previously [6].

In general, tenascin expression in skin appendage tumours was related to the tissue of origin. Syringomas are derived from eccrine duct cells in or near the acrosyringium, and eccrine hidrocystoma is of dermal duct origin [5]. Dense tenascin staining was observed in sweat gland tumours of ductal origin. In the present study, the fibromyxochondroid matrix of all cases of the chondroid syringomas showed heavy anti-tenascin immunostaining. Tenascin can promote chondrogenic differentiation and is detectable in the condensing mesenchyme of cartilage anlagen [9]. In pleomorphic adenoma of the salivary gland, the matrix around the early epithelial as well as the mesenchymal component showed similar staining [7]. In hair follicle tumours, tenascin expression was ob-

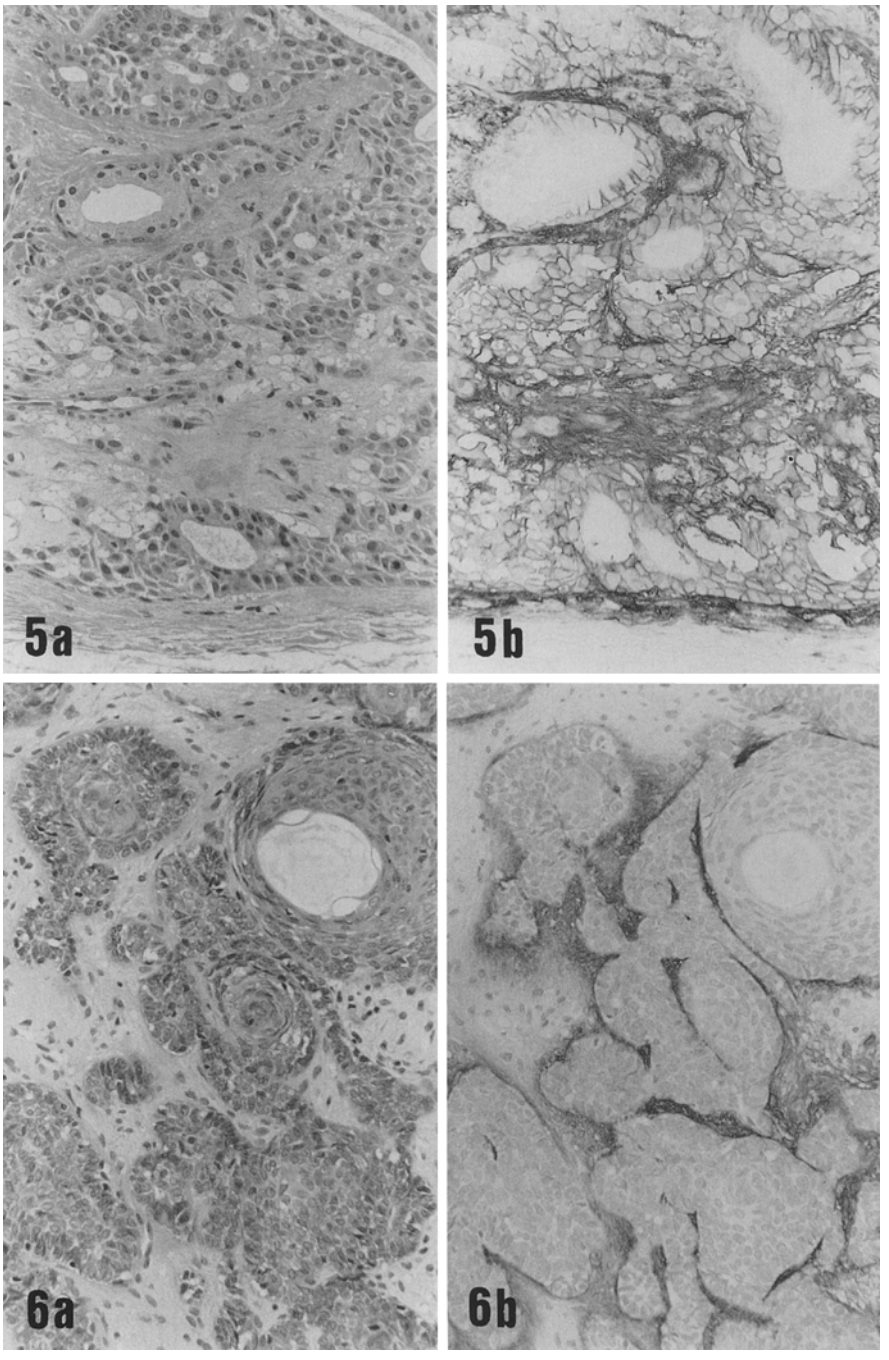

Fig. 5a, b Chondroid syrin goma. **a.** H&E, × 200. **b.** Tenascin immunoreactivity completely surrounded some of the cells but did not extend to the lumen. Areas of chondroid matrix were also stained, × 200

Fig. 6a, b Trichoepithelioma. **a.** H&E, × 200. **b.** Tenascin immunoreactivity was positive in the stroma around basaloid epithelial cells, × 200

served mainly around primitive basaloid epithelium or around cells resembling the lower portion of the hair follicle. All the sweat gland tumours and hair follicle tumours maintained tenascin expression patterns similar to those observed in their cells of origin. In contrast, no tenascin expression was seen in sebaceous gland tumours. Tenascin has been proposed as a mesenchymal marker for epithelial malignancy, and has been reported to be expressed in fetal, juvenile, and neoplastic mammary glands [11]. However, we found that sebaceous carcinomas did not express tenascin and expression is unlikely to be related to malignancy in this system.

The biological function of tenascin in skin appendage tumours remains unclear and its expression and role in skin appendage tumours requires further investigation.

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