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Localization of Ca^{2+}-binding S100 proteins in epithelial tumours of the skin

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Abstract The Ca^{2+}-binding proteins S100A1, S100A2, S100A4, S100A6 and S100B were evaluated immunohistochemically in normal skin and skin appendage tumours. Epidermal basal cells, epithelial cells of sebaceous glands, hair follicle sheet epithelia and eccrine duct reacted strongly with an antiserum against human S100A2 but were nonreactive or weakly reactive to S100A1, S100A4, S100A6 and S100B. Varying types of skin appendage tumours and most peripheral cells in tumour nests of basal cell carcinoma and squamous cell carcinoma showed positive S100A2 immunoreactivity in neoplastic cells corresponding to basal cells but were nonreactive or faintly reactive for other S100 proteins. Langerhan's cells and melanocytes were labelled by S100B. Basophilic cells of calcifying epithelioma were occasionally stained with S100A2 antiserum. Eccrine poroma did not react with any S100 antiserum. Mixed tumours of the skin containing neoplastic myoepithelial cells stained strongly for S100A2 and S100B but only faintly for S100A1, S100A4, S100A6. This is the first report on selective evaluation of different S100 proteins in normal skin. These antibodies are valuable tools for better characterization of skin appendage tumours.

Key words Ca^{2+}-binding S-100 proteins · Epithelial tumours · Skin · Immunohistochemistry

Introduction

S100 proteins are low-molecular-weight Ca^{2+}-binding proteins, which are involved in signal transduction processes and consequently in the regulation of proliferation, differentiation, transcription, and apotosis [15]. So far, 13 different S100 genes have been found in a clustered organization on human chromosome 1q21, leading to the introduction of a new nomenclature for these genes and their corresponding proteins [16]. Interestingly, genes coding for S100 proteins form a tight cluster with genes encoding structural proteins required for epidermal cornification, the epidermal differentiation complex [8]. Since all these structural proteins are expressed within the epidermis, it is conceivable that S100 proteins are also expressed within the epidermis. Consistent with this hypothesis, expression of the novel S100A2 is found in normal epithelial cells, whereas S100A4 and S100A6 are predominantly expressed in tumorigenic epithelial cells, suggesting an involvement of these proteins in tumour suppression or progression [15]. Supporting this notion, the chromosomal region 1q21 is frequently rearranged in human neoplasms [2], possibly influencing the altered expression of some S100 proteins in tumour cells.

In most previous immunohistochemical studies on salivary pleomorphic adenomas [9, 11], skin mixed tumours [4, 10, 12, 13] and many other tumour types, commercially available antisera raised against an ill defined mixture of bovine brain S100 proteins were used for the classification of tumours. Recently we demonstrated that these antisera against bovine S100 proteins reacted mostly against S100A1 and A100B but not against the novel S100A2, S100A4, or S100A6 proteins. In culture cells and tumour tissue [6] and that in salivary gland tumours, duct cells and luminal tumour cells were stained markedly for S100A2, and weakly for S100A1, S100A4, S100A6, and S100B [5]. Therefore, we raised specific antisera against a number of human recombinant S100 proteins to find their immunohistochemical localization, to achieve a more highly differentiated classification of tumours and to monitor subtle pathologic changes in tumour tissue and body fluids [6]. These novel antisera against S100A1, S100A2, S100A6, and S100B were used in this study for the first time to examine the selective distribution of S100 proteins in normal skin and to characterize skin appendage tumours.

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Materials and methods

Tissue specimens of clinically normal skin adjacent to benign lesions (n = 14), and of skin tumours such as steatocystoma (n = 2), trichoepithelioma (n = 2), calcifying epithelioma of Malherbe (n = 2), cylindroma (n = 2), syringoadenoma papilliferum (n = 2), syringocystoma (n = 2), skin mixed tumour (n = 5), eccrine poroma (n = 2), basal cell carcinoma (n = 2), squamous cell carcinoma (n = 3), Langerhans cell histiocytosis (n = 1), malignant melanoma (n = 2), sebaceous adenoma (n = 2), and sebaceous carcinoma (n = 2) were used. The tissues were fixed in 10% formalin and embedded in paraffin, and 4-µm sections were evaluated for histopathology and immunohistochemistry.

Antiserum against human recombinant S100A2 was raised in rabbits. Antisera against human S100A1, S100A4 and S100A6 were raised in goats. The specificity of the antibodies was examined by Western blot analysis as described elsewhere [6]. Monoclonal antibody against human S100B was purchased from Sigma Chemicals (USA).

Deparaffinized sections were treated with methanol containing 0.3% H_2O_2 for 30 min to inhibit nonspecific peroxidase activity. Following extensive rinsing in phosphate-buffered saline, normal swine or rabbit serum (diluted 1:20) was overlaid on the section for 30 min to reduce nonspecific staining.

Primary antibodies were applied for 1 h at room temperature. After three rinses in PBS, sections were incubated with either biotinylated anti-mouse, anti-rabbit, or anti-goat immunoglobulin fraction (1:200, Dako, Copenhagen) for 30 min at room temperature. After washing in PBS, strept–AB complex (1:500, Dako, Copenhagen) was applied for 30 min at room temperature, and diaminobenzidine solution was used for visualization. In control sections, the same procedures were used except that the antibodies were replaced by PBS.

Results

Normal tissue (Table 1)

Basal cells and parabasal cells of the skin epidermis were reactive for S100A2 (Fig. 1A, B) whereas spinous and upper strata were unlabelled. No immuno-reaction of S100A1, S100A4, S100A6 and S100B was found in the epithelial layers. The Langerhan's cells and melanocytes were labelled by S100B.

Sebaceous glands consist of acinous cells, undifferentiated flatted epithelia and short ducts. S100A2 staining was restricted to flattened epithelia and ducts (Fig. 1C).

In hair follicles, epithelial cells were stained with S100A2. External sheaths showed a faint reaction for S100A2, but the internal sheath cells were strongly positive (Fig. 1D). Cuticle and cortex were devoid of all other S100 proteins.

In sweat glands, prominent S100A2 staining was observed in ductal epithelia (Fig. 1E), while strong S100B staining was present in terminal secretory segment (Fig. 1F). In addition, nerve fibres were highly reactive for S100B. No staining for the other S100 proteins was observed.

Skin tumours of hair follicle origin (Table 2)

Steatocystoma multiplex (multiple follicular cyst or cystadenoma) consists of multiple layered epithelium, including intensely keratinized cells. Basally located tumour cells of steatocystoma displayed a moderate reaction with S100A2 antiserum and no S100A1, S100A4 and S100A6 reactivity. In staining for S100B, tumour epithelia were negative while Langerhans cells stained positively.

In the case of trichoepithelioma, epithelial components form nests of basaloid cells and occasionally small keratotic cysts. Proliferating tumour cells, or peripheral cells of tumour foci, corresponding to the basal cells, usually stained for S100A2 immuno-reactivity (Fig. 2A). However, tumour cells were also occasionally negative for this protein. In contrast, parakeratinized cells stained with S100A2 antibodies. The other S100 antisera were nonreactive.

Calcifying epithelioma malherbe (pilomatrixoma) consists of basophilic cells and shadow cells without nuclei, and also transitional cells between the two. Basophilic cells probably arise from trichilemmal epithelium, and shadow cells may correspond to hair cortex. Strong immunostaining was only found for S100A2 and was confined to occasional basophilic cells (Fig. 2B).

Skin tumour of eccrine sweat gland origin (Table 3)

Dermal cylindroma is composed of basophilic cell cords and duct-like or cystic structures and fibrous stroma. Basophilic cell were was grouped forming lobules, while duct-like and cystic structures consist of cuboidal or columnar cells sometimes resembling sweat gland ducts. In tumor masses, a spot like staining for S100A2 was observed with an irregular distribution (Fig. 2E). Basally located tumour cells exhibited conspicuous reaction for S100A2 as well as parakeratinized zones in keratinized focus (Fig. 2E). The staining intensities of S100A2 varied from almost negative to strong, while those of S100A1, S100A4 and S100A6 were usually nonreactive. S100B reactivity in the tumour tissue was restricted to dendritic Langerhans cells.

Syringadenoma papilliferum proliferates with papillary projection consisting of two layers of epithelial cells. Basal layer cells are cuboidal or flat, whereas the luminal layer is columnar in shape. Stroma of papillary tumours characteristically show infiltration of inflammatory and plasma cells. Tumour basal cells expressed S100A2 (Fig. 2C). S100B immunoreactivity in spiroadenoma was mostly negative, but Langerhans cells showed S100B immunoreaction (Fig. 2D).

Syringocytoma cells showed a faint reactivity for S100A2 in basal cells in one case but were negative in another case. Immunoreactivities for the other S100 protein were weak or, mostly, negative.

Skin mixed tumor (chondroid syringoma) is a histopathological counterpart of salivary pleomorphic adenoma and shows a tubuloductal structure proliferating in a variety of so-called stromal tissue. The tumour may show epithelial cords, and hyalinous or chondroid changes are frequent. Tubular or duct-like structures consist of

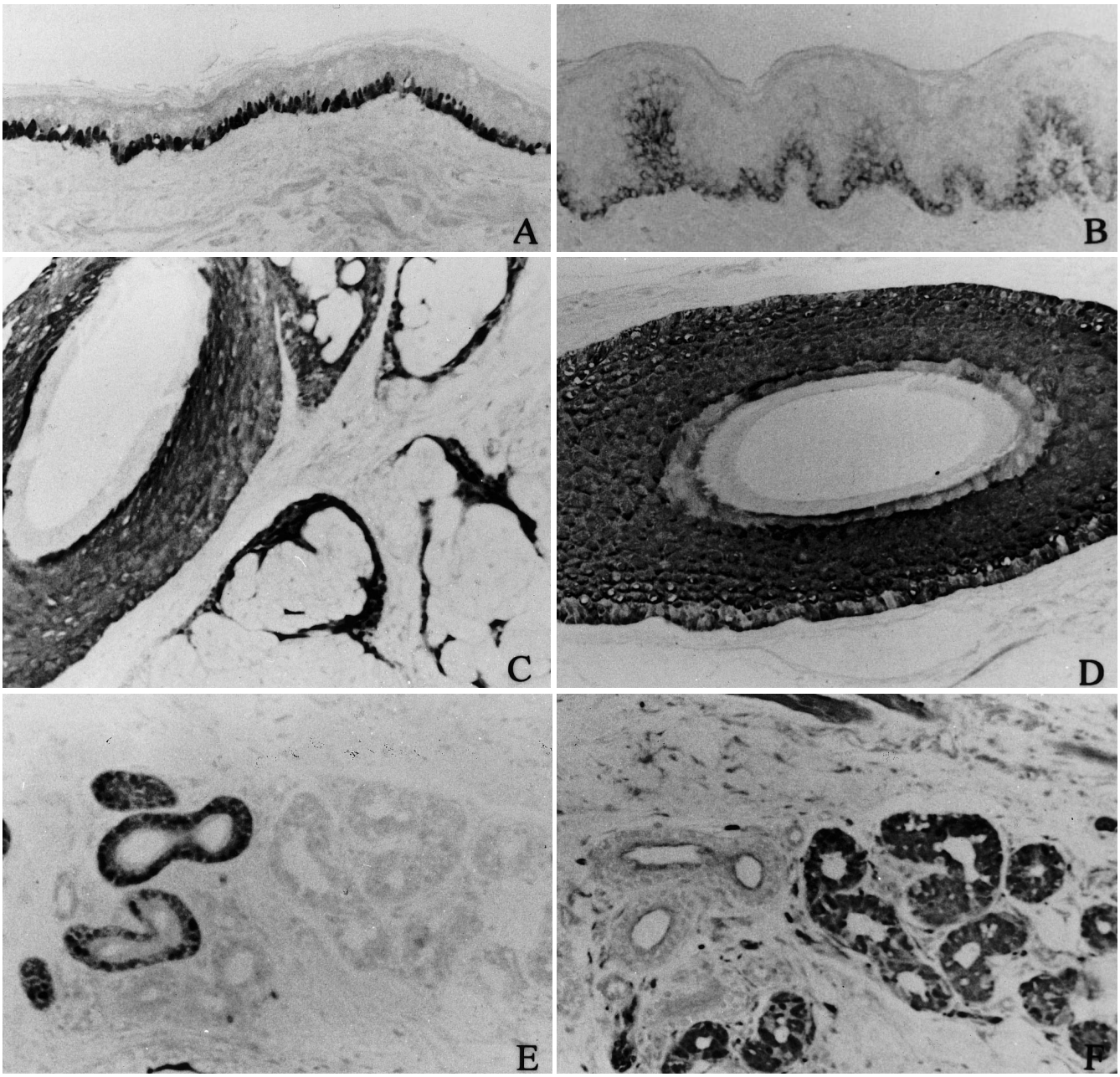

Fig. 1A–F S100 protein immunohistochemistry of normal skin and skin appendages. ×100. **A** S100A2 staining is restricted to basal cells of the epidermis. **B** S100 A2 staining in the basal and few parabasal cells in the epidermis. **C** Sebaceous gland and hair follicle show marked staining of S100A2 in hair sheath and epithelial cells of sebaceous glands. **D** Hair follicle reveals an intense S100A2 immunoreactivity of inner sheath cells and a faint reaction of outer sheath cells. **E, F** Sweat gland staining for **E** S100A2 in duct epithelia and **E** S100B in terminal secretory segments

luminal cells and nonluminal cells. Most prominent reactions were found for S100A2 and S100B in modified myoepithelial cells, which form the outer or nonluminal layer of duct-like structures (Fig. 3A, B). Plasmacytoid neoplastic myoepithelial cells reacted strongly to S100B and relatively weakly to all S100As. In hyalinous, myxomatous and chondroid tissues, spindle-shaped tumour cells and chondroid cells, a prominent S100B action was found. Tumour cells were also reactive for S100A1 (Fig. 3C), and S100A2, S100A4 and S100A6.

In eccrine poroma no immunostaining was found for any of the S100 proteins in the present study.

Sebaceous neoplasm

Sebaceous adenoma had numerous masses of hyperplastic sebaceous lobules showing epithelial cells with an intense immunostaining for S100A2, while displaying almost no or very weak reaction for all other S100A proteins.

In sebaceous carcinoma the tumour foci are composed of lobules and nests consisting of atypical sebaceous cells and basaloid or epitheloid cells. S100A2 immuno-

Table 1 Localization of S100 proteins in 14 specimens of normal skin (– negative staining, + positive staining, ++ strongly positive staining, x subset of immunoreactive cells only)

	S100A1	S100A2	S100A4	S100A6	S100B
Skin epidermis					
basal cells	–	++	–	–	–
Parabasal cells	–	+	–	–	–
Spinous cells	–	–	–	–	–
Granular cells	–	–	–	–	–
Horrified cells	–	–	–	–	–
Hair follicle					
Outer zone	–	+	+	+	+
Inner zone	–	++	+	–	++
Eccrine sweat gland					
Terminal secretory segment	–	–	–	–	++
Coiled duct	–	++	–	–	–
Straight duct	–	x (Basal cells)	–	–	–

Table 2 Localization of S100 proteins in skin tumour of hair follicle origin (– negative staining, + positive staining, ++ strongly positive staining, x subset of immunoreactive cells only)

	S100A1	S100A2	S100A4	S100A6	S100B
Steatocystoma multiplex (*n* = 2)					
Tumour cells	–	++	–	–	–
Keratinized cells	–	++	–	–	–
Tricho-epithelioma (n = 2)					
Tumour cells	–	x	–	–	–
Keratinized cells	–	++	–	–	++
Calcifying Epithelioma Malherb (n = 2)					
Basophilic cells	–	x	–	–	–
Shadow cells	–	–	–	–	–

Table 3 Localization of S100 proteins in skin tumour of eccrine sweat gland origin (– negative staining, + positive staining, ++ strongly positive staining, x subset of immunoreactive cells only)

	S100A1	S100A2	S100A4	S100A6	S100B
Cylindroma (*n* = 2)	–	x (basal cells)	–	–	x (Langerhans cells)
Syringo-adenoma papilliferum (*n* = 2)	–	x (basal cells)	–	–	x (Langerhans cells)
Syringocytoma (n = 2)	–	x	–	–	–
Skin mixed tumour (n = 5)					
Luminal cells	+	–	–	–	–
Nonluminal cells (modified myoepithelial cells)	+	++	–	–	++
Chondroid cells	++	++	+	+	++
Poroma (n = 2)	–	–	–	–	–

staining of sebaceous carcinoma was found in basaloid or epithelial tumour cells (Fig. 2F), but no reaction was found against the other S100 proteins. Sebaceous acinous elements in both normal tissues and tumours were devoid of all S100 proteins.

Other tumours

Langerhans cell histiocytosis, consisting of abnormal Langerhans cells, showed an intense reaction for S100B and a faint reaction for S100A6.

Malignant melanoma showed spindle cells and epithelioid cells: both cell types were intensely stained for S100B, and the epithelioid cells were occasionally stained for S100A2.

The most peripheral cells in the clusters of basal cell carcinoma were labelled by S100A2. No reaction products were seen for S100B in the carcinoma cells.

In the case of squamous cell carcinoma, the invading nests showed the peripheral cells immunostained for S100A2. All other S100A and S100B were nonreactive in the tumour cells.

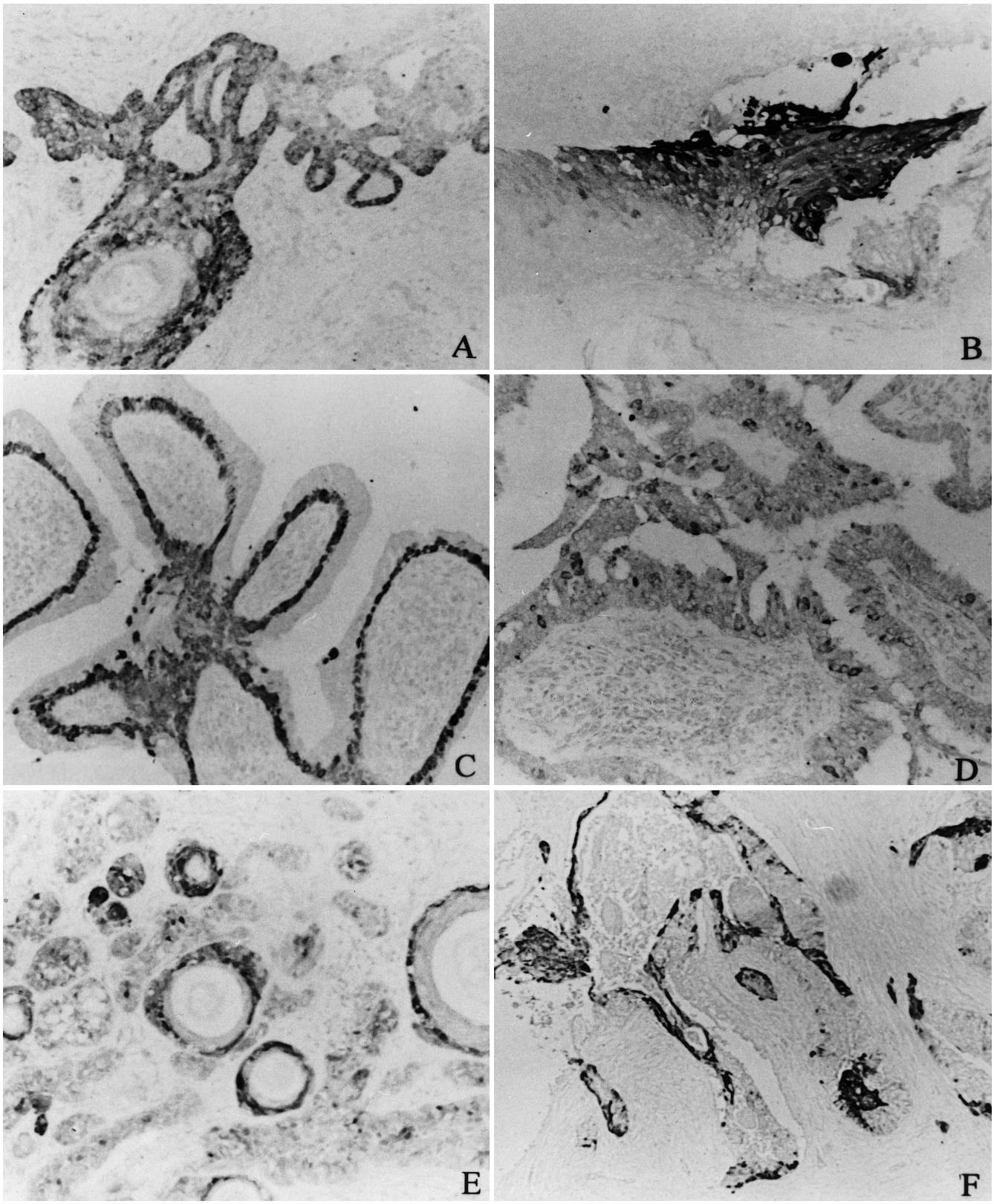

Fig. 2A–F S100 protein immunohistochemistry in skin appendage tumours. ×100. **A** Trichoepithelioma. S100A2 staining is specific for tumour basal cells corresponding to basal cells of the normal epidermis. **B** Calcifying epithelioma Malherbe. S100A2 immunostaining is found in occasional basophilic cells. **C, D** Syringoadenoma papilliferum. S100A2 staining is characteristically present in basal cells of neoplastic epithelium (**C**). S100B staining is only seen in Langerhans cells infiltrating tumour epithelium (**D**). **E** Cylindroma stained for S100A2 in tumour basal cells irregularly distributed in the neoplastic foci. **F** Sebaceous carcinoma. Epithelioid tumour cells stain positively for S100A2

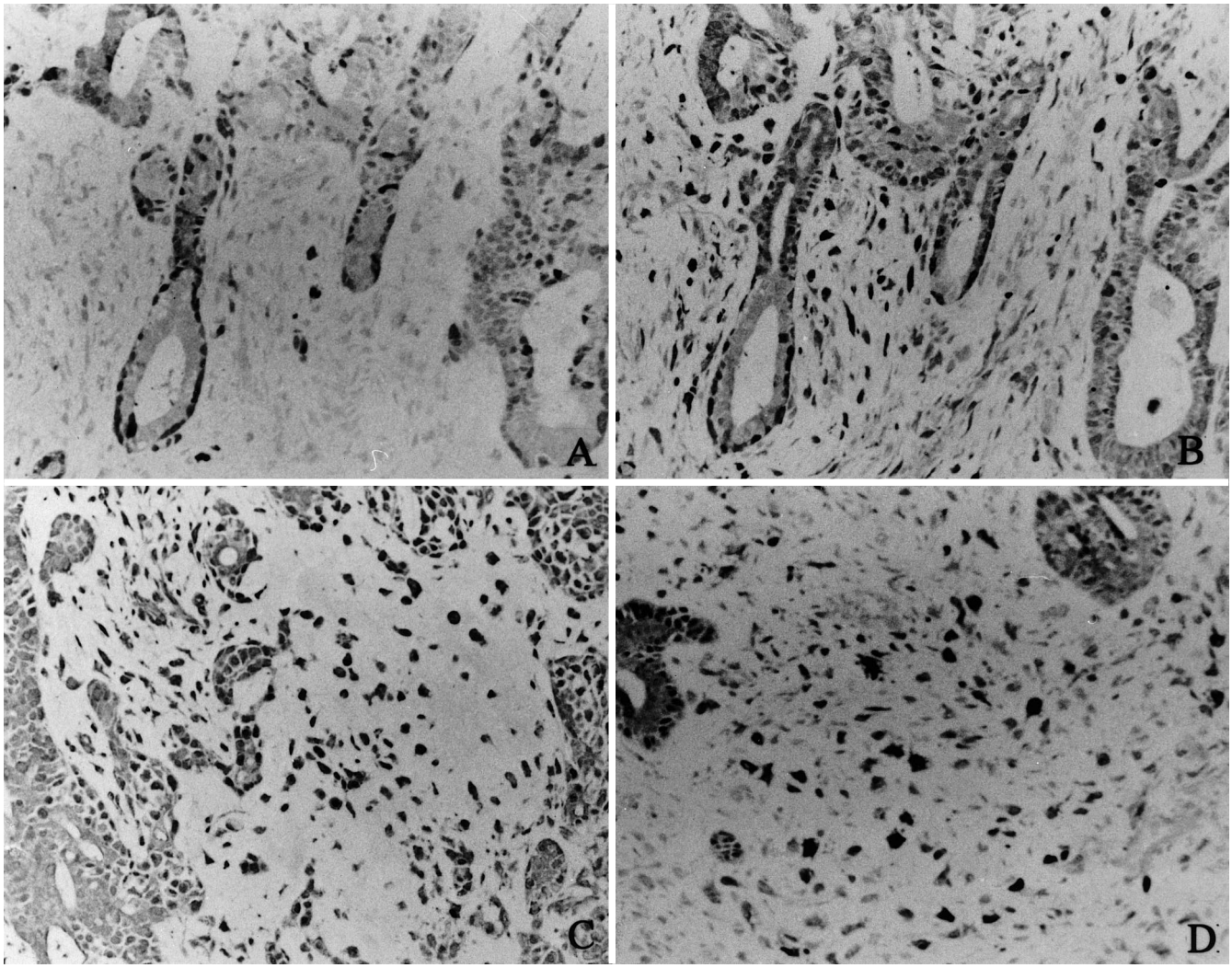

Fig. 3A–D Mixed tumour of the skin ×100. **A** S100A2 staining is confined to nonluminal cells or neoplastic myoepithelial cells forming the tubuloductal structures. **B** S100B staining of neoplastic myoepithelial cells. **C** S100A1 staining is uniformly positive in luminal and nonluminal cells of the tubuloductal structures, and strong in chondroid cells. **D** S100B immunostaining is prominent in chondroid cells

Discussion

Recently, we produced specific antibodies against novel members of the S100 protein family to give a more differentiated localization in normal cells and to characterize and classify solid tumours in greater detail [5, 6]. In the present study we examined the localization of S100A1, S100A2, S100A4, S100A6 and S100B in normal skin epithelium and skin appendage tumours and demonstrated their differential expression in tumour tissues.

In the normal skin, S100A2 was found to be specifically localized in the basal-parabasal cells of the epidermis, hair follicles, epithelial cells of the sebaceous glands and ductal epithelium of the sweat gland. S100B was confined to the terminal secretory segments of the sweat gland and dendritic cells. Tumour tissues including the skin appendage tumours were strongly reactive for S100A2, mostly in the basal cells in trichoepithelioma, syringoadenoma papilliferrum, and cylindroma. Occasional basophilic cells of calcifying epithelioma Malherbe and epithelial tumour cells of sebaceous adenoma and sebaceous carcinoma were also reactive. Mixed tumour of the skin showed a unique distribution of S100 proteins in modified or neoplastic myoepithelial cells and their variants. In contrast, S100A4 staining intensities of skin tumours of hair follicle origin and in skin tumours of eccrine sweat gland origin were rather faint. Hence, we conclude from this study that S100A2 also represents a specific marker of epithelial cells in the content of different skin tumours. Previous studies demonstrated a down-regulation of this protein in several tumour types, including breast carcinoma [7, 14]. In contrast, S100A4 was found to be associated with the metastatic behaviour of tumour cells [3]. Since we found only weak S100A4 expression, but prominent S100A2 staining, it might be assumed that most tumours investigated in this study were still well differentiated and conse-

quently had a low metastatic potential. The expression of S100A2 might therefore indicate a marker for squamous cells. S100A2 immunoreactivity was observed mostly in the cell nuclei of normal and tumorigenic epithelial cells, including the invading neoplastic cells in basal cell and squamous cell carcinoma of the skin, whereas most other S100 proteins were localized in the cytosol. This suggests a nuclear function for S100A2, in agreement with previous studies suggesting a role in suppression of tuomur cell growth [7, 14].

We found low expression of S100A6 in tumours investigated in this study. This is somewhat unexpected, since previous studies show high expression of S100A6 in many tumour tissue and tumours cell lines [6]. The absence of S100A6 in the tumours studied here supports the notion that these tumour represent well-differentiated cells. However, a study of a larger number of tumour samples but, most of all, studies on the physiological function of S100A6 in tumour cells will be required.

S100B staining was quite distinct from all other S100 proteins. Staining was most prominent in dendritic Langerhans cells of the skin tumours, abnormal Langerhans cells in Langerhans cell histiocytosis X and malignant melanoma cells, and not found in the epithelial cell types. Hence, the commercially available antisera recognizing S100B and S100A1 are not well suited to the study of this type of tumours. This is somewhat surprising, since S100B is associated with a number of neoplastic [1] and other [17] diseases. In particular, it is unregulated in melanomas and widely used as a marker for this tumour type. Nevertheless, its functional role in tumour development is still not known.

The work presented in this report demonstrates a distinct cellular and intracellular localization of S100 proteins in normal and human tumour tissue. This palette of well-defined and specific antibodies against the individual S100 proteins is now directly applicable to tumour analysis and classification.

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