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Immunohistochemical study of carbonic anhydrase in mixed tumours from major salivary glands and skin*

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Summary. Immunohistochemical distribution of carbonic anhydrase isoenzyme I and II was studied in mixed tumours of major salivary glands and skin. The normal salivary glands displayed strong carbonic anhydrase activity in both ductal epithelium and serous acinar cells and the serous demilune cells in the submandibular glands, including the eccrine ducts. Pleomorphic adenoma salivary gland origin exhibited positive staining in the innerlayer of epithelial cells of tubular, duct-like and glandular structures. No enzymatic staining was noted in the outer layer of tumour cells in these structures. Spindle tumour cells or the fibroblast-like cells with long cytoplasmic processes identified in the adjacent hyalin and myxomatous stroma were rarely positive, while chondroidal and osteo-chondroidal cells were highly reactive. Mixed tumours of eccrine gland origin showed the most reactive staining cells scattered throughout neoplastic epithelium in all tissues examined. Immunohistochemical stainability was usually higher for carbonic anhydrase II than I for both normal and tumour tissues. The biological roles of the distribution profiles of carbonic anhydrase are discussed.

Key words: Carbonic anhydrase – Pleomorphic adenoma-salivary gland – Mixed tumour – Eccrine gland

Introduction

The immunohistochemical identification of carbonic anhydrase isoenzymes I and II have been described for both salivary glands and other mammalian organs (Gay et al. 1974; Spicer et al. 1979; Kumplainen 1981; Kumplainen and Vaananen 1982; Spicer et al. 1982; Briggman et al. 1983; Hennigar et al. 1983), however their biological roles have not been clearly defined in salivary gland lesions. There are no studies on inflammatory or neoplastic conditions in salivary glands or for mixed tumours of skin origin. The

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biochemical significance of carbonic anhydrase enzymes, which are widely distributed in mammalian organs and tissues, are well established (Yoshimura et al. 1958; Carter 1972; Spicer et al. 1979; 1982). Recently studies on the histochemical properties of carbonic anhydrase in organs, including the salivary glands, have suggested a role in either membrane transport or macromolecular secretion (Spicer et al. 1979; 1982; Hennigar et al. 1983). In the salivary glands, the ductal epithelium of striated and excretory ducts including the serous acinar cells from acini and demilune structures, have demonstrated positive reactivity for carbonic anhydrase which also supports a role in the secretion of bicarbonate ions and macromolecular materials (Spicer et al. 1979; 1982; Hennigar et al. 1983).

The present immunohistochemical study describes the distribution of carbonic anhydrase isoenzymes I and II in salivary gland pleomorphic adenoma (mixed tumour of salivary glands) and mixed tumours of skin.

Materials and methods

Materials. A total 25 cases of pleomorphic adenoma of major salivary gland origin and 5 cases of mixed tumours from eccrine glands of the skin were studied. The biopsies obtained were fixed in 10% neutral buffered formalin for 12 h, embedded in paraffin, and 4 μm sections cut for the immunohistochemically localization of carbonic anhydrase I and II. Sections were also stained by haematoxylin and eosin. Normal tissues adjacent to the salivary gland adenomas were obtained for comparison of effectiveness of fixation solutions. Stainability by CA-II was compared in formalin and Carnoy's fixed paraffin sections and non fixed cryostat sections in normal submandibular gland.

Immunohistochemical methods. Deparaffinized and cryostat sections were immersed in 0.3% H_2O_2/methanol solution for 30 min at 20° C to inactivate endogenous peroxidase. The sections were then rinsed with 0.1 M phosphate buffered saline (PBS, pH 7.4) and immersed in normal rabbit serum (1:20, Wheaton, USA) for 30 min. They were immediately reacted with goat anti-human erythrocyte carbonic anhydrase (CA) I and II antiserum (1:100, Green Cross, Japan) for 1 h, rinsed with PBS, and reacted with rabbit anti-goat IgG antiserum (1:20, Cappel, USA) for 30 min. The sections were again rinsed with PBS and reacted with peroxidase-goat antiperoxidase complexes (PAP, 1:100, Cappel, USA) for 30 min. All the above immunohistochemical procedures were done at 4° C. Finally, the sections were rinsed well and reacted with 0.05 M. Tris buffer containing 0.005% DAB and 0.003% H_2O_2 for 10 min at 20° C.

The control tissue sections were subjected to the same procedures as the fixed paraffin sections except for the replacement of goat anti-human erythrocyte CA with normal goat serum and goat anti-human CA-I and II serum absorbed with purified human CA-I and II (Green cross, Japan), which produced negative results.

Results

In general, the immunohistochemical distributions of carbonic anhydrase I and II were similar for each site studied with CA-II being more intense than CA-I.

Normal salivary glands

Fixed paraffin sections. Both parotid and submandibular glands showed strong carbonic anhydrase II reactivity in ductal epithelium and in serous cells of both the acinar and demilune components with moderate or less

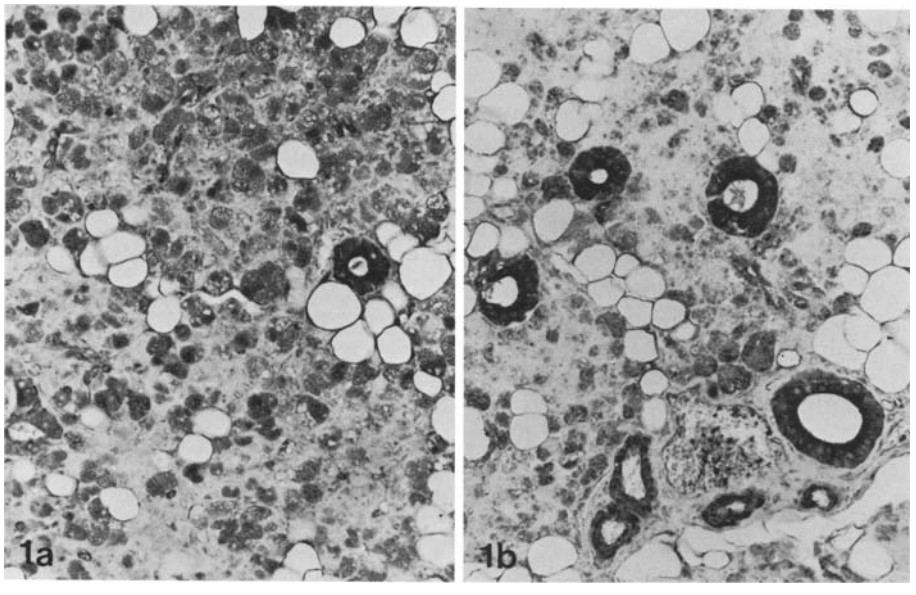

Fig. 1 a, b. Carbonic anhydrase II activity in human parotid glands (×85). **a** Note the strong CA activity in striated duct and serous acinar cells. **b** A strong reactivity for CA is noted in striated and excretory ducts with moderate staining in serous acinar cells

Table 1. CA I and II distribution in normal salivary gland and mixed tumours of salivary gland

	CA I	CA II
Normal salivary gland		
Duct cells	2–3	3
Serous cells	2	3
Mucous cells	0	0
Demilune cells	1	2–3
Mixed tumour		
Duct like cells	1–2 (inner layer)	2–3 (inner layer)
Adenoma type	1	2
Clear cell type	0–±	±–1
Solid type	0	0
Spindle shaped cells	1–2	1–3
Chondroidal and Osteochondroidal tissues	1–2	1–3

0: negative, ±: trace, 1: slight, 2: moderate, 3: strong

intense reactivity for CA-I in the same structures. It was also noted that mucous and myoepithelial cells were devoid of enzyme staining (Figs. 1a, b; Table 3).

Nonfixed cryostat sections. The submandibular glands showed strong CA-II reactivity in serous cells and demilunes of acinar compartments. In contrast

Table 2. CA I and II distribution in normal eccrine and sebaceous gland an mixed tumours of skin

	CA I	CA II
Normal eccrine gland		
Duct cells	2 (inner layer)	3 (inner layer)
Secretory coil	0–2	0–3
Sebaceous gland		
Duct cells	1–2	3
Mixed tumours		
Duct-like cells	2 (inner layer)	3 (inner layer)
Squamous-shaped cells	±–2	±–3

0: negative, ±: trace, 1: slight, 2: moderate, 3: strong

Table 3. Distribution of fixed paraffin and nonfixed frozen sections on CA-II in normal submandibular glands

	Nonfixed	*Fixed*
Serous cells	2–3	2
Mucous cells	0	0
Ducts cells	0–±	2–3

0: negative, ±: trace, 1: slight, 3: strong

with reactivity of serous cells, the striated and excretory ducts gave from negative to trace (Table 3).

Normal eccrine and sebaceous glands

The coiled, secretory eccrine sweat glands consisted of both CA positive and negative cells. The innerlayer of the eccrine sweat ducts was strongly positive, while the outerlayer cells were only slightly reactive.

Examination of the immunohistochemical features of CA in sebaceous gland components showed the outer-layered cuboidal cells of the sebaceous ducts to be strongly positive. It was also noted that the centrally located adult sebaceous cells were negative while the peripherally located basal cells surrounding the aggregated sebaceous component were moderately reactive (Table 2).

Pleomorphic adenoma of salivary glands

The most common type of salivary pleomorphic adenoma consisted of tubular, duct-like and glandular structures which were usually composed of two or multiple layered cellular arrangements. Most of the tubular and duct-like structures demonstrated a positive staining reaction to carbonic anhydrase I and II but only in the inner-layered cells (Fig. 2a). In contrast, the spindle-shaped tumour cells of the outer layer, which are probably myoepithelial in origin, were unreactive.

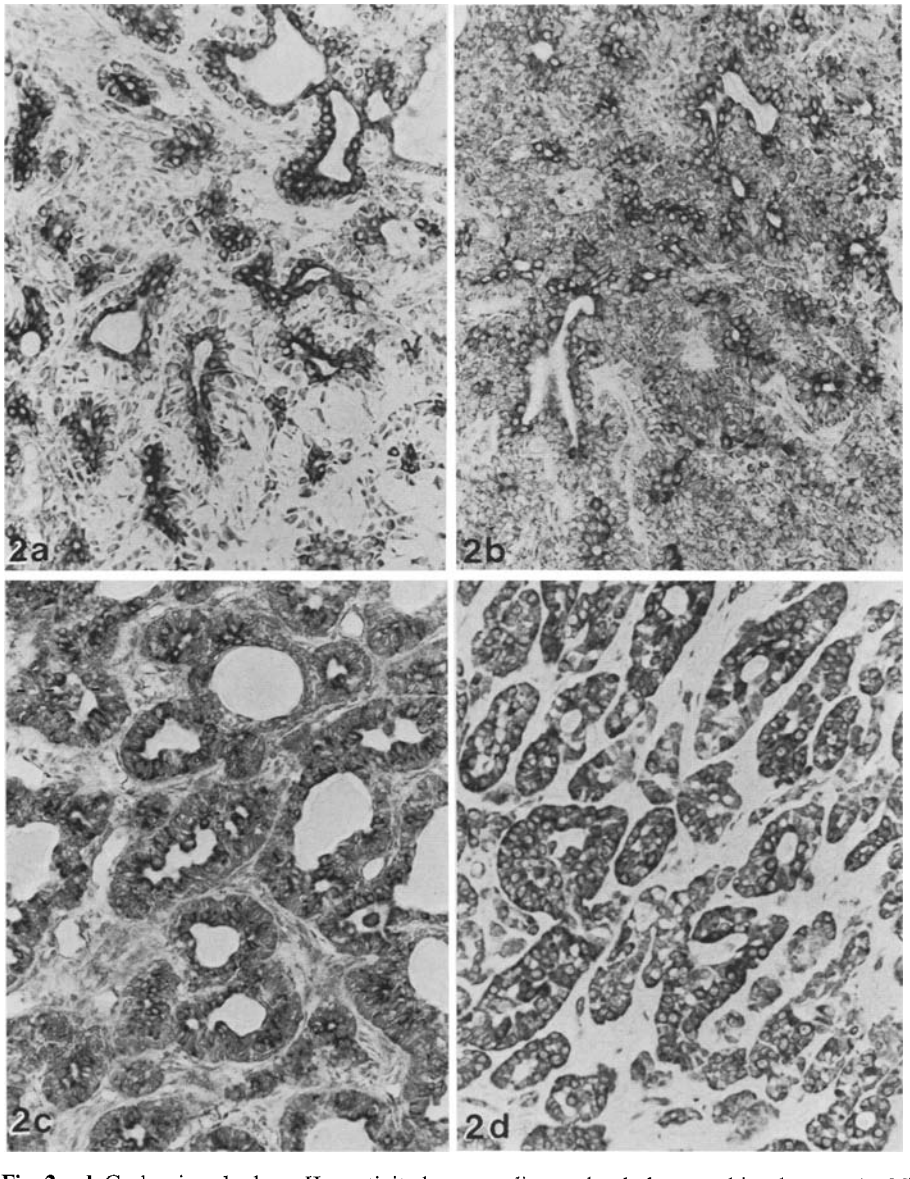

Fig. 2a–d. Carbonic anhydrase II reactivity human salivary gland pleomorphic adenoma (× 85). **a** The tubular structures consist of two epithelial cell layers, an inner layer of cells showing strong enzymatic staining and outer layer of unreactive spindle-shaped tumour cells. **b** Epithelial cells in tubular and solid tumour structures, show a positive CA-II enzymatic reactivity. **c** The inner layer of tumour cells adjacent to the lumen of glandular structures demonstrate a strong carbonic anhydrase II activity. **d** Note the positive CA-II staining in the neoplastic epithelial cells of the glandular tumours

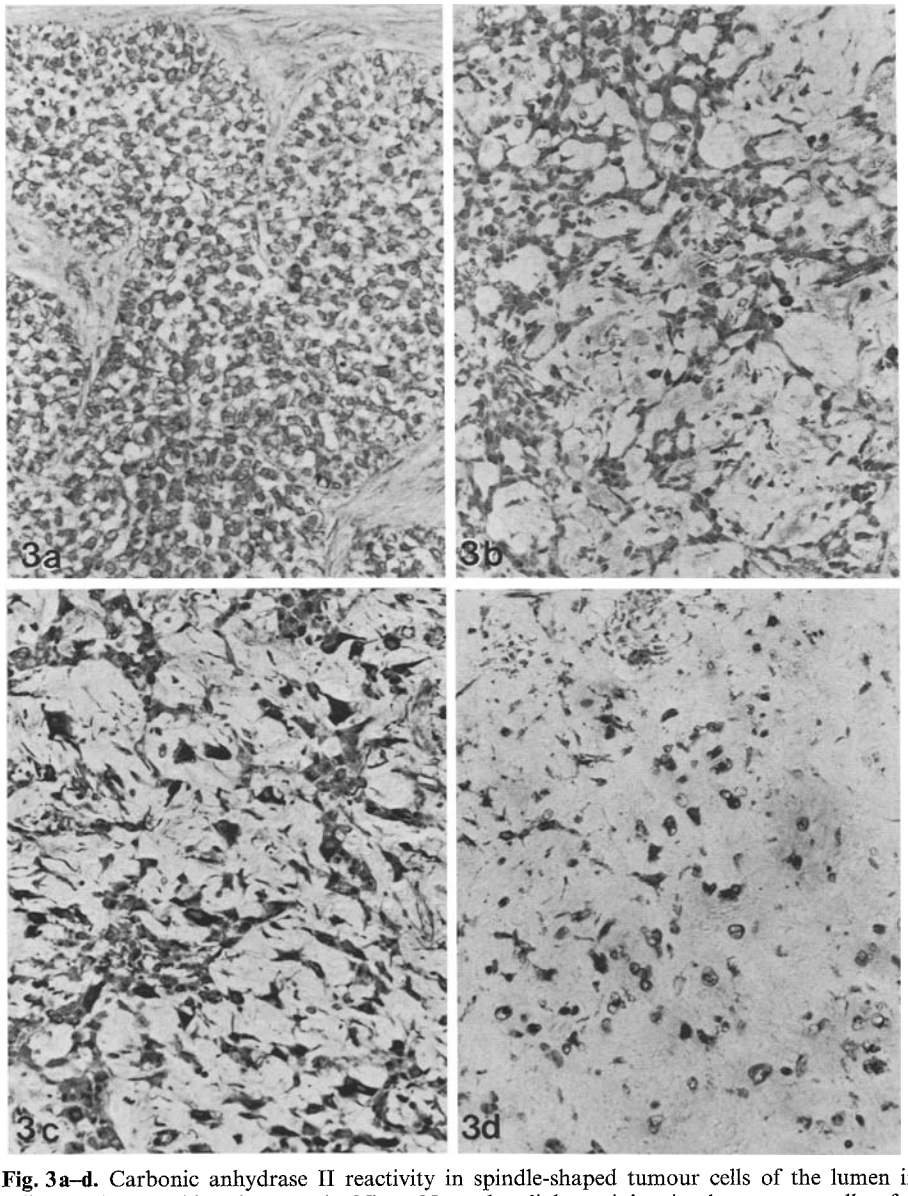

Fig. 3a–d. Carbonic anhydrase II reactivity in spindle-shaped tumour cells of the lumen in salivary pleomorphic adenoma (×85). **a** Note the slight staining in the tumour cells of a clear cell adenoma of myoepithelial cell origin. **b** Generally, the spindle tumour cells of myoepithelial origin show a weak CA-II staining. **c** The spindle-shaped tumour cells display varying degrees of CA-II reactivity ranging from positive to negative staining. **d** This photomicrograph shows the intense reactivity of the chondroidal cells located in the stroma

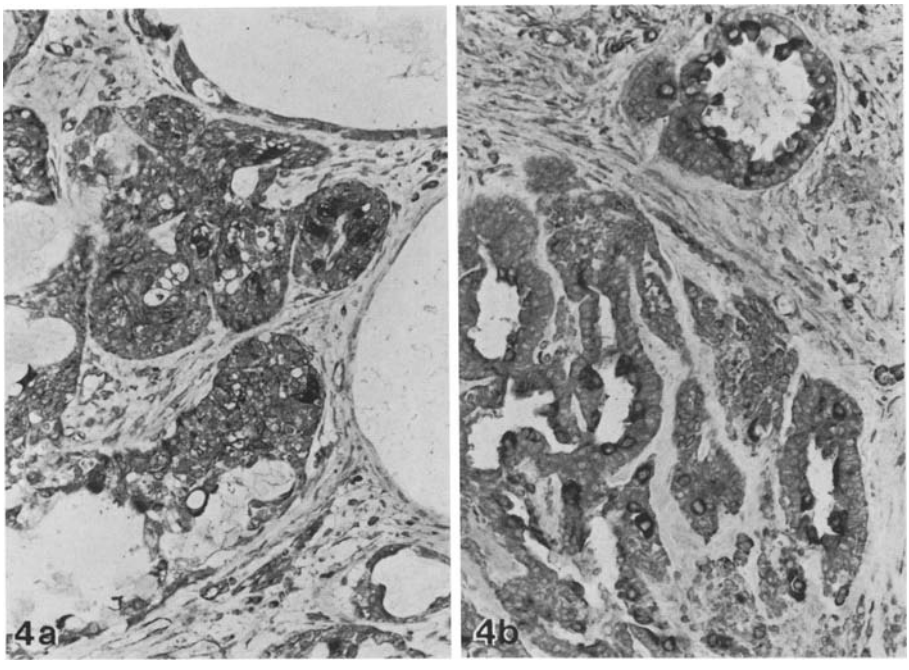

Fig. 4a–b. The carbonic anhydrase II activity in mixed tumour of skin (×85). **a** Strongly reactive cells observed within tumour foci. **b** Marked enzyme activity is distributed in the cells located adjacent to the lumen of duct-like structures

Glandular structures, composed of flattened or squamous-like cells (Fig. 2b), consisted of double or multiple cellular arrangements (Fig. 2c) which included strongly reactive cells located at the luminal surface. Foci of apocrine metaplasia also showed a strong staining reaction for carbonic anhydrase. In addition, the typical neoplastic epithelium (Fig. 2d) of the true adenoma type of tumour reacted positively for carbonic anhydrase enzymatic activity. However, the solid epithelial tumour and areas of squamous metaplasia were unreactive for CA enzymes. In the clear cell type of pleomorphic adenoma, only a slight or minimal staining reactivity was noted in all tumour cells (Fig. 3a). In general, pleomorphic adenoma was usually composed of spindle-shaped tumour cells which were located at the periphery of tubular and duct-like structures together with fibroblast-like or mesenchymal cells, with long anastomosing processes,in a hyalin or myxomatous stroma. The carbonic anhydrase staining in such spindle tumour cells, which have been referred to as modified myoepithelial cells, showed variable reactivity in which those associated with duct-like structures were minimally reactive compared with those in hyaline or myxomatous tissue which were strongly positive (Figs. 3b, c). In addition, it was also noted that the CA reactivity of these large spindled-shaped tumour cells from salivary pleomorhic adenoma, also demonstrated a wide range enzyme activity from strong to minimal (Fig. 3c).

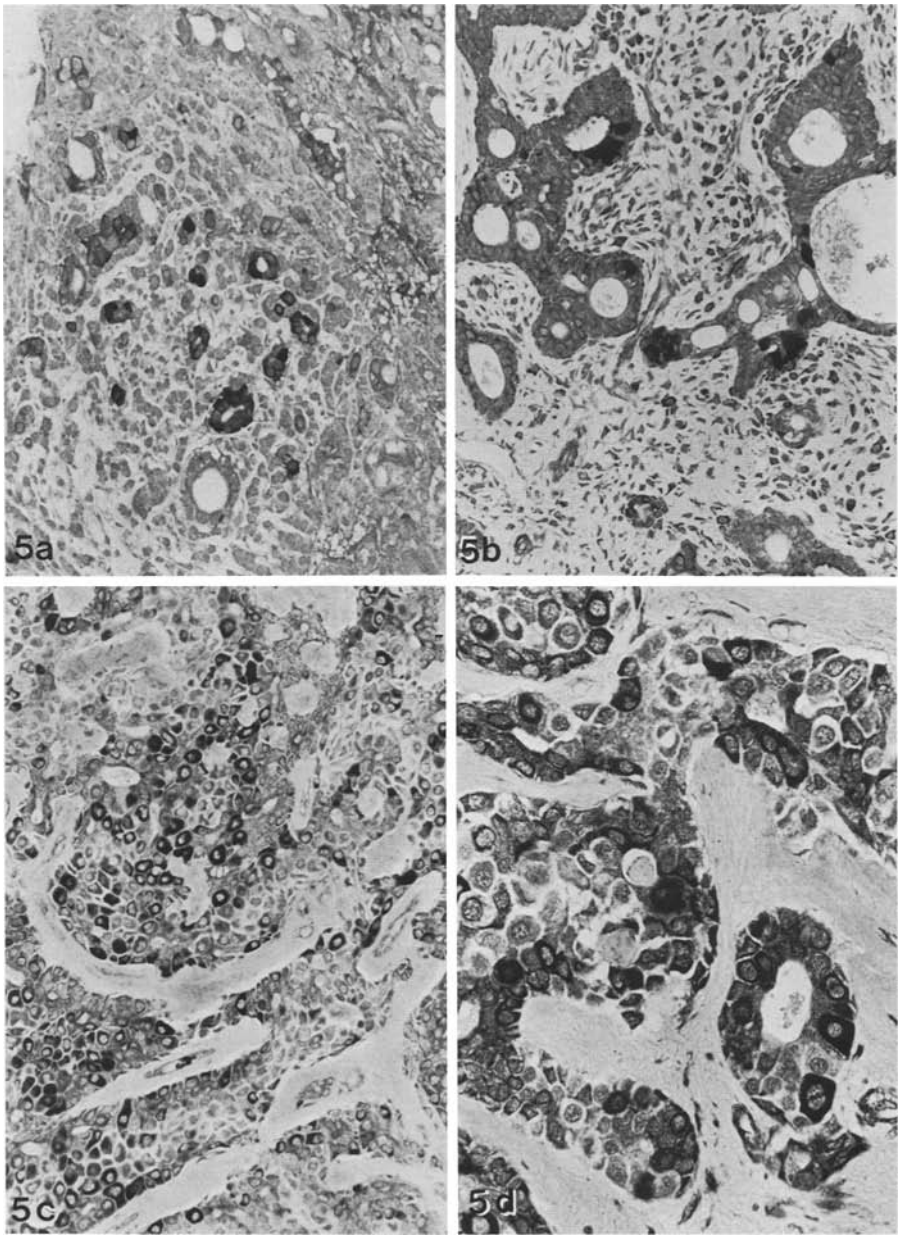

Fig. 5a–d. Carbonic anhydrase enzyme distribution in mixed tumour of skin (×85). **a** Variable carbonic anhydrase II reactivity is noted in the duct-like tumour cells. Some are strongly positive which others are weak or devoid of enzyme activity (×85). **b** Intensely reactive cells are observed scattered throughout the tumour epithelium (×85). **c** Highly reactive tumour cells are distributed throughout the solid tumours cords (×85). **d** Higher magnification of the epithelial cells in solid tumour cords similar to those described for **c**. The tubular structures present within tumour cords contain epithelial cells which show an intense staining for CA-II while others lack enzyme activity (×170)

Chondroid or osteochondroid formation was occassionally seen. Both chondroid and osteoid producing cells displayed a marked staining reactivity to carbonic anhydrase I and II (Table 1).

Mixed tumour of skin

The CA activity of the mixed tumour of eccrine sweat glands was similar to salivary gland pleomorphic adenoma. However, the carbonic anhydrase immunohistochemistry in this tumour showed the highest degree of stainable tumour cells, although several showed poor reactivity. These cells were usually distributed within the inner most layer of cells adjacent to the lumen of duct-like structures as well as within solid tumour cords (Figs. 4a, b; 5a, b). Tubular or glandular types of growth in this mixed tumour disclosed squamous-shaped cells with an abundant CA stainability localized within and scattered throughout the tumour mass (Figs. 5c, d; Table 2).

Discussion

It was not known until this study, that immunohistochemically detectable carbonic anhydrase existed in the tubular and ductal epithelium in tumour tissues. The histochemical observations noted for carbonic anhydrase reactivity in pleomorphic adenoma have not been reported to date. These findings also provided support for the role of CA in biological systems in membrane transport and/or the synthesis and secretion of bicarbonate ions and other macromolecules in both normal and neoplastic epithelium. It has recently been shown that the biological role of carbonic anhydrase in salivary glands is also related to bicarbonate secretion into saliva, and that the immunohistochemical identification of this enzyme in the striated ducts provides additional evidence for the regulation of CO_2 exchange and transport of ions (Spicer et al. 1979; 1982). In the present study, immunohistochemically detectable carbonic anhydrase reactivity occurred in the inner-layer of epithelial cells of the tubular and duct-like structures in salivary gland pleomorphic adenoma. This feature suggests that this type of epithelial cell, located adjacent to the lumen, may function in ion transport or bicarbonate secretion. No ductal or tubular components of the tumour including the solid epithelial foci, squamous metaplastic areas and clear cell adenoma, lacked carbonic anhydrase enzyme reactivity.

The outer layer of tumour cells in the same tubular and ductlike structures, which were composed of spindle shaped tumour cells of myoepithelial cell origin, exhibited a negative response to this enzyme. This finding suggests that these cells may not be involved in carbonate synthesis or ion transport. In contrast to the above, fibroblast-like cells with long processes or spindle shaped tumour cells in the stroma displayed, on occasion, a higher reactivity to carbonic anhydrase. These spindle shaped cells or modified myoepithelial cells have shown a positive staining to S-100 protein (Takahashi et al. 1984; Nakazato et al. 1985) or keratin proteins, as previously reported (Takai et al. 1985). In the stroma of pleomorphic adenoma,

the matrix substances may be synthesized from transformed myoepithelial cells. One of the biological roles of carbonic anhydrase to a relationship to calcification (Kumplainen and Vaananen 1982). In the present study, chondroid or osteochondroid cells in the tumour stroma demonstrated a strong histochemical reactivity to carbonic anhydrase including the spindle tumour cells which are all probably related to chondroid or osteoid formation as well as some components of indirect mineralization.

Strongly positive carbonic anhydrase cells have been found to be limited to epithelial cells from eccrine mixed tumour of the skin. The significance of strong enzyme reactivity of cells in this lesion is probably similar to that in pleomorphic salivary adenoma. It has been shown that clear cells in eccrine sweat glands are involved in the secretion of fluid and ions while dark cells participate in the macromolecular synthesis of precursor sweat. These findings are supported by the present immunohistochemical investigation of CA localization in sweat glands (Montaguna et al. 1953; Briggman et al. 1983). The additional finding that epithelial cells from eccrine mixed tumours have demonstrated high levels of carbonic anhydrase was not known and also supports the above.

Secretion of macromolecules from glandular acinar cells has also been described as one of the functions of carbonic anhydrase. In mouse submandibular glands, numerous well developed granular convoluted tubules, which accumulate and secrete epidermal growth and nerve growth factor, have been identified (Gresik and Barka 1977; Barka 1980; Mori et al. 1983). These granular convoluted tubule cells also contained carbonic anhydrase including the striated ductal cells, although acinar cells werde devoid of enzyme activity. These particular finding may be associated with secretion of growth factors including lectin binding, complex carbohydrates as well as higher molecular weight materials (Schulte and Spicer 1983; 1984; Naito et al. 1983). Salivary gland pleomorphic adenoma and skin mixed tumour occasionally exhibited apocrine like metaplasia in duct-like epithelial structures. In the present investigation, apocrine metaplasia was identified within the inner-layered cells showing positive carbonic anhydrase staining. The biological significance of this finding may be related to the complex phenomena of either macromolecular secretion into luminal structures or bicarbonate and/or other ion exchange.

References

Barka T (1980) Biologically active polypeptides in submandibular gland. J Histochem Cytochem 28:836–859

Briggman JV, Tashian RE, Spicer SS (1983) Immunohistochemical localization of carbonic anhydrase I and II in eccrine sweat glands from control subjects and patients with cystic fibrosis. Am J Physiol 112:250–257

Carter MJ (1972) Carbonic anhydrase; isoenzymes, properties distribution, and functional significances. Biol Rev 47:465–513

Gay CV, Faleski EJ, Schraer H, Schraer R (1974) Localization of carbonic anhydrase in avian gastric mucosa, shell gland and bone by immunohistochemistry, J Histochem Cytochem 22:819–825

Gresik EW, Barka T (1977) Immunohistochemical localization of epidermal growth factor in mouse submandibular gland. J Histochem Cytochem 25:1027–1035

Hennigar RA, Schulte BA, Spicer SS (1983) Immunolocalization of carbonic anhydrase isoenzymes in rat and mouse salivary and exorbital lacrimal glands. Anat Rec 207:605–614

Kumpulainen T (1981) Human carbonic anhydrase isoenzymes C. Effect of some fixatives on the antigenicity and improvements in the method of localization. Histochemistry 72:425–431

Kumpulainen T, Vaananen KH (1982) Immunohistochemical demonstration of extracellular carbonic anhydrase in epiphyseal growth cartilage. Calcif Tissue Int 34:428–430

Montaguna W, Chase HB, Lobitz WC (1953) Histology and cytochemistry of human skin: IV The eccrine sweat glands. J Invest Dermatol 20:415–423

Mori M, Hamada K, Naito R, Tsukitani K, Asano K (1983) Immunohistochemical localization of epidermal growth factor in rodent submandibular glands. Acta Histochem Cytochem 16:536–548

Naito R, Takai Y, Tsukitani K, Asano K Mori M (1983) Use of lectins for differential localization of secretory materials of granular convoluted tubules and ducts in the submandibular gland. Acta Histochem Cytochem 16:483–493

Nakazato Y, Ishida T, Takahashi K, Suzuki K (1985) Immunohistochemical distribution of S-100 protein and glial fibrillary acidic protein in normal and neoplastic salivary glands. Virchow Arch [Pathol Anat] 405:299–310

Schulte BA, Spicer SS (1983) Light microscopic detection of sugar residues in glycoconjugates of salivary glands and the pancreas with lectin-horseradish peroxidase conjugates. I. Mouse. Histochem J 15:1217–1238

Schulte BA, Spicer SS (1984) Light microscopic detection of sugar residues in glycoconjugates of salivary glands and the pancreas with lectin-horseradish peroxidase conjugates. II. Rat. Histochem J 16:3–20

Spicer SS, Stoward PJ, Tashian RE (1979) The immunohistolocalization of carbonic anhydrase in rodent tissues. J Histochem Cytochem 27:820–831

Spicer SS, Sens MA, Tashian RE (1982) Immunohistochemical demonstration of carbonic anhydrase in human epithelial cells. J Histochem Cytochem 30:864–873

Takahashi K, Isobe T, Ohtsuki Y, Sonobe H, Takeda I, Akagi T (1984) Immunohistochemical study on the distribution of α and β subunits of S-100 protein in lumen neoplastic and normal tissues. Virchow Arch [Cell Pathol] 45:385–396

Takai Y, Sumitomo S, Noda Y, Hikosaka N, Mori M (1985) Comparison of effectiveness of different fixatives for keratin distribution in ductal segments of salivary glands. Acta Histochem Cytochem 18:141–148

Yoshimura H, Iwasaki H, Nishikawa T, Matsumoto S (1958) Role of carbonic anhydrase in the bicarbonate excretion from salivary glands and mechanism of ionic excretion. Jpn J Physiol 9:106–123

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