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Immunohistochemical Evaluation of Transglutaminase C in Tumours of Salivary Glands

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Transglutaminase C (TGase C), a family of Ca^{2+}-dependent enzymes and an essential component in the cross-linking of peptide bonds, has been found to be a marker of epithelial differentiation with a possible role in cellular apoptosis, extracellular matrix stabilisation and Ca^{2+} binding, thereby having a potential role in tumour growth, differentiation and invasive behaviour. The expression of TGase C was evaluated in normal human salivary glands and their neoplastic lesions which included pleomorphic adenoma ($n = 30$), Warthin's tumour ($n = 5$), adenoid cystic carcinoma ($n = 10$), acinic cell carcinoma ($n = 5$), mucoepidermoid carcinoma ($n = 5$) and control tissue specimens of normal oral mucosa and squamous cell carcinoma, using polyclonal antibody, the specificity of which was determined by Western blotting, generated by immunising rabbits with purified transglutaminase. The TGase C was observed in the epithelial cells in the control tissue specimens examined. Pleiomorphic adenoma revealed reaction products in luminal tumour cells, the non-luminal or modified myoepithelial cells and their plasmacytoid variants, squamous metaplastic cells and chondroid cells. Adenoid cystic carcinomas had tumour cells in the luminal cells of tubular and cribriform structures and the acinic cell carcinoma had from low to moderate immunoreactivity in the tumour cell component and a diffuse immunoreactivity in the stroma for TGase C. Mucoepidermoid carcinoma showed no reaction products in the mucous-producing cells, while intermediate and epidermoid cells had immunoreactivity in the cell cytoplasm. As the presence of TGase C in salivary gland tumours was confined to those tumour cells which form the predominant histomorphology in each tumour subtype, it may be suggested that these enzymes may have a potential role in the regulation of cellular function in neoplastic salivary tissues affecting tumour growth, differentiation and neoplastic behaviour. Copyright © 1996 Elsevier Science Ltd

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INTRODUCTION

The complex embryogenesis of salivary tissues and most heterogeneous histomorphology of the neoplastic salivary lesions present an annoying dilemma in the study of histogenesis and morphogenesis of salivary tumours. The histogenetic concept of salivary tumours, yet to be substantiated fully, has been more often referred by the semipleuripotential bicellular reserve cell theory which postulates that the basal cells in excretory ducts are the principal reserve of stem cells in the histogenesis of the various tumour subtypes, which are numerous, with a diverse histomorphology and biological behaviour [1]. Studies to evaluate distribution of markers in normal salivary glands and their neoplastic lesions are appropriate to evaluate the normal and neoplastic lesions of salivary glands as biochemical and cytochemical changes associated with tumorigenesis are poorly understood.

Transglutaminase is a family of Ca^{2+}-dependent enzymes which catalyse the cross-linking of proteins during the formation of the *N*-glutamyl-lysyl bond between two peptide

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chains [2–5]. Several types of TGases have been described where TGase C, also known as tissue or type II TGase, has been found to catalyse the protein cross-linking and G-protein mediated activation in normal tissues [6–8] with a possible role in cellular growth in normal development and the proliferation and differentiation of neoplastic cells [9–11]. Much wider interests on TGase C have been recently recognised through its implication in apoptosis and cell adhesion [12–14], tumour growth and differentiation [15, 16] and invasive and metastatic behaviour [15, 17].

A possible involvement of TGase C in morphogenesis and cytodifferentiation during development of prenatal human fetal salivary glands [18] led us to investigate their involvement in neoplastic salivary lesions. The present study, therefore, aimed to evaluate the distribution of TGase C in neoplastic lesions of salivary glands where the results were compared with those in the normal glands and the possible implications of its distribution in the tumour tissues are discussed.

MATERIALS AND METHODS

Antibodies

Human anti-transglutaminase C rabbit antiserum was a kind gift from Dr S. I. Chung, NIDR, Maryland, U.S.A. The preparation and specificity of the antibody has been described previously [19]. Western blots of human fetal salivary glands using the antibody have shown a single band at 76 kDa [18].

Tissue specimens and immunohistochemistry

A total of 55 cases of salivary gland tumours were examined by immunohistochemical methods to detect TGase C using the three-stage streptavidin biotin immunoperoxidase method. The salivary gland tumours were pleiomorphic adenoma ($n = 30$), Warthin's tumour ($n = 5$), acinic cell carcinoma ($n = 10$), adenoid cystic carcinoma ($n = 10$) and mucoepidermoid carcinoma ($n = 5$). The tissue obtained from surgery was fixed in 10% formalin for 6–12 h and embedded in paraffin. Paraffin sections at 4 μm were used in the immunohistochemical method as described elsewhere [18]. The primary antibody was used at a dilution of 1:100 and the sections were incubated with the primary antibody overnight at 4°C. Sections of normal oral mucosa and oral squamous cell carcinoma were used as the positive control and omission of the primary antibody by non-immunised rabbit serum as the negative control.

RESULTS

The reaction product for TGase C was localised mainly in the epithelial component of normal oral mucosa ($n = 3$) and oral squamous cell carcinoma ($n = 10$), and a subjective evaluation on intensity of immunoreactivity failed to demonstrate any difference between the normal and neoplastic squamous epithelia.

Normal salivary glands

Immunostaining of TGase C in the normal gland was observed in ductal segments, the intercalated, striated and excretory duct epithelium and in numerous instances in the capillary vessels with a varying intensity of reaction (Fig. 1a, b).

Neoplastic lesions of salivary glands

Pleomorphic adenoma. The epithelial tumour component forming the duct-like and tubular structures of pleiomorphic adenoma showed intense staining for TGase C (Fig. 1c, d). The duct-like and tubular structures were usually composed of an inner layer of luminal cells which were flat or cuboidal in shape and the outer layer(s) of non-luminal cells, the neoplastic myoepithelial cells, which were usually spindle-shaped with long anastomosing processes. The neoplastic myoepithelial cells and those with squamous metaplasia consistently showed TGase C immunoreactivity (Fig. 2a). The hyaline, myxomatous and chondroid areas produced by the neoplastic myoepithelial cells also had an intense immunoreactivity (Fig. 2b). The plasmacytoid variants of the neoplastic myoepithelial cells were also intensely reactive (Fig. 2c, d).

Warthin's tumour. The eosinophilic epithelial tumour component showed an intense immunoreactivity for TGase C (Fig. 3).

Adenoid cystic carcinoma. Tumours showing tubular and cribriform or a combination of these two structures showed TGase C immunostaining in the luminal tumour cells of tubular adenoid cystic carcinoma (Fig. 4a). The non-luminal cells in the cribriform structures also had a moderate immunoreactivity (Fig. 4b).

Acinic cell carcinoma. The tumour cells of acinic cell carcinoma composed of dark basophilic cells showed a moderate but diffuse immunoreactivity for TGase C in all cells (Fig. 4c).

Mucoepidermoid carcinoma. The mucous-secreting cells were unreactive, the intermediate and epidermoid cells showed a moderate immunoreactivity for TGase C (Fig. 4d).

DISCUSSION

Transglutaminases may have an important role during organogenesis and carcinogenesis. Recent studies have shown the presence of TGase C in the fetal salivary gland epithelium during the intermediate developmental stages of the gland which is associated with active proliferation of the ductal–acinar unit and cytodifferentiation [18]. During the later stages of development, in which the salivary gland epithelium has already undergone maturation, the intensity of reaction for TGase C was found to be reduced in the ductal cells. The presence of TGases coincided with the stages when the intermediate filaments of the cytoskeleton in the fetal glands begin to appear. The stages of increased accumulation of the intermediate filaments, on the other hand, were associated with a decreased immunoreactivity of TGase C. It has thus been concluded that TGase C, in the developing fetal salivary glands, may have a role in assembly of skeletal protein or their organisation and cross-linking to maintain the stable tissue morphogenesis [18].

The potential role of TGase, in normal and neoplastic tissues, to regulate normal development and affect tumour growth, differentiation and invasive behaviour have been demonstrated in recent studies. The functional role of TGase in the formation of isopeptide bonds in protein

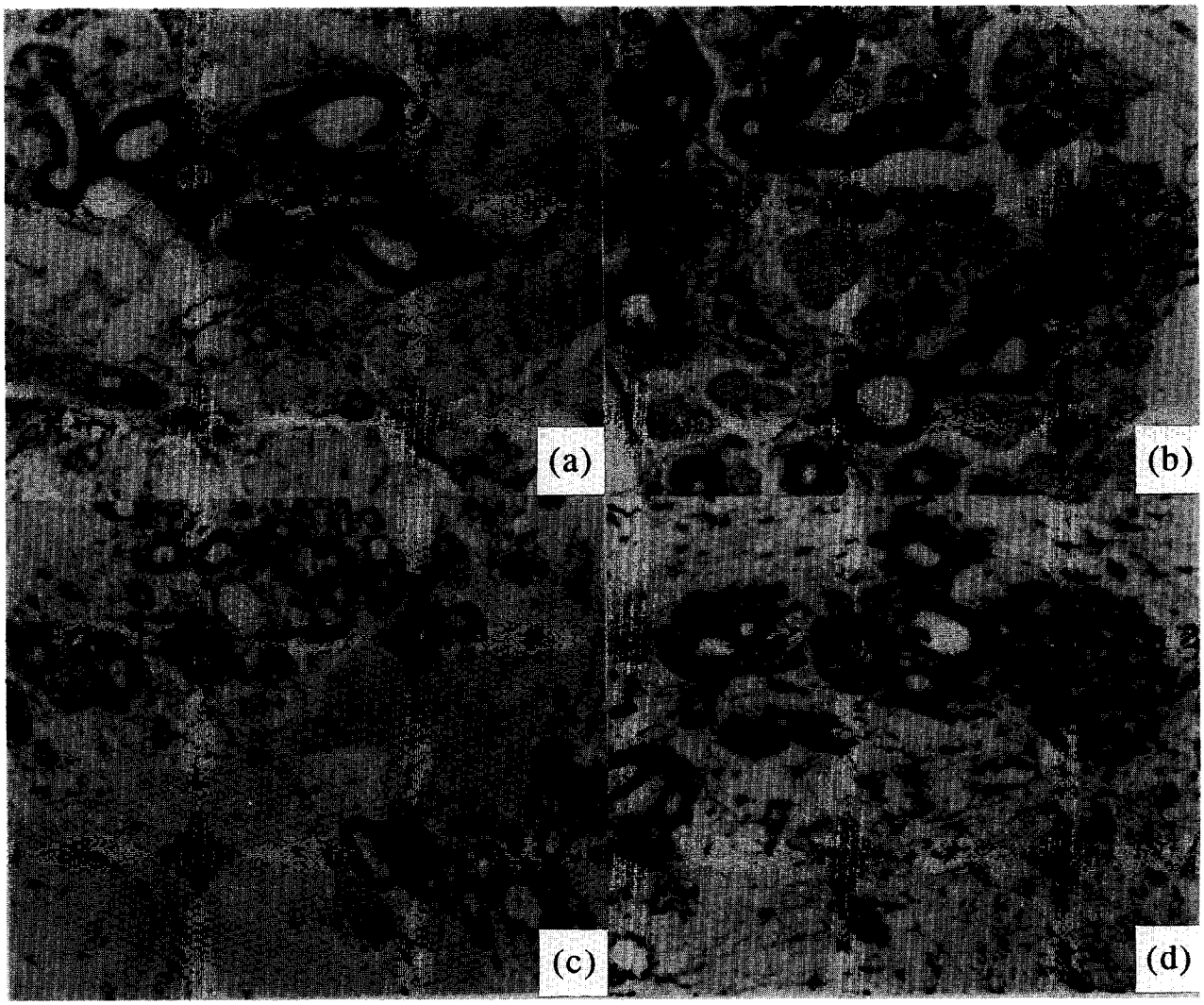

Fig. 1. (a) The TGase C immunoreactivity in normal parotid gland ×100. (b) The ductal cells show immunoreactivity for TGase C (×100). (c) and (d) TGase C immunoreactivity in tumour cells forming small duct-like structures in pleiomorphic adenoma of the parotid gland. The tumour cells show positive immunoreactivity for TGase C; however, the extracellular component shows no reaction.

cross-linking such as keratin intermediate filaments–filaggrin network [20], calcium binding [21], fibronectin-binding [22], extracellular matrix cross-linking in differentiating cartilage [23] and matrix processing [15] and apoptosis [16], metastatic properties [17] may have a significant implication in tumour growth and behaviour. The specific roles in neoplastic salivary lesions are far from clear; however, the present study demonstrates for the first time the expression of TGase II in the neoplastic salivary lesions.

The salivary gland neoplasms, such as pleomorphic adenoma, adenoid cystic carcinoma, the intermediate and epidermal cells of mucoepidermoid carcinoma, show a population of cells that are located at the luminal side of the tubuloductal structure and show a characteristic homology with the normal salivary gland ducts with respect to distribution of intermediate filament proteins [24–26]. The other group of tumour cells in those tumours, the modified myoepithelial cells or their counterparts, shows a most heterogeneous distribution of intermediate filament, often with coexpression of various intermediate filament proteins, and are the possible derivatives of ductal basal or myoepithelial cells [27]. The mucous cells of mucoepidermoid carcinoma, however, do not express keratins [28]. The squamous metaplastic cells of pleiomorphic adenoma and the intermediate or epidermoid cells of mucoepidermoid carcinoma, on the other hand, show a characteristic distribution of keratins [27]. Cytokeratins or other intermediate filament-containing cells in salivary tumours were found to show TGase C. The present study may suggest that various expressions of TGase coincide with the expression of keratins and a possible link between TGase C expression and cross-linking of intermediate cytoskeleton may affect their assembly and organisation and, in turn, the tumour cell growth and differentiation in salivary tumours.

Pleiomorphic adenomas of the salivary glands often show chondroid cells adjacent to hyaline or myxomatous tissues and the TGase C was frequently localised in those areas. The chondroid cells in pleiomorphic adenoma usually show S100 proteins, a group of calcium-binding proteins with a possible role in calcium signalling [29, 30]. The S100 proteins are abundant in the chondrocytes of normal bony epiphysis and chondroma or chondrosarcoma [31, 32]. The

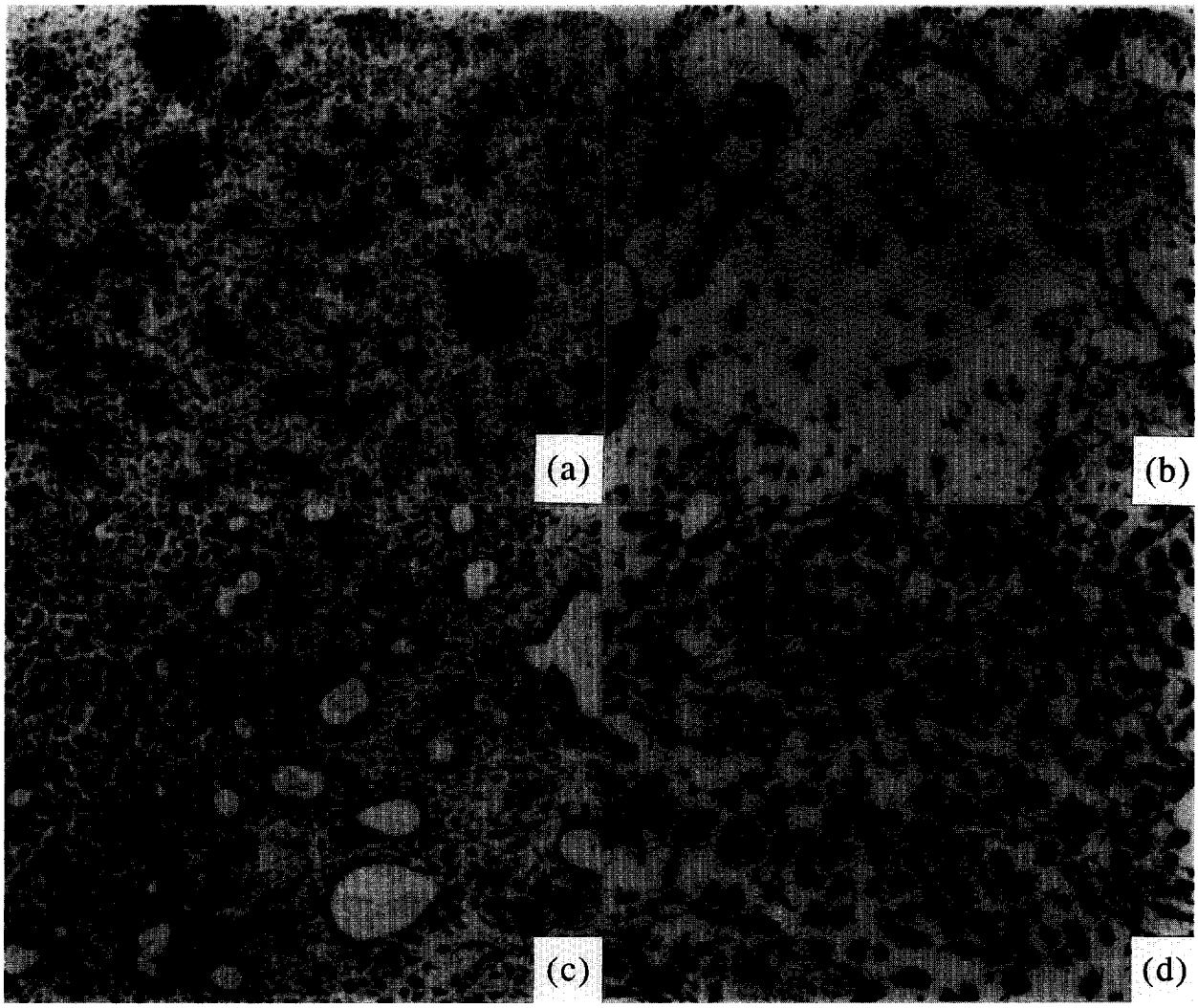

Fig. 2. (a) The TGase C in pleiomorphic adenoma ×100. Tumour cells in tubuloductal structures have luminal and non-luminal or modified myoepithelial cells with positive immunoreactivity for TGase C immunoreactivity. The squamous metaplastic cells are intensely reactive for TGase C. (b) The chondroid cells in hyalinous and chondroid areas of pleiomorphic adenoma show an intense immunoreactivity for TGase C. (c) and (d) The TGase C immunoreactivity is seen in the plasmacytoid variety of modified myoepithelial cells of pleiomorphic adenoma (c) ×100 and (d) ×200.

Fig. 3. Warthin's tumour ×100: immunoreactive TGase C is seen in tumour epithelial cells.

S100 protein, *in vitro*, has been found to control microtubule assembly and disassembly in the presence of Ca^{2+} and Zn^{2+} [33–35]. Interestingly, the highest intensity of S100 protein staining has been found in the hypertrophic chondrocytes of the zone of provisional calcification in the growth plate [36]. Since TGase C is also a calcium ion-dependent enzyme, possessing potential binding sites for Ca^{2+}, there may be interactions between TGase and S100 proteins and a possible role in the calcium signalling mechanism in the differentiation of chondroid cells in pleiomorphic adenomas.

An increased tissue transglutaminase level, significantly higher in the invasive tumour component compared with the adjacent tissues in experimental cancer of the colon in rats, has suggested a potential role for TGase C in intracellular processes during the early proliferative phase, as well as matrix processing during tumour growth and differentiation [15]. An increase in Tgase II has been found to be associated with an induction of apoptosis in the rat tracheobronchial cell line [16]. On the other hand, tissue

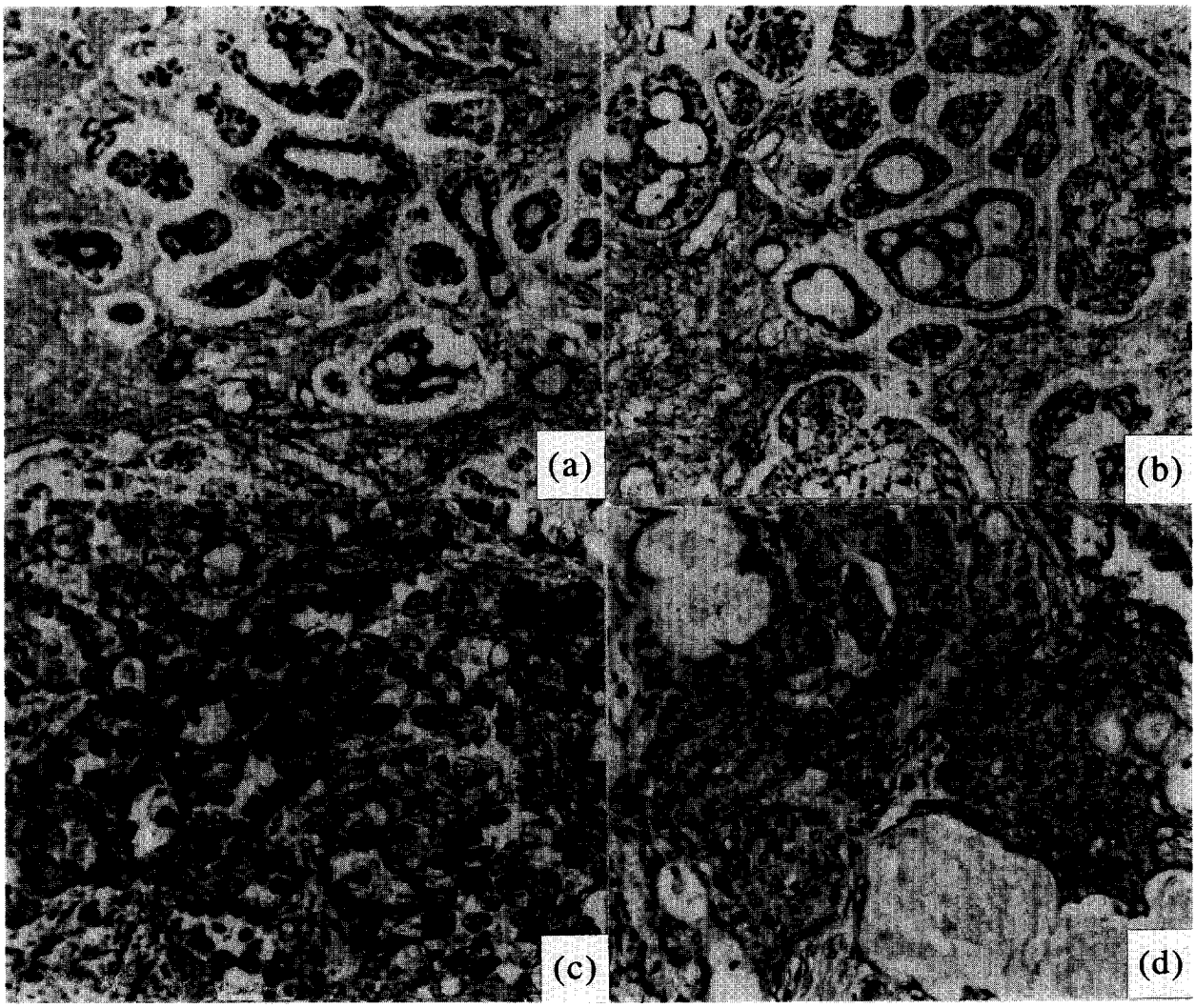

Fig. 4. The TGase C immunoreactivity in adenoid cystic carcinoma ×100. Luminal aspects of tubular structures show a marked immunostaining for TGase C (a) and cribriform foci shows positive immunoreactivity (b). (c) Acinic cell carcinoma ×100. Acinic cell carcinoma tumour cells show moderate and diffuse immunostaining for TGase C. (d) The TGase C staining in mucoepidermoid carcinoma—the tumour cells are moderately positive ×100.

transglutaminase (TGase II) RNA expression has been found to be elevated in metastatic cell lines compared with weakly metastasised human melanoma cell lines [17]. Although the multiple functional role and possible implication of TGase C, in the neoplastic lesions of salivary gland, remain to be elucidated, the observations on localisation of TGase C and its significance in positive or negative induction of growth, differentiation, apoptosis and invasive or metastatic behaviour of tumour cells, in particular, merits further study.

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