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Distribution of immunoreactive transforming growth factor-alpha in non-neoplastic human salivary glands

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Summary. The distribution of immunoreactive transforming growth factor-alpha (TGF-α) was studied in non-neoplastic human major and minor salivary glands using an immunoperoxidase assay in conjuction with an antiserum to human TGF-α. The ductal cell components of all major and minor salivary glands were found to contain significant amounts of TGF-α immunoreactivity. In contrast, acinar and myoepithelial cells consistently lacked immune reaction product in both types of glands. Occasionally, an asynchronous pattern of TGF-α ductal cell immunoreactivity was observed in specific ducts within a section. Also, intraductal secretions, when present, were found to contain TGF-α immunoreactive material. Ductal cells and connective tissue from salivary glands samples showing significant lymphocytic infiltration and loss of acinar cells exhibited higher levels of TGF-α immunoreactivity than normal salivary gland samples. These observations demonstrate, for the first time, the presence of TGF-α immunoreactivity in specific structural components of non-neoplastic human major and minor salivary glands. It will be important in future studies to determine whether alterations in TGF-α expression are detectable in diverse types of salivary gland tumors.

Key words: Transforming growth factor-alpha, Salivary glands, Immunocytochemistry

Introduction

Much evidence has accumulated pointing to the essential role of growth factors as signaling molecules in normal cell growth and differentiation. Transforming growth factor-alpha (TGF-α) is one of the better characterized mammalian growth factors which is synthesized as a 160 amino acid precursor molecule (Lee et al., 1985). This molecule can be biologically active in either the unprocessed or fully processed form (50 amino acids) (Lee, 1990). Many tumor cells have been shown to produce TGF-α; however, in recent years it has become apparent that this growth factor is also produced by many normal cell types (Derynck, 1988). Diverse cell types in the adult animal which express TGF-α include pituitary and brain cells, mammary epithelial cells, skin keratinocytes, oral mucosa, tracheal epithelial cells, macrophages and neutrophils. Expression of TGF-α has also been documented for various tissues during embryonic development (Lee, 1985; Han et al., 1987). Thus, the view has gained support that TGF-α is a molecule that plays an important role in normal physiological events as well as in the development of malignancy.

The signals that regulate proliferation and differentiation of salivary gland epithelial cells have not been clearly elucidated. TGF-α is a good candidate as a potential regulator of salivary gland physiology. To begin to address this issue, the present study examined non-neoplastic human major and minor salivary glands for the presence and distribution of immunoreactive TGF-α.

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Materials and methods

The study was carried out on archival material comprising biopsy sections of non-neoplastic human salivary glands maintained in the files of the Oral Pathology Divisions of the University of Texas at Houston, Dental Branch and the M.D. Anderson Cancer Center. Sections of the submandibular glands were made from whole glands recovered from surgical resections of oral malignancies in continuity with neck dissections. Details of the pathology relating to the one case involving the parotid were not available in the computerized pathology records. Sections of sublingual glands were made from tissue available from excisional biopsies for ranula. Minor salivary glands were obtained from excisional biopsies of labial glands for suspected cases of mucocele or other lesions (Table 1). Case

Table 1. Clinical and histological information of human salivary gland samples.

CASE No.	SEX	AGE	GLAND	CLINICAL COMMENT	HISTOLOGICAL DIAGNOSIS
1	F	71	SSG	Ca-Ton	SCC-FOM+Ton
2	M	67	SSG*	Ca-FOM-Ton	SCC-FOM+Ton
3	M	60	SSG*	Ca-FOM	SCC-FOM
4	F	48	SSG	Ca-BUM	SCC-BUM
5	M	45	SSG	Ca-VC	SCC-VC
6	M	35	SSG	Ca-Ton	SCC-Ton
7	M	47	SSG	Ca-LAL	SCC-LAL
8	?	?	PAR	?	?
9	M	09	SLG*	Lesion-FOM	Ranula
10	M	30	SLG*	Lesion-BUM	Fibroma
11	F	20	MnG	Lesion-RLL	Mucocele
12	M	14	MnG	Lesion-RLL	Mucocele
13	F	37	MnG	Lesion-FOM	Unremarkable
14	F	25	MnG	Xerostomia	Nevus
15	M	31	MnG*	Lesion-Mand	Abscess
16	F	35	MnG	Lesion-BUM	Fibroma

SSG: submandibular salivary gland; SLG: sublingual gland; PAR: parotid gland; Ca: carcinoma; MnG: minor salivary gland; Ton: tongue; FOM: floor of mouth; RMT: retromolar trigone; Mand: mandible; BUM: buccal mucosa; VC: vocal cord; RLL: right lower lip; LAL: lower gingiva; SCC: squamous cell carcinoma; ?: clinical and histologic records were not available; *: presence of considerable inflammatory infiltrates.

selection included only those tissues that were histologically free of primary or secondary tumors as confirmed in the original biopsy reports. The histopathologic diagnosis and related comments of the reporting pathologist were also noted. Five salivary gland samples were characterized by the presence of considerable inflammatory infiltrates (Table 1).

Paraffin sections (5 µm) from each case were prepared. For immunostaining, a standard avidin-biotin-peroxidase complex assay using the VectaStain ABC kit (Vector Laboratories, Burlingame, CA) was employed as previously described (Durban, 1990). Sections were dewaxed, endogenous peroxidase activity blocked with 0.2% (v/v) solution of hydrogen peroxide in methanol (30 minutes), incubated with 10% chicken serum (30 minutes) to reduce non-specific binding and incubated with primary antibody diluted 1:300 in blocking buffer. The primary antibody was a commercial goat antiserum preparation (Biotope, Wilmington, DE) made against a synthetic peptide encompassing human TGF-α residues 1-50. Diaminobenzidine applied for 10 minutes was used as substrate to visualize antigen-antibody complexes (brown precipitate). Sections were counterstained with Harris Haematoxylin. Controls included substitution of primary antibody with either phosphate buffer saline or non-immune goat serum.

Grading of the immunohistochemical reaction was carried out using the following scale: 4+ (highly positive), 3+ (positive), 2+(weakly positive), 1+ (minimally positive) and -(undetectable reaction). This grading system was used to define immunoreactivity of specific structural components such as ducts, acini, connective tissue and blood vessels.

Results

Histologic sections of major and minor non-neoplastic human salivary glands from 16 patients were immunostained with antiserum to human TGF-α. The cases were numbered 1 to 16 for ease of reference and identification. The clinical and histologic data relating to each case, including clinical impression or diagnosis necessitating gland excision, are summarized in Table 1.

Presence of immunoreactive TGF-α in major salivary glands

Observations on the distribution of immunoreactive TGF-α in major salivary glands are summarized in Table 2. Ductal components of all samples studied showed TGF-α immunoreactivity. Acinar cells consistently showed absence of immune reaction and thus, served as an additional built-in-negative control. This pattern of immunoreactivity is illustrated in Fig. 1A with a submandibular salivary gland section (structure labeled d). With the exception of three cases (6, 8 and 10), ductal cell immunoreactivity was intense and ranged between 3+ and 4+. The overall intensity of ductal cell immune reaction varied slightly among cases; for example, compare structure labeled d in Fig. 1A with similarly labeled one in Fig. 1D. However, within a given salivary gland specimen, no significant variation was apparent when the various ductal components (interlobular, striated and intercalated) were compared (Table 2). On occasion, as illustrated in Fig. 1C (arrows), an asynchronous pattern of immunoreactivity within a given duct was observed, with only a few cells showing immunoreactivity. Most of the sections did not have obvious ductal intraluminal secretions (e.g., Figs. 1D, 2B); however, when present, secretions were often TGF-α immunoreactive (Figs. 1C, 2C). Immunoreactivity of connective tissue stroma was also observed in most of the samples, but was most intense in cases in which an inflammatory cell infiltrate was present (Fig. 1B; c).

Also blood vessels, where visible, showed TGF-α immunoreactivity comparable in intensity to that observed in ductal cells (Table 2). However, the human salivary gland myoepithelial cell component was not discernible on the basis of TGF-α immunoreactivity.

Table 2. Distribution of TGF-α immunoreactivity in major salivary glands.

CASE No.*	ACINAR	EXCRETORY DUCTS	STRIATED DUCTS	INTERCALATED DUCTS	BLOOS VESSELS	CONNECTIVE TISSUE
1	-	3+	3+	2+	2+	2+
2	+	4+	4+	3+	3+	4+**
3	-	3+	3+	3+	3+	4+**
4	-	4+	4+	4+	3+	4+
5	-	3+	3+	3+	2+	2+
6	-	2+	2+	2+	2+	2+
7	-	2+	3+	3+	3+	3+
8	-	2+	2+	2+	2+	3+
9	-	4+	3+	+	+	4+**
10	-	2+	2+	NP	NP	2+**

*: reflects patient's description summarized in Table 1; **: lymphocytic infiltrate present; NP: not present on section.

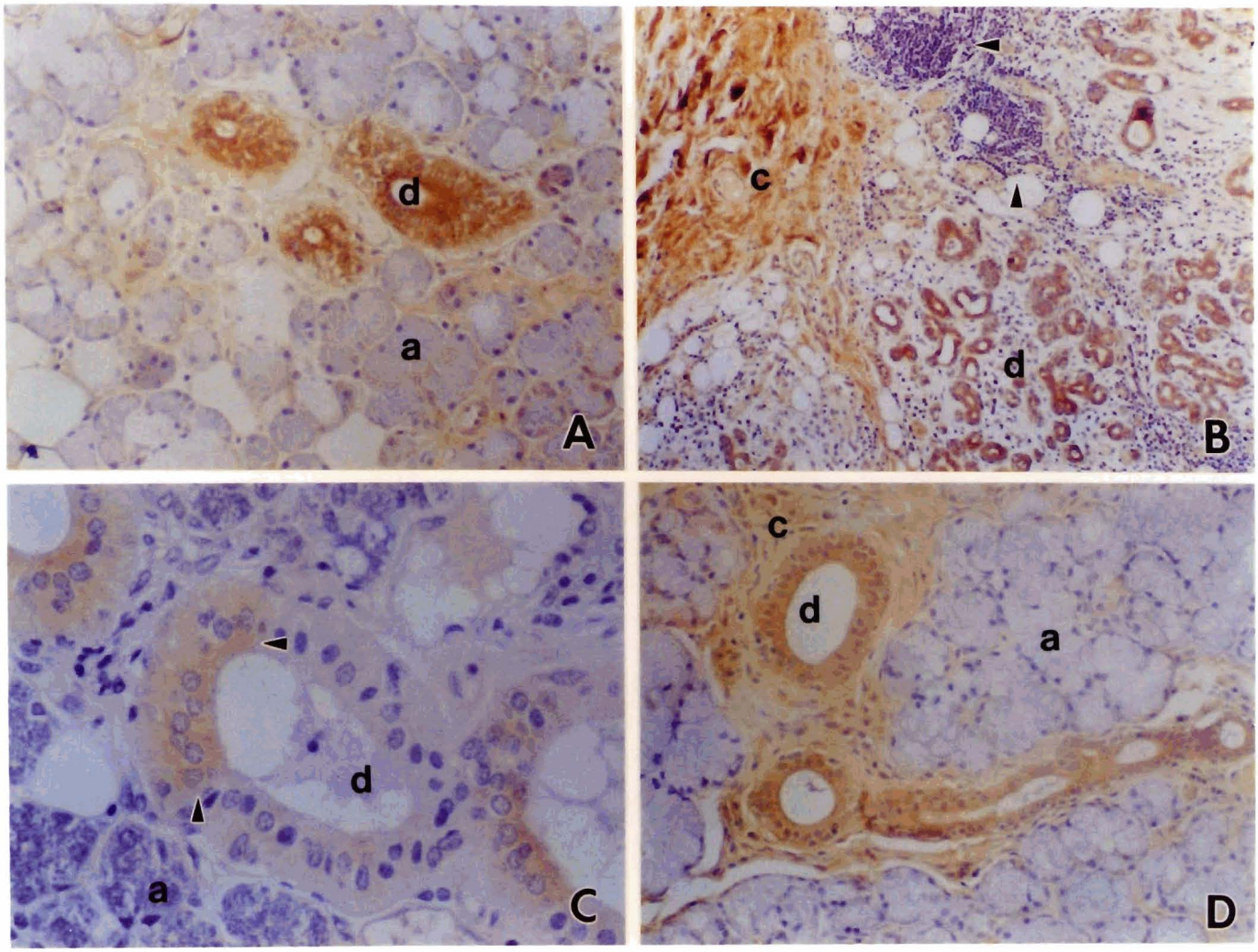

Fig. 1. Distribution of TGF-α immunoreactivity in human major salivary glands detected by immunoperoxidase staining. **A.** Human submandibular salivary gland. **B.** Human submandibular salivary gland lymphocyte-infiltrated. **C.** Human parotid salivary gland. **D.** Human sublingual salivary gland. Note asynchronous TGF-α immunoreactivity in C (delineated by arrows). d: ductal elements; a; acinar cells; c; connective tissue stroma. Magnification: A and C, x 340; B, x 85; D, x 170

TGF-α immunoreactivity in minor salivary glands

A summary of the distribution of TGF-α immunoreactivity in minor salivary glands is shown in Table 3. Like the major salivary glands, all ductal structures of the minor glands also showed TGF-α immunoreactivity. Overall, the immune reaction visualized in minor glands was less intense than in major glands (Fig. 2A-C). Acinar cells were consistently negative for TGF-α immunoreactivity. Immune staining of connective tissue and blood vessels was also apparent in most of the sections. As illustrated in Fig. 2D, controls for reaction

Table 3. Distribution of TGF-α immunoreactivity in minor salivary glands.

CASE No.	ACINAR	INTERLOBULAR DUCTS	INTRALOBULAR DUCTS	INTERCALATED DUCTS	BLOOS VESSELS	CONNECTIVE TISSUE
11	-	3+	2+	2+	+	3+
12	-	2+	2+	2+	2+	3+
13	+	NP	2+	2+	NP	3+
14	-	2+	2+	2+	2+	3+
15	-	3+	3+	2+	2+	4+
16	-	+	+	+		4+

NP: interlobular duct and blood vessels not present on section.

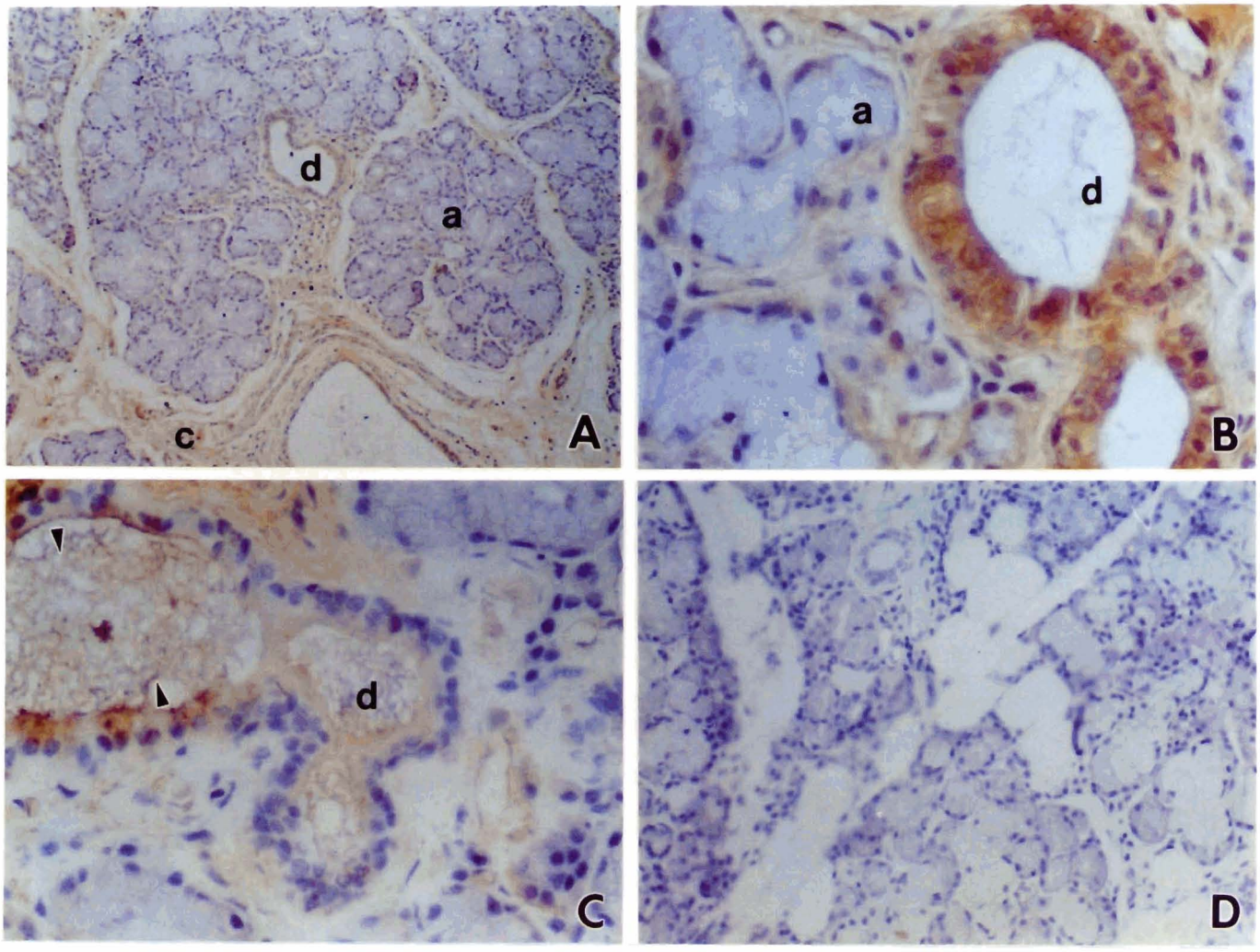

Fig. 2. TGF-α immunoreactivity in human minor salivary glands. **A.** Human minor salivary gland. **B.** Higher magnification of a human minor salivary gland TGF-α immunoreactive duct (d). **C.** TGF-α immunoreactive intralobular secretions in human minor salivary gland section. **D.** Control for reaction specificity where primary antibody was omitted from the assay. a: acinar cells; c: connective tissue stroma. Magnification: A, x 75; B and C, x 295; D, x 145

specificity failed to reveal background immunoreactivity.

Pattern of TGF-α immunoreactivity in lymphocyte-infiltrated salivary glands

Four major salivary glands (cases 2, 3, 9 and 10) and one minor salivary gland (case 15) showed significant chronic inflammatory cell infiltrate. TGF-α immunoreactivity of ductal cell components in these samples, particularly those appearing in focal areas such as that illustrated in Fig. 1B, appeared to be of stronger intensity than in samples free of inflammatory infiltrates. Likewise, connective tissue stroma showed an intense immune reaction in these samples (Fig. 1B; c). Acinar cells were largely unapparent in these samples but the few identifiable ones were negative for TGF-α.

Discussion

The presence and distribution of immunoreactive TGF-α in human major and minor salivary glands has not been examined previously. Using a radioimmunoassay, Yeh et al. (1989) detected TGF-α in human saliva, a finding which was recently confirmed and extended by Humphreys-Beher et al. (1994) while our own studies were in progress (personal communication). However, the potential cellular sources which contribute to the presence of TGF-α in human saliva had not been determined and thus, the present study sought to establish whether detectable levels of immunoreactive TGF-α are associated with specific human salivary gland cells. The observations reported here establish the ductal cell phenotype of major and minor human salivary glands as a source of immunoreactive TGF-α. The presence of immunoreactive TGF-α within ductal luminal secretions also supports the view that the salivary glands are a source for salivary TGF-α. TGF-α immunoreactivity was observed to be weaker in ductal cells of minor salivary glands and this may be due to active TGF-α secretion by these glands rather than to reduced expression. Given the extensive number of human minor salivary glands, their contribution to the total saliva TGF-α pool could be significant.

Our observations with human major and minor salivary glands are in agreement with recent observations with the major salivary glands of rodents where immunoreactive TGF-α was also found to be present throughout the entire duct system (Wu et al., 1993; Humphreys-Beher et al., 1994; Lindsay and Durban, 1994). However, unlike the observations with rodent salivary glands, we did notice an occasional asynchronous pattern of TGF-α ductal cell immunoreactivity in specific ducts within a section (e.g., Fig. 1c). Using approaches and reagents similar to those utilized in our study, myoepithelial cells of the human mammary gland (both normal and benign breast disease) and the mature resting, pregnant and lactating rat mammary gland were found to be strongly TGF-α immunoreactive (McAndrew et al., 1994). In contrast, we found no clear evidence that the salivary gland myoepithelial cell component is TGF-α immunoreactive. While it is difficult to predict with certainty the potential significance of tissue-specific differences in myoepithelial cell TGF-α immunoreactivity, such finding may reflect either distinct functional roles or physiological/proliferative states of myoepithelial cells in different organs.

In recent years, evidence has accumulated indicating that extracellular matrix components can sequester and concentrate a number of growth factors (Fava and Mcclure, 1987; Masumoto and Yamamoto, 1991; Yayon et al., 1991; Vukicevic et al., 1992). Interaction of growth factors with the extracellular matrix could serve to regulate their stability, diffusion and possibly their biological activity. This provides one mechanism whereby extracellular matrix-growth factor interactions may modulate cell growth and differentiation. In this context, our observation that immunoreactive TGF-α is consistently associated with salivary gland connective tissue matrix, and in greater amounts with the matrix of lymphocyte-infiltrated glands (eg., Fig. 1B), could be important. Future studies will attempt to determine whether this reflects sequestration of TGF-α by the matrix or rather actual growth factor synthesis and secretion by connective tissue cells.

Also potentially important was the finding that TGF-α immunoreactivity is increased in ductal cells of salivary glands infiltrated with lymphocytes. This observation at the one cell level is in agreement with and complements recent radioimmunoassay measurements by Humphreys-Beher et al. (1994) which showed increased concentration of TGF-α in saliva from xerostomic and Paget's disease patients. Thus, it will be important to determine whether the observed increased level of ductal and connective tissue matrix immunoreactive TGF-α in salivary glands which are undergoing an inflammatory response is related to progression of the disease.

While the presence of TGF-α immunoreactivity does not establish a direct role for this growth factor in regulating normal human salivary gland cell physiology, TGF-α has been shown to play a role in the growth of some normal and tumor cells through autocrine and paracrine mechanisms (Sporn and Todaro, 1980; Sporn and Roberts, 1985; Coffey et al., 1987; Mead and Fausto, 1989; Luetteke et al., 1993). TGF-α is coupled to intracellular pathways of signal transduction through binding to the epidermal growth factor receptor (reviewed in Carpenter, 1987). Epithelial cells from human major salivary glands and their tumors have been shown to express epidermal growth factor receptors Yamada et al., 1989). Thus, in theory, normal and neoplastic salivary epithelial cells could potentially be stimulated in an autocrine fashion by TGF-α. The TGF-α immunoprofile reported here provides a framework for the design of additional studies to clarify this important issue.

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