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Expression of transforming growth factor alpha in human tissues: immunohistochemical study and Northern blot analysis

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Summary. The expression of transforming growth factor alpha (TGF-α) was examined in various human tissues and the fetus, using immunohistochemistry and Northern blot analysis. TGF-α immunoreactivity was detected mainly in the epithelial cells of the digestive tract, liver, pancreas, kidney, thyroid, adrenal, skin, mammary gland and genital organs. In the digestive tract, epithelial cells with regenerative change or hyperplastic change showed strong immunoreactivity to TGF-α. Peripheral nerve, vessels, megakaryocytes and macrophages in the lung and spleen were also positive for TGF-α. By Northern blot analysis the expression of TGF-α mRNA was confirmed in the digestive tract, salivary gland, thyroid, kidney and mammary gland. In the human fetus, the nerve tissues, liver, adrenal and kidney were positive for TGF-α. Strong immunoreactivity to TGF-α was observed in the hepatocytes of the fetus. These findings indicate that TGF-α is produced by a variety of non-neoplastic cells in both adult and fetal tissues.

Key words: Transforming growth factor-α – Human tissues – Immunohistochemistry – Northern blotting

Introduction

Transforming growth factor alpha (TGF-α) is a mitogen originally found in the culture supernatants of transformed fibroblasts (Delarco and Todaro 1978; Roberts et al. 1980). Human TGF-α shares 40% homology with human epidermal growth factor (EGF) and binds to the same cell-surface receptor as EGF (Massague 1983; Derynck et al. 1984; Marquardt et al. 1984). EGF receptor has tyrosine-specific protein kinase activity and shows autophosphorylation as well as phosphorylation of target molecules in response to EGF or TGF-α (Hunter 1984; Ullrich et al. 1984). TGF-α is produced by a number of transformed cells, and increased levels of this peptide have been detected in a variety of tumour tissues, including oesophageal, gastric, renal and mammary carcinomas, as well as melanomas, when compared with corresponding non-neoplastic counterparts (Todaro et al. 1983; Tahara 1990; Yoshida et al. 1990a). We have also reported that TGF-α produced by tumour cells acts as an autocrine growth factor for gastric carcinomas (Yoshida et al. 1990b, c). Overexpression of TGF-α in transgenic mice induces epithelial hyperplasia, pancreatic metaplasia and carcinoma of the breast (Sandgren et al. 1990) and overexpression of TGF-α in keratinocytes is responsible for the initiation or maintenance of psoriasis (Elder et al. 1989). Although many of its properties in pathological conditions are understood, the site of production of TGF-α in normal tissues has not been elucidated. A number of studies have shown that TGF-α is found in normal tissues or cells such as gastrointestinal mucosa, anterior pituitary glands, keratinocytes and alveolar macrophages (Coffey et al. 1987; Kobrin et al. 1987; Madtes et al. 1988; Cartlidge and Elder 1989; Kudlow et al. 1989). TGF-α mRNA is expressed in rat embryo (Lee et al. 1985). No detailed immunohistochemical observations, however, have been reported for the localization and distribution of TGF-α in normal human tissues of adults and fetuses.

In this paper, we examined the localization of TGF-α in several human tissues by immunohistochemistry. Expression of TGF-α mRNA was investigated by Northern blot analysis.

Materials and methods

Human adult tissues from 35 different sites were obtained during surgical operations or autopsies (performed within 1.5 h after death) at Hiroshima University Hospital. Human fetuses (7–17 weeks of gestation) were obtained by legal elective abortions. They were fixed in 10% neutral formalin and embedded in paraffin. For Northern blot analysis, tissue samples obtained by surgery were frozen in liquid nitrogen immediately after removal and stored at −80° C. All tissues were confirmed histologically as showing no pathological changes. Clinical data showed no endocrine disorder in any of the patients from whom the samples were taken.

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For immunohistochemistry, a modification of the immunoglobulin enzyme bridge technique (ABC method) was used as described previously (Yasui et al. 1988a). Deparaffinized tissue sections were immersed in methanol containing 0.03% hydrogen peroxide for 30 min to block the endogenous peroxidase activity and incubated with 0.05% saponin (Wako Pure Chemical Industries, Osaka, Japan) for 30 min. The sections were then incubated with normal horse serum (diluted 1:20) for 30 min to block the non-specific antibody binding sites. The sections were treated consecutively at room temperature with: anti-TGF-α monoclonal antibody (0.125 μg/ml) for 2 h; biotinylated anti-mouse IgG horse serum (diluted 1:100, Vector, Burlingame, Calif.) for 45 min; and avidin DH-biotinylated horseradish peroxidase complex (Vectastain ABC kit, Vector) for 45 min. Anti-TGF-α antibody (Clone 213-4.4) was obtained from Oncogene Science (Manhasset, N.Y.). This antibody is a mouse monoclonal IgG_{2a} that reacts with denatured and native TGF-α of human and rat origin (Sorvillo et al. 1990). Peroxidase staining was performed for 10–15 min using a solution of 3,3′-diaminobenzidine-tetrahydrochloride in 50 mM Tris-HCl (pH 7.5) containing 0.001% hydrogen peroxide. The sections were counterstained with 3% methyl green. The specificity of the reaction was determined as follows: anti-TGF-α antibody was absorbed at 4° C overnight or room temperature for 2 h with excess TGF-α (Wakunaga Pharmaceutical, Hiroshima, Japan); a negative control monoclonal antibody (TrpE; Oncogene Science) was used at the same concentration as in the primary reaction.

For Northern blot analysis, RNAs were extracted by the standard guanidium isothiocyanate/cesium chloride method (Maniatis et al. 1989). Ten micrograms of poly(A)$^+$ selected RNA was electrophoresed on 1% agarose/formaldehyde gel and blotted onto Zeta-probe nylon filter membrane (Bio-Rad, Richmond, Calif.). Filters were baked for 2 h at 80° C under vacuum. After prehybridization, hybridization was performed at 42° C for 12–15 h using ^{32}P-labelled human TGF-α cDNA probe (Yoshida et al. 1990b). Hybridization solution contained 0.1 M PIPES-NaOH (pH 6.8), 0.65 M NaCl, 5× Denhardt's solution (1× Denhardt's solution contains 0.02% w/v each of bovine serum albumin, Ficoll and polyvinyl pyrolidine), 0.1% sodium dodecyl sulphate (SDS), 50% deionized formamide, 10% dextran sulphate and 100 μg/ml salmon sperm DNA. Filters were washed twice with 0.1 × SSC-0.1% SDS (1 × SSC consists of 0.15 M NaCl and 15 mM sodium citrate) for 30 min at room temperature, followed by two washes in 0.1 × SSC-0.1% SDS for 60 min at 65° C and a rinse in 0.1 × SSC. The filters were exposed to X-ray film. 1.4 kb Human TGF-α cDNA was kindly provided by Dr. R. Derynck (1984). β-Actin probe was purchased from Oncor (Gaithersburg, Md.).

Immunoblot analysis was performed as described previously (Yasui and Ryoji 1989). Recombinant human TGF-α or EGF (100 ng; Wakunaga Pharmaceutical) was applied to 17.5% SDS-polyacrylamide gel electrophoresis, followed by electrotransference onto nitrocellulose filter. To visualize the immune complex, the ECL Western blotting detection system (Amersham) was used.

Results

First, we tried to confirm the specificity of the antibody for TGF-α using human gastric carcinoma sections – many gastric carcinomas show overexpression of TGF-α mRNA and protein. As a positive control for immunostaining, we then selected a representative case of gastric carcinoma, which expressed mRNA for TGF-α at high level [case 198 in Yoshida et al. (1990b)]. Strong cytoplasmic staining was observed in most tumour cells with the anti-TGF-α antibody (Fig. 1a). Cell staining was completely abolished when the antibody was preincubated with excess recombinant TGF-α (Fig. 1c), whereas the staining was not affected by preincubating with excess EGF (Fig. 1b). Reactivity to TGF-α was also confirmed by immunoblot analysis. The antibody specifically reacted with 6 KDa TGF-α, but not with EGF (Fig. 2).

The results of immunostaining of TGF-α in adult human tissues are summarized in Table 1. In the circulatory and respiratory systems, endothelial cells and smooth muscle cells of the vessels, bronchial glandular cells and alveolar macrophages were positive for TGF-α. Squamous metaplasia of the bronchial epithelium also

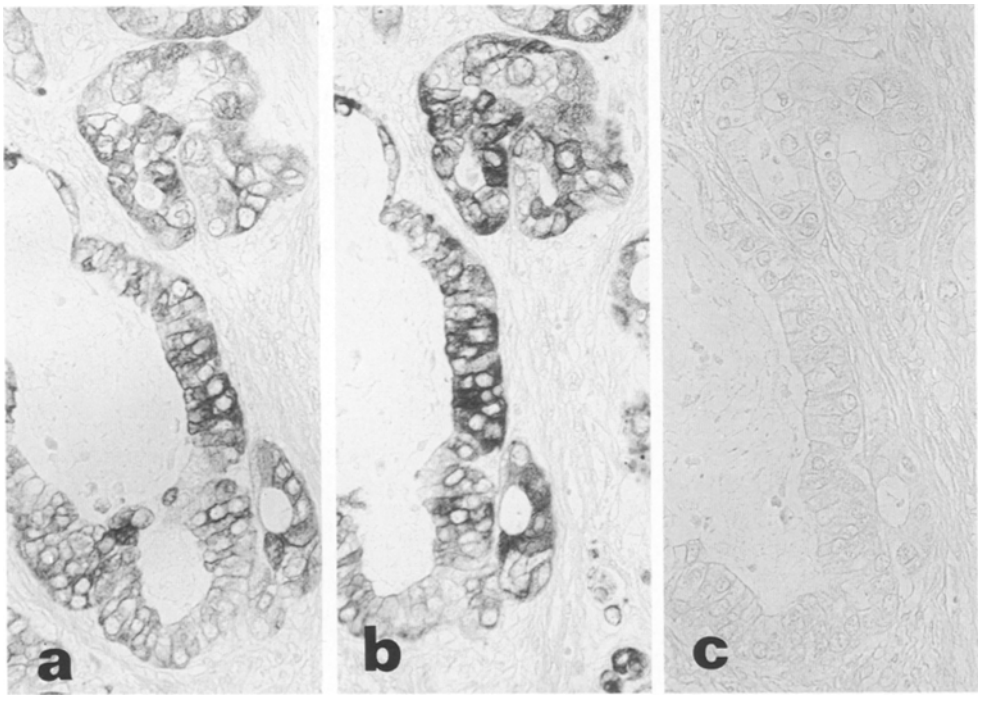

Fig. 1a–c. Immunohistochemical staining of transforming growth factor alpha (TGF-α) in a gastric carcinoma. **a** Strong TGF-α immunoreactivity was detected in most of the tumor cells by anti-TGF-α antibody, ×240. **b** The staining was not affected by preincubating the antibody with excess EGF, ×240. **c** The immunoreactivity was completely abolished when the antibody was preincubated with excess TGF-α, ×240

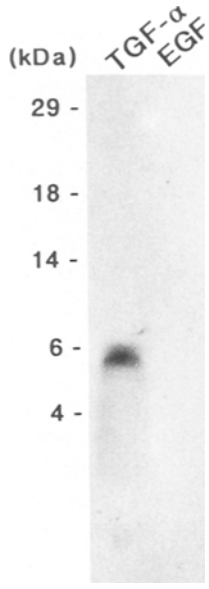

Fig. 2. Immunoblot analysis of TGF-α and EGF using anti-TGF-α antibody. TGF-α or EGF (100 ng) was subjected to 17.5% SDS-polyacrylamide gel electrophoresis, followed by immunoblotting as described in "Materials and methods"

showed TGF-α immunoreactivity. In the digestive organs, surface epithelial cells of the digestive tract were positive for TGF-α. When associated with regenerative change or hyperplastic change, the immunoreactivity was stronger (Fig. 3b). The epithelial cells of the neonatal stomach had very strong immunoreactivity to TGF-α (Fig. 3a). Most of ductal cells of the liver and pancreas showed weak-to-moderate positivity for TGF-α (Fig. 3d, e). Weak immunoreactivity to TGF-α was found in ductal cells of the salivary gland (Fig. 3c). In the urinary organs, epithelial cells of the renal tubules in both cortex and medulla displayed strong positivity for TGF-α (Fig. 3f). In the endocrine organs, weak immunoreactivity for TGF-α was detected in some follicle cells of the thyroid gland and medulla cells of the adrenal gland. In the reproductive organs, oocytes in the ovary showed strong positivity for TGF-α (Fig. 3h). Quite strong immunoreactivity to TGF-α was seen in glandular and ductal cells in a part of the mammary gland that showed no histological alterations (Fig. 3g). Certain cells of the bone marrow, spleen and skin were positive for TGF-α. Schwann cells and ganglion cells in peripheral nerve had weak immunoreactivity to TGF-α.

The expression of TGF-α mRNA in some tissues available was examined by Northern blotting. As shown in Fig. 4, all the tissues examined, including the salivary gland, oesophagus, gastrointestinal tract, thyroid gland, kidney and mammary gland, expressed TGF-α mRNA. The level of TGF-α mRNA expression in the gastrointestinal tract varied in case to case, which might depend on the degree of regenerative change or hyperplastic change in the epithelium.

Immunohistochemical localizations of TGF-α in human fetal tissues are summarized in Table 2. In ecto-

Table 1. Immunohistochemical localization of TGF-α in adult human tissues

Tissue	TGF-α immunoreactivity
1. Cardiovascular system	
– Heart	ND[a]
– Artery	Endothelial cells
	Smooth muscle cells
2. Respiratory system	
– Trachea	Bronchial glandular cells
– Lung	Alveolar macrophage
	Squamous metaplasia
3. Digestive organ	
– Salivary gland	Ductal cells
– Oesophagus	Squamous epithelium
– Stomach	Foveolar epithelial cells (with regenerative or hyperplastic change)
	Intestinal metaplasia
– Duodenum	Surface epithelial cells
– Small intestine	Surface epithelial cells
– Large intestine	Surface epithelial cells (with regenerative change)
– Appendix	Surface epithelial cells
– Liver	Epithelial cells of bile duct
– Gallbladder	Epithelial cells with regenerative or hyperplastic change
– Pancreas	Ductal cells
	Centroacinar cells
4. Urinary organ	
– Kidney	Epithelial cells of renal tubules
– Ureter	ND
5. Endocrine organ	
– Thyroid gland	Follicular epithelial cells
– Parathyroid gland	ND
– Adrenal gland	Medulla cells (weakly)
6. Reproductive organ	
– Testis	Seminiferous epithelial cells
– Prostate	Glandular cells (weakly)
– Ovary	Oocytes
– Salpinx	ND
– Uterus	Epithelial cells of endometrial gland (proliferative phase, weakly)
– Mammary gland	Glandular and ductal cells
7. Haematopoietic system	
– Bone marrow	Megakaryocytes
– Lymph node	ND
– Spleen	Macrophage
– Thymus (involuted)	ND
8. Central nervous system	
– Cerebrum	ND
– Cerebellum	ND
– Spinal cord	ND
9. Other	
– Skin	Epidermal cells
	Sweat gland cells
– Muscle	Smooth muscle cells
– Peripheral nerve	Schwann cells
	Ganglion cells

[a] Not detected

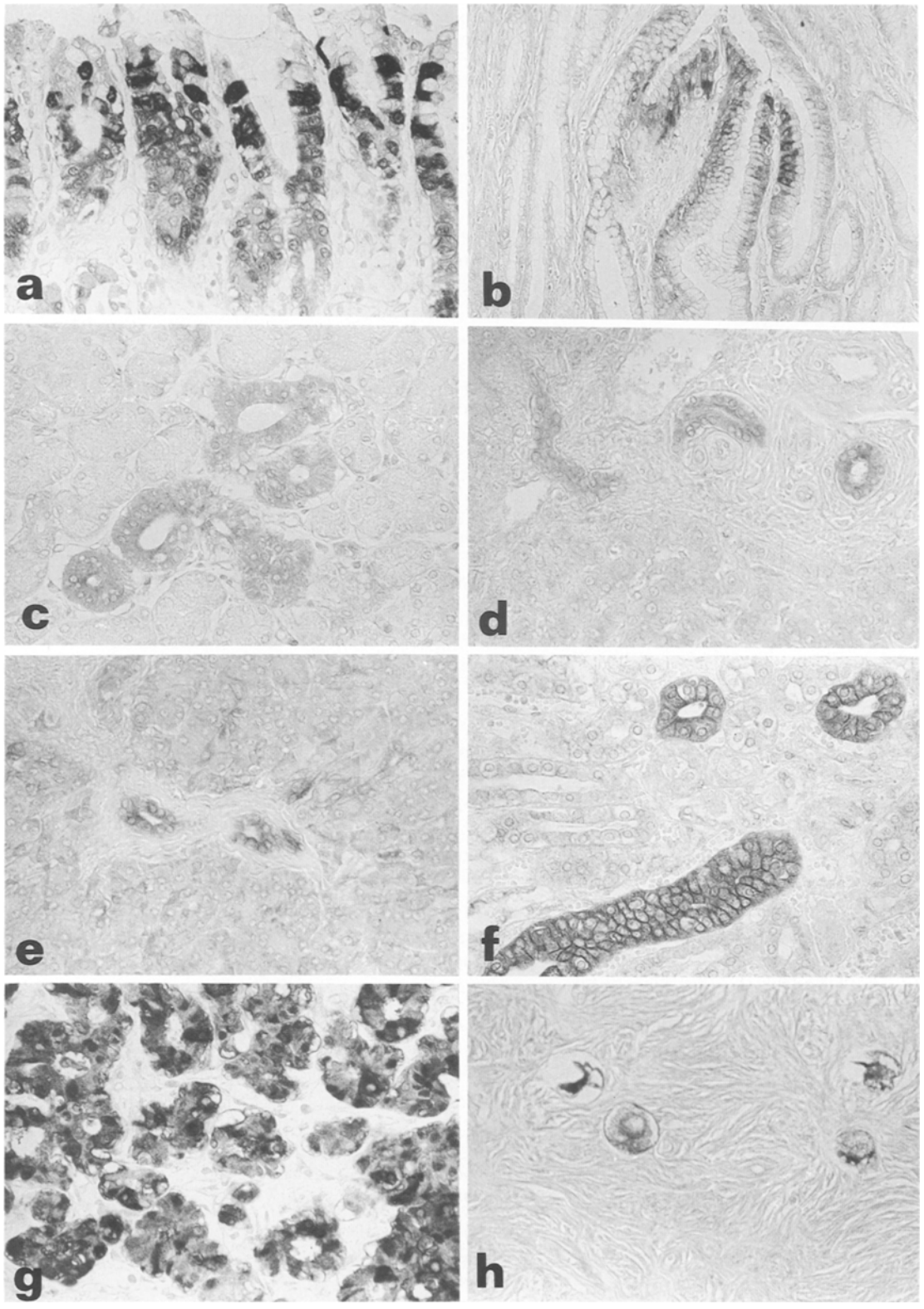

Fig. 3a–h. Immunohistochemical staining of TGF-α in several human tissues. **a** Neonatal stomach. Epithelial cells in the upper one-third of the fundic gland show strong TGF-α immunoreactivity, ×330. **b** Fundic mucosa of the adult stomach. The surface epithelial cells with hyperplastic change are positive for TGF-α, ×170. **c** Salivary gland. TGF-α immunoreactivity is restricted to ductal cells, ×260. **d** Liver. All epithelial cells of interlobular bile ducts show TGF-α immunoreactivity, ×280. **e** Pancreas. TGF-α immunoreactivity is detected in ductal cells and some centroacinar cells, ×220. **f** Kidney. The epithelial cells of renal tubules are strongly positive for TGF-α, ×260. **g** Mammary gland. In a part of the mammary glands, the glandular cells show very strong immunoreactivity to TGF-α, ×280. **h** Ovary. TGF-α immunoreactivity is observed in the oocytes, ×260

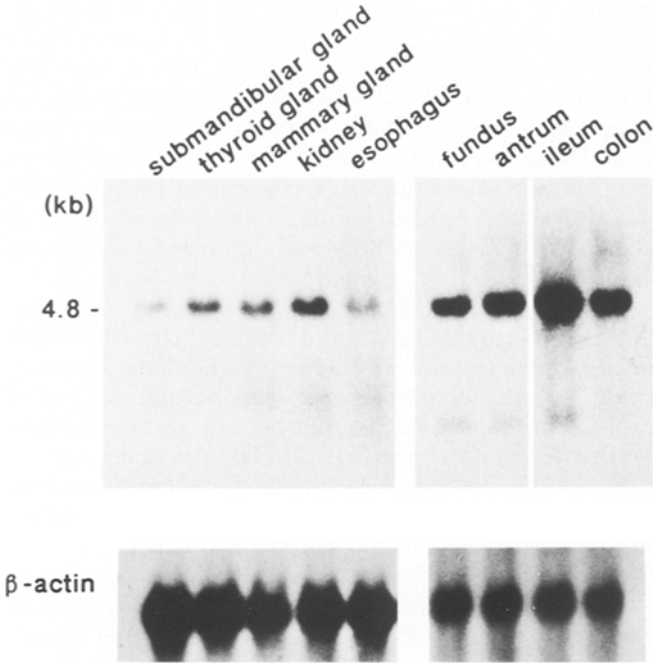

Fig. 4. Northern blot analysis of TGF-α mRNA on several human tissues. Ten micrograms of poly(A)$^+$ selected RNA was subjected to Northern blotting, as described in "Materials and methods". Beta-actin probe was used as an internal control

Table 2. Immunohistochemical localization of TGF-α in human fetal tissues

	Gestational weeks	
	7–10	12–17
1. Ectoderm-derived tissue		
– Skin (Epidermis)		–
– Hair follicle		–
– Central nerve	++	+
– Peripheral nerve	+	+
2. Endoderm-derived tissue		
– Thyroid gland		–
– Respiratory system	–	–
– Stomach	–	–
– Intestine	–	–
– Liver	++	++
– Pancreas	–	–
3. Mesoderm-derived tissue		
– Bone (osteocytes)		–
– Cartilage	–	–
– Muscle tissue		–
– Adrenal cortex	++	+
– Kidney		
– – Glomerulus	–	–
– – Tubules	–	+
– Genital system	–	

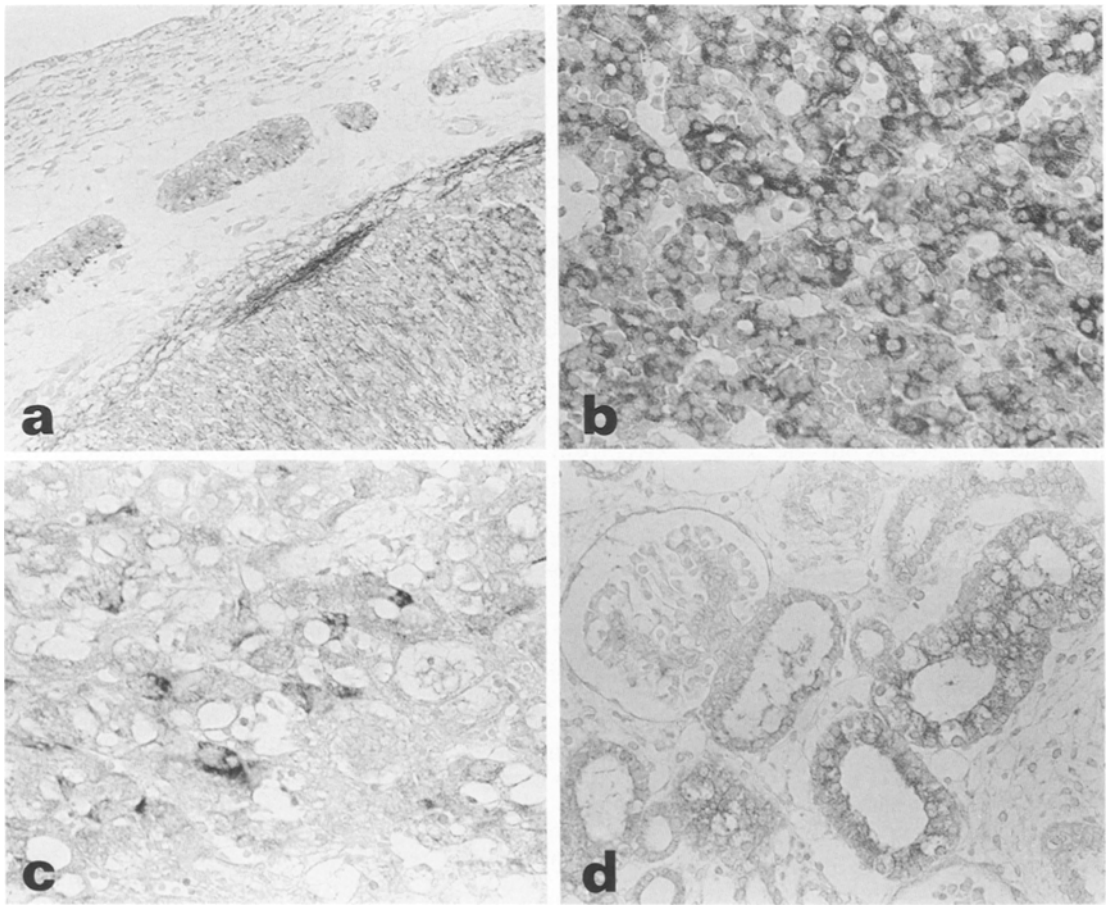

Fig. 5a–d. Immunohistochemical staining of TGF-α in human fetal tissues. **a** The spinal cord and root ganglion at 8 weeks of gestation are positive for TGF-α, ×270. **b** Most of the hepatocytes at 8 weeks of gestation show strong immunoreactivity to TGF-α, ×300. **c** TGF-α positive cells are found in the adrenal cortex at 12 weeks of gestation, ×270. **d** The renal tubules are weakly positive for TGF-α at 13 weeks of gestation, ×250

derm-derived tissues, the central nervous system, including spinal cord and root ganglion, showed TGF-α immunoreactivity (Fig. 5a). Peripheral nerve cells, including schwann cells, were weakly positive for TGF-α. In endoderm-derived tissues, strong TGF-α positivity was detected in the liver from 7 weeks of gestation (Fig. 5b). No immunoreactivity to TGF-α was found in the epithelial cells of the digestive tract. The respiratory system, including the trachea, bronchus and bronchiole, were also negative. Of the mesoderm-derived tissues, the adrenal cortex showed moderate-to-strong positivity for TGF-α (Fig. 5c). Renal tubules were weakly positive for TGF-α from 12 weaks of gestation (Fig. 5d). TGF-α immunoreactivity was not observed in the ovary at 10 weeks of gestation. Bone, cartilage, skeletal muscle, cardiac muscle and smooth muscle were all negative for TGF-α.

Discussion

Although the expression and biological role of TGF-α in a variety of tumours have been reported, the production site of TGF-α in normal tissues has not been examined in detail. Here, we examined the expression of TGF-α on various human tissues by immunohistochemistry and Northern blotting and found that TGF-α is produced by non-neoplastic cells of various organs. TGF-α was found mainly in the epithelial cells of the digestive tracts, liver, pancreas, kidney, mammary gland and so on. EGF, which has a sequence homology with TGF-α and shares the EGF receptor, has also been demonstrated to be present in the gastrointestinal tract, pancreas, kidney and mammary gland (Fukuyama and Shimizu 1991; Kajikawa et al. 1991). However, there are some differences in localization of EGF and TGF-α in these organs. For instance, in the gastrointestinal tract, TGF-α was detected in many surface epithelial cells, especially in the presence of hyperplastic or regenerative change, while EGF was found in a small number of epithelial cells in the pyloric glands of the stomach and in Brunner's glands of the duodenum. In the pancreas, TGF-α was found in epithelial cells of the pancreatic duct, while EGF was expressed in some acinar cells. In the liver, TGF-α was expressed in epithelial cells of the interlobular bile duct, whereas EGF was not.

As we have reported previously, the kidney expresses exceptionally high levels of EGF mRNA, suggesting that most of EGF in the urine or serum is produced by the epithelial cells of renal tubules (Kajikawa et al. 1991). This situation is similar to mouse EGF production by the submandibular glands (Cohen and Savage 1974). In the present study, although the level of TGF-α mRNA expression varied and many organs expressed TGF-α protein, no organs produced huge amounts of TGF-α in adults.

In considering the action or role of TGF-α in proliferation of normal tissues or organs, the localization and quantity of EGF receptor should be taken into account. In general, EGF receptor is widely distributed in the epithelial tissues, especially in basal cells (Fukuyama and Shimizu 1991). In the gastrointestinal tract, since mucosal cells possess a certain level of EGF receptor, both EGF and TGF-α may regulate physiological proliferation of the mucosal cells, including hyperplasia and regeneration, in an autocrine or paracrine manner (Yasui et al. 1988a, b). Furthermore, it seems likely that TGF-α may play a role in stomach carcinogenesis because many cases of superficial carcinoma of the stomach are also positive to TGF-α, as previously reported (Yamamoto et al. 1988).

In fetal tissues, TGF-α was preferentially expressed in the liver, adrenal cortex and nerve system, which may be major sources of TGF-α. The present study, however, could not elucidate when the fetal pattern of TGF-α expression changed to an adult pattern. In the gastrointestinal tract, no expression of TGF-α was detected in fetus, whereas very strong expression was found in neonatal stomach. Physical stimulation by milk may induce the expression of TGF-α in neonatal stomach.

What is the mechanism of differential expression of TGF-α among various organs? The transcription of TGF-α is regulated by a variety of *trans*-acting factors that interact with *cis*-elements in the promoter region of TGF-α gene (Jakobovits et al. 1988). Sp-1 and GC factor (GCF) bind to GC-rich sequences in the promoter (Briggs et al. 1986; Kageyama and Pastan 1989). In case of EGF receptor expression, Sp-1 enhances transcription, whereas GCF suppresses it (Kageyama et al. 1988; Kageyama and Pastan 1989). We have found that the combination or balance of Sp-1 and GCF might regulate the expression of TGF-α in gastric carcinoma cell lines (unpublished). The balance of transcription factors such as Sp-1 and GCF may be critical in the regulation of TGF-α expression in various organs and tissues.

In examining the site of TGF-α production, the in situ hybridization technique is important and there are reports concerning the expression of TGF-α mRNA in neoplastic and non-neoplastic tissues (Wilcox and Derynck 1988; Chung and Antoniades 1992). In the present study, we did not perform in situ hybridization in parallel with immunohistochemistry but did Northern blot analysis to detect mRNA expression. In the gastrointestinal mucosa, the level of TGF-α mRNA expression varied from case to case, which was compatible with the degree of positive reaction in immunohistochemistry. Therefore, it is likely that TGF-α immunoreactivity found in this study is TGF-α production by the cells rather than TGF-α uptake. We should perform the combination study of in situ hybridization and immunohistochemistry in the near future.

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