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Short Contribution

Production of alpha-fetoprotein by human submandibular gland

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ABSTRACT Alpha-fetoprotein (AFP) is a 590 amino acid polypeptide that was initially defined as an embryonal serum globulin. The yolk sac endoderm, fetal liver and fetal gut were shown to be the main sites of AFP synthesis in the embryo (Gitlin and Boesman, *J. Clin. Invest. 46:* 1010-1016, 1967). AFP synthesis is still continued in human adults (Ruoslahti and Seppala, *Int. J. Cancer 8:* 374-383, 1971) although the physiological level of serum AFP is lower than 10 ng/ml. AFP was also demonstrated in certain tumors and in various diseases or conditions such as yolk sac tumor, hepatoma, hepatoblastoma, acute and chronic liver cirrhosis, pregnancy and so on (Abelev, *Adv. Cancer Res. 14:* 295-358, 1971; Ruoslahti and Seppala, *Cancer Res. 29:* 275-346, 1979). Salivary glands have not been implicated in AFP synthesis. We investigated the expression of AFP in normal human salivary glands by immunohistochemistry with a monoclonal antibody against AFP, and documented immunoreactivity in intercalated and striated ducts of adult human submandibular glands.

KEY WORDS: *alpha-fetoprotein, human, submandibular gland, immunohistochemistry, monoclonal antibody*

Production of onco-fetal antigens such as carcinoembryonic antigen and alkaline phosphatase, has been well documented in salivary glands. However, AFP, one of the onco-fetal antigens, has never been implicated in salivary glands. We studied the expression of AFP in human salivary glands by immunohistochemistry with a monoclonal antibody against AFP.

We found that all the normal human adult submandibular glands examined exhibited strong positivity in intercalated ducts and striated ducts, but mucous and/or serous acinar cells, as well as myoepithelial cells, were negative. Moreover, other salivary glands, i.e., parotid glands, sublingual glands, palatal glands, also were completely devoid of immunoreactivity. Figures 1a and 2a demonstrate positively stained portions of submandibular glands. Figure 1a shows positively stained striated ducts of submandibular gland. All the epithelial cells of the striated ducts are uniformly stained. Figure 2a shows the relationship between the mucous acini and the adjacent intercalated ducts at the secretory endpieces of submandibular gland. AFP-positive staining sites are limited to the intercalated duct cells. It has been shown that intracellular localization of AFP was diffusely spread over the Golgi complex and RER areas in hepatocytes (Baranov and Engelhardt, 1987). The existence of membrane-bound AFP was recently reported (Hosokawa *et al.*, 1989). Our results are consistent with these data. AFP has been considered as a stage-specific antigen in the development of hepatocyte (Sell, 1978). In salivary glands, the intercalated duct cell possibly acts as a kind of stem cell having the capacity to differentiate either into secretory cells or into myoepithelial cells or into striated duct cells (Tandler and Riva, 1986). The positive reactions we observed might be stage specific. In the present study we could detect AFP-positive cells only in submandibular glands. These glands may be specifically involved in AFP synthesis as are the submandibular glands in the synthesis of epidermal growth factor.

Experimental Procedures

Three each of adult human submandibular glands, parotid glands, sublingual glands and palatal glands, surgically dissected at the Hospital of Okayama University Dental School, were subjected to immunohistochemical study. These salivary glands were fixed with 10% neutral buffered formalin solution and embedded into paraplast wax in a routine manner. 3-5 μm sections of each salivary gland tissues were subjected to staining for AFP using commercially available monoclonal antibody (Cosmo Bio, Tokyo).

Dewaxed sections were treated with 0.3% H_2O_2 in methanol alcohol for 30 minutes at room temperature in order to block endogenous peroxidase and subsequently pretreated with 0.1% trypsin solution for 60 min at 37°C. Following a pre-treatment with normal rabbit serum, sections were incubated overnight at 4°C in a moisture chamber with anti-AFP monoclonal antibody (1:50, 4 μg/ml). For signal detection, we employed a Vectastaine

Abbreviations used in this paper: AFP, alpha-fetoprotein; RER, rough endoplasmic reticulum.

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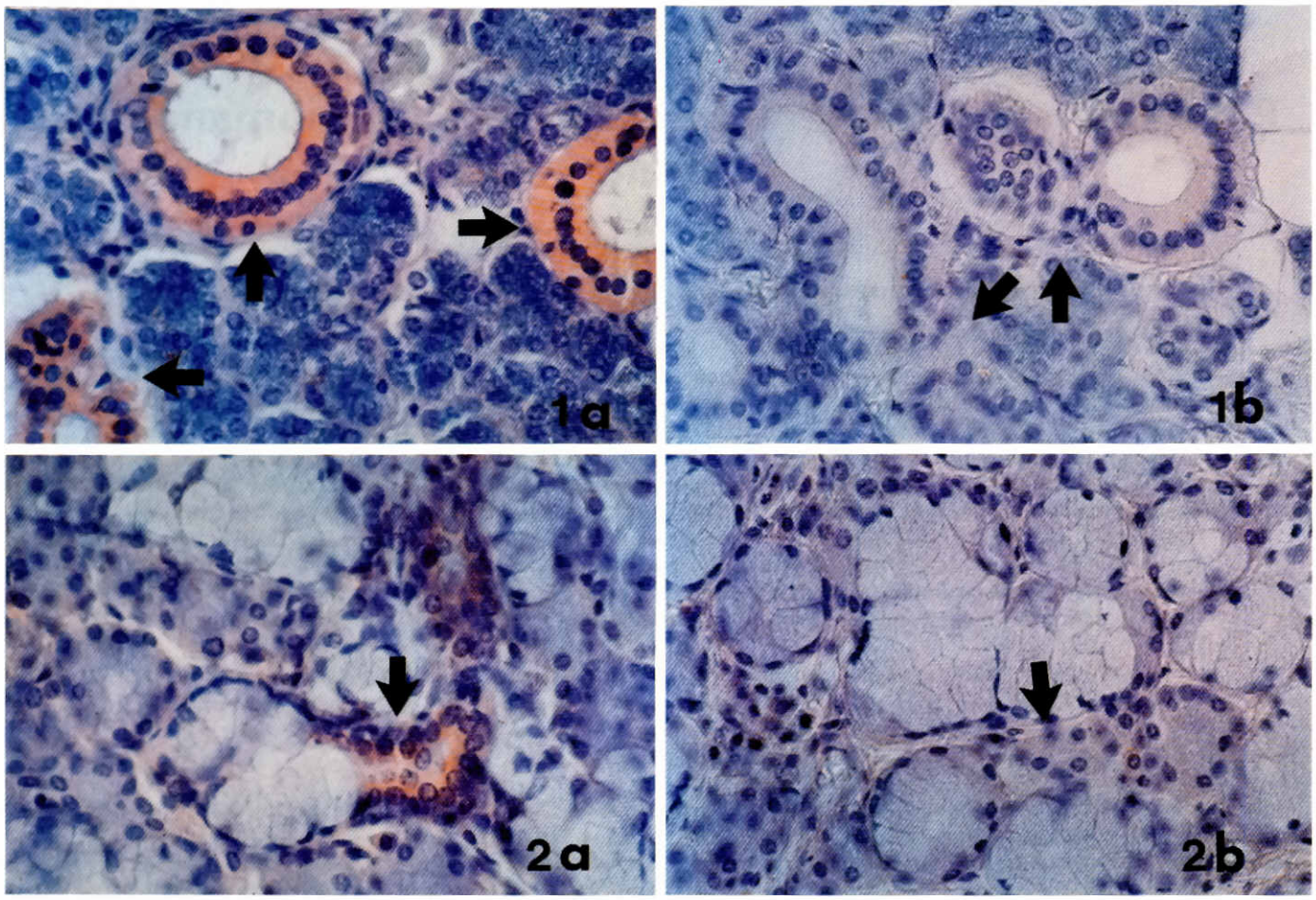

Fig. 1. Striated ducts of human submandibular gland. (a) *Note the intense staining of the striated duct epithelial cell cytoplasms for AFP (arrows). x400.* **(b)** *Negative control of the striated duct epithelial cells (arrows). x400.*

Fig. 2. Intercalated ducts adjacent to the mucous acini of human submandibular gland. (a) *Note the intense staining of the intercalated duct epithelial cells (arrow). x400.* **(b)** *Negative control of the intercalated duct epithelial cells (arrow). x400.*

ABC-Elite Peroxidase Kit (Mouse IgG, Vector Lab. Burlingame, CA). As a negative control, normal rabbit serum was substituted for the primary antibody. Sections were slightly counter-stained with a Mayer-Hematoxylin solution to identify histological structures.

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