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Immunohistochemical expression of epidermal growth factor receptor in salivary gland tumours

Kazuto Yamada, Katsumasa Iwai, Yukihiro Okada, and Masahiko Mori
Department of Oral Surgery, Asahi University School of Dentistry, 1851-1 Hozumi, Hozumi-cho, Motosu-gun, Gifu 501-02, Japan

Summary. Immunohistochemical localization of epidermal growth factor receptor (EGFR) in normal salivary glands and tumours (108 cases) was studied using a monoclonal antibody. In the normal salivary glands, EGFR was occasionally detected in ductal segments of intercalated, striated, and excretory ducts, but not in acinar cells. The frequency of positive EGFR staining in salivary gland tumours was not high: pleomorphic adenoma, 33.8%; mucoepidermoid tumour, 25.0%; adenolymphoma, 44.4%; and sialoadenocarcinoma, 66.6%. Pleomorphic adenomas showed positive staining for EGFR on the luminal side of luminal cells and in squamous metaplastic cells of tumour tissue. Some modified myoepithelial cells were also reactive whereas outer spindle tumour cells were unstained. Adenolymphomas regularly exhibited positive EGFR staining in the cell membrane; mucoepidermoid carcinoma displayed positive staining in cell membranes in epidermoid tumour cells and cytoplasmic staining in mucous-secreting tumour cells. Sialocarcinomas revealed cell membrane staining and whole cytoplasmic staining for EGFR. The immunohistochemical localization of EGFR could be classified into two types, one the cell membrane-positive type found in epithelial tumour cells, and the second the cytoplasmic positive type seen in normal ductal cells, the luminal tumour cells of pleomorphic adenomas and mucous-secreting tumour cells.

Key words: EGF receptor – Human salivary tumour – Monoclonal antibodies

Introduction

Epidermal growth factor (EGF) was originally isolated from mouse submaxillary glands (mEGF) and localized immunohistochemically in granular convoluted tubule cells as the site of highest concentration (Barka 1980; Mori et al. 1983). Human EGF (hEGF) is also known as urogastron; and is a polypeptide functionally and biochemically similar to mEGF. Recently, hEGF has been reported to be widely distributed in human tissues and organs and their neoplastic lesions, including normal salivary glands and tumours (Eldder et al. 1978; Heitz et al. 1978; Waterfield et al. 1982; Kasselberg et al. 1985; Mori et al. 1989, 1989; Tatemoto et al. 1988; Tsukitani et al. 1987). The biochemical action of EGF requires the binding of the factor to specific receptors located in the plasma membrane (Carpenter and Cohen 1979; Barka 1980). This EGF receptor (EGFR) is a 170KD glycoprotein which has thyrosine kinase activity and ligand-stimulated receptor autophosphorylation, and is structurally similar to the avian erythroblastosis virus (AEV) v-erb-B oncogene transforming protein (Downward et al. 1984). Physiological effects of EGF include stimulation and proliferation of many types of cells in vivo and in vitro, and either a reduction in EGF binding to EGFR or over-expression of EGFR has been noted in keratinocytes and squamous cell carcinomas (Ozanne et al. 1985, 1986). EGF, transforming growth factor-α (TGF-α), and EGFR might play important roles in malignant transformation or gene amplification.

Many recent studies on immunohistochemically detectable EGFR have been carried out in normal human tissue and tumours (Gusterson et al. 1984;; Damjanov et al. 1986), including human salivary gland tumours (Caselitz and Seifert 1987; Seifert et al. 1987) gastric cancer (Sakai et al. 1986; Yasui et al. 1988), lung tumour (Berger et al. 1987; Hendler and Ozanne 1984; Hwang et al. 1986), brest cancarcinoma (Fitzpatrick et al. 1984; Sainsburg et al. 1985; Ozawa et al. 1988; Horne

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et al. 1988; Wrba et al. 1988) squamous cell carcinomas (Hender et al. 1984; Cowley et al. 1984; Kamata et al. 1986; Merlino et al. 1985; Ozanne et al. 1986; Ozawa et al. 1987, 1988; Möller et al. 1989), and meningiomas (Shiurba et al. 1988), and nervous system tumours (Reifenberger et al. 1989).

The present study was conducted in order to identify EGFR immunohistochemically in normal salivary glands and in several types of neoplastic lesions of salivary glands, and to compare the distribution patterns found with the previously described distribution of hEGF (Mori et al. 1987). Immunohistochemical localization of EGFR has also been reported in normal human tissues (Damjanov et al. 1986).

Materials and methods

Tissue materials consisted of normal salivary glands (7 cases) and salivary gland tumours: pleomorphic adenomas (80 cases), adenolymphomas (18 cases), mucoepidermoid carcinomas (8 cases), and sialoadenocarcinomas (3 cases). In order to examine the effect of the fixative solution, normal submandibular salivary gland tissue was fixed in 10% formalin (5 cases) for 12 h, Bouin's (6 cases) for 18 h, PLP (periodate-lysine-paraformaldehyde) (3 cases) for 24 h, or Carnoy's solutions (6 cases) for 4 h. The fixed tissue specimens were embedded in paraffin, and 4 μm sections were made for immunohistochemical staining and for H&E staining for routine histology.

Paraffin sections were used to detect EGFR immunohistochemically with the two techniques; the indirect and ABC methods using monoclonal antibodies. Specificity of EGFR from human A-431 cells and biological effects of monoclonal anti-EGFR antibody has already been described (Schreiber et al. 1983; Yarden et al. 1985). After deparaffinization of the sections, following inactivation of endogenous peroxidase (ethanol including 0.3% H_2O_2) and removal of nonspecific background staining (1/20 rabbit serum), the sections were examined by the EGFR (Bio-Markor) reaction, immunohistochemically. In the indirect method, the sections were treated with the antibody (1/20) for 1 h, reacted with HRP-conjugated rabbit anti-mouse IgG (1/20) for 30 min, and visualized with 0.03% 3-3 diaminobenzidine tetrahydrochroride (DAB) containing 0.005% H_2O_2 solution. In the ABC method, they were treated with primary antibody (1/120) for 1 h, reacted with biotin labelled rabbit IgG anti-mouse IgG (1/200) for 30 min, treated with AB complex (10 μl/ml) for 30 min, and with DAB solution.

Result

From the comparative study of fixative solutions for immunohistochemical identification and localization of EGFR, 10% formalin-fixed sections were found to be the most reactive among the 4 fixatives examined. Therefore, 10% formalin solution was used as the fixative for all specimens in this study.

Comparison of the two methods showed that EGFR staining by the indirect method was the same as that seen by the ABC method. However, dilution of the primary antibody differed in order to obtained the same staining intensity to EGFR; dilution of the indirect method was 1:20, and that of the ABC method 1:120.

Table 1. Immunohistochemical staining for salivary gland tumours

	Positive case	Total	Percentage
Pleomorphic adenoma	31	80	38.8%
Mucoepidermoid tumour or carcinoma	2	8	25.0%
Adenolymphoma	8	18	44.4%
Sialoadenocarcinoma	2	3	66.6%

Immunohistochemically detectable EGFR in the normal salivary glands was usually confined to the ductal segments, including intercalated (Fig. 1B), striated (Fig. 1C, 1D) and excretory duct cells (Fig. 1E), and the staining intensity varied greatly. In some cases, certain cells of the striated duct were positive for EGFR, whereas other cells of the duct were negative or other striated ducts were totally devoid of EGFR staining (Fig. 1A). In large excretory ductal epithelium, there was an irregular distribution of EGFR, with some cells showing very strong staining, and others none (Fig. 1E). Capillary vessels also indicated positive staining.

Irrespective of the histological variations among pleomorphic adenomas, EGFR staining was detected in 38.8% of the cases (31/80). It was distributed in the luminal borders of luminal cells, and in luminal cells of tubulo-ductal structures (Fig. 2A, B, C). Luminal tumour cells of some duct-like structures were strongly stained, but unstained cells were also present (Fig. 2D, E, F).

In an occasional pleomorphic adenoma, an intense immunohistochemical staining of EGFR was noted in a limited number of tubulo-ductal cells, whereas only slight staining was found in most luminal tumour cells (Fig. 3A). This EGFR staining pattern of such luminal tumour cells resembled that seen in the normal striated duct. In rare instances, slight staining of EGFR was detected in the border zone between luminal and outer tumour cells in tubulo-ductal structures (Fig. 3B). Pseudoglandular structures of pleomorphic adenoma exhibited positive staining for EGFR in centrally located tumour cells (Fig. 3C). Squamous metaplasia was usually present in the central part of tumour foci, and their cytoplasm membranes were stained (Fig. 3D). No reaction product of the EGFR staining reaction was found in outer tu-

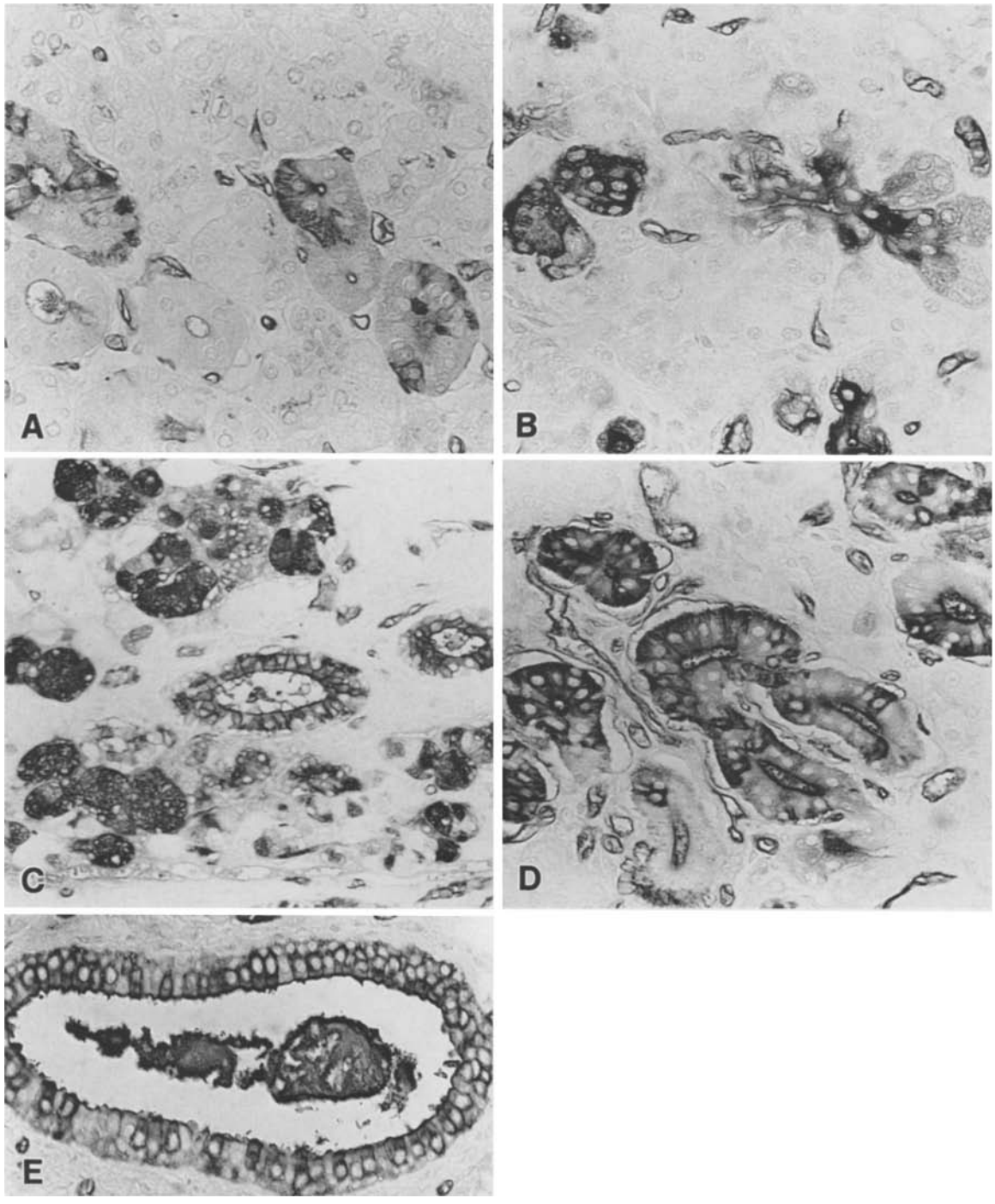

Fig. 1A–E. EGFR staining in the normal salivary glands of humans. 10% formalin fixed paraffin sections, ×200. **A** Monoclonal antibody to EGFR stains certain parts of some cells in the striated duct. **B** Strong staining for EGFR is present in intercalated duct cells and certain ductal cells. **C** Strong staining for EGFR is confined to some acinar cells only rarely, and positive EGFR staining is localized in ductal epithelium. **D** In striated duct cells of the submandibular gland, the degree of EGFR staining varies in certain ductal cells, but not all the ductal cells are positive. Cytoplasmic-positive type of EGFR stain is seen. **E** Large excretory duct epithelium shows cytoplasmic-positive type of EGFR staining, and EGFR is distributed mainly in luminal epithelial cells

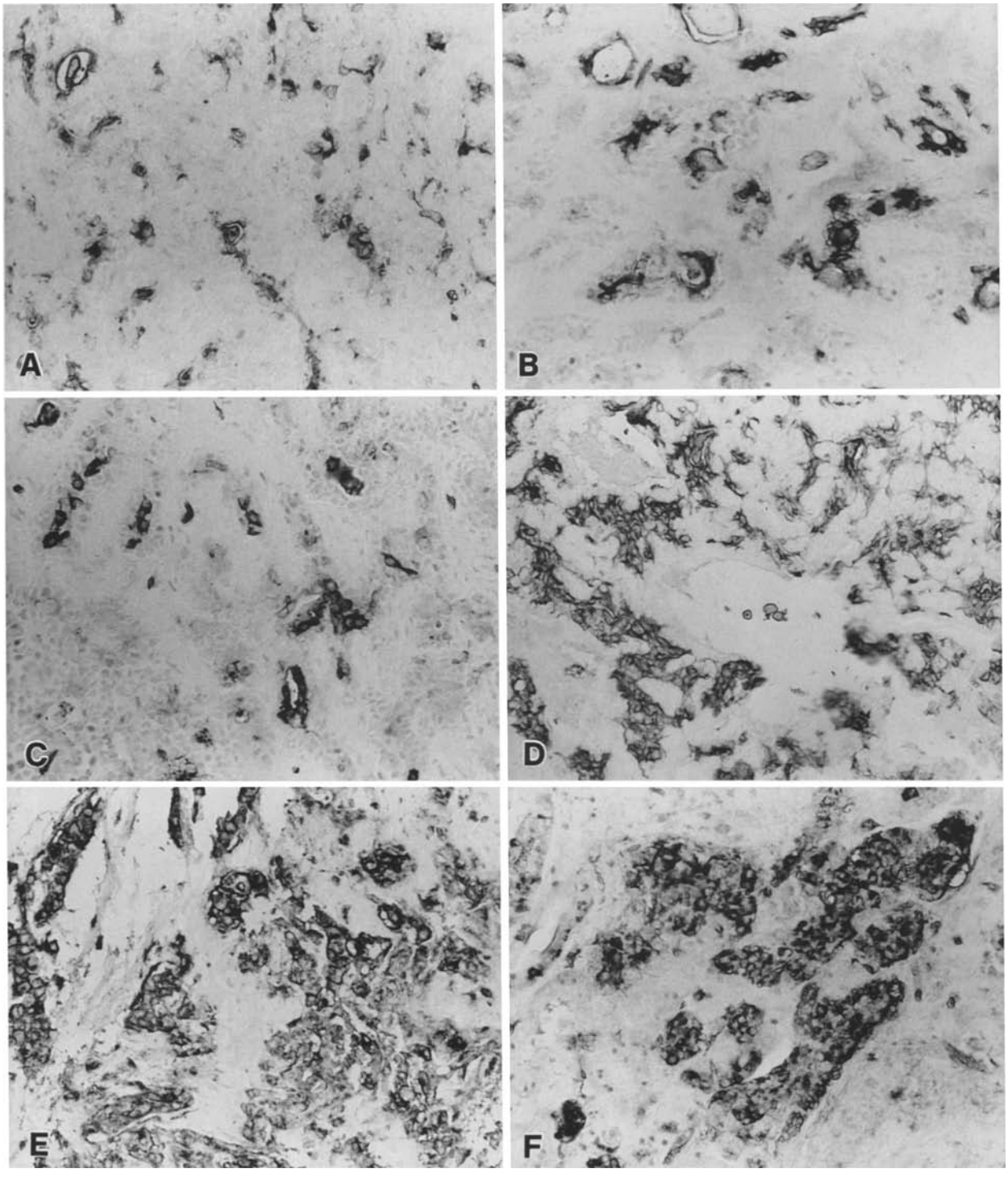

Fig. 2A–F. EGFR staining in variant histologic types of pleomorphic adenomas. ×100. **A** EGFR staining is confined to luminal borders of tubulo ductal structures. **B** The strongest staining for EGFR is restricted to luminal borders or luminal side of luminal tumour cells. **C** The strongest staining for EGFR occurs in certain tumour cells located on the luminal side of tubulo-ductal structures. **D** Positive EGFR staining is present in luminal tumour cells of tubulo-ductal structures. **E** Positive EGFR staining is distributed in squamous metaplastic tumour cells in some of the tubular structures. **F** Strong EGFR staining is confined to squamous metaplastic tumour cells located in central parts of solid foci

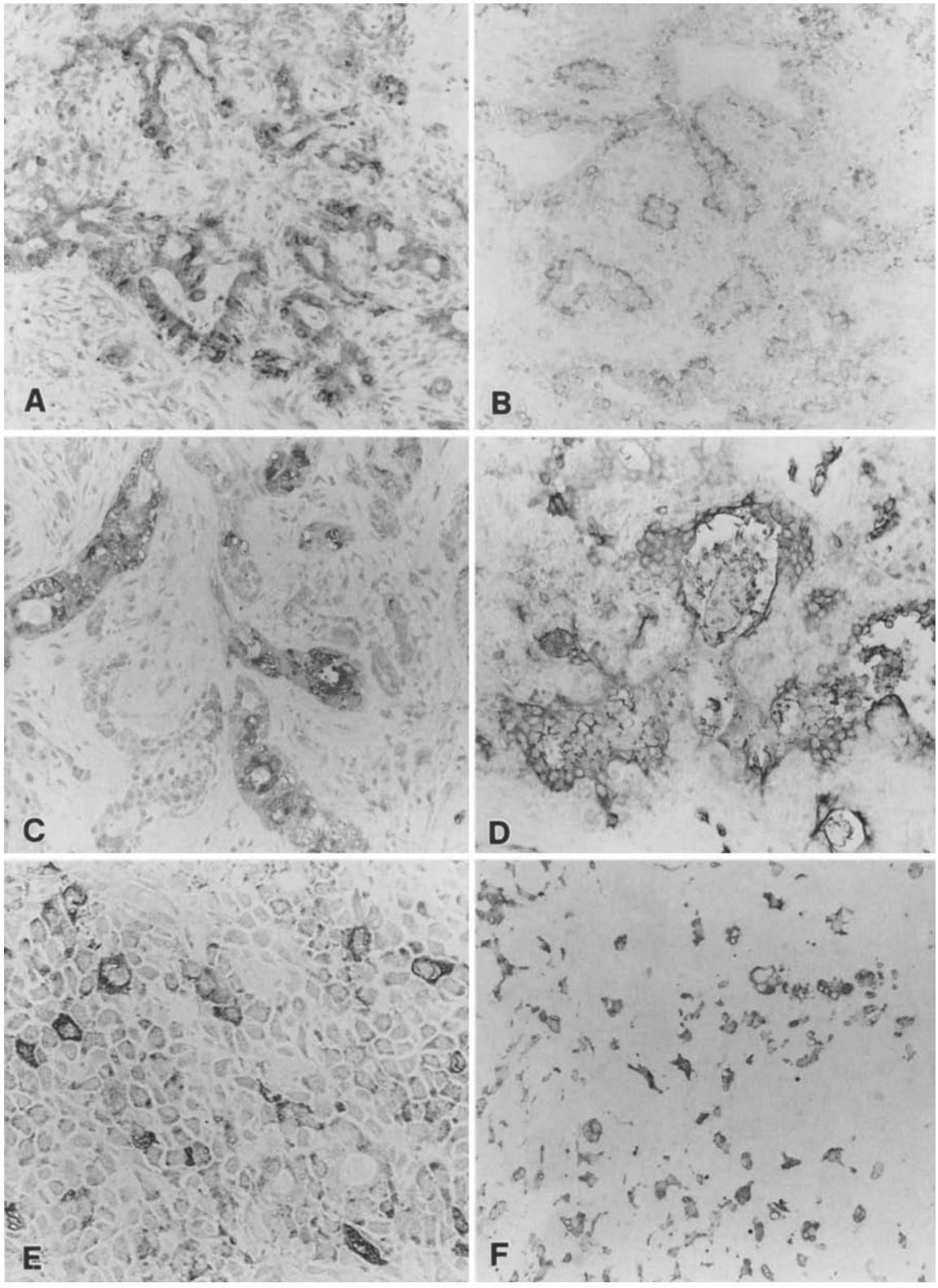

Fig. 3A–D. EGFR staining in pleomorphic adenomas, ×100. **A** Comparatively intense staining for EGFR is present in certain tumour cells located in cyst-like epithelium. **B** Slight EGFR staining is localized in the border zone between luminal tumour cells and outer tumour cells of tubulo-ductal structures. **C** Luminal tumour cells of glandular structures give the cytoplasmic positive type of staining for EGFR. **D** Luminal tumour cells of large cyst-like structure express EGFR staining as the cytoplasmic-positive type. **E** EGFR staining in modified myoepithelial cells. Some tumour cells contain EGFR in their cytoplasm. ×200 **F** Chondroidal changed cells in myxomatous tissue of pleomorphic adenomas stain intensely for EGFR

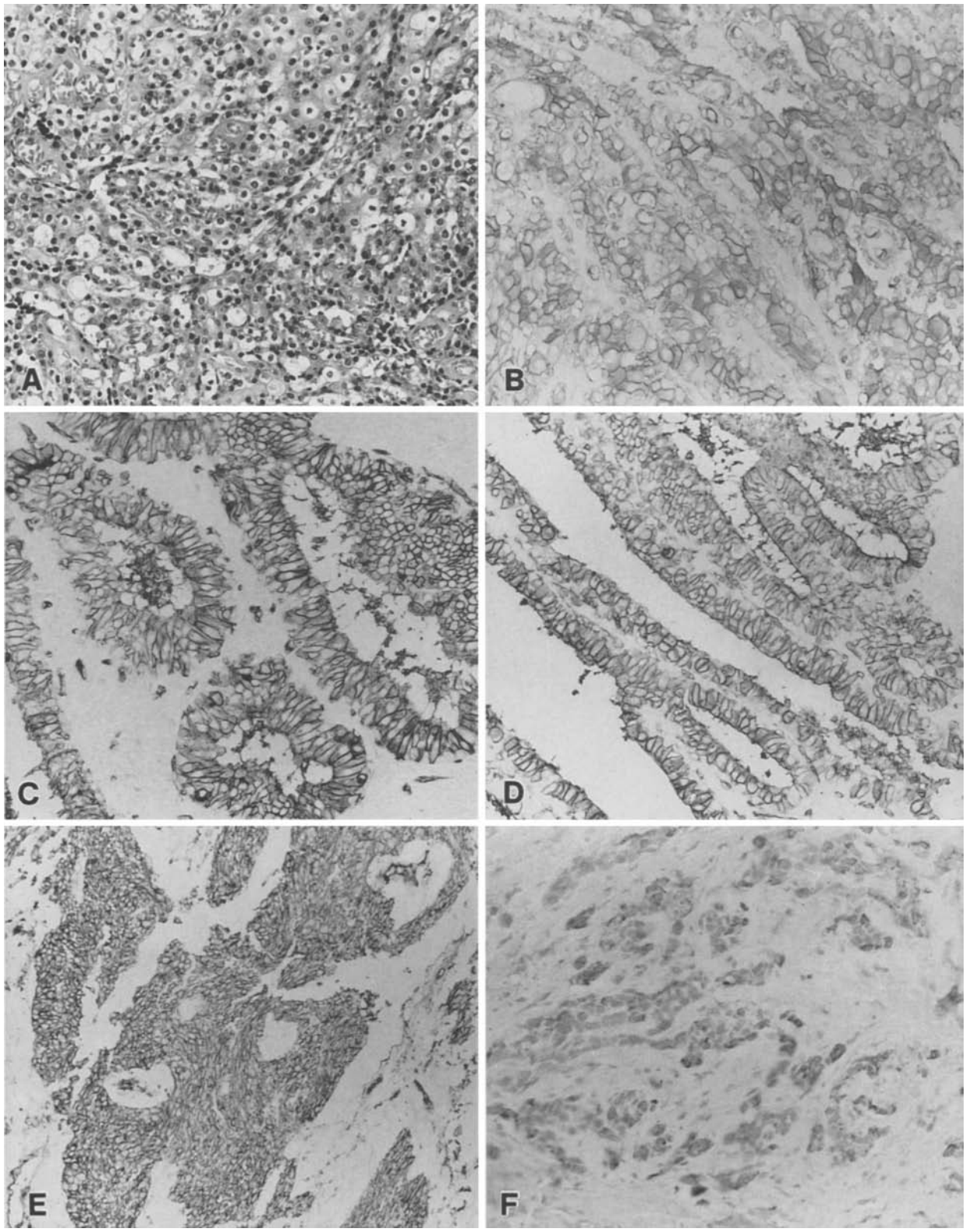

Fig. 4A, B. Mucoepidermoid carcinoma, intermediate type (G-II), ×100. **A** Mucoepidermoid carcinoma composed of epidermoid and intermediate type of tumour cells. **B** EGFR staining shows cell membrane-positive type in epidermoid tumour cells, and cytoplasmic-positive type in intermediate cells. **C, D** Adenolymphomas, ×100. EGFR staining in eosinophilic tumour epithelia is found in the plasma membrane at the apex of occasional columnar cells. Basal tumour cells do not display a positive EGFR reaction. **E** Sialoadenocarcinoma, ×100. EGFR staining is limited to cell plasma membrane (cell membrane-positive type of EGFR staining). **F** Sialoadenocarcinoma, ×100. Scattered adenocarcinoma cells show diffuse, positive staining for EGFR (cytoplasmic-positive type)

mour cells of tubulo-ductal or other structures, or in any type of modified myoepithelial cell.

Some plasmacytoid myoepithelial cells manifested positive EGFR staining in their cytoplasm, whereas all myoepithelioma types of tumour were unstained (Fig. 3E). Chondroidal changed cells in hyalinous or myxomatous structures of pleomorphic adenomas regularly showed restricted, positive EGFR staining (Fig. 3F).

There are three types of neoplastic cells found in mucoepidermoid tumours: epidermoid tumour cells, mucous-secreting cells, and intermediate tumour cells. The incidence of positive EGFR staining was found to be 25% (2/8) of the cases. Immunostaining for EGFR was usually confined to the plasma membrane of epidermoid tumour cells (cell membrane-positive type); whereas in mucous cells a diffuse weak reactivity was found in the cytoplasm (diffusely positive cytoplasmic type) (Fig. 4A, B).

The incidence of positive staining for EGFR in adenolymphomas was 44.4% (8/18). All epithelial tumour cells, irrespective of cell shape, showed positive EGFR staining cell membrane positive type in apical columnar tumour cells. Basal tumour cells of adenolymphomas were unstained (Fig. 4C, D).

There were great variations among the specimens in terms of histopathology in sialoadenocarcinomas; epidermoid, clear, mucous, and intermediate cells were seen. EGFR staining was noted in 66.6% (2/3) of the cases. It was limited to the plasma membrane in epidermoid-type tumour cells, whereas staining was diffuse and restricted to the cytoplasm in other sialoadenocarcinoma cells (Fig. 4F).

Discussion

Gusterson et al. (Gusterson et al. 1984) were the first to describe the immunohistochemical localization of EGFR in salivary glands in their study of the EGFR in normal tissues and some malignant tumours. Seifert et al. (1987) and Caselitz and Seifert (1987) have described EGFR immunohistochemistry in large numbers of salivary gland tumours in the salivary gland registry of UKE. The present study is the first detailed report of EGFR expression in several histological types of salivary gland tumours and in the normal gland. Most previous reports have stated that cell membrane staining was given by epidermal and glandular epithelial cells (Cowley et al. 1984; Gullick et al. 1986; Hwang et al. 1986; Kamata et al. 1986; Ozanne et al. 1986; Sakai et al. 1986; Ozawa et al. 1987, 1988; Wrba et al. 1988). Gusterson et al. (1984) reported that, in exceptional cases, cytoplasmic staining was noted in salivary gland ducts, but that no staining could be found in acinar cells. In the present results, ductal segments of salivary glands were occasionally positive for EGFR, with varying levels of cytoplasmic staining, and excretory duct cells showed irregular staining from negative to strong. Immunohistochemical expression of EGFR from the present result was similar to staining patterns of the reports from Seifert et al. (1987), and Caselitz and Seifert (1987). However, Gusterson et al. (1984) stated that ductal basal cells (reserve cells) indicated selective staining for EGFR. This discrepancy of EGFR staining in ductal basal cells might be related to the different MoAb used to detect EGFR. In this regard, hEGF was localized immunohistochemically on the luminal side of ductal cells with the use of MoAb to hEGF generated by the synthetic gene technique (Tsukitani et al. 1987; Mori et al. 1987), and hEGF/γ-urogastrone was confined to intercalated duct cells and luminal borders of ducts with the use of hEGF/γ-urogastrone purified from human urine (Tatemoto et al. 1988). As for the comparison of histochemical localization of hEGF and EGFR, there is a difference in distribution between hEGF and EGFR in ductal segments: sites positive for hEGF, in striated duct cells and on the luminal sides of duct cells, are not always positive for EGFR staining; thus EGFR staining in ductal segments shows a lower incidence than hEGF staining.

Pleomorphic adenomas of salivary gland origin are the most common benign tumour and manifest both hEGF and EGFR when examined immunohistochemically. hEGF is always restricted to luminal tumour cells of tubulo-ductal structures, whereas staining for it is negative in outer tumour cells of the structures (Mori et al. 1987). In contrast, EGFR staining was not always positive on the luminal side of luminal tumour cells, and only 38.8% of pleomorphic adenomas were EGFR positive. Thus immunohistochemical distribution between hEGF and EGFR is not the same. Squamous metaplasia was occasionally found focally in epithelial tumour structures of pleomorphic adenomas, and metaplastic epithelial cells displayed the cell membrane positive type of EGFR staining as found in squamous-cell carcinomas or epidermal keratinocytes. Caselitz and Seifert (1987) stated that the polygonal cells in chondroid matrix of pleomorphic adenoma were positive for EGFR reaction, and in the present study chondroidal cells were also reactive to EGFR as reported by Caselitz

and Seifert. In this study, similar immunohistochemical localization in pleomorphic adenoma was identified (Fig. 3 B).

EGFR expression in neoplastic epithelial cells in mucoepidermoid carcinomas, adenolymphomas, and sialoadenocarcinomas, coincided with the cell membrane-positive type as seen in normal epidermal cells and squamous cell carcinomas (Ozawa et al. 1987, 1988). It is reported that overexpression of EGFR or a high level of EGFR occurs in squamous cell carcinoma and keratinocytes, and this phenomenon suggests that EGF has an important role in the regulation of growth and differentiation of epithelial cells. Berger et al. (1987) described that the frequency of EGFR-positive tumour cells was more often high in various histological types of lung tumours than in other tumours. EGFR in squamous cell carcinoma type of tumour cells could be attributable to abnormal expression of the v-erb-B gene. The frequency of EGFR expression varied with the different histological types of salivary gland tumours; the higher frequency of EGFR staining in squamous type tumour cells of salivary gland lesions would be valuable in retrospective studies.

Recently, Shiurba et al. (1988) noted that strong staining for EGFR (monoclonal antibody from A431 cells) was found in endothelial cells of meningiomas, and they suggested that the receptor may participate in tumour angiogenesis. In the present study, vascular endothelial cells of pleomorphic adenoma and sialocarcinomas exhibited varied staining for EGFR with the use of similar monoclonal antibody. As angiogenesis is usually required for growth of epithelial tumour cells, the EGFR in endothelial cells may participate indirectly in tumour growth by signaling proliferation of the vascular endothelium.

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