Acta histochemica **109** (2007) 37—44

Available online at www.sciencedirect.com
ScienceDirect

acta histochemica
www.elsevier.de/acthis

Immunohistochemical detection of epidermal growth factor and epidermal growth factor receptor in the lingual mucosa of rats during the morphogenesis of filiform papillae

Shin-ichi Iwasaki[a,*], Hidekazu Aoyagi[a], Hideki Yoshizawa[b]

[a]*Advanced Research Center, The Nippon Dental University School of Life Dentistry at Niigata, 1-8 Hamaura-cho, Niigata 951-8580, Japan*
[b]*Department of Biology, Matsumoto Dental University School of Dentistry, 1780 Hirookagouhara, Shiojiri, Nagano 399-0781, Japan*

Received 30 May 2006; received in revised form 31 July 2006; accepted 1 September 2006

KEYWORDS
Filiform papillae;
Morphogenesis;
Epidermal growth factor (EGF);
Epidermal growth factor receptor (EGFR);
Immunohistochemistry;
Confocal laser-scanning microscopy

Summary

We examined the immunofluorescence labelling epidermal growth factor (EGF) and epidermal growth factor receptor (EGFR), as well as differential interference contrast (DIC) images, during the morphogenesis of filiform papillae and the keratinization of the lingual epithelium of rats on semi-ultrathin sections of epoxy resin-embedded samples using laser-scanning microscopy. We also examined semi-ultrathin sections of epoxy resin-embedded, toluidine blue-stained samples by light microscopy to obtain details of cell histology and morphology. No immunoreactivity specific for EGF and EGFR was detected on the lingual epithelium of fetuses on days 12 and 16 after conception (E12 and E16), during which time the number of layers of cuboidal cells in the lingual epithelium increased from one to several. Immunoreactivity specific for EGF and EGFR was first detected on the lingual epithelium of fetuses at birth or on postnatal day 0 (P0). Immunoreactivity specific both for EGF and EGFR appeared in the connective tissue and the basal cells of the papillary and interpapillary cell columns. The lingual epithelium was composed of stratified squamous cells. The rudiments of filiform papillae were compactly arranged and interpapillary cell columns were very narrow. Immunoreactivity specific for EGF and EGFR was distinct on the cell membrane of basal cells of the papillary cell column and weakly positive on the cell membrane of basal cells of the interpapillary cell column on postnatal day 21 (P21). Thus, the patterns of immunoreactivity of EGF and EGFR differed as the filiform papillae developed. Filiform papillae developed gradually from P0 to P21. The width of interpapillary spaces also increased during

*Corresponding author. Tel.: +81 25 267 1500; fax: +81 25 267 1134.
E-mail address: iwasaki@ngt.ndu.ac.jp (S. Iwasaki).

0065-1281/$ - see front matter
doi:10.1016/j.acthis.2006.09.004

this period. These observations indicate a possibility that EGF might affect the expression of keratins in the lingual epithelium via epithelium–mesenchymal interactions.

Introduction

Analysis by scanning electron microscopy of the tongues of rats and mice reveal that filiform papillae form just before birth (Iwasaki et al., 1996, 1997). Light and transmission electron microscopy demonstrate that there are no rudiments of filiform papillae and no signs of keratinization in the dorsal lingual epithelium of rats and mice during the middle and late periods of gestation. Keratinization of the dorsal lingual epithelium is clearly recognizable in newborn rats and mice, together with the rudiments of filiform papillae. Thus, the filiform papillae of rats and mice seem to develop rapidly and exclusively during the 2 or 3 days before birth, in parallel with the keratinization of the dorsal epithelium of the tongue (Iwasaki et al., 1999a, b).

Iwasaki et al. (2003, 2006) reported that keratins 13 (K13) and 14 (K14) are expressed with specific timing and in specific regions of the lingual epithelium during the morphogenesis of lingual papillae. K14 is located exclusively on the basal and suprabasal cells, and K13 is located on the intermediate layer of the interpapillary cell column. K14, in particular, is considered to be a marker of mitotically active cells in the oral epithelium. However, the triggers for mitotic activity of basal and suprabasal cells remain to be identified. Thus, it is important for a full understanding of the morphogenesis of lingual papillae, to identify the factors that affect the expression of keratins in the lingual epithelium. In the present study, we examined the timing of the appearance and the distribution of epidermal growth factor (EGF) and epidermal growth factor receptor (EGFR) as possible factors related to the immunohistochemically detected changes in levels and distribution of keratins.

EGF is a potent mitogen that plays an important role in controlling growth by binding to cell surface receptors on both normal and neoplastic cells (Stoscheck and King, 1986). The EGFR is a phosphoglycoprotein that has tyrosine kinase activity and undergoes ligand-stimulated autophosphorylation (Cohen, 1983). Some studies on immunohistochemically detectable EGF and EGFR have been performed in normal human oral tissues (Shirasuna et al., 1991; Whitcomb et al., 1993; Araujo et al., 2003). The cited reports indicate that EGF is located in the connective tissue near the epithelium of the oral tissue (Shirasuna et al., 1991) and/or in the cytoplasm and cell membrane of the basal and suprabasal cells of the epithelium (Araujo et al., 2003). These reports also showed that EGFR is clearly detectable only in the cell membrane of the basal and suprabasal cells of the epithelium (Shirasuna et al., 1991; Whitcomb et al., 1993; Araujo et al., 2003). Humphreys-Beher et al. (1994) indicated that transforming growth factor-α (TGF-α), which is homologous to EGF in terms of amino acid sequence, has activity identical to that of EGF, and that it is also produced in the salivary glands of rats.

The present study was designed to clarify the timing of the appearance of, and changes in the pattern of, immunoreactive EGF and EGFR. It is likely that EGF and EGFR play important roles in the mitotic activity of basal and suprabasal keratinocytes during the morphogenesis of lingual papillae. Using a previously described method (Iwasaki et al., 2006), we examined the localization of EGF and EGFR by immunofluorescence labelling of semi-ultrathin sections of epoxy resin-embedded specimens. We discuss our results in terms of the morphogenesis of filiform papillae on the rat tongue and the keratinization of the lingual epithelium in rats.

Materials and methods

Animals

Sprague–Dawley rats (SPF; Japan SLC, Hamamatsu, Japan) were used for all observations. Sixteen-week-old females were caged with breeding males of the same age and examined for vaginal plugs; the day that a plug was observed was considered to be the 1st day of gestation (E1). The gestation period of this strain of rats is approximately 21 days. Fetuses were removed on E12 and E16 from pregnant female rats after they had been killed by an intraperitoneal overdose of sodium pentobarbital (200 mg/kg body weight). Tongues were also removed postnatally from rats just after birth (P0) and on postnatal day 21 (P21). Each experimental