IMMUNOHISTOCHEMICAL EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGF-R) IN FETAL HUMAN OLFACTORY MUCOSA

Çiğdem ELMAS, M.S., Deniz ERDOĞAN, Ph..D., Candan ÖZOĞUL, Ph.D.

Gazi University Faculty of Medicine, Department of Medical Histology and Embryology, Ankara, Turkey
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SUMMARY

Purpose: *In this study, it was aimed to investigate the expression of EGF-R in different developmental stages of fetal olfactory epithelium and also to see the developmental changes in olfactory mucosa.* ***Method:*** *EGF-R expression was investigated immunohistochemically on human fetal olfactory mucosa, aged between 7 to 30 weeks.* ***Results:*** *It was demonstrated that EGF-R was present in adult and growing olfactory neurons, sustantacular cells, and basal cells. Immunoreactivity of these cells, however, was changing in different weeks of fetal growth.* ***Conclusion:*** *Our results demonstrate that human olfactory mucosa expresses EGF-R, which my help understand the development of olfactory mucosa.*

Key words: *EGF-R, Immunohistochemistry, Olfactory Mucosa.*

INTRODUCTION

Epidermal growth factor (EGF) has mitotic activity on epithelial and mesothelial cells (1,2). It increases ion uptake, glycolysis and synthesis of DNA and RNA in these cells (3). A sequence of events that end with DNA synthesis and mitosis occur in the EGF-stimulated cells . In the G1 phase of cell cycle, the cell collects all the knowledge from the environment and decides to grow or not (3,4). If EGF is present in the environment, the cell prepares itself physiologically and biochemically to grow, and divides after 10 to 24 hours .

EGF binds to its receptors on the cell surface called epidermal growth factor receptors (EGF-R) (4,5). EGF-R is related both structurally and functionally to transforming proteins (6,7). When EGF combines with its receptor on the cell surface, it activates the protein kinase area of the receptor, causing aggregation and subsequent phosphorylation (4).

EGF-R was studied in many different tissues but the publications relating EGF-R and olfactory mucosa were very few in number. Recently, olfactory mucosa gained an important role in diagnosis of degenerative illnesses due to the conformational changes in its structure (8,6). Therefore, we need more studies to explain the development of olfactory mucosa and its structure from embryo to adult. In this study, the expression of EGF-R in 7, 9.5, 17, 18, 23 and 30 weeks old human olfactory mucosa was investigated. The expression of EGF-R in the tissues may be important for a better

understanding of the development of olfactory mucosa.

Olfactory epithelium (OE) consists of sensory neurons projecting to the olfactory bulb, sustentacular cells, cytokeratin containing horizontal basal cells (HBCs) and cytokeratin lacking globose basal cells (GBCs) (9,10). In many studies, it has been reported that the cells within the GBC population give rise to neurons in OE (10, 11). Other cells in the normal OE continue to divide and act as transit amplifying cells. Olfactory receptor neurons continue to grow and regenerate until the last differentiation of GBCs occur.

Effects that regulate this event are not known in vivo, but in vitro studies recently showed that members of growth factor family including EGF-R, TGF-alpha and NGF are effective in the proliferation of these cells. This study focuses on the transforming growth factor-alpha (TGF-alpha) and nerve growth factor (NGF). We searched for the immunohistochemical expression of EGF-R on olfactory epithelium under light microscopy.

MATERIALS AND METHODS

A consecutive series of 7, 9.5, 17, 18, 23 and 30 weeks old human olfactory mucosas were obtained from spontaneous abortus material used for diagnostic purposes. Immediately after surgical removal, olfactory mucosas were fixed in 10% neutral formalin for about 72 hours. Then, they were dehydrated by insertion in increasing series of alcohol. After this, each sample was parafin embedded for conventional histological study. Cross sections (3-4 µm) were fixed on polilyzine covered slides. Each section of tissue blocks were deparaffinized with xylene and rehydrated. Endogenous peroxidase activity was blocked in 0.1 % hydrogen peroxide (Fisher Scientific, Melrose Park. IL) for 10 minutes and they were incubated with saponin to help easy binding of primary anticore to antigenic areas. Nonspecific labelling was blocked in serum blocking solution (20 minutes). Sections were incubated with EGF-R rabbit polyclonal antibody Ab-4 (100 µg/ml), diluted 1:20 in PBS (Oncogene Science, Manhasset, New York USA) over night at +4°C. Without washing, the secondary antibody, a 1% diluted biotin labeled anti rabbit total Ig, was applied for 30 minutes at room temperature. A negative control was revealed by the avidin-biotin-complex-peroxidase technique (ABC Histostatin, Zymed California, USA) using diaminobenzidine (DAB, Sigma) as chromogen. Afterwards, the slides were counterstained with hematoxyline for one minute, dehydrated in graded ethanol and mounted in a conventional medium. The intensity of the immunoperoxidase reaction was classified as follows: Negative (-); when the cells were devoid of any detectable EGF-R expression; slightly positive (+); moderately positive (++); strongly positive (+++). The entire case series was then evaluated by separating all the samples in two categories for EGF-R expression: Negative (absence of reaction) and positive (+,++,+++) Table 1.

RESULTS

Expression of EGF-R on olfactory mucosa of different embryonic weeks were examined immunohistochemically under light microscopy.

7 weeks fetal human olfactory mucosa:

OE was not completely differentiated and a single layer squamous epithelium was recognized. Laminae propria (Lp) had a mesenchimal appearance. Blood vessels were well developed and there were erythrocytes in their lumen in different stages of development. Glands were not differentiated. Mild immunoreactivity was defined on the apical membrane and the cytoplasm of OE. Immunostaining on the mesenchymal cells of Lp were weak. Immunoreaction on the erythrocyte varying from strong to weak was interesting. (Fig.1)

9.5 weeks fetal human olfactory mucosa:

Olfactory epithelium had a completely differentiated appearance. It was detected that differentiation from single layer squamous epithelium to epithelium pseudostratificatum occurs between the 7^{th} and 9^{th} weeks. A strong immunoreactivity was detected on the apical cell membrane and cytoplasm. Positivity of apical cell membrane in some areas was stronger than the cytoplasm. Connective tissue cells and the fibers on the Lp showed a mild immunoreactivity. A mild immunorection was also seen in the

Table-1 : Distribution of reactivity pattern in fetal olfactory mucosa.

Weeks	7	9.5	17	18	23	30
Pattern	**Reactivity with EGF-R**					
Apical cell membrane	++/+++	+++	+++	+++	+++	++
Apical cytoplasm	++	++	++	++ +	+++	++
Basal cells		+	++	++	+++	++
Fibroblasts		++	+++	+++	++	++
Gland cells		++	++ +	+++	+++	++
Mesenchymal cells	++					
Blood vessels	++	++	++	+++	+++	++
Erytrocytes	+++/+	+/++	+	+	+	+

Negative(-); when the cells were devoid of any detectable EGF-R expression; slightly positive (+); moderately positive (++); strongly positive (+++).

glands' cell cytoplasm (Fig 2a). Fig. 2b represents the negative control of this week.

17 weeks fetal human olfactory mucosa:

Strong membranous immunoreactivity was seen in OE. Mild positivity was detected on the apical cytoplasm. Immunostaining was decreased from apical to basal in OE. However a mild positivity was seen in the cytoplasm of basal cells (Fig.3a and 3b).

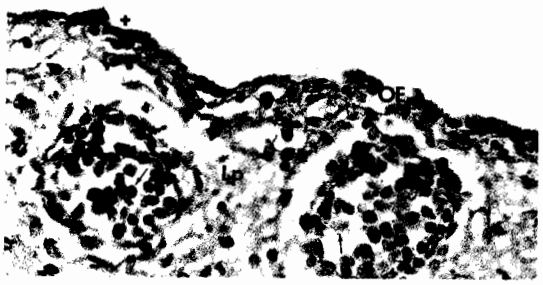

Fig. 1: (Immunperoxidase, Hematoxylin X 400) Nondifferentiated olfactory epithelium (OE) is seen in 7 week old fetuses. Laminae propria (Lp), apical membrane (+) and cytoplasm (*) of the OE are seen. Varying immunoreaction from strong to weak on the erythrocyte (►) is interesting.

18 weeks fetal human olfactory mucosa:

All structures in olfactory mucosa of 18 weeks old fetuses were identical with the adult shape. In the epithelium, beside a strong membraneous immunoreactivity, there was mild positivity recognized in all types of cell cytoplasm. Connective tissue stained weakly. A quite strong reaction was detected in the glands. Positivity of EGF-R was slightly stronger on the apical cell membrane. Despite the systematic immunoreaction on the olfactory epithelium, a weak immunostaining was detected in the fibers and fibroblast cytoplasm of Lp. (Fig. 4)

23 weeks fetal human olfactory mucosa:

The strongest membraneous reactivity was detected in this week. In addition, the positivity on the apical cytoplasm was mild. The immunoreactivity was decreased from apical to basal epithelium.

However, a mild positivity was detected on the basal cell cytoplasm. Negative immunostaining was seen in Lp. (Fig. 5)

30 weeks fetal human olfactory mucosa:

The most detectable feature of this week was the increased height of OE. The reactivity on OE was prevalent. But this reactivity was mild in comparison to other weeks. The reactivity of apical cell membrane was stronger but not detectable as in the other weeks. In the connective tissue, a weaker reactivity was observed on the cell cytoplasm and fibers (Fig 6).

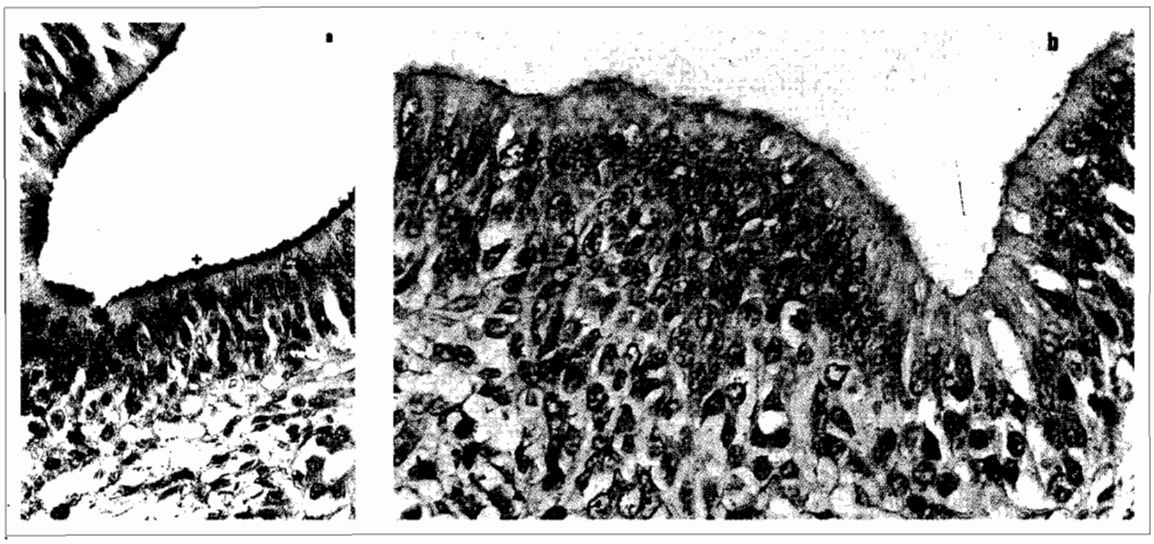

Fig. 2 : (a) (Immunperoxidase, Hematoxylin X 400) 9.5 week old olfactory mucosa is seen. Apical cell membrane (+), cytoplasm (*), and glands cell cytoplasm (➡) show different immunoreaction. (b) (Immunperoxidase, Hematoxylin X 400) No reaction is seen in negative control of this week.

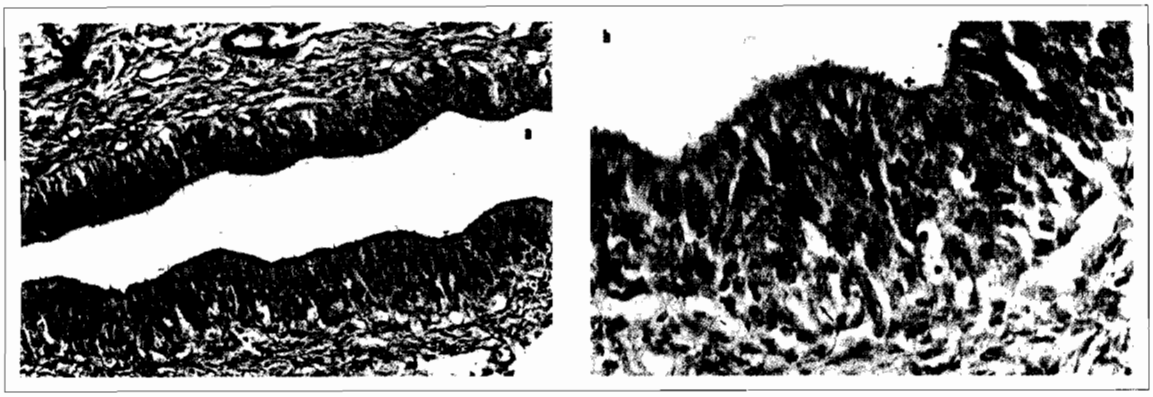

Fig. 3 : (a) (Immunperoxidase, Hematoxylin X 200) 17 week old olfactory mucosa is seen. Immunoreaction of strong apical membrane (+) and mild apical cytoplasm (➡) are seen. (b) (Immunperoxidase,Hematoxylin X 400) Mild (➡) to strong (+) staining in the olfactory epithelium of the same week with a higher magnification.

DISCUSSION

Olfactory receptor neurons are continuously replaced postnatally through the initiation of division and terminal differentiation of progenitor cells located in the basal layer of OE. Although the factors that regulate this process in vivo are not known, recent in vitro studies demonstrated that the members of the growth factor family including EGF and TGF-alpha, are highly potent in promoting the proliferation of progenitor cells, suggesting a role for the EGF-R, which is the molecular receptor for both mitogens (12).

Some investigators have identified EGF-R in olfactory cells and developing neurons. Ezeh et al. reported that TGF-alpha and EGF stimulated and regulated cell division in OE and TGF-alpha was a more potent activator of EGF-R than EGF (6). Mahanthappa et al. reported that EGF acted as a mitogen for the basal cells that give rise to olfactory neurons and that transforming growth factor- beta s (TGF-beta s)

Fig. 4 : (Immunperoxidase, Hematoxylin X 400) 18 week old olfactory mucosa is seen with strong membraneous (+) and mild positivity in all types of cell cytoplasm (→) Weak reaction is seen on connective tissue (→). Almost strong reaction is seen in the glands (double arrow).

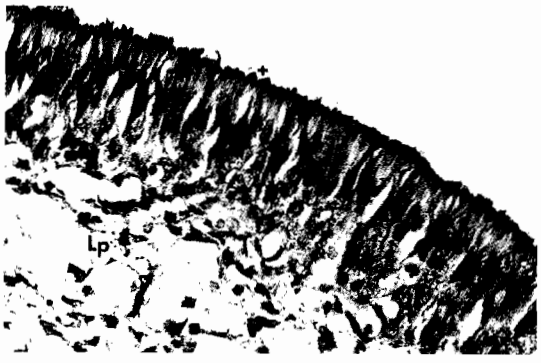

Fig. 5 : (Immunperoxidase, Hematoxylin X 400) Most stronger membraneous (+) and mild apical cytoplasm staining (*) are seen on the on 23 week old olfactory mucosa Mild positivity is also seen on the basal cell cytoplasm (→). Lp has no reaction.

promoted neurogenesis (13). This study supported their view on EGF-R. However we can not say anything about TGF-beta s, as it was absent in our study. In early weeks EGF-R expression was stronger in basal cell cytoplasm and it increased in 17, 18 and 23 week old fetuses. But in 30 week old fetuses reactivity in the basal cells were equal to other cell cytoplasms.

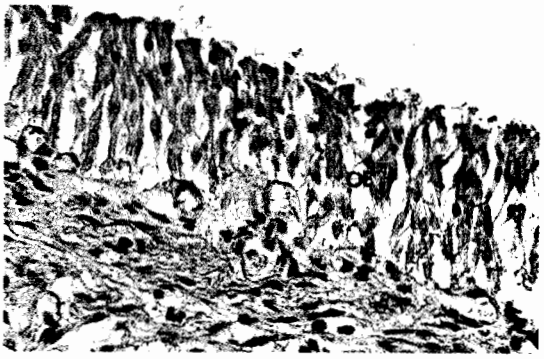

Fig. 6 : (Immunperoxidase,Hematoxylin X 400) Prevalent (↕) reactivity is seen on 30 week old olfactory mucosa (OE).

Immunohistochemical results obtained by Farbman et al. on adult rat olfactory epithelium showed that basal cells, supporting cells and acinar cells of Bowman's gland were immunoreactive with antibody to TGF-alpha but not with antibody to EGF (7). However, in our study in fetal human olfactory mucosa, we found that all of these cell types had shown strong EGF-R expression. Especially, basal cell and gland cell cytoplasms showed increasing immunoreaction from early weeks to further weeks of development. On the other hand, Balboni et al. have reported that a positivity for EGF-R on human OE, increasing with age was detected in the apical portion of the sensory epithelium (14). The results of our study generally agree with Balboni's findings in OE during the perinatal period. According to Salehi-Ashtian et al., EGF receptor and TGF-alpha were detected primarily in horizontal basal cells but rarely in globose basal cells, suggesting EGF-R is not likely to be the mitotic regulator of sensory neurons (15). In another study in adult brain regions, while both EGF and TGF-alpha mRNA's were detected in all regions, highest regional EGF-R concentrations were observed in the olfactory bulb, basal hypothalamus and cerebellum (16). Our study only included olfactory mucosa and we saw that the whole structure of this mucosa, especially cells of epithelium, glands and blood vessels have shown highest EGF-R concentrations where as connective tissue cells had lesser concentrations.

In this study it was shown that EGF-R was expressed in the early postmitotic period and increased in fully mature olfactory cells. Its expression period indicates that it starts the cell growth and differentiation. In relation to its mitotic activitiy, EGF-R showed strong immunoreactivity on the basal cells and the strongest activity was recognized on the apical membrane of the cells. That is the reason for EGF and EGF-R to bind there.

Correspondence to: Dr. Çiğdem ELMAS
Turgutreis cad. 72 / 4
Maltepe
06570 ANKARA - TÜRKİYE
Phone : 312 229 41 00
E-mail: diamondcigdem@hotmail.com

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