IMMUNOHISTOCHEMICAL LOCALIZATION OF THE EPIDERMAL GROWTH FACTOR RECEPTOR IN NORMAL MOUSE TISSUES

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SUMMARY

***Purpose** : The levels of epidermal growth factor (EGF) receptors were investigated in normal mouse tissues. **Methods** : PAP method was used for immunstains. Intensity of immunohistochemical staining was subjectively estimated. **Results** : Two patterns of reactivity were recognized; strong membraneous or diffuse cytoplasmic staining. The basal epithelial cells of the skin and the collecting tubule cells of the kidney showed membraneous reactivity whereas the bronchial epithelial cells, parietal cells of the stomach and tubular cells of the kidney showed cytoplasmic reactivity predominantly with the antibody. **Conclusion** : The distribution of the epidermal growth factor receptors in different cell types and cellular compartments may implicate varying activation states of the growth factor and its interaction with a milieu available ligand.*

***Key Words:** Epidermal Growth Factor-Receptor, Mouse Tissue, Immunohistochemistry, Expression.*

INTRODUCTION

Epidermal growth factor (EGF) was originally described as a substance isolated from the mouse salivary glands which initiated premature eyelid opening and incisor eruption when injected into the neonatal mouse (1). These physiological events are the net results of the proliferation and differentiation of the cells on EGF- specific receptor molecules at the cell surfaces (2, 3). EGF - EGF receptor interactions are a multistep process that can be controlled by the ligand that is biologically active in optimal concentration, and by expression of the receptor on the surface of some specific cell types (4).

Binding of EGF to the EGF receptor can initiate a mitogenic response (5, 6), but the mechanism of intracellular signalling is not certain. EGF binding activates the tyrosine protein kinase which results in the phosphorylation of the receptor and other cellular substrates (7-9).

Receptor ligand complexes cluster within clathrin-coated pits, are endocytosed following accumulation in endosomes, and can than be delivered to another subcellular compartment, presumably the lysosome, where they are both degraded (10-12). Before binding to the ligand, EGF receptor should be expressed on the cell surface, and this process is controlled at various levels (4).

EGF receptor expression in normal tissue has been investigated by both analysis of the specific binding of labelled EGF and the receptor- specific

monoclonal antibodies. By comparing the distributional disparities that arise from combined use of these methods, the location and activity of the receptor can be deduced. Analysis of the cellular site of receptor localization can also provide information regarding the activity of the receptor. In this study, we examined the distribution of the EGF receptor expression immunohistochemically in mouse tissues.

MATERIALS AND METHODS

Preparation of tissue samples

Male Swiss albino mice (n=4),weighing approximately 30 g were fixed with 4 % paraformaldehyde by cardiac perfusion. After perfusion for 10 min, the mice were further perfused with 10 % neutral formaline for 5 min. Thereafter tissues were removed. The stomach, lung, liver, kidney, testis, thymus, lymph node and skin of the mice were sampled and postfixed in 10 % neutral formaline for 24 h. After processing the specimens, they were embedded in paraffin. 4-5 μm thicksections were used for stainings.

Antibodies and staining procedure

PAP method was used for immunstains. The monoclonal antibody (Sigma anti mouse EGF-R) was diluted (1:200) and performed for 60 min at room temperature. After washing in 0,01 M phosphate buffered saline (PBS,pH:7.4), the slides were covered with rabbit anti-mouse Ig which was labeled with peroxidase and diluted (1:200) (CBL) in PBS containing 0.2 % BSA and normal human serum. After washing in PBS the slides were stained with 3,3'-diaminobenzidine - tetrahydrochlorid (DAB, Sigma) containing 0.01 % H2O2 for peroxidase activity. Counter staining was done with hematoxylen. Negative control staining was run by ommitting the first (primary antibody) step (13).

Intensity of immunohistochemical staining was subjectively estimated and expressed as negative (-), questionably or weakly positive (+/-), definitively positive (+) or very strongly positive (++).

RESULTS

The distribution of the EGF-R in the mouse tissues is shown in table-1.

Two reaction patterns were recognized: membraneous or diffuse cytoplasmic staining. Membraneous staining pattern was observed in the basal layer of the skin. The epithelial cells of proximal and distal tubules of the kidney showed an intense diffuse cytoplasmic reactivity and

	Reactivity with EGF-R	
Tissue	**Intensity**	**Pattern**
Skin (Epidermis)	+	Membraneous in basal layer
Lung		
Branchial epithelium	++	Diffuse cytoplasmic
Alveolar epithelium	++	Diffuse cytoplasmic
Stomach (Fundus)		
Mucus secreting cells	–	–
Gastric glands	–	–
Parietal cells	+	Diffuse cytoplasmic
Kidney		
Glomerula	-	–
Proximal tubules	++	Diffuse cytoplasmic
Distal tubules	++	Diffuse cytoplasmic
Collecting tubules	++	Diffuse in luminal half of the cytoplasm
Liver		
Hepatocytes	-/+	Diffuse cytoplasmic
Bile duct cells	-/+	Membraneous
Testis	–	
Lymph node	–	
Thymus	–	

Table - 1 : Distribution of EGF receptors in normal mouse tissues.

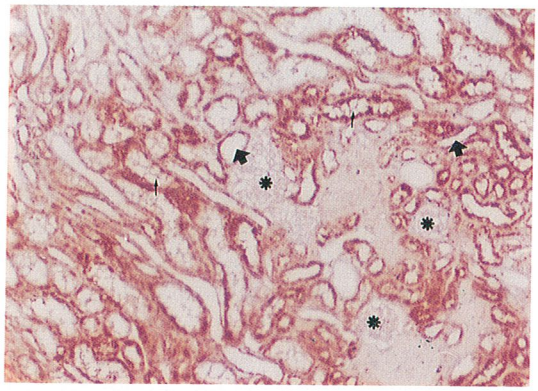

Fig. 1 : Immunohistochemical staining for EGF-R in mouse kidney. The cells of proximal (thin arrows) and distal tubule (thick arrows) stained diffuse cytoplasmic. Glomerula did not stain (*). (Immunoperoxidase; Hematoxylen, X 120).

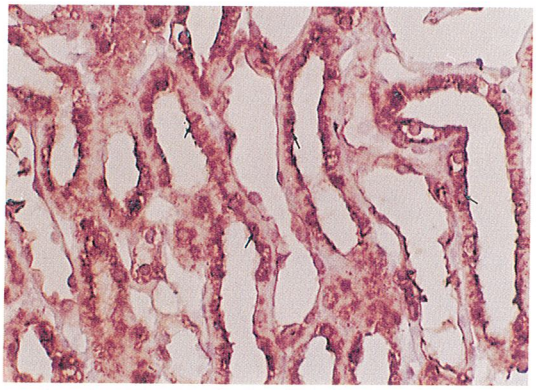

Fig. 3 : Mouse kidney showing positive immunoreaction in the collector tubules following incubation with anti-mouse EGF-R antibody. The supranuclear portion of the cytoplasm showed diffuse cytoplasmic reactivity (thin arrows) (Immunoperoxidase; Hematoxylen, X 480).

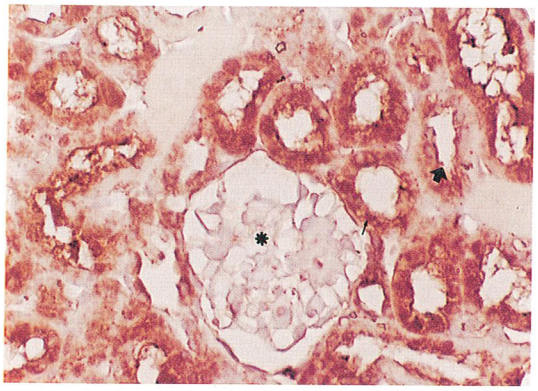

Fig. 2 : Immunohistochemical staining for EGF-R in mouse kidney. The cells of proximal (thin arrows) and distal tubule (thick arrows) stained diffuse cytoplasmic. Glomerula did not stain (*). (Immunoperoxidase; Hematoxylen, X 480).

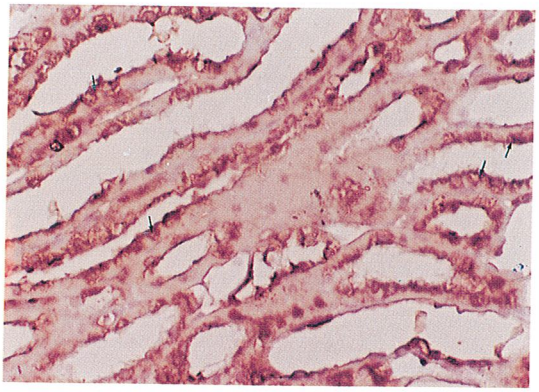

Fig. 4 : Mouse kidney showing positive immunoreaction in the collector tubules following incubation with anti-mouse EGF-R antibody. The supranuclear portion of the cytoplasm showed diffuse cytoplasmic reactivity (thin arrows) (Immunoperoxidase; Hematoxylen, X 480).

glomerula did not stain (Fig. 1, 2).

Diffuse cytoplasmic staining was seen in luminal half of the collecting tubules of the kidney (Fig. 3, 4).

Diffuse cytoplasmic reactivity was observed in the bronchial epithelium (Fig. 5).

Coarse diffuse dense cytoplasmic reactivity was also found in the parietal cells of the stomach (Fig. 6).

In the liver, all hepatocytes and the bile duct cells showed weak positivity. Testis, thymus and lymph node cells were found to be negative.

In the negative materials control, no specific staining was observed.

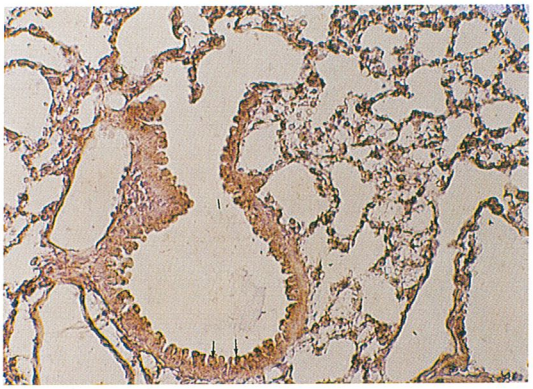

Fig. 5 : Immunoperoxidase staining of lung by anti-mouse EGF-R. The branchial and alveolar epithelium are indicated. The branchial epithelium showed intense diffuse cytoplasmic immunoreactivity (arrows). (Immunoperoxidase; Hematoxylen, X 240).

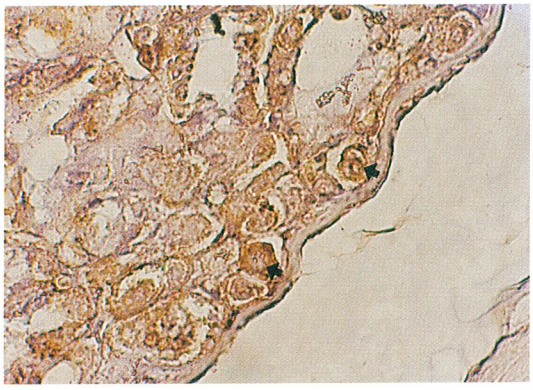

Fig. 6 : In the parietal cells of the stomach appeared diffuse dense cytoplasmic reactivity (arrow). (Immunoperoxidase; Hematoksilen X 480).

DISCUSSION

In the present study, EGF receptor expression in normal mouse tissues has been investigated by analysis of receptor- specific monoclonal antibodies.

We observed that epithelial and parietal cells of the stomach, the bronchial cells the proximal, distal and collecting tubular cells, expressed receptor in their cytoplasm. Localization of EGF receptor in the cytoplasm may reflect internalization of the receptor, a rapid process that occurs after ligand binding (10, 12). Rapid degradation of the internalized receptor was shown in a mast cell culture system (10). However, it is suggested that some of the internalized receptor in EGF treated hepatocytes (11, 14) may recycle to the cell surface. Intracellular receptor localization also represent the newly synthesized molecules in the synthetic machinary of the cells prior to their insertion into the cell membrane. Internalized receptor may also represent post-translationally modified receptor; phorbol ester-treated cells reportedly internalize receptor into a cellular compartment from which it may recycle to the cell surface (15, 16). Investigation of the EGF receptor in the isolated perfused rat liver has revealed a large pool of latent receptor with low affinity for EGF (11, 14). Although cell fractionation studies localized this low affinity class of receptors as a distinct population of intracellular vesicles, all of the immunohistochemically detected receptor was at the cell surface (11). Since ligand affinity and cellular localization can be rapidly changed by receptor phosphorilation (15-17), one could hypothesize that a rapid flux between latent internalized and high-affinity membrane localized receptor could be induced by local stimulation. Damjanow et al. reported that the majority of the receptors is in the internalized form in the cells of mice tissues (4).

In addition, we detected EGF receptor expression on the cell surface in the basal epithelial cells of the skin and the collector tubule cells of the kidney. Especially in the skin, the cells that continue to proliferate express the EGF receptor, while cells of the stratified cornified epithelium do not. Thus, we propose that receptor expression on the cell surfaces a consequence of differentiation, may be one of the mechanisms that regulates the EGF inducible proliferation of cells in a particular tissue that exposed to a constant source of growth factor.

Lack of detection of EGF receptor on the membrane by immunohistochemical methods cannot be interpreted as total absence of EGF receptor.

EGF is mainly synthesized the submaxillary glands in rats and mice. But prepro-EGF (the EGF precursor) messenger RNA (mRNA) is surprisingly abundant in the kidneys (18). Thus, EGF plays a number of important roles in renal

physiology. Recently, EGF was found to be important in initiating controlateral renal hyperthrophy in uninephrectomized mice (19, 20). In this study, presence of EGF receptor is determined at significantly high levels in the proximal, distal and collector tubules. In other words, this finding reflects that EGF has the ability to induce mitogenesis in the renal tubule cells in mouse kidney under normal conditions.

We observed three distinct patterns of EGF receptor in various epithelia. In the first group, the cells were not expressing EGF receptor at probably immunohistochemically detectable levels.

The second pattern, a strong membraneous and also cytoplasmic expression, was observed in actively proliferating cells like the basal layers of stratified epithelia.

The third pattern predominantly cytoplasmic expression was observed in certain epithelia like kidney tubule reflecting the intensity of internalized receptor and/or newly synthesized molecules.

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