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The Role of Epidermal Growth Factor (EGF)/Transforming Growth Factor (TGF)-α in the Development of Peptic Ulcer Disease, Malignant Tumors and Malignant Ascites.

Eiji Ohmura M.D.

Department of medicine I, Saitama Medical School, Saitama Medical Center, 1981 Tsujidoh-cho, Kamota, Kawagoe-city, Saitama.
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Growth factors are proteins that modulate cell proliferation and other cellular functions. Many of these factors are polypeptides, and they include epidermal growth factor (EGF) and transforming growth factor (TGF)-α. EGF and TGF-α are members of EGF family, which also contains heparin-binding EGF-like growth factor (HB-EGF), amphiregulin and betacellulin (1, 2). The mature 53-amino-acid EGF is released from the 1,217 amino acid transmembrane precursor by proteolytic cleavage steps. TGF-α mRNA encodes a 160-amino acid transmembrane precursor which contains the mature 50-amino- acid TGF-α. The amino acid sequence homology between EGF and TGF-α is 35%. They both bind to the glycosylated 170-kDa cell surface membrane receptor, and their biological activities are exerted through this receptor. EGF is mainly synthesized by the salivary glands and the kidney (1). TGF-α, on the other hand, was initially thought to be produced by transformed cells and embryonic tissues. However, surveys of TGF-α mRNA expression and protein localization have shown widespread distribution of TGF-α in normal cells and tissues, including activated macrophages, keratinocytes, mammary epithelial cells, the anterior pituitary and most gastrointestinal cells and tissues (2).

This report summarizes our studies, which have focused on the role of EGF and TGF-α in the development of diseases, such as peptic ulcer disease, malignant tumors and malignant ascites.

Key words: EGF, TGF-α, peptic ulcer diseases, cancer, ascites

(1) Salivary immunoreactive human epidermal growth factor (IR-hEGF) in peptic ulcer patients

It has been shown that EGF, which inhibits gastric acid secretion and stimulates DNA synthesis in the gastric mucosa of the rat, is localized in the submandibular glands and secreted into saliva. Subsequent in vivo studies have revealed that EGF applied topically has a cytoprotective effect on the rat gastric and duodenal mucosa, suggesting that salivary EGF plays a pathophysiological role in the development of gastroduodenal lesions (3, 4). We therefore investigated the difference in salivary levels of EGF in normal subjects and patients with gastroduodenal lesions (5). The results show that the concentration of hEGF was lower in patients in the active and healing stage of peptic ulcer disease than in normal subjects (Table 1). There were no significant differ-

Table 1. IR-hEGF levels in saliva in normal subjects and patients with gastro-duodenal diseases.

Saliva	IR-hEGF (ng/ml, $\bar{m}\pm SE$)	
Normal Subjects	3.19±0.46	n=47
Atrophic or superficial gastritis	2.31±0.34	n=28
Peptic ulcer		
┌active stage	0.96±0.26*	n=4
├healing stage	1.06±0.24**	n=8
└scaring stage	2.40±0.42	n=21
Gastric cancer	2.44±0.27	n=21

ences in salivary EGF levels between normal subjects and patients in the scaring stage of peptic ulcer disease, or those with gastric cancer and atrophic or superficial gastritis. Similar results have also been reported by other investigators (6). Thus, it is speculated that in subjects whose hEGF levels in saliva are low, the resistance of the gastric or duodenal mucosa to physicochemical stress may decrease and such condition could promote the development of peptic ulcer diseases. Very recently Playford et al. showed that EGF is cleaved into smaller, less active forms in acidic (pH<4) gastric juice (7). For this reason, the increases in the pH of gastric juice in response to medical therapy may promote the healing of gastric injury by EGF.

On the other hand, recent reports suggest that TGF-α produced locally in the normal gastric mucosa inhibits gastric acid secretion and stimulates proliferation of the mucosa (8), implying that TGF-α mediates repair of acute gastric injury.

(2) The roles of insulin-like growth factor (IGF)-I and TGF-α in human pancreatic cancer cell growth

It is generally assumed that the autonomous growth of tumor cells is maintained by growth factors secreted by tumor cells themselves and/or by the abnormal activation of intracellular signaling pathways which is normally initiated by the growth factors. Such activation of the signaling system is commonly caused by the activated oncogenes or inactivated tumor-suppresser genes, such as ras, myc, p53, Rb genes.

We have demonstrated factors involved in the proliferation of human pancreatic cancer cells (MIA PaCa-2;PC cells) (9). PC cells grew autonomously even in serum-free medium (0.3% bovine serum albumin), and serum-free conditioned medium from PC cells had a stimulatory action on the growth of the cells, implying that a factor (s) produced and released by PC cells stimulates their own growth. Immunoreactive (IR)-IGF-I and IR-TGF-α were detected in the serum-free conditioned medium of PC cells. The specific binding sites for these growth factors were found in PC cells, and authentic IGF-I and TGF-α stimulated PC cell growth. These findings suggest that IGF-I and TGF-α act on PC cell growth as autocrine factors. Similar mechanisms are found in thyroid cancer cells (10). The relationship between the overproduction of these growth factors and the abnormal genes (i.e., activated oncogenes and/or inactivated tumor suppressor genes) should be clarified.

(3) Induction of ascitic fluid by EGF and TGF-α in mice.

The precise mechanism for the development of peritoneal or pleural effusions associated with malignancy is unknown. However, it has been suggested that certain mediators produced by the tumor cells induce the malignant ascites (11). It has been well established that malignant cells produce a number of peptide growth factors, including TGF-α(2, 12). TGF-α has also been found to be present in ascitic fluid and pleural effusion specimens obtained from patients with malignant tumors (13). On the other hand, we were surprised to find that EGF administered subcutaneously by an osmotic minipump induced fluid retention round the minipump in rats (unpublished observation). Since EGF and TGF-α are thought to bind to the same receptor, it is speculated that the TGF-α produced by tumor cells is involved in the formation of abnormal fluid. We therefore studied the ability of EGF/TGF-α to produce ascites in mice (14).

Peritoneal administration of EGF (10-40 μ

Table 2. Effects of EGF and TGF-α on ascites production in mice.

hEGF/TFG-α added (μg/mouse/week)		Ascites (ml, $\bar{m}$±SE, n=6)
Experiment 1		
Vehicle		0.05±0.01
hEGF,	12.5	0.86±0.18*
	25	1.41±0.26*
	50	1.51±0.10*
Experiment 2		
Vehicle		0.07±0.01
TGF-α,	12.5	0.67±0.12*
	25	1.28±0.20*
	50	5.11±0.88*

Mice were administered with EGF or TGF-α intraperitoneally by the osmotic minipump, and volumes of ascitic fluid were measured one week after. (* ; $p<0.01$ v.s. control)

g/mouse/week) or TGF-α (10-40 μ g/mouse/week) by osmotic minipumps resulted in the formation of bloody ascites, which was similar to malignant ascites (Table 2). The amount of ascites produced was dependent on the dose of the growth factors. Vehicle (mouse serum albumin) alone or insulin-like growth factor-I (40 μ g/mouse/week) had no effect. Thus, it is possible that peritoneal effusion associated with disseminated tumor is, at least partly, due to TGF-α produced by the tumor cells. The activity of TGF-α for neo-vascularization and/or increased vascular permeability may be related to the action of the growth factor. In addition to TGF-α, vascular permeability factor (VPF), a tumor -secreted heparin-binding growth factor responsible for increased vessel permeability and microvascular angiogenesis, may also be involved in the formation of pleural or peritoneal effusions associated with malignancy.

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