Effects of epidermal growth factor on the growth of human gastric cancer cell and the implanted tumor of nude mice

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INTRODUCTION

Growth factors are found in a variety of adult and embryonic tissues. They are important regulators of cell differentiation and proliferation, and play an important role in maintaining the integrity of the epithelium. They have also been implicated in malignancy. Epidermal growth factor (EGF), a single-chain polypeptide of 53 amino acid residues, is found mainly in the submandibular glands and Brunner’s gland of the gastrointestinal tract. It can be combined with the specific receptor (EGF-R) of the target cell membrane[1]. Some studies suggested that the expression of EGF-R was increased in gastric cancer tissue. It was also reported that EGF can increase the mitosis in vitro[2]. Patients with EGF receptor-positive gastric cancer may have a poorer prognosis than those with EGF receptor-negative cancers. So, EGF has the function to influence the tumor cell growth. At present, the effect of EGF in this process has been unclear yet.

In this report, we seek to determine the effect of EGF on the growth of human gastric cancer cell (MKN-28, SGC-7901 and MKN-45) in vitro. In nude mice which underwent surgical implantation of the same gastric cancer cells, EGF was injected intraperitoneally to investigate the influence of EGF on tumor cell growth, so as to confirm the safety of EGF in the treatment of peptic ulcer[3-14].

MATERIALS AND METHODS

Materials

Gastric cancer cell lines, MKN-28, SGC-7901 and MKN-45, are well-differentiated, moderate-differentiated and low-differentiated human adenocarcinoma cell lines respectively. 3T3 cell is normal human epithelial cells 3T3 were assessed when incubated with recombinant human EGF (rhEGF, 0.05, 0.1, 0.5, 1.0, 10, 50, 100μg.L-1) using MTT method. The cells of MKN-28, MKN-45, SGC-7901 (gastric cancer tissue 1.5mm3) were implanted in the BALB/cA nude mice for 10 days. The EGF was given intraperitoneally (15, 30, 60μg.kg-1) for 3 weeks. The body weights of the tumor-bearing animals and their tumor mass were measured afterwards to assess the mitogenic effect of rhEGF in the nude mice.

RESULTS: Within the concentration range of 0.05-100μg.L-1, rhEGF could increase the cell growth of normal 3T3 cells (cell growth rate 100% vs 102.8%, P<0.05), but partially restrain the gastric cancer cell growth. The latter effect was related to cell differentiation. In 15-60μg/kg rhEGF groups, the mean implanted tumor mass of MKN-28 cell were 1.75g, 1.91g, 2.08g/NS group 1.97g (P>0.05), the mean tumor mass of SGC-7901 cell were 1.53g, 1.07g, 1.20g/NS group 1.07g (P>0.05), and for MKN-45 cell, the tumor mass were respectively 1.92g, 1.29g, 1.77g/NS group 1.82g (P>0.05). So rhEGF had no obvious effect on implanted MKN-28, SGC-7901 and MKN-45 tumor growth.

CONCLUSION: EGF has no stimulating effect on the human gastric cancer cell growth neither in vitro nor in vivo.

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positive control. Experimental controls were treated with DMSO only. **Tumor implantation into nude mice** Gastric cancer tissue (1.5mm³) were implanted s.c. in the right dorsal area of 4-6wk old male nude mice. Animals were fed with an autoclaved diet and tap water (acidified to pH 2.5). After 10d, the animals were assigned into the rhEGF treatment groups (15,30,60µg.kg⁻¹, intraperitoneally, 5 times per week for 3wk), negative control group (saline, 2ml intraperitoneum) and positive control group (MMC, 2mg.kg⁻¹, twice every week, 6 times altogether). The body mass of Balb/c tumor-bearing animals and their tumor weights were measured using anesthesia with ether.

Inhibitory rate (IR) of tumor growth = \( \frac{m(tumor)_C - m(tumor)_T}{m(tumor)_C} \) (\( m(tumor)_C \): mean tumor weight of negative control group; \( m(tumor)_T \): mean tumor weight of rhEGF treatment group).

**Statistical analysis**

Student’s *t* test was performed to assess potentially significant differences between individual groups of observations. The test statistics were then compared with values obtained from standard two-tailed tables. A *P* value of <5% was accepted as indicating probable significance when comparing the various groups.

**RESULTS**

**Mitogenic effects of EGF in vitro**

We found that EGF had no significant growth-stimulatory effects on gastric cancer cells in a dose-dependent manner (Figure 1). The lowest cell growth rates in MKN-28, S-7901 and MKN-45 cell lines were 81.7%, 80.7% and 86.1% respectively, compared with the positive control group. EGF could inhibit the cancer cell growth within the level of 0.05 to 100µg.L⁻¹. But there was no probable significance within the same group. In contrast, for the normal 3T3 cells, EGF could increase the cell growth significantly after the coincubation (*P*=0.0008). We also found that the influence of EGF on the gastric cancer cell growth was dependent on the differentiation of the cell. Under the same concentration, the inhibition was greater in well-differentiated cells.

**DISCUSSION**

We have examined the effect of EGF on the established cell line, MKN-28, SGC-7901 and MKN-45, derived from human gastric adenocarcinoma, both in vitro and in vivo. The results may be somewhat controversy to those formerly reported, that EGF had no obviously effect on the gastric cancer growth[15,16]. Growth factors are components of signal transduction pathways that have a considerable spectrum of biological activity, such as control of cell proliferation, differentiation, apoptosis and transformation[17,18]. Of these growth factors, EGF family are important agents for gastric mucosa. The EGF family include at least seven mammalian polypeptides: EGF, TGF-α, amphireguin (AR), cripto heregulin, betacellulin and heparin-binding epidermal growth factor (HB-EGF). Except cripto and heregulin, all of these proteins have been shown to bind and activate the 170-kilodalton EGF receptor tyrosine kinase[19,20]. They share a similar spectrum of biological activities exerted through interaction with EGF-R. EGF-R is a transmembrane glycoprotein, which can stimulate cell proliferation mainly through induction of the proto-oncogenes c-fos and c-myc, and of molecules such as polyamines.

The TGF-α can cause morphological transformation and promote anchorage independent growth in vitro. Although there is no evidence of TGF-α secretion from nonneoplastic adult tissue, it is synthesized during fetal development and produced by many tumor tissues[21,22]. TGF-α is frequently produced by malignant as well as normal cells and may stimulate their own proliferation. However, less is known about the role of EGF in oncogenesis[23-25]. The importance of growth factors in the healing and oncogenesis of gastrointestinal diseases has recently received much attention. In inflamed mucosa, EGF is found primarily in the cytoplasm of the superficial epithelial and submucosal layers.
cells, as in the normal mucosa[26]. In addition to providing a mitogenic stimulus, EGF may also help the proliferating cells to migrate into the superficial epithelium during the process of “cytoprotective” epithelial repair[27].

The development of monoclonal and polyclonal antibodies against EGF has allowed studies of the localization of EGF in normal and neoplastic tissues to be performed[28-31]. Immunocytochemical staining has shown distribution of epidermal growth factor and transforming growth factor α(TGF-α) in the gastrointestinal tract with high levels[32-33]. Normal epithelial cells secrete such growth factors to regulate cell replacement by autocrine or paracrine mechanisms. It is speculated that these growth factors may regulate the transition rate between G2-phase and mitosis of the cell cycle[34]. It has reported that HB-EGF is mitogenic for some types of cells, such as fibroblasts, vascular smooth muscle cells, keratinocytes and rat hepatocytes, but not endothelial cells[35].

The mitogenic action of EGF and TGF-α in vitro has been reported in many gastrointestinal tissues, including esophagus, stomach and intestine, and there is little information about the association between the mucosal expression of these peptides and indices of cellular proliferation in vivo[36]. It was reported that EGF immunoreactivity was present in 26-37% of gastric cancers, and the presence of EGF in gastric cancer correlated with the degree of gastric wall invasion, lymph node metastasis and disease progression[37-39]. Although the epidermal growth factor/receptor system has been found abnormal in intestinal type gastric cancer, overexpression of EGF-R, erbB-2 and erbB-3 receptor genes was mainly found. There has been some controversy in the literature whether EGF-R overexpression related to tumor progression or to early stages of gastric carcinogenesis[40-41]. The study had shown that overexpression of the EGF-R gene was infrequent in the metaplastic gastric mucosa. A major problem in gastric carcinogenesis is to determine the changing point from benign pre-neoplastic lesions to malignancy. There is a general agreement that this process involves different steps in cellular changes, requiring both activation and inhibition of specific genes, but there is still no evidence to support EGF or EGF-R overexpression to be a reliable marker of increased cancer risk in patients[42-44]. The present study has sought to clarify their effect on the growth of gastric cancer cell in vitro and in vivo. In this study, we have found that there was no effect of EGF on the growth of established cell lines, MKN-28, SGC-7901, MKN-45, derived from human gastric adenocarcinoma, both in vitro and in vivo. Further study is headed to elucidate whether EGF could cause abnormal differentiation of the cells during the treatment of peptic ulcer for a long period.

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