Abnormal expression of growth regulatory factors in Barrett's oesophagus

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SUMMARY

1. In order to assess potential abnormalities in the control of mucosal proliferation, 30 patients with Barrett's oesophagus were studied in order to evaluate the presence and distribution of epidermal growth factor, transforming growth factor-α and epidermal growth factor receptor to determine the Ki-67 labelling index in the affected oesophageal mucosa. Serial sections were analysed immunohistochemically. Ten of the patients had adenocarcinoma in the Barrett's mucosa and the other 20 had differing histological types of Barrett's mucosa (10, intestinal-type; 10, fundic- or cardiac-type).

2. The expression of transforming growth factor-α, epidermal growth factor and epidermal growth factor receptor was increased and the Ki-67 labelling index was higher in Barrett's mucosa compared with normal gastric mucosa. The 'intestinal-type' of Barrett's mucosa had the greatest expression of transforming growth factor-α, epidermal growth factor receptor and the highest Ki-67 labelling index compared with the other types of Barrett's metaplasia. Five cases of 'intestinal-type' Barrett's metaplasia had especially high Ki-67 labelling indices and these patients over-expressed both transforming growth factor-α and epidermal growth factor receptor. The patients with adenocarcinomas in the Barrett's mucosa also over-expressed transforming growth factor-α and epidermal growth factor receptor, but not epidermal growth factor, compared with normal gastric mucosa.

3. In conclusion, both normal gastric mucosa and Barrett's mucosa have potential autocrine growth regulatory mechanisms, but Barrett's mucosa has increased expression of both of the measured ligands and of the epidermal growth factor receptor.

Key words: Barrett's mucosa, cell proliferation, epidermal growth factor, epidermal growth factor receptor, transforming growth factor.

Abbreviations: EGF, epidermal growth factor; EGF-R, epidermal growth factor receptor; TGF-α, transforming growth factor-α.

INTRODUCTION

In 1950, Barrett [1], in his paper "Chronic peptic ulcer of the oesophagus and 'oesophagitis'", drew attention to a condition in which the lower portion of the oesophagus was lined by a columnar epithelium. Subsequently, it was recognized that Barrett's mucosa has a 30-40-fold increased risk of adenocarcinoma compared with the normal oesophagus [2-5]. As a result, many attempts have been made prospectively to assess these lesions for malignant potential [6-9]. Unfortunately, none of the available screening procedures have had any documented impact on the prognosis of this disease [2, 4, 5, 10-12]. This is, in part, because the events that control cellular proliferation, differentiation and oncogenesis in the oesophagus have until recently been poorly understood. The importance of growth factors [for example, epidermal growth factor (EGF) and transforming growth factor-α (TGFα)] and their receptor [epidermal growth factor receptor (EGF-R)] in controlling cellular growth has been documented in other gastrointestinal tissues [13-15].

EGF and TGFα are part of the EGF family because they have three common characteristics: high-affinity binding to EGF-R, production of mitogenic responses in EGF-sensitive cells, and a similar primary structure containing six half-cystine residues [16, 17]. It is believed that EGF and TGFα exert their oncogenic effects primarily by induction of intranuclear c-fos and c-myc genes, which are 'proto-oncogenes' [18-20], but many other potential oncogenic proteins are also stimulated, including polyamines [10].

EGF-R is a transmembrane glycoprotein with intrinsic tyrosine kinase activity [21]. The truncated receptor has sequence homology with the product of the c-erbB2 oncogene. Over-expression of EGF-R in tissues has been detected in human carcinomas, including oesophageal, colonic and breast cancer [22-26].
sought to determine the presence and distribution of 
EGF, TGFα and EGF-R in Barrett's mucosa in an 
attempt to define the role of these growth factors in 
the development of oesophageal cancer. This study has 
therefore assessed: (1) the expression of EGF, TGFα and 
EGF-R in Barrett's mucosa and adenocarcinomas arising 
from Barrett's mucosa; and (2) cellular proliferation by 
using the Ki-67 labelling index [27].

METHODS

Patients

Thirty patients with histologically proven Barrett's 
columnar-lined oesophageal mucosa, who were attending 
an annual endoscopic follow-up screening programme, 
were studied. Eighteen were women and 12 were men, 
with a mean age of 65 years (range 32–74 years).

Biopsies and immunohistochemistry

The patients were endoscoped and biopsies were taken 
from areas of columnar (Barrett's) mucosa and any areas 
of frank mucosal neoplasia. In addition, all patients had a 
biopsy taken from benign cardiac gastric mucosa [28].

Three immediately adjacent biopsies were taken from 
each oesophagus (and gastric cardia). The biopsies were 
snap-frozen in the endoscopy room, and 6 μm cryostat 
sections were cut. Four serial sections were stained 
immunohistochemically to demonstrate EGF, TGFα, 
EGF-R and the Ki-67 labelling index. The antibodies 
were supplied by Oncogene Science (Manhaset, NY, 
U.S.A.); all were IgG monoclonal antibodies which had 
specific binding to their substrate (no cross-reactivity of 
EGF with TGFα or vice versa) [29]. The antigen–anti-
body reaction was visualized by the modified strepavidin 
technique [30].

The biopsies were classified according to the type of 
metaplasia [2] and dysplasia [28]. Mucosa with fundic- 
or cardiac-type metaplasia were grouped together 
because they have a low risk of malignant transformation, 
unlike intestinal-type metaplasia [2, 28]. Therefore three 
categories of Barrett's mucosa comprised: (i) fundic- 
or cardiac-type Barrett's mucosa, (ii) intestinal-type Barrett's 
mucosa, and (iii) adenocarcinoma. In these categories 
mucosa demonstrating dysplasia was noted separately.

Positive EGF and TGFα staining was graded according 
to criteria which we developed. Five randomly viewed 
areas of at least 200 consecutive nucleated epithelial cells 
were independently assessed by two observers. The 
sections were considered positive only if the number of 
epithelial cells which had at least 1 + staining was > 10% 
of the total epithelial cells. The intensity of staining was 
graded as 0–3 [0, no staining, 1 +, weakly positive, 2 +, 
moderately positive (cytoplasm positive but unstained 
cytoplasm also visible), 3 +, strongly positive (entire cyto-
plasm densely stained)]. The grade considered to be 3 +
was stained as densely as simultaneously stained (control) 
submandibular gland tissue. Some sections of differing 
staining intensities exhibited membranous staining in 
addition to cytoplasmic staining, and this finding was 
noted separately. In some cases, the staining intensity was 
variably and a mean intensity per section was 
calculated. For example, if 100 cells were counted in one 
section and the number of cells of each intensity was as 
follows: 0 = 4, 1 = 14, 2 = 78, 3 = 4, the mean count was 
calculated as (0 × 4) + (1 × 14) + (2 × 78) + (3 × 4) = 182/ 
100. Therefore the mean intensity in this section was 
rounded up to '2'.

EGF-R-positive staining was graded by a modified 
method described previously for bladder epithelium [31]. 
Sections (consisting of five randomly viewed areas of 100 
consecutive nucleated epithelial cells) were considered 
positive only if > 10% of all cells had at least 1 + staining, 
and negative if <10% of cells stained positively. The 
intensity of surface membrane staining was graded as 
0–3+ [3 + being considered equal to the simultaneously 
stained positive control tissue (oesophageal squamous 
carcinoma) and 2 + equal to benign squamous oesophae-
gial mucosa]. Some sections had a heterogeneous staining 
intensity and the mean intensity was calculated per 
section, as above. Although diffuse cytoplasmic staining 
was visible in many epithelial cells, there was little 
difference between sections.

The Ki-67 labelling index was calculated by expressing 
the number of positively stained epithelial nuclei as a ratio 
of the total population of epithelial cells. A minimum of 
500 epithelial cells was counted [27, 32].

The discrepancy between the two sets of independent 
cOUNTS for the 60 sections (30 Barrett's mucosa +30 
cardiac mucosa) was less than 5%.

Statistics

Spearman rank analysis was used to assess the correla-
tion between histological appearances and expression of 
EGF, TGFα and EGF-R. The data were not normally 
distributed, so that Kruskal–Wallis analysis was used to 
compare the variance of each of the variables (EGF, 
TGFα, EGF-R expression and the Ki-67 labelling index) 
with the different histological groups. Wilcoxon's paired 
test compared the expression of EGF, TGFα and EGF-R 
in Barrett's biopsies with corresponding biopsies from 
normal cardiac mucosa in the same patient.

RESULTS

Ten patients were proven to have adenocarcinoma arising 
from areas of Barrett's mucosa. Ten patients had distinct 
tive 'intestinal-type' Barrett's mucosa and another ten had 
fundic- or cardiac-type Barrett's mucosa.

The histological grading of the three categories of 
Barrett's mucosa was significantly correlated with TGFα 
and EGF-R expression (P<0.05) and the Ki-67 labelling 
index (P<0.01), but not with EGF expression (P=0.4). 
Similarly, the Ki-67 labelling index was correlated with 
TGFα and EGF-R expression (P<0.05), but not with 
EGF expression (P=0.2).

The mean expression of EGF did not change signifi-
cantly between the three histological groups (P=0.5)
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However, TGFα expression increased stepwise from low levels of fundic- or cardiac-type mucosa, to moderate levels in intestinal-type mucosa, and moderate to high levels in adenocarcinoma (P<0.05) (Fig. 2). Similarly, EGF-R expression was low in fundic- or cardiac-type mucosa and high in both intestinal-type mucosa and adenocarcinoma (P<0.05) (Fig. 3). The Ki-67 labelling index was very low in fundic- or cardiac-type mucosa and very high in adenocarcinoma (P<0.01) (Fig. 4).

There was no statistically significant difference between EGF, TGFα and EGF-R expression in fundic-type Barrett's mucosa and in normal cardiac gastric mucosa (P>0.5). However, in intestinal-type Barrett's mucosa TGFα and EGF-R expression was significantly increased compared with normal cardiac mucosa (P<0.05 and P<0.01, respectively).

We found that the Ki-67 labelling index was low in some sections which stained strongly for the two peptides but showed little staining for EGF-R.

Five of the intestinal-type Barrett's mucosa showed moderate to severe dysplasia and had moderate or strong expression of both TGFα and EGF-R. These five mucosae also had very high Ki-67 labelling indices with a mean of 29% (range 20–36%) compared with normal gastric mucosa (mean 6%), fundic-type Barrett's mucosa (mean 8%) and the other five intestinal-type Barrett's mucosa (mean 15%) and Barrett's adenocarcinoma (mean 27%). All other cases of Barrett's mucosa had at most mild dysplasia.

DISCUSSION

It has been reported previously that EGF, TGFα and EGF-R are associated with mitogenesis and oncogenesis [13, 14, 22]. It is therefore of considerable interest that we have demonstrated the presence of these growth-controlling peptides and their receptor in normal gastric mucosa, Barrett's columnar-lined mucosa and Barrett's neoplasia.

It has been reported in other tissues that co-expression of TGFα or EGF and EGF-R may be associated with autocrine growth regulation in normal gastrointestinal mucosa [33–35] and in neoplasia [36]. On the other hand, it seems that the tissue concentrations of TGFα and EGF alone do not elicit neoplastic change. It has been suggested that the oncogenic response also depends on the 'intrinsic biological features of the target tissue' [37]. It may be that this 'intrinsic sensitivity' is accounted for, in part, by the surface density of EGF-R expression [38].

Our results are compatible with a recent report indicating that gastrointestinal mucosal cells which express ligand alone may not show evidence of proliferation [39, 40].

Our finding of over-expression of both ligand and receptor in intestinal-type Barrett's mucosa and Barrett's neoplastic mucosa also characterize other neoplastic
tissues [41, 42]. This is inappropriate because over-expression of TGFα should down-regulate EGF-R expression (partly by increasing internalization and degradation of receptors) [43, 44]. Loss of the 'normal' negative feedback loop [45] may be present in transformed cells.

We found a stepwise increase in expression of TGFα and EGF-R from fundic-type Barrett's mucosa through intestinal-type Barrett's mucosa to Barrett's adenocarcinoma. It is interesting to note that the five patients with moderate to severe dysplasia showed high TGFα and EGF-R expression as well as high Ki-67 labelling indices, compatible with involvement of the ligand and receptor in the pre-neoplastic changes of the oesophageal mucosa. These findings are consistent with previous reports which indicate that intestinal-type Barrett's mucosa is more liable to undergo increased proliferation and neoplastic change compared with either fundic- or cardiac-type mucosa [2, 3, 9, 46, 47].

The simultaneous over-expression of TGFα and EGF-R may not be by chance. TGFα is known to increase the level of TGFα mRNA in an 'autocrine-loop' in neoplastic cells [41] and it has been postulated that under certain circumstances TGFα may induce EGF-R expression also [48]. These changes may reflect the failure of autoregulation of TGFα expression by molecules such as anti-oncogenes [14].

There are three mechanisms whereby co-expression of TGFα and EGF-R can lead to increased mitogenesis and oncogenesis [49]. TGFα may stimulate receptors on the same cell, activating an intracellular cascade of cytoplasmic and nuclear proliferation-inducing effects (autocrine mechanism). It has also been reported that cell-cell adhesion, mediated by binding of membrane-anchored TGFα to EGF-R, may promote cell proliferation ('juxtacrine' mechanism) [50]. In addition, TGFα from one cell can bind to the EGF-R in nearby cells, although it has been suggested that expression of TGFα and EGF-R had to be very high, since there is much dilution of ligand in the extracellular space (paracrine mechanism) [37]. Our study does not permit conclusions about which mechanism is predominant in the growth regulation of normal or abnormal oesophageal mucosa.

In the present study we have found a significant correlation between the Ki-67 labelling index and the expression of TGFα and EGF-R, but not the expression of EGF, consistent with the finding that staining for EGF does not differ significantly between the different types of Barrett's mucosa or Barrett's neoplasia. However, EGF is demonstrable and it is not possible to exclude the possibility that EGF may be exerting a synergistic action, because EGF-dependent stimulation also increases the level of TGFα mRNA (auto-induction) [51]. In addition, EGF may induce signal amplification once cells have already been transformed by TGFα [52]. In general, it is likely that EGF and TGFα augment or amplify each other's actions [14, 12, 49, 53].

Although the two peptides bind to EGF-R, TGFα has different conformational, receptor-binding and process-


