Gut

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Growth factors in the gastrointestinal tract

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Cancer cells grow at a rate more rapid than that at which they die thus progressively accumulating as a mass or tumour. This imbalance of growth is a result of both excessive growth stimulation and of delayed cell death. Research into growth factors and their receptors focuses on the mechanisms which promote the growth of cells and seeks to explain the overstimulation observed in many cancer cells. The information gained allows the design of drugs directed at reducing tumour cell growth rate or eradicating cancer cells by interfering with or exploiting their aberrant growth regulatory systems. In addition, it identifies those tumour types where these treatments may be appropriate.

Type 1 growth factor receptors and their ligands

Many polypeptide growth factors and receptors have now been identified. These fall into three broad classes, those with tyrosine kinase activity, those composed of seven transmembrane sequences, and the haemopoietic cell growth factor receptors. The type 1 growth factor receptors consist of the epidermal growth factors receptor, *c-erbB-2* and *c-erbB-3*.¹ These molecules consist of a single polypeptide chain with an extracellular N-terminus which traverses the cell membrane once. The extracellular domain is glycosylated with high mannose and complex N-linked carbohydrate chains. The cytoplasmic domain can be divided into two main regions, a sequence which encodes a tyrosine kinase and a C-terminal region containing tyrosine residues which are substrates for phosphorylation. In their unliganded form the receptors exist principally as monomers in the cell membrane. When a ligand binds, at least in the case of epidermal growth factor receptor, this promotes or stabilises receptors as dimers.² Coincident with dimerisation is activation of the receptors catalytic activity. The C-terminal tyrosine residues are transphosphorylated by the adjacent receptor of the dimer. Several proteins have now been identified that are substrates for tyrosine kinase growth factor receptor. These include GAP (the growth factor receptor activating protein of *ras*), phospholipase C-gamma, and phosphatidylinositol 3 kinase.³ Each of these proteins possesses a region called an SH2 domain that allows it to bind to a phosphorylated tyrosine residue in a growth factor receptor. After binding, the substrate itself becomes phosphorylated and in the case of phospholipase C-gamma this now becomes more enzymatically active. One of the most active current subjects of research in this area is to

identify useful interactions between receptors and second messenger generating systems, in part to explain the extensive diversity of receptor types.

An even more perplexing problem is why a single receptor such as epidermal growth factor receptor has at least four ligands, all of which seem to bind to a similar site and stimulate very similar responses. Those ligands so far identified that are known to bind are epidermal growth factor itself, transforming growth factor-alpha, and two newly discovered molecules, amphiregulin and heparin binding epidermal growth factor. Thus, in principle, there is no more justification in terming the epidermal growth factor receptor by its current name than in calling it the amphiregulin or heparin binding epidermal growth factor receptor.

The *c-erbB-2* protein probably also possesses several ligands, although only two have so far been cloned, and thus it is still not clear whether partially purified binding activities in the published reports are the same or distinct. The factors most well characterised are gp30, p75, Neu-activating factor (NAF), heregulin, and Neu-differentiating factor (NDF). The latter two have now been cloned and sequenced.^{4,5} The *c-erbB-3* receptor has no identified ligands yet, but several laboratories are seeking one (or more!). One approach to bringing some order to this very rapidly expanding family of molecules is to examine their normal pattern of distribution. This can be done at the protein level principally by immunocytochemical staining but also by mRNA expression, either by in situ hybridisation or northern blotting. Examining mRNA is more important for the diffusible growth factors since their site of synthesis may be distinct from their site of protein accumulation (however, so far these seem to be much the same).

Immunocytochemical staining has shown that the distribution of epidermal growth factor in the gastrointestinal tract is rather well defined with high levels appearing only in salivary glands and Brunner's glands of the duodenum. Transforming growth factor-alpha protein and mRNA, however, is much more broadly expressed. It is largely absent in submandibular gland but is present in all epithelia of the gastrointestinal tract. In the oesophagus, staining is primarily in the superficial layers of epithelium, and in the stomach surface enterocytes express the protein. In the duodenum, the villi are positive but Brunner's glands are negative. Staining is present in the luminal epithelium of the colon, appendix, and rectum. Throughout the bowel, staining is less pronounced

towards the base of the villi or crypts.⁶ The pattern of staining for heparin binding epidermal growth factor is not known. The distribution of amphiregulin is not well defined but low levels are present in colon. An additional possible member of the type 1 receptor family of ligands has recently been identified and called cripto. It is not known if this binds to any of the receptor types so far described. We are currently examining in detail its distribution in normal tissues including those of the gastrointestinal tract where it is expressed at low levels. The distribution of the putative *c-erbB-2* ligands is wholly unknown.

The type 1 receptors are all expressed in epithelial cells in the normal gastrointestinal tract. Briefly, epidermal growth factor receptor is found in oropharynx, the ducts of salivary glands, and in oesophagus. In the stomach, only mucous neck cells of gastric glands are positive and low levels of expression are found in the small and large intestine. In the adult, *c-erbB-2* is expressed at fairly uniform levels in the gastrointestinal tract epithelium throughout. *c-erbB-3* is expressed in oropharynx, salivary gland ducts, oesophagus, stomach, and at somewhat lower levels in small and large intestine.⁷

Members of the type 1 growth factor and growth factor receptor family are often expressed at raised levels in human cancers. This over-expression can be modelled in experimental systems, which have shown that it will transform immortalised cells. Over-expression of epidermal growth factor receptor, but possibly not *c-erbB-2*, requires the presence of an activating ligand to make cells malignant. It is not known if over-expression of *c-erbB-3* is transforming. Over-expression of each receptor is now known to occur in specific tumour types of the gastrointestinal tract. The mechanism of this increase is either increased mRNA transcription or gene amplification, or both. The epidermal growth factor receptor is over-expressed in head and neck cancers, stomach cancers, and possibly oesophageal cancers but not significantly in colorectal cancers.⁸ *c-erbB-2* protein is over-expressed in stomach cancers and rarely in colorectal cancers.⁹ Recent work on rather modest numbers of cases suggests that *c-erbB-3* is over-expressed in oesophageal, stomach, and colorectal cancers but the detailed prevalence figures and mechanism of activation are not yet known (unpublished results). Again, preliminary experiments suggest that amphiregulin and cripto are also over-expressed in some tumours of the gastrointestinal tract, particularly those of the colon.

Some attempts to examine the relationship of growth factor receptor over-expression in the gastrointestinal tract cancers and prognosis have been made. Generally, over-expression of epidermal growth factor receptor or *c-erbB-2* seem to be indicative of short relapse free interval and overall survival as they are in other cancers such as those arising in breast and lung. No studies have yet examined *c-erbB-3* in this context.

Finally, if over-expression of a growth factor or a growth factor receptor is at least partly responsible for transformation, these systems represent targets for new anticancer drugs. Many approaches are being explored in the laboratory, including growth factor antagonists, monoclonal antibodies, dimerisation inhibitors, kinase inhibitors, antisense oligonucleotides, and transcription inhibitors.¹⁰ Clinical trials of monoclonal antibodies to *c-erbB-2* are underway in breast cancer patients in the United States¹¹ and these results may encourage similar trials, for example, in stomach cancer, where a proportion also over-express the protein. Only time will tell the value of all or any of these particular strategies but the type 1 systems currently represent the most promising test of new 'designer' anticancer drugs.

Trefoil peptides

The trefoil peptides¹² are members of a growing family of proteins that show tissue specific and cell type specific expression in the gastrointestinal tract. These proteins are strongly associated with mucosal repair after ulceration, but may also be involved in some neoplasms of the gastrointestinal tract. The peptides so far identified include (i) pS2, which is an oestrogen responsive gene product found in the normal stomach¹³; (ii) hSP,¹⁴ which is the human homologue of porcine pancreatic spasmolytic polypeptide¹⁵ and *Xenopus* spasmolysin¹⁶; and (iii) rat intestinal trefoil factor.¹⁷

The importance of epidermal growth factor in maintaining the integrity of the gastrointestinal mucosa has long been recognised but until recently the mechanism of this effect was not understood. However, a new model for mucosal repair involving epidermal growth factor receptor ligands and also trefoil peptides has been proposed following the description of a specific ulceration association cell lineage (UACL)¹⁸ and its association with cells producing hSP and pS2. It has been proposed that after ulceration cells of the UACL secreting epidermal growth factor and transforming growth factor alpha grow out from adjacent glands and ramify to form a new gland. Groups of cells adjacent to the UACL are then stimulated to produce hSP and pS2, to divide, and to repopulate the ulcerated mucosal surface.^{19,20} Involvement of the UACL and cells expressing trefoil peptides has been found in peptic ulcers,¹⁸ in intestinal ulcers associated with Crohn's disease and ulcerative colitis,^{19,20} and also in chronic pancreatitis.^{19,21} Discovery of this system may have important clinical implications. Immunochemical measurement of the level of trefoil peptides in serum or other body fluids might be useful for the diagnosis and monitoring of ulcerative conditions.²⁰ Use of epidermal growth factor receptor ligands as agents to stimulate ulcer healing should be investigated further; a small scale clinical trial of daily urogastrone treatment showed encouraging results in gastric ulcer patients,²² and new studies could benefit from the availability of recombinant epidermal growth factor.²³⁻²⁵

The trefoil peptides are also expressed in some forms of neoplasia, although their role is not yet clear (while all the peptides possess a broad range of biological activities, only porcine pancreatic spasmolytic polypeptide and amphibian spasmolysin have been formally shown to be mitogenic for epithelial cells). In normal conditions, the pS2 and hSP proteins are expressed only by the surface epithelial cells of the antral region and fundus of the normal stomach and nowhere else on the gastrointestinal tract.^{13,14} However, pS2 and hSP expression is found in 40% of stomach cancers (more strongly in tumours of diffuse type than those of intestinal type),²⁶ in 85% of biliary tract cancers,²⁷ in 75% of pancreatic cancers,²⁸ and in 85% of colorectal cancers.²⁹ The mechanism by which transcription of these genes is activated has not been determined, but it is notable that the pS2 gene promoter contains an epidermal growth factor responsive element, and potential autocrine loops for stimulation of the epidermal growth factor receptor have been identified in some of these tumour types.^{21,30}

The fibroblast growth factors (FGFs) and their receptors (FGFRs)

The seven recognised members of the FGF family are acidic FGF (FGF1), basic FGF (FGF2), the proteins encoded by *INT2* (FGF3)³¹ and *HST1* (FGF4)^{32,33} genes, FGF5,³⁴ FGF6³⁵ and keratinocyte growth factor (FGF7).³⁶ Fibroblast growth factors are expressed during fetal development with tight temporal and spatial control, and also in some tumour types (indeed the genes encoding FGFs 3-6 are dominant oncogenes capable of transforming cells in vitro). While FGF7 is a potent mitogen specific for epithelial cells,³⁶ acidic and basic

FGFs (FGFs 1 and 2) are mitogenic for a wide range of cell types as well as having powerful angiogenic effects. The properties of these factors make them candidates for involvement in inflammation and tissue repair as well as in neoplasia.

There are two distinct families of FGF receptors: high affinity transmembrane receptors with tyrosine kinase activity and low affinity heparan sulphate proteoglycans. The high affinity receptor family has five members, currently known as FGFR-1 (*flg*),³⁷ FGFR-2 (*bek*),³⁸ FGFR-3,³⁹ and FGFR-4,⁴⁰ KGFR.⁴¹ All the receptors have two or three extracellular immunoglobulin domains, a single transmembrane domain, and a conserved cytoplasmic tyrosine kinase component split by a short tyrosine kinase insert (as in the receptors for platelet derived growth factor and colony stimulating factor 1). It seems likely that there are differences in interaction of individual FGFs with the different FGFRs, but evidence for this is so far fragmentary. Keratinocyte growth factor (FGF7), unlike FGF1 and FGF2, binds to FGFR-2 but not well to FGFR-1. FGFR-3 is stimulated by both FGF1 and FGF2 equally,³⁹ while FGFR-4 binds FGF1 but not FGF2.⁴⁰ The heparan sulphate proteoglycans in the extracellular matrix were originally considered to be a system to store and stabilise the levels of ligands, but recently a series of interesting observations have led to a revision of this view. Association of FGFs with proteoglycans has been shown to modify their interaction with the high affinity cell surface receptors⁴²⁻⁴⁴ and for FGF3 seems to be essential for its transforming effect.⁴⁵ It is now believed that the low affinity receptor system may allow fine tuning of ligand action locally, as well as controlling the release of secreted FGFs to remote targets.

The FGF system is involved in a number of infectious and inflammatory conditions of the gastrointestinal tract. For instance, it has recently been found that herpes simplex virus type 1 gains entry to cells through the association of viral particles with FGF2 and subsequent binding to FGFR-1 on the surface of target cells.⁴⁶ It is possible that viral infectivity could be reduced by use of heparin related compounds to sequester FGFs. Whether other viruses use a similar system to pass cellular defences is not yet known. The powerful angiogenic and mitogenic effects of the FGFs might be exploited in a variety of clinical conditions, particularly those involving ischaemia. The discovery that the structure of basic FGF (FGF2) can be altered to increase its resistance to acid without loss of function means that this factor may have a therapeutic application in peptic ulcer disease since it has been found to be more effective than cimetidine in promoting vascularisation and healing of cysteamine induced ulcers in rats.⁴⁷

Fibroblast growth factors and their receptors are involved in some forms of gastrointestinal cancer. The gene encoding FGF4 (*HST1*) was originally isolated as a transforming oncogene from a human gastric cancer, and co-amplification of the FGF4 gene with that encoding FGF3 (*INT2*) is associated with poor survival in oesophageal cancer.⁴⁸ An oncogenic sequence (K-sam) isolated from the KATO III gastric cancer cell line is the result of rearrangement of the FGFR-2 gene, and amplification of this sequence has been found in primary gastric cancer.⁴⁹ Hence abnormalities of both FGFRs and their ligands have already been identified in gastric cancer, and it is highly likely that such events occur in other tumours of the gastrointestinal tract. The FGF receptors may represent targets for novel forms of therapy, perhaps with basic FGF (or other FGFs as appropriate) complexed to toxins such as saporin⁵⁰ or *Pseudomonas* endotoxin. The use of heparin related compounds to sequester FGFs and reduce ligand availability to receptors on tumour cells also has potential clinical applications and exciting new developments can be anticipated in this field.

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