The possibility that gastrin may play a role in the development of colorectal carcinoma has aroused considerable interest over the past decade. In early reports some colorectal carcinomas and colorectal carcinoma cell lines were shown to produce gastrin,1 to express gastrin receptors,2 and to respond mitogenically to exogenous gastrin.3 The most favoured explanation for these observations was an autocrine or paracrine loop in which gastrin was produced by the tumour, bound to tumour receptors, and stimulated tumour growth. However, gastrin may also have acted as an endocrine agent as hypergastrinaemia has been reported in a number of patients with colorectal carcinoma, although the source of the gastrin has not been defined.4-7 Further studies of the expression of gastrin5-7 and gastrin receptors8-10 in colorectal carcinomas and colorectal carcinoma cell lines, and of hypergastrinaemia in patients with colorectal carcinoma,11-13 indicated that the early positive reports were not universally correct. Recent information on the nature and source of the gastrin produced, and the discovery of novel receptors selective for non-amidated gastrins, makes it timely to reconsider the involvement of progastrin derived peptides in colorectal carcinoma.

Synthesis, storage and secretion of gastrin in colorectal carcinomas

There is now abundant evidence that most colorectal carcinomas synthesise progastrin. Gastrin mRNA has been detected by both polymerase chain reaction and northern hybridisation in colorectal carcinoma cell lines, normal human colonic mucosa and colorectal carcinomas.6-10-16 The gastrin mRNA is of low abundance but the major band of 0.7 kilobases is identical in sequence to antral gastrin mRNA.5-16-17 There is, however, considerable disparity in the literature on the proportion of colorectal carcinomas that contain amidated gastrin (table 1). The variable efficiency of translation and extent of posttranslational processing of gastrin in peptide producing tumours12 offers an explanation for some of the contradictory reports as assays for gastrin have frequently been confined to amidated forms. The processing of gastrin from progastrin to the amidated product involves a number of steps, which include endopeptidase and carboxypeptidase mediated cleavages, and which end in conversion of glycine extended gastrin to the amidated forms (fig 1).21-22 Using either region specific antisera or processing independent analysis,22 which quantitates all molecular forms of gastrin irrespective of the degree of processing, progastrin or progastrin derived peptides are now being detected in 80-100% of colorectal carcinomas (table 1). The presence of non-amidated gastrin peptides in colorectal carcinomas has assumed greater importance with the recent reports that gastrin-gly has a growth promoting effect in a non-transformed colonic epithelial cell line,23 as well as in a pancreatic cancer cell line24 and in fibroblasts,25 and with the description of receptors capable of binding non-amidated gastrin.21-24-27 In addition, transgenic mice expressing human progastrin in the liver had raised concentrations of plasma progastrin and a hyperplastic colonic mucosa.28 The nature of the receptor responsible for the proliferative effects of progastrin is completely unknown.

Hypergastrinaemia in patients with colorectal carcinoma has also been controversial. Early studies were not controlled for Helicobacter pylori status (a known cause of hypergastrinaemia), had small sample sizes with the results skewed by apparent outliers, and measured only amidated gastrin.14-15 After controlling for these factors, we have confirmed that circulating amidated gastrin is not raised in patients with colorectal carcinoma, but have made the intriguing finding that circulating non-amidated gastrin is raised both in H pylori positive (5.2-fold) and negative (2.3-fold) patients with colorectal carcinoma.17 The initial reports of a decrease in plasma gastrin following surgical resection of the colorectal carcinoma18 have not been confirmed however.14-15 The low gastrin concentration in colorectal carcinomas, together with the increase in circulating partially processed gastrin, is consistent with constitutive secretion, whereby gastrin is not stored in a processed form in tumour cells, but is secreted from peptide containing vesicles by exocytosis.29 An alternative explanation is that colorectal carcinomas are secreting an agent such as gastrin-releasing peptide which stimulates the secretion of antral gastrin.4

Gastrin/cholecystokinin receptors in carcinoma tissue and on cell lines

For gastrin to function as a growth promoting agent, specific receptors capable of transducing a mitogenic signal must be localised on the tumour. At least four receptors exist for gastrin and cholecystokinin (CCK).30 The classic CCK-A receptor on pancreatic acinar cells is selective for sulphated CCK 

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\text{dissociation constant (Kd) = 20 pM},
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whereas the gastrin/CCK-B receptor on gastric parietal cells recognises gastrin17 and both sulphated and unsulphated CCK, with approximately equal affinity (Kd = 2-6 nM). Both A and B receptors are members of the family of receptors with seven transmembrane segments. Despite sharing 50% identity in sequence, the A and B receptors can be clearly distinguished by several selective antagonists. A low affinity gastrin,31 binding site (Kd = 200 nM) has also been described on colorectal carcinoma cell lines,31 identified as a 78 kDa protein,32 and called the gastrin/CCK-C receptor.32 Surprisingly, the gastrin/CCK-C receptor is unrelated to the A and B receptors in sequence, but belongs to a family of enzymes involved in the β-oxidation of fatty acids.33 The C receptor can also be distinguished from the A and B receptors by the fact that addition of a C-terminal glycine residue to gastrin17 does not affect binding.27 A novel high affinity receptor selective for glycine extended gastrin, with a different antagonist profile from the A and B receptors, has also been described on a rat pancreatic carcinoma cell line (AR4–2J),34 and on a non-transformed colonic epithelial cell line.21 A slightly different receptor, recognising both glycine extended and amidated gastrins with similar high affinity, has been reported on the mouse colon cell line CA, and on Swiss 3T3 fibroblasts.26 Definition of the relation of the novel receptors to each other, and to the gastrin/CCK-A, -B, and
Gastrin as an autocrine growth factor

Gastrin is an established growth factor for the non-antral gastric mucosa, but proliferative effects on the normal mucosa of the small and large intestine, or on the development of colorectal carcinoma, are controversial. For example, patients with hypergastrinaemia associated with pernicious anaemia do not have an increased long term risk of colorectal carcinoma, although the risk may be increased in the first five years after diagnosis. Variations in the source and forms of gastrin may be responsible for these contrasting viewpoints as the observation that overexpression of intact progastrin in transgenic mice increased colonic mucosal proliferation suggests that biological activity is not confined to the amidated or glycine extended forms.

The growth of some colorectal carcinoma cell lines both in culture and as xenografts is stimulated by exogenous and endogenous hypergastrinaemia, and considerable interest has therefore been generated by the hypothesis that progastrin derived peptides may be acting as autocrine growth factors in colorectal carcinoma. In the autocrine model (fig 2) a cell synthesises its own growth factor, which is released into the surrounding medium. Binding of the growth factor to cell surface receptors then results in the transmission of a mitogenic signal to the cell nucleus, with a consequent increase in cell proliferation. The most convincing evidence for the involvement of progastrin derived peptides in an autocrine loop is the observation that expression of antisense gastrin mRNA reduced both growth and tumorigenicity of the colorectal carcinoma cell lines Colo 320 and HCT 116, and growth of the conditionally immortalised mouse colon cell line YAMC.

In contrast, growth of the colorectal carcinoma cell line Colo 205A, which expressed negligible amounts of gastrin mRNA, was unaffected by expression of antisense gastrin mRNA. The antisense experiments demonstrate that progastrin derived peptides act as autocrine growth factors in colorectal carcinoma cell lines, but do not define the form of progastrin derived peptides or the nature of the receptor involved in the loop.

The existence on colorectal carcinoma cell lines of an autocrine loop involving amidated gastrin and the gastrin/CCK-B receptor (fig 2A) does not seem to be a common phenomenon. As discussed in the last section, most colorectal carcinoma cell lines do not express gastrin/CCK-B receptors. In addition the selective gastrin/CCK receptor antagonists L364,718 and L365,260 had no effect on proliferation of colorectal carcinoma cell lines at concentrations sufficiently high to saturate CCK-A and gastrin/CCK-B receptors, respectively.

Antisera directed against the common C-terminal tetrapeptide of gastrin and CCK inhibit the proliferation of only a small proportion of colorectal carcinoma cell lines, but do not affect the proliferation of other colorectal carcinoma cell lines. The possibility of an alternative autocrine loop which utilises non-amidated gastrins (fig 2B) should also be considered. Such peptides have been demonstrated in colorectal carcinoma specimens, as discussed in the section on synthesis and secretion. In the case of the mouse colon cell line YAMC, proliferation is partially inhibited by an antibody selective for glycine extended gastrin, but not by an antibody selective for amidated gastrins. Furthermore, YAMC cells do not bind amidated gastrin, but do express the antisense experiments demonstrate that progastrin derived peptides act as autocrine growth factors in colorectal carcinoma cell lines, but do not define the form of progastrin derived peptides or the nature of the receptor involved in the loop.

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In contrast, antibodies directed against gastrin may be useful for treatment of colorectal carcinoma. As mentioned earlier, antibodies recognising the C-terminal amidated tetrapeptide common to both gastrin and CCK inhibit the proliferation of some, but not all, colorectal carcinoma cell lines. Conversely, proliferation of the mouse colon cell line YAMC is inhibited by antibodies recognising glycine extended, but not amidated, gastrins. A particularly promising development has been the demonstration that proglumide, a C-terminal amidated tetrapeptide common to both gastrin and CCK, inhibited the growth of colorectal carcinoma cell lines grown as xenografts in nude mice and rats, but have not yet been subjected to clinical trials. The observation that most colorectal carcinomas do not express gastrin/CCK-B receptors, however, indicates that it will be unlikely that gastrin/CCK-B receptor selective antagonists will be a general treatment for colorectal carcinoma.

Therapeutic possibilities

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Gastrin, gastrin receptors and colorectal carcinoma

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