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The C-terminal flanking peptide of progastrin induces gastric cell apoptosis and stimulates colonic cell division in vivo

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ABSTRACT

Progastrin (PG) is processed into a number of smaller peptides including amidated gastrin (Gamide), non-amidated glycine-extended gastrin (Ggly) and the C-terminal flanking peptide (CTFP). Several groups have reported that PG, Gamide and Ggly are biologically active in vitro and in vivo, and are involved in the development of gastrointestinal cancers. CTFP is bioactive in vitro but little is known of its effects in vivo. This study investigated the bioactivity of CTFP in vivo in normal tissues using gastrin deficient (GASKO) mice and in two mouse models of cancer (SCID mice bearing xenograft tumors expressing normal or knocked-down levels of gastrin and a mouse model of hepatic metastasis). As with Ggly, CTFP treatment stimulated colonic proliferation in GASKO mice compared to control. CTFP also significantly increased apoptosis in the gastric mucosa of male GASKO mice. CTFP did not appear to effect xenograft growth or the incidence of liver metastases. This is the first demonstration that CTFP has specific biological activity in vivo in the colon and stomach.

1. Introduction

Amidated gastrin (Gamide), which is synthesized predominantly in the G cells of the gastric antrum, is the fully processed gastric hormone derived from preprogastrin (Fig. 1) [8]. Gamide was originally assumed to be the only biologically relevant and active form of progastrin (PG), as it acts on the cholecystokinin 2 receptor (CCK2R) to stimulate gastric acid secretion and proliferation in the stomach and proximal small intestine [8,19,22]. However, PG and several PG-processing intermediates, including glycine-extended gastrin (Ggly) and the C-terminal flanking peptide (CTFP) of progastrin, are now known to also be biologically active. These non-amidated PG-derived peptides have negligible affinity for the CCK2R [1,19,32]. Putative receptors have been reported for PG [34], and glycine extended gastrin (Ggly) [39], but not yet for CTFP.

PG, Gamide, Ggly and CTFP were identified in the gastric antrum, plasma and colorectal cancer extracts [25,35,36]. Pauwels et al. reported that circulating levels of CTFP were similar to those of Gamide [31], however Smith et al. later demonstrated that CTFP was the predominant peptide in the antrum and circulation, with concentrations between 4- to 60-fold higher than Gamide, respectively [35]. A correlation between circulating Gamide and cancer was observed, with high Gamide associated with a 3.9 fold increased risk for developing colorectal carcinomas [36]. Immature gastrin peptides (non-amidated forms) were also identified in primary tumors and resected colorectal cancer [25,35]. Ciccotosto et al. found elevated concentrations of PG-derived peptides in resected colorectal tumors and in the circulation of patients with colorectal carcinoma [3]. Many colorectal carcinomas express the gastrin gene; however they lack the enzymes to fully convert PG to Gamide, resulting in immature precursors [25]. CTFP was found to be the main PG form in resected colorectal tumors, but was not necessarily elevated in the plasma from patients with colorectal cancer compared to disease-free patients [35].

PG-derived peptides are linked to the development of cancer [10]. In vitro evidence demonstrates PG, Gamide, Ggly and CTFP act independently as growth factors in gastrointestinal cancer cell lines [1,35], and CRC cells expressing antisense gastrin can lose their tumorigenic potential, which can be restored with

Abbreviations: CTFP, C-terminal flanking peptide; Ggly, glycine extended gastrin; Gamide, amidated gastrin; PG, progastrin; GASKO, gastrin knockout; AS, antisense; VO, vector only; ECL, enterochromaffin-like; RPMI, Roswell Park Memorial Institute medium; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; PBS, phosphate buffered saline; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Gy, Gray; Ci, curie; SEM, standard error of the mean.

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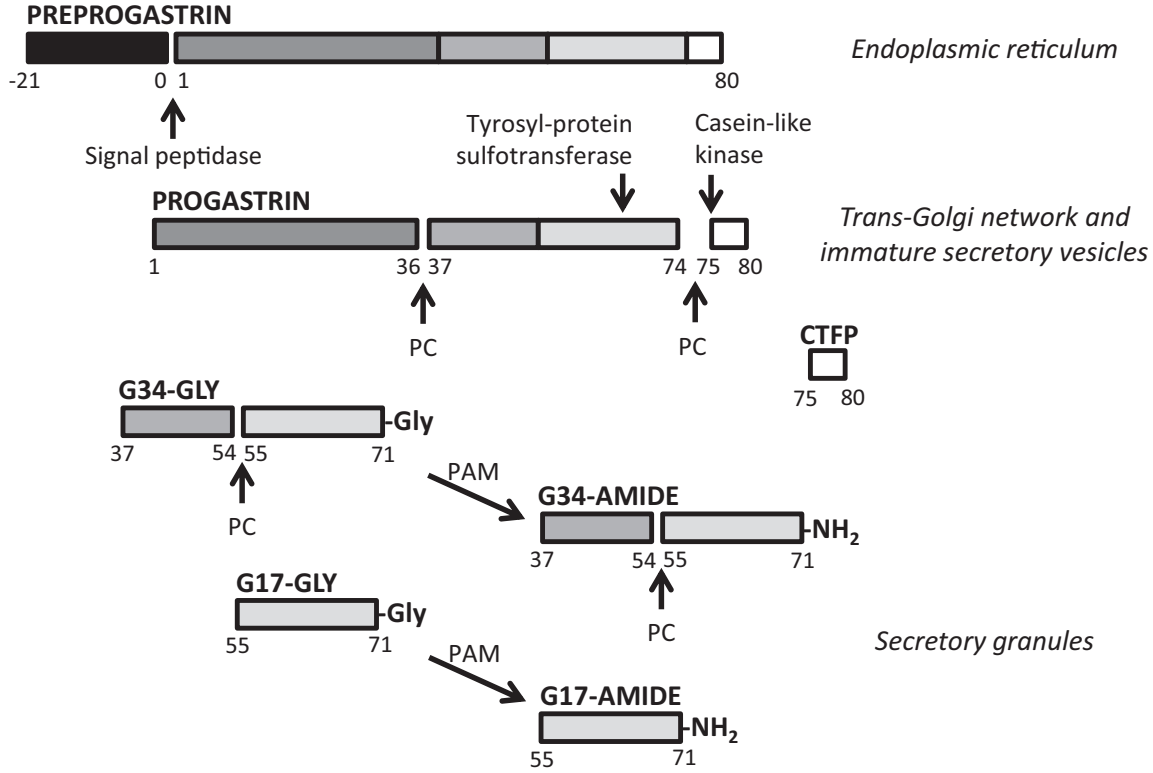

Fig. 1. Processing of human progastrin in the antral G cell. Preprogastrin, the precursor to progastrin, is 101 amino acids long. Progastrin translocates to the trans-Golgi network after its signal sequence (−21 to 0) is cleaved in the endoplasmic reticulum. Progastrin undergoes modifications (sulfation or phosphorylation) and cleavage of the modified peptide by the endopeptidase prohormone convertase (PC) releases CTFP. Carboxypeptidase E removes the C-terminal arginines from the immature gastrins. PC further cleaves glycine extended gastrin-34 (G34-gly) to Ggly (G17-Ggly), both of which can be amidated by peptidyl-α-amidating mono-oxygenase (PAM) to give Gamide (17 or 34).

exogenous PG [17]. PG stimulates proliferation [23,33], while Gamide and Ggly activate both cell proliferation and migration [16,22,27,28]. Ggly inhibits apoptosis, whereas Gamide may be anti- [2] or pro-apoptotic [6,24], depending on the cell line studied. CTFP activates MAP-kinase signaling and stimulates both proliferation and migration in non-transformed gastric epithelial cells (IMGE-5) as well as stimulating proliferation in gastric (LIM1839) and colonic carcinoma cell lines (SW1222, HCT-15) [35]. Patel et al. showed that inhibition of apoptosis by CTFP in human gastric cancer (AGS) cells was dependent on the PI3-kinase pathway [30].

Mice over-expressing different forms of PG have significantly different phenotypes compared to wild type littermates. Mice with elevated Gamide levels (INS-GAS mice) have increased acid secretion, thickened oxyntic mucosa, and decreased apoptosis and increased proliferation in the gastric mucosa [24,38], and are prone to develop gastric cancer spontaneously after reaching 20 months in age [12,37]. Mice with increased circulating Ggly (MTI/G-gly [5,20]) or PG (hGAS [38]) concentrations provide evidence that non-amidated forms of gastrin primarily act in the colon to stimulate mitosis and inhibit apoptosis [20,38]. Experiments with mice over-expressing PG-derived peptides either endogenously or exogenously or with wild type mice treated with excess peptides have increased understanding of the potential roles PG-derived peptides may play in vivo. However, such mice are unable to define exactly which forms are responsible for specific activities, as different processing intermediates can also be found in the over-expressing mice. There are currently no mice that overexpress CTFP without overexpressing other PG peptides also.

Gastrin knockout (GASKO) mice provide an important model, since infusion of individual PG fragments in vivo allows comparisons with results identified in overexpressing mice [14,20]. In the stomach, mice deficient in gastrin displayed decreased parietal and enterochromaffin-like (ECL) cells, and had a thinner fundic mucosa and impaired acid secretion. In the colon, mucosal height and proliferation were also decreased. The GASKO phenotype could be fully or partially rescued when the mice were crossed with mice over-expressing different forms of PG or treated with exogenous peptides [10,20]. Gamide infusion was able to restore the altered gastric phenotype [6,14,20], while PG and Ggly appeared to predominately act in the colon [14].

Ottewell et al. compared the effects of PG, Ggly, PG55-80, PG72-80 and CTFP (PG75-80) treatment on colonic mitosis. When gastrin-deficient mice were treated with peptides 30 min prior to exposing them to 8 Gy of γ-irradiation, PG or PG55-80 (but not Ggly, PG72-80 or CTFP) could stimulate colonic mitosis 4.5 h post irradiation [29]. The contrast between this study and previous findings where Ggly injection alone could significantly increase colonic proliferation in GASKO mice [20] suggests that longer-term infusion of CTFP may be needed to see a biologically relevant response. The long term effects of CTFP on the stomach and colon of GASKO mice have not been determined.

While the in vitro work has provided strong evidence that CTFP has growth factor activities similar to other bioactive PG-derived peptides, the preliminary in vivo data was inconclusive in establishing whether or not CTFP was also active in animals. Here, we have examined the in vivo effects of CTFP on normal tissue using GASKO mice and two mouse models of cancer (xenograft and metastasis). Xenograft mouse models are useful to determine if any cell line has the ability to develop into a tumor in vivo. Ferrand et al. found that the human CRC cell line DLD-1 transfected with antisense gastrin (AS), in contrast to DLD-1 cells transfected with vector only (VO), could not establish tumors in vivo [9]. Metastases models aid the study of cancer spreading from a primary site to a secondary site, via the blood or lymph. Kuruppu et al. developed a