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New approaches to therapy of cancers of the stomach, colon and pancreas based on peptide analogs

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Abstract. Cancers of the stomach, colon and exocrine pancreas are major international health problems and result in more than a million deaths worldwide each year. The therapies for these malignancies must be improved. The effects of gastrointestinal (GI) hormonal peptides and endogenous growth factors on these cancers were reviewed. Some GI peptides, including gastrin and gastrin-releasing peptide (GRP) (mammalian bombesin), appear to be involved in the growth of neoplasms of the GI tract. Certain growth factors such as insulin-like growth factor (IGF)-I, IGF-II and epidermal growth factor and their receptors that regulate cell proliferation are also implicated in the development and progression of GI cancers. Experimental investigations on gastric, colorectal and pancreatic cancers with analogs of somatostatin, antagonists of bombesin/GRP, antagonists of growth hormone-releasing hormone as well as cytotoxic peptides that can be targeted to peptide receptors on tumors were summarized. Clinical trials on peptide analogs in patients with gastric, colorectal and pancreatic cancers were reviewed and analyzed. It may be possible to develop new approaches to hormonal therapy of GI malignancies based on various peptide analogs.

Key words. Gastrin; gastrin-releasing peptide (GRP); bombesin/GRP antagonists; somatostatin analogs; targeted cytotoxic peptides; GH-RH antagonists.

Introduction

In spite of recent advances in diagnosis and treatment, gastric carcinoma, ductal adenocarcinoma of the pancreas and colorectal cancer remain among the leading causes of cancer-related deaths worldwide [1–3]. The pathogeneses of these cancers remain elusive, and therapeutic options are limited. Notwithstanding the progress in our understanding of the molecular biology of these cancers, the systemic treatment of this group of diseases remains unsatisfactory. Conventional chemotherapy has not produced dramatic improvements in response rates or patient survival, and new treatment modalities are urgently needed. It is expected that new therapeutic procedures based on gene therapy will eventually emerge, but advances in proteomics research may have an even greater impact than genomics in our understanding of the mechanisms of diseases and designing new drugs. The analysis of gene products and the identification of functional protein networks within cells are among the initiatives or objectives of proteomics [4]. The progress in this field can lead to the development of new synthetic ligands that act as agonists or antagonists on specific receptors, hence modifying intracellular pathways important in proliferation and differentiation.

In the past several decades great improvements in the treatment of breast and prostate cancer have been achieved by endocrine manipulations which comprise the

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use of peptide analogs. Various new approaches, including the inhibition of growth factors, their receptors or related oncogene products, are also being implemented. In this article we have attempted to reexamine the involvement of certain neuropeptides and their receptors in the tumorigenesis of the stomach, colon and pancreas. Thus, growth hormone-releasing hormone (GH-RH), mammalian bombesin-like peptides, such as GRP and neuromedin B, and their receptors are present in various tumors, and appear to be involved in mitogenic effects [5–10]. The receptors for somatostatin and luteinizing hormone-releasing hormone (LH-RH) have also been found in many tumors [5, 6, 11, 12]. These findings are being used for the elaboration of new therapeutic approaches. An abridgement of the status of somatostatin, bombesin/GRP, GH-RH and their various analogs is followed, for heuristic reasons, by a brief description of some other gastrointestinal hormone peptides, a number of which are also found in the brain. Experimental and clinical studies with peptide analogs aimed at the development of new methods for treatment of gastrointestinal cancers are then summarized and reviewed.

Somatostatin and its analogs

The hypothalamic neuropeptide somatostatin exists in two main active forms, a 14-amino acid peptide (somatostatin-14) and an amino terminally extended version consisting of 28 amino acids (somatostatin-28) (table 1) [5, 12, 13]. Both somatostatin-14 and somatostatin-28 are present in the gastrointestinal (GI) tract, exhibit inhibitory effects on a wide variety of cells, and appear to be endogenous growth inhibitors [5, 14]. Somatostatin inhibits the secretion of many hormones, including growth hormone, insulin and glucagon, gastrin, secretin and cholecystokinin (CCK). Somatostatin can also suppress various exocrine secretions, including gastric acid and pancreatic enzymes and bicarbonate secretion [5, 14, 15]. Somatostatin may similarly serve as an autocrine/paracrine regulator. The targets of somatostatin action are often the same tissues in which the peptide is produced, and somatostatin present in discrete cells of the pancreas, gastric mucosa and duodenum may, through paracrine control, regulate the endocrine pancreas and GI tract [5, 12]. Because of the short plasma half-life of somatostatin-14, more stable synthetic somatostatin analogs were designed and developed for therapeutic purposes [14], including octreotide (Sandostatin) [16], vapreotide (RC-160) [17] and lanreotide [5, 12] (table 1). These analogs have a longer plasma half-life and are about 50 times more potent than somatostatin. Somatostatin and its octapeptide analogs exert their effects through specific membrane receptors [5, 6, 12]. At least five distinct receptor subtypes ($sst_{1–5}$) have been cloned and characterized [18, 19]. These receptors are distributed in normal and cancerous tissues, but found in higher density in the latter [11, 12, 20]. While native somatostatin shows similar high affinity to $sst_{1–5}$, the synthetic octapeptides such as octreotide, RC-160 and RC-121 bind preferentially to sst_2 and sst_5, display moderate affinity to sst_3, and a low affinity to sst_1 and sst_4 [5, 18, 19]. Thus, the effects of somatostatin analogs may be direct and mediated by somatostatin receptors present on tumor cells or induced by inhibition of the release or action of GI hormones and growth factors such as insulin-like growth factor (IGF)-I and epidermal growth factor (EGF). Somatostatin octapeptide RC-160 also stimulates tyrosine phosphatase activity [21] and inhibits the proliferation of the cells expressing the sst_2 gene [22]. Therefore, tyrosine phosphatase appears to be a transducer of the growth inhibition signal in sst_2-expressing cells [22]. However, in sst_5-expressing cells, the phosphatase pathway is not involved in the inhibitory effect of RC-160 on cell growth,

Table 1. Structures of somatostatin 14 and 28 and somatostatin octapeptide analogs.

Somatostatin-14	H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH
Somatostatin-28	Ser-Ala-Asn-Ser-Asn-Pro-Ala-Met-Ala-Pro-Arg-Glu-Arg-Lys-
	Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH
Octapeptide analogs	
SMS-201-995 *Sandostatin (Octreotride)*	D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-ol
RC-160 *Octastatin (Vapreotide)*	D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2
RC-121	D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2
BIM 23014 *Somatulin (Lanreotide)*	D-Nal(2)-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2

and the inositol phospholipid/calcium pathway is implicated [23, 24]. Thus, sst_2 and sst_5 bind octapeptides such as RC-160 with high affinity and mediate the inhibition of cell growth by distinct mechanisms.

In contrast, other studies showed that sst_5 can also activate protein tyrosine phosphatases, which consequently leads to the rise in retinoblastoma protein (Rb) and G1 cell cycle arrest [25]. Different signaling mechanisms may be involved for other receptor subtypes. Ligand binding to sst_1 can activate the mitogen-activated protein kinase cascade and limit the proliferative signals generated by growth factor receptors [26]. Somatostatin receptors undergo internalization after ligand binding (fig. 1). Various sst subtypes differentially internalize somatostatin and its analogs [27]. A more detailed presentation of the various signaling pathways modulated by somatostatin receptors can be found in a review by Csaba and Dournand [28]. There are additional mechanisms that can contribute to the growth inhibitory action of somatostatin, such as the effects on angiogenesis. Somatostatin analogs can act directly on endothelial cells that express somatostatin receptors and also indirectly by inhibiting the production of growth factors such as IGF-I and vascular endothelial growth factor (VEGF) [5, 12, 29].

Somatostatin analogs are used for the treatment of acromegaly, neuroendocrine tumors of the gastroenteropancreatic system, including carcinoid tumors, insulinomas, glucagonomas, gastrinomas and VIPomas [5, 12, 14, 30]. However, detailed coverage of the use of somatostatin analogs for the therapy of endocrine gastroenteropancreatic tumors is beyond the scope of this review, and the reader is referred to other articles [30, 31]. Somatostatin analogs, including RC-160, are powerful tumor growth suppressors in experimental models of various cancers, including pancreatic, colorectal and gastric [5, 12, 14]. Consequently, attempts have been made to use modern somatostatin analogs for the therapy of various human cancers, but relevant palliative benefits have been obtained only in hepatocellular carcinoma [32]. To improve the efficacy of cancer therapy based on somatostatin, new analogs are being designed. Thus, recently a new cyclohexapeptide analog of somatostatin, designated SOM230, was synthesized [33], which binds with high affinity to $sst_{1,2,3}$ and sst_5, inhibits GH secretion and decreases circulating IGF-I levels more effectively than octreotide. [33]. In addition, several peptidic and nonpeptidic selective ligands for sst_{1-5} are being developed [34, 35].

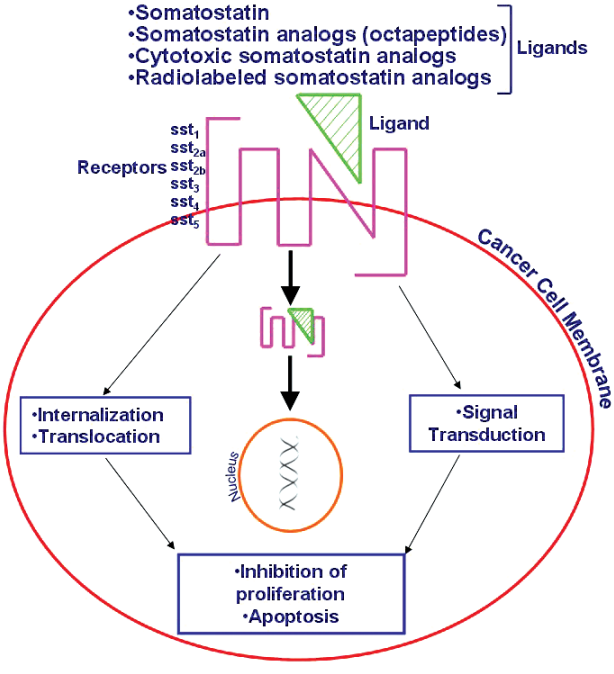

Figure 1. Schematic representation of mechanisms of binding to the receptors and internalization of somatostatin, its octapeptide analogs, radiolabeled analogs and cytotoxic conjugate of somatostatin AN-238, based on Viguere et al. [239] and McCarthy et al. [285]. Some of the mechanisms are still hypothetical.

Localization of tumors and metastases by scintigraphy with radiolabeled somatostatin analogs

The presence of somatostatin receptors, mainly of sst_2 on tumors, makes possible the binding and internalization of labeled somatostatin analogs (fig. 1) and permits localization of some primary tumors and their metastases using scintigraphy techniques [11, 20, 30, 36, 37]. Radiolabeled analogs of somatostatin, such as [^{111}In-DTPA-D-Phe^1]-octreotide (OctreoScan) are used clinically for localization of tumors expressing receptors for somatostatin [20,30]. ^{111}In-DOTA-lanreotide, which has high affinity for sst_2, sst_3 and sst_5, has also been used [38]. In addition, various radiolabeled somatostatin analogs have been designed and synthesized that show high specificity to sst subtypes. These analogs contain ^{64}Cu, ^{67}Ga, ^{111}In, ^{177}Lu, ^{188}Re or ^{99m}Tc as radionuclides for detection and /or targeted radiotherapy of tumors [39–41]. The compounds are injected intravenously (i.v.), and gamma camera pictures are obtained. Krenning et al. [20] carried out somatostatin receptor scintigraphy in more than 1000 patients and reported that various primary tumors and their metastases, both neuroendocrine and nonneuroendocrine, can be localized in vivo. Neuroendocrine tumors that could be localized with OctreoScan include gastrinomas, insulinomas, glucagonomas, neuroblastomas, carcinoids and small cell lung carcinoma (SCLC) [20]. A positive scintigram may predict a good response to treatment with octreotide [20]. Some nonneuroendocrine tumors expressing sst_2 such as breast and prostate cancers and brain tumors can be also localized by receptor scintigraphy [30]. A loss of sst_2 gene expression was found by Buscail et al. [42] in human pancreatic cancer. Accordingly, no positive images of pancreatic adenocarcinomas can be obtained with Oc-

treoScan. However, Raderer et al. [38] were able to locate pancreatic lesions in three patients using ^{111}In-DOTA-Lanreotide, which could be due to the expression of sst_3 and/or sst_5 in the pancreatic cancers of these patients. Investigations on the expression of messenger RNAs for the five somatostatin receptor subtypes led to diverse results. Laws et al. [43] and Pinzani et al. [44] found that almost 100% of colon cancer specimens express mRNA for sst_2, and mRNA for sst_5 is less prevalent with the progression of the disease [44]. Buscail et al. demonstrated a loss of mRNA for sst_2 in more advanced stages and metastases in patients with colorectal cancers [42]. However, mRNA for sst_5 was found in these tumors by the same group [42]. While Radulovic et al. [45] found high affinity receptors for radiolabeled [Tyr^{11}] somatostatin-14, which binds to all five receptor subtypes, in 8 of 15 specimens of colorectal cancer, Reubi et al. [46] reported binding sites for somatostatin in only 2 of 25 specimens. Virgolini et al. [47] showed an in vivo accumulation of ^{111}In-DOTA-lanreotide in liver or lung metastases in three of three patients with colorectal cancers. At present, only limited information is available on the expression of somatostatin receptors on gastric cancers. Miller et al. [48] found low-affinity, high-capacity receptors in 27 of 28 gastric carcinoma specimens and in corresponding normal tissues, and high-affinity binding sites in 1 of the 28 tumors.

Cytotoxic somatostatin analogs

The presence of receptors for somatostatin on various neuroendocrine malignancies and many other solid tumors motivated us to develop somatostatin octapeptides as carriers to deliver cytotoxic agents specifically to cancerous cells (fig. 1) [6, 11, 49, 50]. Thus, we synthesized a series of novel targeted cytotoxic somatostatin conjugates that consist of carriers RC-121 and RC-160 (table 1) coupled to doxorubicin (DOX) or its superactive derivative, 2-pyrrolino-DOX (AN-201) [5, 6, 51, 52]. Of these hybrid cytotoxic conjugates, AN-238 containing AN-201 was demonstrated to be very effective on a variety of human experimental cancer models [5, 6]. Some of these studies on gastric, colorectal and pancreatic cancers are described in the respective sections. Since many normal tissues, such as those of the gastroenteropancreatic system, the kidneys and the pituitary, also express high-affinity receptors for somatostatin, toxic side effects with AN-238 were expected. However, in our studies, no receptor-specific toxicity was observed after treatment with cytotoxic analog AN-238, possibly because it is used in relatively low doses [5, 6, 52]. Thus, no significant changes were found in the basal or GH-RH-stimulated GH release from the pituitary in mice after treatment with AN-238 [5, 6]. This could be explained by the fact that AN-201, the cytotoxic radical in AN-238, affects mainly neoplastic cells with high mitotic activity, and damage to well-differentiated cells with a slow turnover ratio is probably smaller than that inflicted on neoplastic tissue. In addition, the resting cells of the GI tract can eventually replace the damaged cells, restoring normal organ function [5, 6]. Appropriate supportive care and replacement therapy could further alleviate the symptoms of dysfunction of the endocrine and alimentary systems. On the whole, targeted somatostatin analogs would be much less toxic than adjuvant chemotherapy.

Bombesin GRP and their analogs

The bombesin-like peptides comprise three subfamilies of peptides found in amphibians and mammals, including humans [5, 7, 8]. The first member of this family, the tetradecapeptide bombesin, was purified from frog skin [53] and identified (table 2). Subsequently, two mammalian bombesin-like peptides, GRP, which is related to bombesin and neuromedin B, were isolated from porcine stomach and spinal cord [54, 55]. GRP is a 27-amino acid peptide, and its carboxyl-terminal decapeptide is similar to that of bombesin [7], except for one amino acid, His in place of Gln. Bombesin-like peptides are not classical hypothalamic hormones, since they play only a minor role in the release of pituitary hormones [5]. However, bombesin-like immunoreactivity, which appears to be due mostly to GRP-27 and its cleavage product GRP-10, is present in mammalian brain, including the hypothalamus, as well as in lung and GI tract [8]. Bombesin and GRP may function as GI hormones and neurotransmitters [5, 12]. In the digestive tract, bombesin and GRP stimulate the contraction of smooth muscles, the proliferation of intestine and pancreas, the exocrine secretion of the stomach and pancreas, and the release of gastrin, somatostatin, pancreatic polypeptide and other gastrointestinal hormones. GRP appears to control gastrin release in a paracrine fashion. From the oncological perspective, the most important action of bombesin/GRP and neuromedin B is their ability to function as growth factors and modulate the proliferation of tumors through autocrine or paracrine mechanisms [5, 9, 12, 56]. This action was first discovered in SCLC [56], but later it became clear that bombesin-like peptides are also produced in other cancers, such as breast, prostatic and pancreatic cancer [9], and act as growth factors involved in tumor progression [5, 12]. Other data show that GRP and its receptors, expressed aberrantly in several types of tumors, regulate morphology and differentiation, rather than proliferation [57, 58].

Four receptor subtypes for the bombesin-like peptides have been identified and cloned, subtypes 1-3 being found in mammals [7, 59–61]. Receptor subtype 1 binds bombesin and GRP with high affinity, and subtype 2 prefers neuromedin B [5, 6, 12]. The bombesin receptor

Table 2. Structures of gastrin-cholecystokinin peptides, bombesin, GRP and bombesin/GRP antagonists.

Human G-17 (little gastrin)	Glp-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2 SO_3H
Pentagastrin	N-t-butyloxycarbonyl-β-Ala-Trp-Met-Asp-Phe-NH_2
Cholecystokinin (CCK)-33	Lys-Ala-Pro-Ser-Gly-Arg-Val-Ser-Met-Ile-Lys-Asn-Leu-Gln-Ser-Leu-Asp-Pro-Ser-His-Arg-Ile-Ser-Asp-Arg-Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2 SO_3H
CCK-8	Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe- NH_2
Bombesin	pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
Gastrin-releasing peptide (GRP)	Val-Pro-Leu-Pro-Ala-Gly-Gly-Gly-Thr-Val-Leu-Thr-Lys-Met-Tyr-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
Bombesin/GRP antagonists	
RC-3095	D-Tpi^6,Leu^{13} ψ [CH_2NH]Leu^{14}-Bombesin(6–14)
RC-3940-II	Hca^6,Leu^{13} ψ [CH_2N]Tac^{14}-Bombesin(6–14)

Hca, hydrocinnamic acid; Tpi, 2,3,4,9 tetrahydro-1H-pyrido-[3,4-b]indole-3-carboxylic acid;
Tac, thiazolidine carboxylic acid; ψ, pseudo peptide bond.

subtype 3 is an orphan receptor because its natural ligand has not yet been identified [5, 6, 12]. The bombesin/ GRP receptor subtype 1 has been detected on various human malignancies, including SCLC, breast, ovarian, prostate, pancreatic, gastric and colon cancer as well as brain tumors [5, 6, 12]. In contrast, neuroendocrine tumors express mostly subtype 2 or 3 of bombesin receptors [62]. The Neuromedin B receptor and bombesin receptor subtype 3 also seem to be expressed in various human cancers [62].

The finding that bombesin or GRP can function as an autocrine growth factor for SCLC and other tumors, including pancreatic cancers, motivated various groups to develop bombesin/GRP antagonists [5, 12, 63–67]. Diverse bombesin/GRP antagonists were synthesized in our laboratory (table 2). Among these compounds was RC-3095 [D-Tpi^6,Leu$^{13}\psi$(CH_2NH)Leu^{14}]bombesin(6–14) which showed a strong inhibitory effect on many experimental cancers [5, 12, 63]. Later, we modified C- and N-terminal amino acids, and produced the antagonist RC-3940-II [Hca^6,Leu$^{13}\psi$(CH_2N)Tac^{14}]bombesin(6-14) with increased binding affinity to the receptors and stronger antitumor activity. These potent antagonists block GRP-stimulated amylase release from rat pancreatic acini, show strong binding affinity to Swiss 3T3 and H-346 SCLC cells and inhibit GRP-stimulated growth of these cells in vitro. The tumor inhibitory mechanism of bombesin/GRP antagonists appears to be more complex than a simple competitive action on the receptor. Bombesin/GRP antagonists affect intracellular second messengers, causing changes in calcium concentrations [5]. In addition to intracellular calcium mobilization and protein kinase C (PKC) activation, bombesin-like peptides also affect extracellular receptor kinase (ERK) and C-jun N-terminal kinase mitogen-activated protein kinase (JNK MAP) kinase pathways [68]. In this way antagonists can interfere with growth-promoting signals originated from other receptors. The main mechanism of tumor inhibitory action of bombesin/GRP antagonists appears to involve the reduction in levels of EGF receptors on tumors [5, 12, 69]. After administration, bombesin/ GRP antagonists are rapidly eliminated from the bloodstream, but EGF receptors remain downregulated for many hours. Thus, a single daily injection of the antagonists can maintain tumor growth inhibition [5]. Although bombesin receptors are widely expressed in the GI tract and other tissues, no side effects of treatment with bombesin/GRP antagonist RC-3095 have been detected so far in clinical phase I/II trials.

Cytotoxic bombesin analogs

Since bombesin/GRP receptors are expressed in various tumors, the application of radiolabeled bombesin analogs for tumor detection has been proposed [5, 6, 12, 70]. ^{111}In-labeled analogs of bombesin are rapidly internalized and stored in the receptor-expressing cells, allowing a more specific and longer-lasting visualization of tumors. Recently, a ^{188}Re(I)-radiolabeled bombesin analog that maintains high specificity for the GRP receptor in vivo was developed [71]. This analog [^{188}Re(H_2O)(CO_3)-diaminopropionic acid-SSS-bombesin(7-14)NH_2] was reported to specifically target GRP receptors on human prostate cancer and PC-3 cells [71]. A 99m-Technecium-labeled bombesin analog has also been used successfully for the scintigraphic imaging of breast cancers in patients [72].

We synthesized targeted cytotoxic bombesin analogs using as carriers bombesin-like antagonists, which have high binding affinity to bombesin/GRP receptor subtype 1 [73]. Doxorubicin or AN-201 were used as cytotoxic agents and were linked to carriers such as RC-3095 and its amino terminally truncated analog to form cytotoxic bombesin-like hybrids. Thus, cytotoxic bombesin analog AN-215 was prepared by linking AN-201-14-*O*-hemiglutarate to the amino terminal of des-D-Tpi-RC-3095 (table 2). This analog showed high binding affinity to bombesin/GRP receptors on Swiss 3T3 cells [73]. We then demonstrated that bombesin receptors present on experimental human tumors could be used for targeting cytotoxic bombesin analogs in vivo. The effectiveness of cytotoxic bombesin analog AN-215 was evaluated in nude mice bearing various human cancers, including gastric. As in the case of the receptors for somatostatin, the receptors for bombesin/GRP are present in various normal tissues, but in our experimental studies with cytotoxic bombesin analog AN-215 we did not observe any receptor-specific toxicity on GI function [6].

GH-RH

GH-RH is secreted by the hypothalamus and stimulates the synthesis and the release of GH from the pituitary. GH-RH is also present in various extrahypothalamic tissues, including the gastrointestinal tract and in tumors of neuroendocrine origin [74]. An ectopic production of GH-RH by carcinoid and pancreatic cell tumors was demonstrated in 1981 by Frohman and Szabo [10]. The 44- and 40-amino acid forms of GH-RH were first purified from human pancreatic tumors and identified and only subsequently isolated from hypothalamic tissue [75]. GH-RH is structurally related to vasoactive intestinal peptide (VIP). The full biological activity is contained in the NH_2-terminal 29-amino acid sequence [GHRH(1-29)NH_2] (table 3).

GH-RH and GH-RH receptors in human cancers

The expression of messenger RNA (mRNA) for GH-RH and the presence of biologically or immunologically active GH-RH was demonstrated in various human tumors, including cancers of the breast, endometrium and ovary, prostate and lung [5, 12]. GH-RH was also found in human gastroenteropancreatic cancers [76]. We detected mRNA for GH-RH in pancreatic (SW1990, PANC-1, MiaPaCa-2, Capan-1, Capan-2 and CFPAC1), gastric (NCI-N87, HS746T and AGS) and colonic (COLO 320DM and HT-29) cancer lines. In studies in vitro with pancreatic, colonic and gastric cancer lines, exogenously added GH-RH(1-29)NH_2 increased the rate of cell proliferation.
Peptide receptors on tumors that might mediate the effects of GH-RH, and its antagonists were identified recently [77, 78]. The isolation and sequencing of complementary DNAs (cDNAs) encoding tumoral GH-RH receptors revealed that they are splice variants (SVs) of the pituitary GH-RH receptors [77]. Reverse transcriptase-polymerase chain reaction (RT-PCR) analyses revealed the expression of receptor SVs in several cancers. The major part of the cDNA sequence of SV_1 is identical with the corresponding sequence of pituitary GH-RH receptor cDNA, but the first 334 nucleotides of SV_1 and SV_2 are different from those of the pituitary GH-RH receptor. SV_1 appears to be the major isoform of GH-RH receptors expressed in neoplastic tissues [77, 78]. mRNA for the SV_1 isoform of GH-RH receptors is expressed in tumors of pancreatic (SW1990, PANC-1, MiaPaCa-2, Capan-1, Capan-2 and CFPAC1), colorectal (COLO 320DM and HT-29) and gastric (NCI-N87, HS746T and AGS) cancer cell lines [76] (see sections on respective tumors). These findings suggest that in some tumors, GH-RH and its tumoral receptor could form an autocrine/paracrine mitogenic loop, which might be involved in the control of the malignant growth. The inhibitory effect of GH-RH antagonists on cancers could be based in part on the interference with the local stimulatory GH-RH system.

Table 3. Structures of human secretin, VIP, GH-RH and its antagonistic analogs.

Secretin	His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-Leu-Ser-Arg-Leu-Arg-Glu- Gly-Ala-Arg-Leu-Gln-Arg-Leu-Leu-Gln-Gly-Leu-Val-NH_2
VIP	His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH_2
hGH-RH(1-29)NH_2	Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Val-Leu-Gly- Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg-NH_2
GH-RH antagonists	
JV-1-38	[PhAc-Tyr^1,D-Arg^2,$Phe(4\text{-}Cl)^6$,Har^9,$Tyr(Me)^{10}$,Abu^{15},Nle^{27},D-Arg^{28},Har^{29}]hGH-RH(1-29)NH_2
JV-1-63	[PhAc-Tyr^1,D-Arg^2,$Phe(4\text{-}Cl)^6$,Har^9,Amp^{10},Abu^{15},Nle^{27},D-Arg^{28},Har^{29}]hGH-RH(1-29)NH_2
JV-1-65	[PhAc-Tyr^1,D-Arg^2,$Phe(4\text{-}Cl)^6$,Amp^9,$Tyr(Me)^{10}$,Abu^{15},Nle^{27},D-Arg^{28},Har^{29}]hGH-RH(1-29)NH_2

PhAc, phenylacetyl; Phe(4-Cl), 4-chlorophenylalanine; Amp, 4-amidino-phenylalanine; Abu, -aminobutyric acid; Nle, norleucine; Har, homoarginine.

Antagonistic analogs of GH-RH

The clinical need for GH-RH antagonists became apparent after it was established that somatostatin analogs do not adequately suppress GH and IGF-I levels in patients with tumors potentially dependent on IGF-I. First, it was found that replacement of Ala^2 by D-Arg^2 within the NH_2 terminal 29-amino acid segment of GH-RH produces GH-RH antagonists [79] as in [Ac-Tyr^1,D-Arg^2]-hGHRH(1-29)NH_2. Systematic efforts to develop better GH-RH antagonists led to potent GH-RH antagonists MZ-4-71 and MZ-5-156 [80]. Other substitutions were then incorporated into GH-RH antagonists, yielding analogs such as JV-1-36, JV-1-38 and JV-1-65 with increased activity [81, 82] (table 3). These compounds show a high binding affinity to pituitary and tumoral GH-RH receptors, and strongly inhibit pituitary GH secretion, in vitro and in vivo [5, 82].

GH-RH antagonists can inhibit tumor growth indirectly through suppression of the endocrine GH/IGF-I axis, and also by direct action on the tumor cells [5, 82]. The indirect mechanism is important for those cancers that depend on IGF-I as a growth factor. A strong positive association was reported between plasma IGF-I levels and the risk of prostate, breast and colorectal cancers [82, 83]. GH-RH antagonists decrease the levels of IGF-I in serum by inhibiting the release of GH from the pituitary, which results in a suppression of hepatic IGF-I production. Direct inhibitory effects of GH-RH antagonists appear to be mediated by the tumoral SVs of GH-RH receptors, through mechanisms that may or may not involve tumoral IGF-I and IGF-II. Thus, in many human cancer lines, including pancreatic and colorectal cancers, GH-RH antagonists inhibit the production of IGF-I and IGF-II and the expression of IGF-II mRNA by an action apparently exerted through SVs of GHRH receptors. Since IGF-I and IGF-II are potent mitogens for many cancers, a suppression of their production would inhibit tumor growth. In addition, IGF-I can enhance angiogenesis by inducing the expression of VEGF [84]. However, in the case of H-69 SCLC and other cancers, tumor inhibition is not associated with a suppression of production of IGF-I and IGF-II, but appears to be due to the blockade of the stimulatory action of tumoral autocrine GH-RH by GH-RH antagonists. In some tumors, more than one of these mechanisms may operate [5, 12, 82]. GH-RH antagonists may offer advantages over other classes of prospective antitumor agents. Therapy with GH-RH antagonists should be devoid of severe side effects typical of chemotherapy. Because GH-RH antagonists inhibit IGF-II-dependent tumors, they should be superior to GH antagonists, as the synthesis of IGF-II is not controlled by GH. GH-RH antagonists could be also used for suppression of tumors that do not express somatostatin receptors or those that contain only low receptor levels. No clinical trials have been carried out so far with advanced GH-RH antagonists. The administration of an early GH-RH antagonist was reported to reduce GH hypersecretion in a patient with metastatic GH-RH-secreting carcinoid tumor, but the effect of large i.v. doses lasted only 3–4 h [85].

Ghrelin

Recently, an endogenous ligand specific for receptors of GH secretagogues has been isolated from the rat stomach and identified as a 28-amino acid peptide having an unusual octanoyl ester on its Ser^3 side chain [86]. It was designated the GH-releasing peptide or ghrelin [86], and it is considered a stomach hormone. Ghrelin and its receptors are also present in the pituitary and hypothalamus [87]. Human ghrelin is homologous to rat ghrelin, except for two amino acids. Ghrelin is produced in mucosal endocrine cells of the stomach and intestine and secreted from the stomach. Ghrelin circulates in the blood stream and is present in human plasma at considerable levels. Ghrelin appears to be involved in the regulation of pituitary GH release and control of appetite. Recently, ghrelin and two types of receptors for ghrelin were identified in some tumors, including breast cancer, prostate cancer and gastrointestinal endocrine tumors [88, 89]. Thus, Papotti et al. [88] recently reported that most gastric carcinoids (and related neuroendocrine cell hyperplasias) and some intestinal carcinoids produce ghrelin. The clinical impact of hypersecretion of ghrelin is unknown, but it may be involved in some malignancies [90]. Plasma ghrelin levels recorded in patients with gastroenteropancreatic tumors, gastrointestinal carcinoids, gastrinomas, insulinomas, VIPomas and glucagonomas were similar to controls [91].

Gastrin

The gastrin molecule first isolated by Gregory et al. from hog antral mucosa, identified and then synthesized, had 17 amino acid residues [92] and it was called little gastrin (G-17) (table 2). This heptadecapeptide is strongly acidic. Other forms of gastrins have been also isolated. A form called big gastrin (G-34) by Yalow and Berson [93] consists of heptadecapeptide G-17 with additional 17 amino acids at the N-terminus. Minigastrin (G-14) has the same C-terminal as G-17 and G-34 [94]. The three molecules, gastrin G-14, G-17 and G- 34, exist in circulation [94]. All gastrins except for synthetic pentagastrin exist in an unsulfated form and a form with sulfated tyrosine in position 6 from the C-terminal [94]. All biologic actions of gastrin reside in the C-terminal tetrapeptide amide group -Trp-Met-Asp-Phe-NH_2, which is the same in all gastrin molecules [94]. Gastrin is made and released from the G cells

of antral mucosa [94]. The main physiologic action of gastrin is the stimulation of acid secretion from the parietal cells of the stomach [94]. Gastrin also evokes pepsin secretion and increases gastric mucosal blood flow. Gastrin stimulates the proliferation of the cells of the stomach, colon and pancreas [94, 95]. Administration of gastrin or increased endogenous serum gastrin levels, as in Zollinger-Ellison syndrome, leads to a greater proliferation of gastrointestinal mucosa [96, 97]. Gastrin release is stimulated by bombesin, GRP and inhibited by secretin, glucagon, gastric inhibitory polypeptide (GIP) and VIP [94]. The effects of gastrin on stomach, colorectal and pancreatic cancers are discussed in specific individual sections.

Cholecystokinin

Cholecystokinin (CCK), previously also called pancreozymin, was first purified from hog small intestine by Mutt and Jorpes [98] and identified as a linear 33-amino acid peptide hormone (table 2). It occurs in several molecular forms, including CCK-4, CCK-8, CCK-33, CCK-39 and CCK-58 [94, 99]. The C-terminal pentapeptide of CCK is identical to that of gastrin, and it also occurs in amphibian caerulein [94]. Like gastrin, cholecystokinin has a sulfated tyrosine in position 7 from C-terminus [100]. CCK is present throughout the small intestine and is also widely distributed throughout both the central and peripheral nervous systems [101]. The chief physiologic actions of CCK consist of stimulation of pancreatic enzyme secretion and activation of contractions of the gallbladder [94]. The release of CCK is induced by the presence of fat and protein within the intestinal lumen, and its levels are increased by bombesin [94]. CCK and other GI peptides can influence the rate of growth of neoplastic tissue [96, 102]. In nude mice bearing transplanted pancreatic cancer tissue, exogenous CCK augments the growth of tumor [102]. In hamsters, CCK stimulates the development of nitrosamine-induced pancreatic cancer [103]. CCK can also increase the growth of human pancreatic cancer cells in vitro [96, 102].

Secretin

Secretin was the first hormone to be discovered [104]. It was isolated by Mutt et al. [105] from pig small intestine and identified in 1965. Secretin is composed of 27 amino acid residues, and its N-terminus is similar to that of glucagon (table 3). Immunoreactive secretin has been detected in the duodenum, jejunum and in the central nervous system (CNS) [94]. The main action of secretin is to increase pancreatic water and bicarbonate secretion [106]. Secretin is released by duodenal acidification [94], but not by GRP/bombesin. In the stomach, secretin can inhibit gastrin-stimulated acid secretion in most species, including man. Somatostatin analogs RC-121 and RC-160 are potent inhibitors of secretin-induced exocrine pancreatic secretion of HCO_3^- [15].

VIP

VIP was first isolated from porcine small intestine [107], and it is a potent vasodilatory peptide containing 28 amino acid residues (table 3). VIP has a considerable degree of homology with GH-RH, secretin and glucagon [94]. VIP is also structurally related to the pituitary adenylate cyclase-activating polypeptide (PACAP), which has 38 amino acids [108]. The N-terminal 28-amino acid sequence of PACAP has 70% sequence homology with VIP [108]. VIP is found throughout the gut and in the CNS. VIP stimulates gastric and intestinal secretion and enzyme release from the exocrine pancreas [108] in man and other mammals. It also increases GH release and inhibits the action of somatostatin. VIP acts as a neurotransmitter or neuromodulator, participates in the regulation of a variety of bodily functions and may be involved in the pathogenesis of several diseases [109]. Overproduction of VIP by tumors is associated with 'pancreatic cholera', the watery diarrhea-hypochloremia-hypochlorhydria (Verner and Morrison syndrome). There are two VIP receptors, $VPAC_1$ and $VPAC_2$, both with high affinity for VIP and PACAP that can be distinguished pharmacologically by the $VPAC_1$-selective analog [110]. Moody et al. [111] reported that VIP and PACAP stimulate the growth of lung cancer cells. Thus, VIP strongly increases colony formation of SCLC and non-SCLC cells [111]. The VPAC1 receptor antagonist VIPhybrid (VIPhybd) inhibits the clonal growth of lung cancer cells [111, 112]. These data suggest that VIP may be a regulatory peptide in SCLC and non-SCLC. However these findings remain controversial, since it has also been reported that VIP suppresses the growth of SCLC tumor implants in athymic nude mice in vivo [113].

Pancreatic polypeptide

Pancreatic polypeptide (PP) has a sequence of 36 amino acids, and its main source is the pancreas [114]. CCK appears to be the regulator of PP release. The principal action of PP is thought to be the inhibition of pancreatic exocrine secretion [94]. For a more comprehensive description of these and other gastrointestinal peptides, the readers are referred to specific reviews [94, 115] and various textbooks.

Gastric carcinoma

In 2003 there were an estimated 22,000 new cases of gastric carcinoma, with 12,000 deaths in the USA [1]. Thus carcinoma of the stomach ranks seventh in the USA among causes of cancer-related deaths. Nevertheless, stomach cancer remains the fourth most common cancer in Europe in incidence, with over 207,000 new cases and 152,000 deaths, the third largest for cancer in 1995 [2]. Moreover, stomach cancer is the leading cause of mortality from cancer in Japan, China and India [3]. It is estimated that in 1990 there were 800,000 new cases and about 630,000 deaths from gastric cancer worldwide [3]. Thus, gastric cancer is number 2 overall in the world, second only to lung cancer and remains a major international health problem [3]. More than 90% of stomach cancers have been reported to be adenocarcinomas, and the remainder are predominately non-Hodgkin's lymphomas [116]. Multiple genetic alterations are responsible for the development and progression of gastric cancer [117]. Gastric carcinoma can be divided into two distinct types, the intestinal type and the diffuse type, that can be distinguished by characteristic histological features [118, 119]. The intestinal type carcinoma is often preceded by multifocal atrophic gastritis and is more common in elderly men. Diffuse type cancer occurs in patients under the age of 50 and mainly in women, and has a less favorable prognosis, but its histologic precursor lesion is usually not identifiable [120, 121]. *Helicobacter pylori* infection is the major risk factor for development of gastric cancer [122]. *H. pylori* has been accepted as a human carcinogen, but it is assumed to have an indirect action as it provokes gastritis and only later metaplasia and dysplasia [120]. However, other bacteria and environmental factors may also play a role in the development of gastric cancer, because ~25% of gastric cancer patients have no evidence of *H. pylori* infection [121].

The prognosis for gastric cancer patients depends on the clinical and pathological stage at diagnosis [1–3, 122–124]. Surgical resection is effective in early-stage cancers. However, despite improved surgical procedures and the widespread use of endoscopy, the prognosis for patients with gastric cancer remains relatively poor as most cases are diagnosed at an advanced stage [2]. Approximately 65% of patients with gastric cancers have regional or distant metastases at the time of diagnosis [123]. Radiation therapy and chemotherapy are of limited use, and only 20–40% of patients at later stages of the disease respond to chemotherapy [124]. The present median survival rate for gastric cancer patients in the USA is only 6–12 months [124]. New treatment modalities are needed for patients with locally advanced and metastatic gastric cancer.

GI hormones, especially gastrin and bombesin/GRP, and growth factors such as EGF, IGF-I and -II and transforming growth factor-α (TGF-α) are implicated in the growth of human gastric adenocarcinoma [5, 12]. Gastrin appears to be an important trophic hormone both for normal and for malignant gastrointestinal mucosal cells, and human gastric tumors show significant responses to human gastrin-17 (G-17), as based on ^{75}Se-selenomethionine uptake [125]. Gastrin also stimulates growth of the MKN-45 human gastric cancer cell line in vitro and in vivo [126]. G-protein-coupled CCK-B/gastrin receptor is essential for the proliferation of gastric mucosal cells in vivo [127]. Proliferative responses to gastrin may be due to paracrine effects, mediated by increased production of growth factors such as members of the EGF family [128].

EGF receptors and IGF-I receptors are expressed on human gastric cancer cell lines. Both TGF-α and IGF-I promote cell growth and increase the response of the cells to gastrin [129]. Gastric cancer patients also show higher serum and urinary c-erbB-2 levels than healthy controls [130]. The overexpression of c-erbB-2 protein and amplification of c-erbB-2 in gastric adenocarcinomas seem similar to the well-established findings in breast cancers [131]. In gastric adenocarcinoma, HER-2/neu positivity appears to be linked to advanced rather than early disease [132]. Most studies suggest a prognostic value of HER-2/neu status for this tumor [132]. Patients having gastric adenocarcinoma with c-erbB-2 amplification are potential candidates for adjuvant therapy using herceptin, a humanized monoclonal antibody to c-erbB-2 gene product [131]. Vascular endothelial growth factor (VEGF) also induces the proliferation of gastric adenocarcinoma cells as well as tumor angiogenic cells [133].

Experimental studies on gastric carcinomas

Our results [134] and those of others [135] indicate that somatostatin analogs such as RC-160 (Vapreotide) and Sandostatin (Octreotide) decrease serum gastrin and inhibit the growth of MKN-45 human gastric cancer xenografts in nude mice. Receptors for the GRP and neuromedin B (NMB) are found throughout the gastrointestinal tract in the submucosal and myenteric plexuses. The receptors for bombesin and GRP are present in human gastric cancers and gastric cancer lines [136]. Preston et al. found that 13 of 23 gastric cancers expressed high-affinity binding sites for bombesin, of which 12 were found to be of the GRP-preferring subtype [136]. Thus GRP may be implicated in the pathogenesis of human GI malignancies, and bombesin/GRP antagonists may have therapeutic potential in human GI cancers [136]. We demonstrated that bombesin stimulates the growth of Hs746T cells in vitro and that the binding of bombesin to Hs746T and MKN-45 cells is inhibited by the antagonist RC-3095 and other bombesin/GRP antagonists [134, 137]. We also showed that the administration of bombesin/

GRP antagonist RC-3095 inhibits the growth of MKN-45 and Hs746T human gastric cancers xenografted into nude mice or cultured in vitro and causes downregulation of EGF receptors on tumor cells [134, 137]. Serum gastrin levels were significantly diminished in RC-3095-treated groups [134].

In view of the presence of the receptors for bombesin and somatostatin in the gastric cancer and gastric cancer lines, we investigated the subtypes of receptors for somatostatin and bombesin in various human gastric cancer lines. We also tested the antitumor effects of cytotoxic somatostatin analog AN-238 and bombesin conjugate AN-215 [138], both containing cytotoxic radical AN-201, on cancer lines xenografted into nude mice. Cytotoxic bombesin analog AN-215 powerfully inhibited the growth of AGS cancers that express high-affinity subtype 1 bombesin receptors. AGS, Hs746T and NCI-N87 human gastric cancers expressed high-affinity sst_2 and sst_5 receptors. Cytotoxic somatostatin analog AN-238 exerted a strong inhibitory effect in vivo on NCI-N87 and Hs746T cancers which displayed a high concentration of somatostatin receptors, but had a weaker effect on AGS tumors which expressed the lowest receptor levels [138]. Cytotoxic radical AN-201 had only insignificant effects. The results indicate that the growth of human gastric cancers that have a high concentration of sst_2 or sst_5 somatostatin receptors is suppressed by cytotoxic somatostatin analog AN-238. Gastric cancers that express subtype 1 bombesin receptors are inhibited by cytotoxic bombesin analog AN-215 [138]. These findings suggest that this class of targeted compounds should be considered for the therapy of advanced gastric cancer.

Reubi et al. [110] demonstrated that 54% of gastric carcinomas express VIP/PACAP receptors. It was also reported that scanning with radiolabeled VIP can visualize gastric cancers and metastases that express receptors for VIP. Thus, Virgolini et al. [139] indicated that primary or recurrent gastric adenocarcinomas were visualized in five of five patients with VIP labelled with iodine-123, and liver metastases were seen in two of two patients.

We examined the expression of mRNA for GHRH and for SVs of its receptors in tumors of human gastric cancer cell lines. mRNA for both GHRH and SV-1 isoform of GHRH receptors was expressed in gastric NCI-N87, HS746T and AGS cancer cell lines [76]. In proliferation studies in vitro, the growth of gastric cancer cells was stimulated by GHRH(1-29)NH_2 and inhibited by GHRH antagonist JV-1-38. This study indicates the presence of an autocrine/paracrine stimulatory loop based on GHRH and SV-1 of GHRH receptors in human gastric cancers. The finding of SV-1 receptor in human cancers provides an approach to an antitumor therapy based on the blockade of this receptor by specific GHRH antagonists.

Clinical trials

There may be an increased risk of stomach cancer in acromegaly [140]. Clinical trials with cytotoxic peptide analogs and GH-RH antagonists in patients with gastric cancers are still in the planning stage. Bombesin/GRP antagonist RC-3095 is in phase II trials. In a trial on somatostatin analog octreotide carried out by Cascinu et al. [141], 15 patients with advanced gastric cancer refractory to chemotherapy were given octreotide at a dose of 200 µg three times a day (t.i.d.) for 5 days a week, while 14 received supportive care only. The duration of survival in patients treated with octreotide was extended to a median time of 16 weeks compared with 8 weeks in the control group. None of the patients showed objective responses, but 7 patients given octreotide had stable disease compared with 3 in the control group [141]. Octreotide was also used as an adjuvant analgesic in the management of gastrointestinal cancer pain [142]. The hormonal approaches for treatment of gastric cancer based on analogs of somatostatin, GH-RH and bombesin/GRP should be actively pursued. Targeted cytotoxic somatostatin analog AN-238 and bombesin conjugate AN-215 may overcome the chemoresistance of patients with advanced, metastatic gastric cancer.

Colorectal cancer

Colorectal cancer is the third most common malignancy among men and women in the USA and overall, combining both sexes, the second leading cause of cancer-related deaths [143]. It is estimated that in 2003 there were about 147,000 new cases of colon and rectum cancer in the USA and that about 57,000 Americans die of these cancers every year [1]. In Europe, colorectal cancer ranks second in frequency of new cases in both men and women, with about 334,000 new cases estimated for 1995, distributed about equally between the sexes [2]. Deaths from cancers of the colon and rectum in Europe (189,000 in 1995) also ranked second after lung cancer [2]. In terms of global incidence, colorectal cancer ranked third in frequency in men and second in women, but it was a fourth leading cause of mortality in both sexes [3]. Thus, worldwide colon and rectal cancer accounted for 783,000 new cases and caused 437,000 deaths in 1990 [3].

Colorectal cancer has a relatively good prognosis, and in Europe 5-year relative survival is approximately 46% for cancers of the colon and rectum [2]. Computed tomography (CT) colonography, immunochemical fecal occult blood tests and stool screening using molecular markers can be used for colorectal cancer screening [143]. Screening can also detect early-stage colorectal cancer when it is responsive to treatment, as shown by the findings that 90% of patients in the USA diagnosed with localized disease are alive 5 years after diagnosis [143]. However, only

about 37% of colorectal cancers are diagnosed at this stage [143]. Colorectal cancer is presently treated by surgical ablation, but many colorectal cancers are detected at a late stage when surgery cannot cure the disease. At least 40% of patients with colorectal cancer develop metastases during the course of their illness [144]. Chemotherapy alone or combined with radiotherapy can be used as a treatment approach for advanced disease and as an adjuvant therapy to surgery [144]. However, none of these approaches is highly effective against disseminated colonic cancer [145]. New treatment modalities are needed for treatment of advanced or metastatic colorectal cancer. The combination of 5-fluorouracil (5-FU) plus levamisole as adjuvant therapy can reduce the recurrence rate and death rate in colon cancer patients after surgery [146]. Adjuvant therapies of colon cancer include new agents such as oxaliplatin and CPT 11 and angiogenesis inhibitor Avastin. Tumor growth requires angiogenesis, which is a complex process regulated by stimulatory and inhibitory factors [147]. The development of anti-angiogenic agents, such as a monoclonal antibody against VEGF, is a promising approach to treat many tumors, including metastatic colorectal cancer [148]. Patients treated with a combination of chemotherapy and monoclonal antibodies against VEGF had a significantly improved response rate over those receiving chemotherapy alone [149].

Resistance to chemotherapy may be the consequence of genetic alterations in tumors, and in colorectal cancer, a poor response to chemotherapy has been associated with mutations in the *p53* gene [150]. Thus, the development of new therapeutic agents that can overcome resistance caused by mutant *p53* is an important goal in the management of this malignancy [151].

Various reports indicate the possible involvement of growth factors such as IGF-I and -II and TGF-α and GI hormones, especially gastrin and bombesin/GRP in the tumorigenesis of the colon [5, 12]. IGF-I receptors are present on primary specimens of human colon carcinomas and colorectal cancer cell lines [152]. IGF-I and IGF-II stimulate growth of HT-29, LS411N, LS513, SW480 and WiDr human colorectal carcinoma cell lines [153]. The incidence of colon cancer is increased in acromegaly, suggesting that excessive secretion of GH, IGF-I or GH-RH may be a factor. Serum IGF-I seems to be implicated in the development of colorectal neoplasia in acromegaly [154]. The results of a nested case-control study show an association between colorectal cancer risk in men and elevated plasma levels of IGF-I [83]. Western life style may result in obesity and elevated insulin levels, which are associated with a decrease in the production of IGF-binding proteins and can consequently lead to an increase in bioavailability of IGF-I. Therefore, diet and associated factors may influence the risk of colorectal cancers [155], and circulating IGF-I levels may regulate colon cancer growth and metastasis [156]. IGF-II mRNA and IGF-II peptide are also overexpressed in primary human colon cancers [157]. The levels of circulating IGF-II are likewise elevated in patients with colorectal adenomas and colorectal cancer [158]. The possible roles of the IGF system in colorectal cancer were reviewed by Hassan and Macaulay [159].

EGF receptors are also expressed in colorectal cancer cell lines. Both TGF-α and IGF-I promote cell growth of colorectal cancer cell lines and increase the response of the cells to gastrin [129]. Karnes et al. [160] showed that monoclonal antibody mAb225 against EGF receptors can inhibit the proliferation of some colorectal carcinoma cell lines that co-express TGF-α and EGFR. There is considerable interest in HER-2/neu as a prognostic factor and target site of therapy in adenocarcinomas of colon [132]. The expression of HER-2/neu may have significant prognostic value in colorectal cancer, and HER-2/neu overexpression appears to be an indicator of adverse outcome [132]. VEGF-C is also expressed in human colorectal cancer tissues and may play an important role in lymphatic spread of colorectal cancer [161].

The gastrin gene is expressed in the normal human colon and colorectal adenocarcinoma [162]. Gastrin-17 stimulates growth of primary human colorectal adenocarcinomas [163], and may function as an autocrine growth factor in some cultured colon carcinoma cell lines [164]. Gastrin receptor antagonist CR2093 reverses this gastrin-stimulated growth and inhibits the basal growth of primary human colorectal cancers [163] expressing gastrin receptors. Pentagastrin also stimulates cancer cell proliferation in chemically induced colon cancer in rats [165]. It was also reported that pentagastrin significantly increases tumor growth of human colon adenocarcinoma cell lines CX1, X56 and HT29 xenografted into nude mice [166]. Somatostatin alone significantly inhibited tumor growth in two of the cell lines and also inhibited gastrin-induced growth [166]. Increased serum gastrin has been detected in patients with adenocarcinoma of the colon [167, 168]. Watson et al. [163] suggested that antigastrin agents may be of clinical value in the treatment of colorectal tumours.

Laws et al. [43] investigated somatostatin receptor subtype mRNA expression in human colorectal cancer and normal colonic mucosae. They found that tumor expression of sst_2 and sst_5 is retained after malignant transformation in colonic epithelium. Other ssts (1, 3 and 4) were expressed infrequently [43]. Human colorectal cancer tissue and human colonic cancer cell lines also express bombesin binding sites of the GRP-preferring subtype [169]. A high GRP receptor mRNA level of human colorectal tumors is related to tumor dedifferentiation and lymphatic vessel invasion [170]. Williams and Schonbrunn [171] characterized functional bombesin receptors in a human duodenal cancer cell line HuTu-80. [^{125}I-Tyr^4]bombesin binding was inhibited by bombesin receptor agonists and antagonists

including GRP-(14-27), [D-Phe^6]bombesin(6–13)ethylamide and neuromedin B. These receptors of GRP subtype are functionally coupled to second messenger production, but do not stimulate cell proliferation [171]. In our studies, 8 of 15 clinical specimens (53%) of human colon cancer showed the presence of somatostatin receptors [45]. Receptors for bombesin were found in 6 (40%) specimens of human colon cancer, but not in normal colonic mucosa specimens. Bombesin antagonists inhibited the binding of ^{125}I-Tyr^4-bombesin [45]. Recent studies also show that most colon cancers, but not normal colonocytes, express GRP, GRP receptor and mRNA for GRP receptor [172]. Thus, GRP could be an autocrine growth factor in colorectal cancer [59].

About 96% of colorectal carcinoma express VIP/PACAP receptors [110]. The high density of VIP receptors in colorectal cancers allows their detection with radioimaging [173]. Scanning with VIP radiolabeled with ^{123}I can visualize intestinal tumors and metastases that express receptors for VIP [174]. In patients with colorectal adenocarcinomas, primary or recurrent tumors were visualized in 10 of 10 cases, and liver metastases in 15 of 18 patients [139]. Among 17 colorectal adenocarcinomas, 4 tumors with positive VIP scans were also visualized with radiolabeled somatostatin analogues [139].

Studies on effects of peptide analogs in experimental colon cancers

Approaches based on hormonal manipulations, such as the use of analogs of somatostatin or antagonists of bombesin/GRP, have been tried in experimental colorectal cancer. In our initial studies on colorectal cancer with peptide analogs, we showed that somatostatin analog RC-160 inhibited in vivo growth of transplanted DHD/K12 rat colon cancer and its hepatic metastases [175, 176]. Chronic administration of RC-160 also significantly inhibited the incidence and growth of liver metastases of 320 DM and WidR human colon cancer cell lines [177]. It was similarly reported that octreotide inhibits in vivo and in vitro growth of human colon cancer cell lines LIM 1215, LIN 2045 and LIM 2412 [178]. We also showed that RC-160 and bombesin/GRP antagonists RC-3095 and RC-3440 inhibit the growth of the HT-29 human colon cancer cell line transplanted into nude mice [179, 180]. In addition, RC-3095 inhibited metastatic tumor growth after intrasplenic injection of HT-29 cells into nude mice [180]. Specific binding sites of somatostatin, bombesin and EGF were detected on intact HT-29 cells or on the membranes from HT-29 tumor xenografts [180]. The inhibitory effects of bombesin antagonists on tumor growth were consistently linked with a significant downregulation of EGF receptors [180]. Somatostatin analogs can potentiate the antitumor effects of cytotoxic agents, as it was shown that lanreotide synergistically enhanced the antiproliferative action of 5-FU and mitomycin C on colon cancer cell lines [181]. In various malignancies, including colorectal cancers, there may be a loss of gene expression for sst_2, which is the preferred subtype for somatostatin octapeptide analogs [23]. However, the expression of sst_5 and sst_3 should make possible the therapy with these analogs labeled with various radioisotopes or linked to cytotoxic agents. Because at least the subtype 5 of somatostatin receptors (sst_5) is present in colorectal carcinomas, we tested the targeted cytotoxic somatostatin analog AN-238 on human colon cancer lines xenografted into nude mice [182]. AN-238 inhibited the growth of HCT-15 and HT-29 colorectal cancers that express mutant *p53*, whereas AN-201 and DOX showed no effect. AN-238, AN-201 and DOX were equally effective on HCT-116 tumors that express wild-type p53. None of the compounds could suppress the proliferation of LoVo tumors that lack functional ssts. Thus, cytotoxic somatostatin analog AN-238 can inhibit the growth of experimental colon cancers that express ssts, regardless of their *p53* status [182].

The colon has a high concentration of immunoreactive VIP [183]. It was shown that VIP inhibits the growth of human colon carcinoma cell lines in monolayer culture. Immunocytochemical studies indicate that VIP induces differentiation-promoting effects in colon cancer cells [184].

In view of the involvement of IGF-I and IGF-II in the growth of colorectal cancers [5, 12, 82], we evaluated the effects of GH-RH antagonists on the growth of experimental colon cancers. Thus, we treated nude mice bearing xenografts of HT-29 human colon cancer with GH-RH antagonists MZ-4-71, MZ-5-156 and JV-1-36 [185]. All the GH-RH antagonists inhibited the growth of HT-29 colorectal cancer and reduced IGF-II concentrations and IGF-II mRNA expression in tumors. In vitro, antagonist MZ-5-156 also decreased IGF-II production and the proliferation of HT-29 cells [185]. These studies demonstrate that GH-RH antagonists can inhibit the growth of HT-29 human colon cancers in part through a reduction in the production and secretion of IGF-II by cancer cells [82, 185]. However, the effect of GH-RH antagonists on tumoral GH-RH may be also involved. Thus, mRNA for both GH-RH and the SV_1 isoform of GH-RH receptors is expressed in tumors of human colorectal COLO 320DM and HT-29 cancer cell lines [76]. In vitro, the growth of colonic cancer cells is stimulated by GH-RH(1–29)NH_2 and inhibited by GH-RH antagonist JV-1-38 [76]. Consequently, an autocrine/paracrine stimulatory loop based on GH-RH and SV_1 of GH-RH receptors could operate in human colorectal cancers. Some of the inhibitory effects of GH-RH antagonists on colorectal cancers could have been exerted through the SVs of GH-RH receptor on tumors. The discovery of the GH-RH SV_1 receptor in human colorectal cancers makes possible a new strategy for therapy based on blockade of this receptor by GH-RH antagonists [5, 82].

Clinical studies

Pollak et al. reported that octreotide in doses of 400 μg t.i.d. can reduce serum IGF-I levels in patients with colorectal cancers [186]. In a trial carried out by Cascinu et al. [141], 46 patients with advanced colorectal cancer refractory to chemotherapy were randomized, and 24 patients received octreotide at a dose of 200 μg t.i.d. for 5 days a week, while 22 were given supportive care only. Patients treated with octreotide showed an improvement in duration of survival for a median time of 24 weeks versus 12 weeks in the control group [141]. In addition, 11 patients given octreotide showed stable disease, compared with only 3 in the control group [141].

Goldberg et al. [187] also showed that octreotide at a dose of 150 μg given t.i.d. is not an effective therapy for patients with advanced asymptomatic colon carcinoma. Other studies with octreotide, summarized in [188], showed no extension of survival in patients with colorectal cancer, although treatment with octreotide lowered serum IGF-I levels and reduced proliferative activity of tumors. Another analog of somatostatin, lanreotide, given to patients with advanced colorectal cancer, in escalating doses up to 6 mg t.i.d. subcutaneously (s.c.) for 2 months lowered serum IGF-I, caused major side effects such as cramps, but had no effect on tumors [189]. Cascinu et al. [190] reviewed these clinical studies and concluded that the results available suggest that octreotide is not effective in the management of untreated patients with advanced GI cancer, but additional studies of octreotide in chemotherapy-resistant patients are needed [190].

Avastin (bevacizumab), a recombinant monoclonal antibody targeting VEGF, is in a phase III clinical trial in patients with advanced colon cancer. Patients who received Avastin plus chemotherapy lived an average of 20.3 months, compared wich 15.6 months for chemotherapy alone [191]. Thus, Avastin markedly improved overall survival when combined with standard chemotherapy [191]. Targeted cytotoxic somatostatin analog AN-238 may be useful for the therapy of chemoresistant colon cancers that express a mutant p53, if sst_5 is also expressed.

Exocrine pancreatic cancer

Adenocarcinoma of the pancreas is the second most common gastrointestinal malignancy in the USA [192]. Statistical estimates indicate that 30,700 new cases of pancreatic cancer occurred in the USA in 2003, and nearly an equal number – 30,000 – died from this malignancy [1]. Thus, pancreatic cancer is now the fourth leading cause of cancer-related deaths among American men and women [193]. Pancreatic cancer ranked eighth in Europe, with 74,000 new cases in 1995 [2]. The prognosis for this disease in Europe, as in the USA, is dismal; the 5-year survival is 3–5% and corresponds to low US rates. Thus, in 1995 there were 75,000 estimated deaths from pancreatic cancer in Europe, a figure somewhat higher than the predicted incidence, probably due to differing practices in defining the cause of death [2]. Global statistics for 1990, compiled in 1999 [3], indicate that worldwide pancreatic cancer ranks 13th in incidence. However, pancreatic cancer is responsible for 168,000 deaths per year and is the ninth most frequent cause of cancer-related deaths in the world [3]. Established risk factors for pancreatic cancer include cigarette smoking, consumption of smoked or processed meats, colonization by *H. pylori*, N-nitrosamine exposure, chronic excess gastric or duodenal acidity [193]. It was stated that the elimination of cigarette smoking would eventually prevent ~27% of pancreatic cancers in the USA [194].

Pancreatic adenocarcinoma is generally thought to arise from pancreatic ductal cells, and more than 90% of malignant pancreatic neoplasms are classified as ductal adenocarcinomas [193], although ductal or ductular epithelial cells form only about 4% of the human exocrine pancreas. Acinar cell carcinomas are rare [193]. Ductal adenocarcinoma has a very poor prognosis, in part because most patients have advanced disease by the time of diagnosis [195]. Early diagnosis and screening for nonendocrine pancreatic cancer is difficult, although the technology for detecting these small tumors is available [195, 196]. The delay in detection is mostly due to the fact that early symptoms are usually vague and nonspecific and very often neglected by the patients and missed by the physicians [195]. The median survival time of patients with pancreatic cancer in the USA is 3 months [197]. About 65% of patients with pancreatic cancer will die within 6 months of diagnosis, and nearly 90% within 1 year. Surgical resection is presently the only effective form of curative therapy [197]. The first pancreaticoduodenectomy was reported in 1935 by Whipple et al. [198]. However, only 15–20% of patients with pancreatic cancer are candidates for a surgical resection [199] because of the spread of the tumor to adjacent tissue [200]. For the patients who undergo potentially curative resection, the 5-year survival is about 20% [198]. An improvement in the outcome may occur in patients who also receive chemotherapy and/or radiotherapy [198].

Nearly all patients with pancreatic cancer develop metastatic disease and become candidates for systemic treatment [192]. Chemotherapy with single or multiple agents for inoperable pancreatic cancer results in only modest improvement in response rates [195]. High-dose external beam irradiation in combination with 5-FU or FAMS (5-FU, adriamycin, mitomycin C, streptozocin) was recommended to palliate patients with inoperable tumors and those unfit for a major pancreatic resection [195]. Extensive efforts were made to develop programs of adjuvant therapy and improve surgical survival rates and programs of neoadjuvant therapy to increase the num-

ber of patients who can be surgically resected [201]. Unfortunately, the efficacy of chemotherapy is very poor, the response rates being only about 20%, and the median survival is 3–5 months [192]. Combination chemotherapy such as fluorouracil, doxorubicin and mitomycin or 5-FU, doxorubicin and cisplatin may not be superior to 5-FU alone [192, 202]. Clinical response and survival have been improved only slightly with the introduction of gemcitabine [192]. Thus, there is a continued need for more effective treatment in pancreatic cancer. The biology of pancreatic cancer was expertly reviewed by Poston et al. [203], and Bardeesy and DePinho [198], and the aberrant signaling pathways in pancreatic cancer cells were elaborately presented [204]. An improved understanding of pancreatic cancer biology might lead to more effective treatment [203]. Various experimental studies are consistent with the view that the exocrine pancreatic carcinomas are sensitive to GI hormones and growth factors [5, 12]. EGF, TGF-α, IGF-I and IGF-II, cholecystokinin, secretin, bombesin-like peptides and gastrin have growth-promoting effects, while somatostatin, pancreatic polypeptide and pancreastatin inhibit the growth of pancreatic cancers [5, 12, 205]. Some of the growth-promoting substances may be derived from islet cells, as it was shown that experimental ductal pancreatic cancers develop in close connection with the islets, and human ductal pancreatic cancers frequently have scattered groups of cells displaying neuroendocrine differentiation [206]. Although the etiology of pancreatic adenocarcinoma is not clear, stimulation of tumor growth by gastrointestinal peptides is likely to be involved [207]. Regimens based on monoclonal antibodies, which may serve as carriers of therapeutic isotopes or protein toxins, are being evaluated [208]. Possible new approaches to treat this malignancy could be based on inhibition of some GI hormones, growth factors or their receptors by administration of hormonal peptide analogs as well as on monoclonal antibodies against oncogene receptors.

Role of IGF-I and -II

Much evidence indicates that growth factors play a role in pancreatic cancer. IGF-I and IGF-II appear to be involved in the proliferation of both normal and neoplastic cells or phenotypic transformation of cells [5, 12, 82]. IGF-I is overexpressed in human pancreatic cancer and may play an autocrine or paracrine role in its progression [209]. IGF-II mRNA is present in about 20% of normal human pancreatic tissue samples, in 66% of pancreatic cancer samples and in the COLO-357 human pancreatic cancer line [210]. IGF-II was also found by immunohistochemistry in malignant pancreatic tissues [210]. A monoclonal antibody (αIR3) that blocks ligand binding to the IGF-I receptor (IGF-IR) inhibited IGF-II-mediated growth stimulation in human pancreatic cell lines [210]. These findings suggest that IGF-II acts through IGF-IR to enhance growth of pancreatic cancer cells [210].

Role of EGF, TGF-α and other growth factors

EGF, TGF-α and other growth factors also appear to be involved in the proliferation of normal and neoplastic cells of pancreas or phenotypic transformation of its cells. PANC-1 and MiaPaCa-2 human pancreatic cancer cell lines have receptors for EGF [211]. EGF stimulates the growth of MiaPaCa-2 pancreatic cancer cells in culture and may act as an autocrine growth factor [211]. EGF also augments pancreatic carcinogenesis induced by nitrosamines [212]. Thus, after pancreatic adenocarcinomas were induced in Syrian hamsters by administration of N-nitrosobis(2-oxopropyl)amine (BOP) [212], additional s.c. injections of EGF were given to some animals. The incidence of pancreatic cancer in the EGF- and BOP-treated animals was 75 versus 44% in those treated with BOP alone. It was also reported that overexpression of EGF receptors (EGF-R) and production of TGF-α enhances the growth of cultured human pancreatic cancer cell lines [213]. Autocrine stimulation of the EGF receptor can contribute to sustained mitogenic activity and proliferation of pancreatic cancer cells [214]. Human pancreatic ductal carcinomas exhibit high EGFR levels, and both EGF-R family members HER-2 and HER-3 are also overexpressed [215]. The overexpression of all three receptors was documented by immunohistochemistry, Northern blot analysis and in situ hybridization, and it was most pronounced in the cancer cells within the tumor mass [215]. The expression of HER-3 was associated with decreased patient survival [215]. Blockade of EGF receptor by monoclonal antibody IMC-C225 (cetuximab) [216] or of EGF receptor signaling by a tyrosine kinase inhibitor [217] inhibits growth of human pancreatic carcinoma xenografts in nude mice. Xiong and Abbruzzese [218] recently reviewed EGF receptor-targeted therapy for pancreatic cancer and summarized preclinical and clinical phase I studies with monoclonal antibodies such as IMC-C225 that inhibit ligand binding to EGF receptors. Tyrosine kinase inhibitors may also become clinically available. Most EGFR inhibitors are in the early phases of clinical development, but a phase II trial with antibody cetuximab IMC-C225 in combination with gemcitabine in patients with advanced pancreatic cancer produced encouraging findings, and a phase III trial is being planned [218]. Acidic and basic fibroblast growth factors (FGFs) are also overexpressed in human pancreatic cancer, and their expression can be correlated with advanced tumor stage [215, 219]. Similarly, the expression of a mutated form of p53 protein in human pancreatic cancer correlates with enhanced biological aggressiveness [220]. Nerve growth factor (NGF) is another important substance in pancreatic carcinogenesis. Some pancreatic cancer cell lines produce

NGF, which can act in a paracrine and/or autocrine way and enhance tumor growth in vivo [221].

Role of GI hormones

Gastrin, CCK, caerulein and secretin promote the growth of exocrine pancreas and increase DNA, RNA and the protein content of rat pancreas [222–224]. The production of hyperplasia and hypertrophy of the exocrine pancreas by gastrin, CCK and secretin is now well established [222–224]. Caerulein, an amphibian skin decapeptide structurally and functionally related to CCK, also produced both hyperplasia and hypertrophy of rat pancreatic acinar cells [225]. It is likely that gastrin and CCK also influence the growth of the malignant cells of the pancreas and phenotypic transformations [96]. Thus, gastrin stimulated growth of AR4-2J pancreatic carcinoma in rats [96]. In nude mice bearing transplanted SW-1990 human pancreatic cancers, CCK increased the growth of the tumors [102]. CCK also stimulated the development of nitrosamine-induced pancreatic cancer in rats and hamsters [96, 103], indicating that it can be a tumor promoter. The increase in serum CCK in rats produced by feeding raw soya flour can lead to pancreatic carcinomas [96]. In vitro, CCK also increases the rate of growth of SW-1990 human pancreatic cancer cells in tissue culture [96,102]. The receptors for CCK are present on pancreatic acinar cells of rats and are overexpressed in malignant azaserine-induced pancreatic tumors [226, 227]. Another study demonstrated that DSL-6 rat pancreatic carcinoma expresses three subtypes of CCK receptors, CCK-B, high-affinity CCK-A and low-affinity CCK-A receptors [228]. Gastrin (CCK-B) receptors, which are not present in normal rat pancreas, constituted ~34% of the total high-affinity CCK receptors in tumors. It was suggested that the expression of gastrin (CCK-B) receptors may be induced by gene mutation or amplification during carcinogenesis and may promote tumor growth [228]. Other studies showed that cholecystokinin and gastrin antagonists lorglumide and loxiglumide inhibit gastrin-stimulated DNA synthesis in a rat tumoral acinar pancreatic cell line AR42J [229]. The coexpression of amidated gastrin ligand and its receptor was reported in human pancreatic carcinoma samples, showing that local gastrin mechanisms might be involved in pancreatic carcinogenesis [230]. Other groups detected CCK-B and bombesin receptors in normal human pancreata, but not in pancreatic cancer samples, or found the coexpression of ligand and receptor only in a low percentage of cancers (reviewed in [227]). Recently Reubi et al. [227] reported that human pancreatic cancers rarely express CCK-2 receptors, formerly called CCK-B receptors, and suggested that CCK analogs may not be of clinical value to treat pancreatic cancers.

Reubi et al. [110] also showed that 65% of pancreatic carcinomas have VIP/PACAP receptors. It was also reported that VIP stimulates in vitro growth of the Capan-2 human pancreatic carcinoma cell line, which expresses VIP-1 receptor [231]. Scanning with radiolabeled VIP can visualize pancreatic cancers and metastases that express receptors for VIP. Thus, primary pancreatic adenocarcinomas were visualized by imaging in 10 of 12 patients, and liver metastases were seen in 7 of 7 patients [139]. A CCK receptor antagonist MK-329 was evaluated in a pilot clinical trial in patients with advanced pancreatic cancer, but it was found to have no effect on tumor progression or pain [232].

Role of sex steroids and LH-RH

Sex steroids may likewise play a role in the growth of the normal and cancerous pancreas [5, 12, 14, 195, 233]. The presence of specific receptors for estrogen and androgen in rat and human pancreatic cells indicates that sex hormones could influence the neoplastic process [5, 12, 14, 195, 233]. Both androgen and estrogen receptors are also found in human pancreatic adenocarcinomas and cancer cell lines. Testosterone stimulates the growth of xenografts of human pancreatic adenocarcinomas in nude mice [234]. In human pancreatic cancers, aromatase and 5α-reductase were detected [234]. These enzymes are found only in sex steroid-dependent tissues [234]. However, tamoxifen treatment had no effect on experimental pancreatic cancers in hamsters [235].

Chronic administration of agonists of LH-RH produces inhibition of the pituitary-gonadal axis, manifested by decreased secretion of LH and FSH, and reduction in plasma levels of sex steroids [5, 12]. Administration of LH-RH antagonists also strongly reduces the plasma levels of LH, FSH and sex steroids [5, 12]. We have shown that treatment with agonist [D-Trp6]LH-RH and antagonist cetrorelix inhibits growth of acinar tumors in rats and ductal pancreatic cancers in hamsters [69, 236, 237]. However, clinical trials with LH-RH agonists in patients with pancreatic carcinoma were unsuccessful [12]. Receptors for LH-RH are present in hamster and human pancreatic cancers as well as in normal pancreata [12], but their function is not known. In hamsters with chemically induced pancreatic cancers, these LH-RH receptors are localized in pancreatic tumor cells [238]. [D-Trp6]LH-RH treatment results in a marked increase in the concentration of LH-RH receptors in the nuclei. It is possible that the increase in the concentration of LH-RH receptors in nuclei might be related to activation of mechanisms involved in increased apoptosis of these cells [238].

Effects of somatostatin and analogs

It has already been cited that somatostatin and its analogs inhibit secretion and/or action of gastrin, secretin, CCK and VIP. In pancreatic acinar cells, receptor-bound so-

matostatin undergoes internalization and degradation [239] (fig. 1). Somatostatin and its analogs reduce the levels of IGF-I and inhibit growth of MiaPaCa-2 pancreatic cancer cells induced by EGF [211]. Somatostatin appears to reverse the stimulatory effects of EGF on the phosphorylation of the tyrosine kinase portion of the EGF receptor in the Mia-PaCa cell line [240]. Thus, somatostatin and its octapeptide analogs such as RC-160 and RC-121 stimulate tyrosine phosphatase activity in the MiaPaCa-2 line and inhibit its growth in vitro [21]. Consequently, somatostatin might inhibit the growth of experimental pancreatic cancers by suppressing the action or secretion of GI hormones and endogenous growth factors. In 1984 we reported that chronic administration of some early somatostatin analogs inhibited the growth of acinar pancreatic carcinomas in rats and of ductal cancers in hamsters [237]. RC-121 and RC-160 also inhibited the growth of transplanted or nitrosamine-induced ductal pancreatic cancers in hamsters [241]. Thus, we reported inhibition of tumor growth and evidence of histological regression of the BOP-induced pancreatic cancer in hamsters after treatment with somatostatin analog RC-160. The combination of RC-160 with [D-Trp^6]LHRH produced the best results in terms of prolongation of survival and histological regressive changes [236, 242]. Combinations of chemotherapy with somatostatin analogs were also investigated. Thus, in hamsters with ductal pancreatic cancers induced by BOP, treatment with RC-160 combined with 5-FU also produced greater tumor inhibition than 5-FU or the analog alone [236]. Another group likewise demonstrated that octreotide decreased the metastatic capability of nitrosamine-induced pancreatic cancers in hamsters [243]. RC-160 also inhibited growth of MiaPaCa-2 human pancreatic cancer xenografted into nude mice [244] and the growth of CFPAC-1 cells in vitro together with a decrease in cyclic AMP (cAMP) production in the cells [245]. Poston et al. [246] studied the effects of octreotide and tamoxifen on the growth of SKI human pancreatic cancers in nude mice. Octreotide, given alone or as a combined regimen with tamoxifen, significantly reduced the rate of growth of SKI tumors and DNA, RNA and protein content of the tumors [246]. Weckbecker et al. [247] similarly found that octreotide at a dose of 5–50 μg twice a day in nude mice bearing MiaPaCa pancreatic tumors induced significant inhibition of tumor growth. On the other hand, it was also reported that RC-160 did not inhibit in vitro growth of two human pancreatic cancer cell lines, MiaPaCa-2 and Panc-1, stimulated with EGF or IGF-1 [248]. No displaceable binding with ^{125}I-Tyr^{11}-somatostatin was observed on MiaPaCa-2 or Panc-1, indicating that somatostatin receptors were absent from these lines. Buscail et al. [42] then demonstrated the loss of sst_2 gene expression in human pancreatic adenocarcinomas and most of the derived pancreatic cancer cell lines. In another study, the same group rectified the sst_2 defect in human pancreatic cancer BxPC-3 and Capan-1 cells by stable transfection with cDNA for human sst_2 [249]. Both BxPC-3 and Capan-1 cells expressing sst_2 showed a significant reduction in cell growth. Thus, the tumorigenicity can be reversed by the restoration of sst_2 expression [249]. In vivo gene transfer of sst_2 was also investigated in two transplantable models of primary and metastatic pancreatic carcinoma in hamsters [250]. Both the adenoviral vector-based, as well as polyethylenimine (PEI) vector-mediated sst_2 gene transfer resulted in a significant reduction of pancreatic tumor growth and in tumors transfected with sst_2 gene the apoptosis was increased [250].

Studies with targeted cytotoxic analogs of somatostatin and analogs labeled with radionuclides

Clinical studies in patients indicate a binding of radionuclide somatostatin octapeptide to pancreatic cancers expressing mRNA for sst_3 and sst_5 [38]. Somatostatin analogs labeled with radionuclides are of considerable interest for the diagnosis and therapy of tumors expressing somatostatin receptors, such as gastroenteropancreatic. A ^{67}gallium-labeled somatostatin analog, suitable for targeting tumors expressing sst, was developed [251] and evaluated in the AR4-2J pancreatic tumor model. Labeling with ^{67}Ga considerably improved the efficacy of the tested somatostatin analogs with respect to sst_2 affinity and tissue distribution. It was also reported that ^{111}indium-DTPA-octreotide is accumulated in the pancreas of rats and in rat pancreatic cancers [252].

The presence of binding sites for somatostatin in certain cancers was utilized by us for targeting various chemotherapeutic agents linked to somatostatin analogs. First, an early cytotoxic analog AN-51 consisting of methotrexate linked to analog RC-121 at the N-terminus was tested in nude mice bearing transplanted MiaPaCa-2 human pancreatic cancers [253]. The treatment with AN-51 inhibited tumor growth, whereas methotrexate or RC-121 administered singly in equimolar doses had no significant effect. However, AN-51 had significant toxicity on the GI tract [253]. We then tested AN-238 on human pancreatic cancer lines xenografted in nude mice that express sst_5 and sst_3 [254]. AN-238 and its radical AN-201 were administered i.v. to nude mice bearing SW-1990 pancreatic cancers which expressed mRNA for sst subtypes 3 and 5. The effects of repeated administration of AN-238 and AN-201 were also evaluated on xenografted Panc-1, CFPAC-1, Capan-1 and Capan-2 pancreatic cancers with various patterns of sst subtypes 2A, 3 and 5 [254]. Repeated administration of AN-238 significantly inhibited growth of SW-1990 cancers and other tumors. AN-201 was toxic and less effective. Pretreatment with RC-160 1 h before administration of AN-238 decreased its antitumor effect and increased the toxicity apparently because RC-160 occupied and blocked binding sites for

AN-238. Thus, growth of experimental human pancreatic cancers that express somatostatin receptors can be inhibited by cytotoxic analog AN-238 [254]. Benali et al. [255] showed that the expression of sst_2 in the hamster pancreatic cancer cells PC-1 and PC-1.0 leads to inhibition of proliferation of these cells in vitro. In vivo studies in hamsters after orthotopic implantation of PC-1.0 cells showed that tumor growth and metastatic progression of allografts containing sst_2-expressing cells were inhibited. After administration of the analog AN-238, the antitumor bystander effect, observed in mixed tumors containing a 1:3 ratio of sst_2-expressing cells and control cells, was significantly extended. The incidence of metastases was also lower in animals with sst_2-expressing tumors treated with AN-238. These results indicate that in vivo sst_2 gene transfer combined with chemotherapy with targeted cytotoxic somatostatin analog can provide a therapeutic approach to pancreatic cancer [255]. Cytotoxic somatostatin analogs could be considered for the treatment of patients with advanced pancreatic cancer who did not respond to other therapies [6]. All these studies demonstrate major differences in efficacy between 'straight' analogs such as RC-160 and cytotoxic analog such as AN-238. Consequently, it is likely that cytotoxic somatostatin analogs such as AN-238 would be targeted even to tumors with a low concentration of somatostatin receptors, producing effective clinical responses [6].

Role of bombesin/GRP and effects of antagonistic analogs

Numerous studies show that bombesin and GRP can influence the release of GI hormones, promote pancreatic secretion and growth, and stimulate pancreatic carcinogenesis [5, 12]. Administration of bombesin to rats produces significant pancreatic hyperplasia (increase in pancreatic weight, protein and DNA content) [256] and hypertrophy. Other studies indicate that bombesin/GRP can stimulate pancreatic carcinogenesis. Bombesin promotes azaserine-induced pancreatic cancers in rats [257], and GRP induces in vivo and in vitro growth of rat acinar pancreatic carcinoma [258]. mRNAs for both bombesin and its receptors were detected in various human pancreatic cancer cell lines showing that bombesin/GRP may be an autocrine growth factor in pancreatic cancer [9]. Normal and tumor pancreatic cells contain a specific GRP receptor that is expressed more on malignant pancreatic tissues [259]. Bombesin stimulates the proliferation of CFPAC-1 and other human pancreatic cancer cells in vitro through its receptors [260]. Bombesin also significantly stimulates the proliferation of HPAF and CD18 human ductal pancreatic cancer cell lines. Bombesin may be involved as a growth factor in the development of pancreatic ductal adenocarcinoma in humans.

We have attempted to develop a hormonal therapy for exocrine cancer of the pancreas based on bombesin/GRP antagonists [9, 12, 260]. The inhibitory effects of RC-3095 on nitrosamine-induced ductal pancreatic cancers in hamster were demonstrated in several studies and were accompanied by a major downregulation of EGF receptors [69, 261, 262]. Antagonists RC-3095 and RC-3940-II also inhibited the growth of CFPAC-1, CAPAN-2 and SW1990 human pancreatic cells xenografted into nude mice [260, 263]. The inhibitory effect of RC-3095 on bombesin-induced growth of CFPAC-1 cells is probably initiated by competitive occupancy of the binding sites for bombesin/GRP on the cells [260]. Nevertheless, the suppressive effects of bombesin antagonists on the growth of cancers appear to involve downregulation of EGF receptors [69]. Thus, it has been found that in vivo treatment with RC-3095 greatly reduced the concentration of EGF receptors in pancreatic, colorectal and gastric cancers [69, 260]. RC-3095 may affect EGF binding through an action on protein kinase C system [264]. Liebow et al. demonstrated that RC-3095 can inhibit phosphorylation responses to EGF and upregulation of EGF receptors produced by bombesin/GRP [265]. It is also possible that the inhibitory action of RC-3095 on growth of pancreatic cancers is mediated through several mechanisms. Other bombesin/GRP antagonists such as RC-3940-II, with high binding affinity to pancreatic cancers, are even more potent than RC-3095 in inhibiting the growth of human pancreatic adenocarcinoma cells [263]. Thus, both RC-3095 and RC-3940-II inhibited growth of SW-1990 pancreatic cancers xenografted into nude mice, but RC 3940-II was more effective [263]. In cell cultures, both RC-3095 and RC-3940-II effectively inhibited the proliferation of SW-1990 cells, inducing a dose- and time-dependent decrease in the number of cells [263]. These findings suggest the merit of continued evaluation of bombesin/GRP antagonists for the possible development of new approaches to the treatment of pancreatic cancer. Clinical phase I/II trials with RC-3095 in patients with exocrine pancreatic cancers are in progress.

Effects of GH-RH antagonists

GH-RH stimulates pancreatic enzyme secretion probably by interacting with VIP receptors [266]. The presence of GH-RH-like peptides in the GI tract and on tumors of neuroendocrine origin has been reported [5, 74, 82]. GH-RH antagonists can inhibit tumor growth by decreasing production of hepatic IGF-I or by directly suppressing the autocrine/paracrine production of IGF-I and IGF-II by the tumor and by inhibiting the action of tumoral GH-RH [5, 82]. As inferred in previous sections, autocrine GH-RH, IGF-I and IGF-II are implicated in the pathogenesis of pancreatic carcinoma [5, 12, 82]. The effects of GH-RH antagonists were evaluated in hamster and human exper-

imental models of pancreatic cancer [267]. GH-RH antagonist MZ-4-71 inhibited the growth of nitrosamine-induced pancreatic cancers in hamsters. MZ-4-71 and MZ-5-156 also reduced growth of SW-1990 human pancreatic cancers xenografted into nude mice and decreased IGF-II concentration in tumors [267]. IGF-I levels in serum and in pancreatic cancer tissue were not changed after therapy, indicating that an effect on IGF-I was not involved in tumour inhibition. However, IGF-II concentrations in tumours were reduced by 50–60% after treatment with GH-RH antagonists. In vitro, the concentration of IGF-II in the culture medium was increased after seeding of SW-1990 cells, indicating that this pancreatic cancer cell line secreted IGF-II. This was supported by the expression of IGF-II mRNA in the SW-1990 cells. MZ-5-156 also decreased SW-1990 cell proliferation in vitro. These results suggest that the inhibitory effects of GH-RH antagonists on the growth of pancreatic cancers may result from a reduction in the production of IGF-II in the tumors [5, 12, 267].

GH-RH antagonists could likewise inhibit pancreatic cancers by direct action on tumor receptors for GH-RH and nullification of the effects of autocrine GH-RH [5]. Thus, the expression of mRNA for both GHRH and GH-RH-receptor isoform (SV_1) was found in tumors of human pancreatic SW1990, PANC-1, MIA PaCa-2, Capan-1, Capan-2 and CFPAC1 cancer lines grown in nude mice [76]. These results indicate the presence of an autocrine/paracrine stimulatory loop based on GHRH and SV_1 of GH-RH receptors in human pancreatic cancers. In vitro, the growth of pancreatic cancer cells was stimulated by GH-RH(1-29)NH_2 and inhibited by GH-RH antagonist JV-1-38. The finding of GHRH receptor SV_1 in human pancreatic cancers provides a new therapeutic approach based on GH-RH antagonists.

Clinical trials

Early clinical trials indicated that some patients with pancreatic cancer may respond to the antiestrogen tamoxifen with a prolongation of survival [233]. On the basis of experimental observations that administration of [D-Trp^6]LH-RH inhibits the growth of pancreatic cancers [237], this analog was tried clinically in patients with inoperable pancreatic cancer (stages III and IV). Thus, patients with unresectable and histologically verified adenocarcinoma of the pancreas were treated with daily s.c. injections of [D-Trp^6]LH-RH 1000 μg/day for the first 7 days and then 100 μg/day. This therapy produced a clinical improvement and an increase in survival in some patients [268]. One patient with stage IV disease survived for 16 months. In addition to subjective and objective improvement which could be observed after 3 weeks of treatment, reduction in tumor mass and liver metastases was noted during laparotomy performed 11 months after the initiation of therapy with [D-Trp^6]LH-RH [268]. However, the median survival time was only 7.2 months [269]. In another study, 36 patients with advanced ductal pancreatic cancer, stages II, III and IV, were treated with the LH-RH analog buserelin. No partial or complete remission was seen, and 26 patients showed tumor progression [270]. Thus, therapy based on LH-RH agonists alone cannot be recommended for patients with advanced pancreatic cancer. A therapeutic regimen based on the antiestrogen tamoxifen or the antiandrogen cyproterone acetate in combination with the LH-RH agonist buserelin was also investigated in 9 patients with unresectable pancreatic adenocarcinoma, but it was found to be ineffective [271].

In phase I and II clinical trials, somatostatin analog RC-160 was used as a single drug in patients with advanced irresectable pancreatic cancer [272, 273]. In the first study, RC-160 was given s.c. three times a day at the dose of 500 μg to 21 patients. Six patients had CT-stable disease 6 months after starting treatment [272]. In the second trial, an escalating regimen up to 6 mg per day by continuous s.c. infusion was used [273]. The treatment was well tolerated, and side effects including cramps were minimal. In both studies, which evaluated more than 40 patients, nearly 30% of patients showed radiological evidence of tumor stabilization for up to 6 months, with measurable improvement in the quality of life, particularly analgesic requirements. None of the 9 patients developed gallstones. However, RC-160 alone, even in large doses, did not seem to be adequate for inducing an effective palliation in most patients [272, 273].

Rosenberg et al. [274] used a combination of Sandostatin with tamoxifen to treat patients with pancreatic cancer. Twelve patients with ductal adenocarcinoma of the pancreas were treated with 100 μg of octreotide t.i.d. and tamoxifen 10 mg twice daily. The survival of the octreotide-tamoxifen group was compared with a matched historic cohort of 68 untreated patients with pancreatic cancer. The median survival time for the group treated with octreotide and tamoxifen was 12 months compared with 3 months for the historic cohort. One-year survival rate for the octreotide-tamoxifen-treated group was 59% compared with 16% for the historic cohort. Thus, patients with ductal adenocarcinoma of the pancreas showed an increased survival after treatment with a combination of octreotide and tamoxifen [274].

Friess et al. [275] used the treatment with octreotide in 22 patients with advanced ductal pancreatic cancer. Octreotide was given by subcutaneous injection at the dose of 100 μg t.i.d., but after tumor progression the dose was increased to 200 μg (t.i.d.). No remissions were seen. Three patients showed no change, and 19 patients showed tumor progression with a median survival time of 17 weeks. In another trial, Cascinu et al. [141] randomized 32 patients with advanced pancreatic cancer refractory to

chemotherapy and treated 16 with octreotide at a dose of 200 μg t.i.d. for 5 days a week, while 16 patients in another group received best supportive care only [141]. No objective responses to octreotide were seen, but patients treated with octreotide showed some prolongation of survival with a median time of 15 weeks compared with 8 weeks in the control group [141]. High-dose octreotide treatment (2 mg t.i.d.) in patients with unresectable pancreatic carcinoma [276] resulted in stable disease in 9 patients (19%), but no complete or partial response was observed, and the median overall survival was 21 weeks [276]. Octreotide combined with LH-RH agonist goserelin was also tried in the therapy of advanced pancreatic cancer, but the objective response rate was low [277]. Reubi et al. [278] did not observe any objective remissions or stabilization in 11 patients with metastatic pancreatic cancer treated with octreotide. Klijn et al. [279] treated 14 patients with metastatic pancreatic cancer with octreotide (200 μg t.i.d.). Plasma IGF levels fell transiently, but EGF levels increased. Ten patients showed progressive disease, and survival time was a disappointing 2 months [279]. Canobbio [280] also treated 19 patients with advanced exocrine pancreatic carcinoma, with 250 μg to 1 mg/day of another somatostatin analog Somatulin (BIM 23014). Six patients showed some improvement in pain and performance status, but on the whole the effects were limited [280]. The overall results indicate that treatment with octreotide and other octapeptide analogs of somatostatin is not effective in patients with advanced pancreatic cancer [192]. Cascinu et al. [190] concluded that although the results of clinical trials suggest that octreotide is not effective in untreated patients with advanced pancreatic cancer, additional details are needed to evaluate octreotide in chemotherapy refractory patients in order to clarify its effects not only on the survival, but also on the quality of life of the patients [190].

Critical role of somatostatin receptors for clinical management of pancreatic cancer

Collectively, the clinical results indicate that somatostatin analogs are inadequate for inducing an effective palliation in patients with pancreatic cancer. Poor therapeutic responses to somatostatin analogs are likely due to low levels or absence of sst_2 receptors in human exocrine pancreatic cancers. Initially, Reubi et al. [278] reported the absence of somatostatin receptors in human exocrine pancreatic adenocarcinomas based on the lack of binding of ^{125}I-labeled analogs of octreotide and somatostatin-28. Subsequently, Buscail et al. [22, 23] determined that the receptor subtype 2 mediated the antiproliferative effect of octapeptide somatostatin analogs. The same group then evaluated the mRNA expression of sst_{1-5} subtypes on both specimens of primary and metastatic pancreatic adenocarcinoma and human pancreatic cell lines, and detected the presence of multiple sst mRNAs, but in comparison with normal pancreatic tissue specimens, there was a loss of sst_2 expression in tumor tissues and pancreatic cell lines tested [42]. They concluded that the loss of sst_2 expression may contribute to the malignancy of human pancreatic cancer and could explain the lack of therapeutic effects of somatostatin analogs in patients with pancreatic cancers. Somatostatin analog scintigraphy in patients with pancreatic cancer also shows a frequent lack of somatostatin receptor expression [281]. Thus, in human exocrine pancreatic cancers there is a loss of gene expression for sst_2, which is one of the preferred subtypes of octapeptide somatostatin analogs. However, the expression of sst_5 and sst_3 in pancreatic cancer should make possible the therapy with somatostatin analogs labeled with various radioisotopes or cytotoxic somatostatin analogs. Fisher et al. [282] examined mRNA expression of the somatostatin receptor subtypes (sst_{1-5}) in 11 specimens of pancreatic adenocarcinoma tissues and human pancreatic cancer cell lines. Eight tumors expressed sst_2 and two tumors sst_5. However, of the nine cell lines, only one (MIA PaCa-2) showed high-affinity binding sites for somatostatin-14. They suggested that a defect in receptor protein expression at the cell surface might explain the lack of effect of somatostatin analogs in patients with pancreatic cancers [282]. Although Fisher et al. [282] reported that the genes for sst_3 and sst_4 were not expressed in any of the cell lines or specimens of human pancreatic cancer, Raderer et al. [38] detected the presence of mRNA for sst_3 in samples of pancreatic cancer as well as in the pancreatic cell lines (ASPC-1, BxPC3, Capan-1 and Panc-1) by Northern blot analysis. ^{111}In-DOTA-lanreotide bound to the cell lines and tumor samples. Primary lesions could be visualized in all three patients with pancreatic cancer who underwent scanning with ^{111}In-DOTA-lanreotide, as well as liver metastases [38].

McCarthy et al. [283] reported responses of 62–69% in 85 patients with metastatic neuroendocrine tumors treated with high doses of ^{111}In-pentetreotide (Octreoscan), targeted to tumor somatostatin receptors. Objective responses observed included biochemical and radiographic responses with prolongation of survival [283]. This supports the view that cytotoxic analogs of somatostatin or bombesin/GRP that can be targeted to tumors could be considered for treatment of patients with advanced pancreatic cancer who do not respond to other therapies. Collectively, basic and clinical findings suggest that targeting of radiolabeled or cytotoxic peptide analogs to receptors on tumors could provide a viable therapeutic modality for gastrointestinal cancers.

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