

Actions and Potential Therapeutic Applications of Growth Hormone–Releasing Hormone Agonists

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In this article, we briefly review the identification of GHRH, provide an abridged overview of GHRH antagonists, and focus on studies with GHRH agonists. Potent GHRH agonists of JI and MR class were synthesized and evaluated biologically. Besides the induction of the release of pituitary GH, GHRH analogs promote cell proliferation and exert stimulatory effects on various tissues, which express GHRH receptors (GHRH-Rs). A large body of work shows that GHRH agonists, such as MR-409, improve pancreatic β -cell proliferation and metabolic functions and facilitate engraftment of islets after transplantation in rodents. Accordingly, GHRH agonists offer a new therapeutic approach to treating diabetes. Various studies demonstrate that GHRH agonists promote repair of cardiac tissue, producing improvement of ejection fraction and reduction of infarct size in rats, reduction of infarct scar in swine, and attenuation of cardiac hypertrophy in mice, suggesting clinical applications. The presence of GHRH-Rs in ocular tissues and neuroprotective effects of GHRH analogs in experimental diabetic retinopathy indicates their possible therapeutic applications for eye diseases. Other effects of GHRH agonists, include acceleration of wound healing, activation of immune cells, and action on the central nervous system. As GHRH might function as a growth factor, we examined effects of GHRH agonists on tumors. *In vitro*, GHRH agonists stimulate growth of human cancer cells and upregulate GHRH-Rs. However, *in vivo*, GHRH agonists inhibit growth of human cancers xenografted into nude mice and downregulate pituitary and tumoral GHRH-Rs. Therapeutic applications of GHRH analogs are discussed. The development of GHRH analogs should lead to their clinical use. (*Endocrinology* 160: 1600–1612, 2019)

This mini-review summarizes the current findings on agonistic analogs of GHRH (1) and their potential therapeutic applications. The demonstration by Frohman and Szabo (2) in 1981 of ectopic production of GHRH by carcinoid and pancreatic tumors made possible the isolation and characterization in 1982 by

the groups of Guillemin *et al.* (3) and Rivier *et al.* (4) of 40 and 44 amino acid forms of GHRH from human pancreatic tumors that caused acromegaly. See the actual recollections by Michael Thorner (5). A decade later, the Thorner group [Gaylinn *et al.* (6)] and Mayo (7) cloned the pituitary type of GHRH receptors (pGHRH-Rs), a

member of G protein-coupled receptors. Our group identified a splice variant of GHRH-Rs (SV1) in tumors and sequenced its cDNA (8, 9). SV1 is found in most tumors and in normal tissues, possesses ligand-dependent and independent activity (10), and was shown to be functional as a receptor. Clinical studies established that GHRH can stimulate GH release in normal subjects (11) and in children with GH deficiency (12). Other investigations (13) proved the role of GHRH in the regulation of the GH secretion in animals, and clinical perspectives of GHRH were summarized by Gelato (14). The expression of GHRH gene and the presence of GHRH peptide were then also found not only in tumors, such as breast, ovarian, and endometrial cancers (15), but also in various extrahypothalamic tissues (16, 17), hinting at other roles for GHRH, in addition to regulation of pituitary GH secretion.

Although GHRH was first identified in tumor tissues (2–5), few investigators tried to explore the possible role of GHRH in carcinogenesis by the mid-1990s. At that time, our group, experienced in oncology from the work on analogs of LH-releasing hormone (LHRH), was already deeply engaged in cancer research, and we decided to initiate work on our project on the synthesis and evaluation of GHRH antagonists for possible use in cancer therapy (9). We also began a systematic synthesis of agonistic analogs of GHRH (18, 19). As the biological activity of GHRH is confined to the N-terminal sequence of 29 amino acids, this sequence was used for the substitution and replacement of various amino acid residues in the solid-phase syntheses aimed at making both agonists and antagonists of GHRH (9). Our task to produce GHRH antagonists was facilitated by the previous demonstration by Robberecht *et al.* (20) that the replacement of Ala in position 2 of GHRH with D-arginine leads to antagonists. Our work between 1994 and 2017 resulted in several series of potent GHRH antagonists intended to inhibit tumor growth (9, 21–23). Antagonists of the Miami (MIA) series suppressed the growth after subcutaneous administration of diverse human cancer lines, xenografted into nude mice in microgram doses (23). Antagonists MIA-602 and MIA-690 were among the most potent antitumor analogs and also displayed anti-inflammatory activities (23).

The activity of the early series of agonists of GHRH with C-terminal agmatine (19) or JI class analogs with ornithine in positions 12 and 21 (18) was evaluated by the stimulation of release of GH *in vitro* and *in vivo* in rats. The agonistic GHRH activity was increased further in our analogs of MR series (24). We synthesized nearly 100 analogs of this series with modifications of the GHRH(1-29)NH₂ backbone, N- and C-terminals, and the substitutions of Apa³⁰ or Gab³⁰ C-terminal groups

(24). Their GHRH activities were compared with the analog JI-38 of the previous series (18) and with GHRH(1-29)NH₂. Agonists with N-terminal N-Me-Tyr and C-terminal methyl amide, including MR-403, MR-406, MR-409, and MR-410, were ~100 times more potent on subcutaneous administration than the parent hormone and had higher binding activity to the pituitary than GHRH(1-29)NH₂ (24). Analogs with C-terminal agmatine, such as MR-356, and analog MR-502 with C-terminal Gab³⁰-NH₂ had much higher intravenous activity than JI-38 (24). In view of high endocrine activity, which would be difficult to increase further, these analogs of the MR series were deemed to be suitable for development in various medical fields and evaluation of their clinical applications. Initially, the JI class and subsequently, the MR series of GHRH agonists were used to evaluate potential clinical applications in diverse fields, including endocrinology and oncology (1), cardiology (25–30), angiogenic therapy with stem cells (31), diabetes (32, 33), wound healing (34), ophthalmology, and other applications. The actions and potential therapeutic applications of GHRH analogs are summarized in Table 1, and key investigations will be now reviewed.

Effects of GHRH Agonists on Tumor Growth

In tests *in vitro*, GHRH agonist MR-409 promoted the cell viability of NCI-H446 small cell lung cancer (SCLC) and HCC827 and NCI-H460 non-SCLC cells, as well as of other cancer lines, including PANC-1 pancreatic, HCT-116 and HCT-15 colorectal, and J-82 bladder cancer cells (1). MR-409 also stimulated G1- to S-phase cell-cycle transition and cyclin D1 and D2 expression and reduced cell apoptosis, whereas GHRH antagonist MIA-602 suppressed cell viability and stimulated apoptosis. The exposure of lung cancer cells to agonist MR-409 significantly stimulated the production of cellular cAMP, whereas antagonist MIA-602 decreased it (1). The levels of pGHRH-R and SV1 in lung cancers were significantly upregulated after *in vitro* treatment with MR-409 (1). The stimulatory activities of GHRH agonist MR-409 on tumor cells *in vitro* appeared to be in accord with its activating effects on various tissues (25–34).

Surprisingly, *in vivo*, the effects of GHRH agonists, such as MR-409, on tumor growth turned out to be opposite (1). Daily subcutaneous administration of 5 µg agonist MR-409 to mice bearing xenografts of NCI-H446, HCC827, and NCI-H460 lung cancers significantly inhibited tumor growth by 48% to 65% after treatment of 4 to 8 weeks (1) (Fig. 1). The reduction in activated signal transducer and activator of transcription 3 (STAT3) and p21-activated kinase 1 in tumors was

Table 1. Actions and Potential Therapeutic Applications of GHRH Analogs

Analog	Code No.	Experimental Effects Demonstrated	Potential Application	References	
GHRH antagonist	JV-1-36	Inhibition of tumor growth	Tumor therapy	(9, 22, 35)	
GHRH antagonist of MIA class	MIA-602	Inhibition of tumor growth	Tumor therapy	(23, 36, 37)	
	MIA-690	Inhibition of ocular inflammation	Treatment of eye diseases	(38–41)	
GHRH agonists of JI class	JI-34	Reduction in ROS, anti-inflammatory effects	Inflammatory diseases	(39–41)	
		Inhibition of amyloid aggregation	Treatment of Alzheimer's disease	(42)	
	JI-36	Decrease in deterioration of cognitive performance		(42)	
	JI-38	Decrease in dyslipidemia	Treatment of dyslipidemia in T1D	(43)	
		Stimulation of β -cell survival and proliferation	Treatment of diabetes	(32, 33, 44)	
	GHRH agonists of MR class	MR-403	Increase in insulin expression/secretion and stimulation of islet engraftment		
Stimulation of cardiomyocyte survival and repair of myocardial infarction			Treatment of heart diseases	(25–27)	
MR-502		Stimulation of survival of mesenchymal stem cells		(31)	
		Stimulation of wound healing	Tissue repair	(45)	
MR-356		Stimulation of β -cell survival and proliferation	Treatment of diabetes	(33, 46)	
		Increase in insulin expression/secretion and stimulation of islet engraftment			
MR-409		Stimulation of cardiomyocyte survival and repair of myocardial infarction	Treatment of heart diseases	(28, 29, 47)	
		Attenuation of cardiac hypertrophy		(30)	
GHRH agonist [Nle27]GHRH(1–29)NH2		TH 9507	Inhibition of aortic calcification	Wound healing	(48)
			Stimulation of fibroblast migration and wound healing	Therapy of some tumors	(34)
	EGRIFTA	Inhibition of tumor growth <i>in vivo</i>		(1, 49)	
		Antioxidative and anti-inflammatory effects	Treatment of eye diseases	(50)	
	GHRH agonist Tesamorelin	Neuroprotective effects in experimental diabetic retinopathy		(50)	
		Immune enhancing effects ^a	Treatment of compromised immune system ^a	(51)	
	GHRH agonist Tesamorelin	Actions on the brain ^a	Improvement of mild cognitive	(85)	
		Decrease of serum cholesterol levels in T2D ^a	Improvement of lipid profile in T2D	(52)	

Abbreviations: ROS, reactive oxygen species; T1D, type 1 diabetes; T2D, type 2 diabetes.

^aClinical trials.

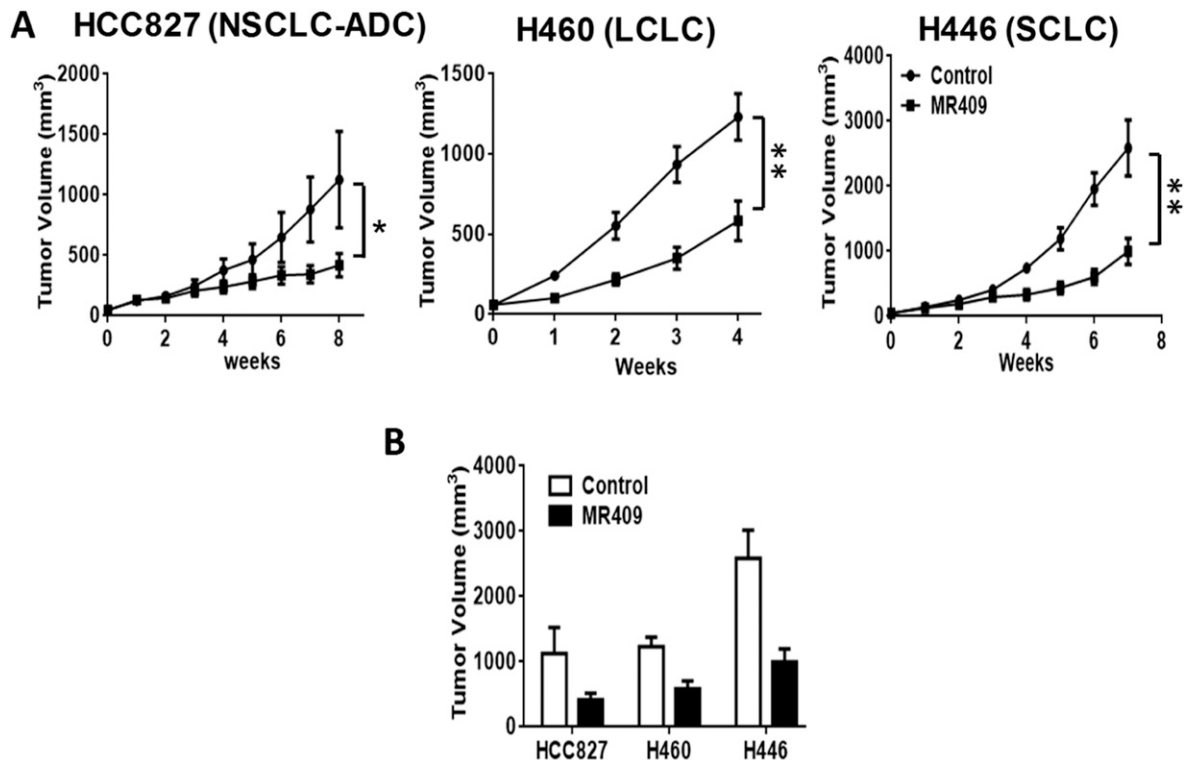


Figure 1. Inhibition of lung tumor growth *in vivo* by treatment of GHRH agonist MR-409. (A) Tumor growth in nude mice xenografted with lung tumors. The average tumor growth of HCC827 ($n = 20$), H460 ($n = 16$), and H446 ($n = 14$) is presented. (B) The average tumor growth (means \pm SEM) at the end of experiments, $*P < 0.05$; $**P < 0.01$. Significant inhibition of tumor growth occurred after therapy with MR-409. LCLC, large cell lung cancer; NSCLC-ADC, non-SCLC-adenocarcinoma. [Reproduced from Schally AV, Wang H, He JL, Cai R, Sha W, Popovics P, Perez R, Vidaurre I, and Zhang X. "Agonists of growth hormone-releasing hormone (GHRH) inhibit human experimental cancers *in vivo* by downregulating receptors for GHRH." *Proc Natl Acad Sci*, November 2018;115(47):12028–12033.]

similar to that produced by GHRH antagonists. In animals with HCC827 and H446 tumors treated with MR-409 for 8 weeks, there was a substantial reduction in serum IGF-1 levels, but no changes were seen in H460 tumors after treatment with MR-409 for only 4 weeks. Likewise, MR-409 decreased serum IGF-1 in C57BL/6 wild-type mice (49). Likewise, treatment of nude mice bearing NCI-N87 human stomach cancers with 5 μ g/day of another GHRH agonist, MR-356, lowered serum IGF-1 levels and markedly decreased tumor growth by day 63 of therapy (49). We also evaluated the effects of agonist MR-409 on *in vivo* tumor growth of other human cancers (1). The cancer lines tested comprised prostatic (PC-3), triple-negative breast cancers (MDA-MB231 and MX-1), colorectal (HCT-116 and HCT-15), as well as pancreatic (CFPAC-1 and PANC-1), gastric (NCI-N87), and bladder (RT-4 and J-82) cancers, which all expressed GHRH-Rs (1). The extent of inhibition of tumor growth was similar to that produced by GHRH antagonists at the same dose (1, 23, 36, 37). Administration of MR-409 to nude mice xenografted with these tumors for 1 to 2½ months markedly decreased tumor growth (1).

Our studies also revealed that the treatment of mice bearing HCC827, H460, and H446 lung cancers with the

agonist MR-409 induced a downregulation of GHRH-Rs in the pituitary gland and tumors, as measured by Western blot (1). The levels of both pGHRH-R and SV1 were reduced (Fig. 2). The reduction of GHRH-Rs in tumors was similar to that produced by GHRH antagonists MIA-602 (23, 36). In contrast to findings observed *in vitro*, the levels of effector proteins of cell cycle (cell-cycle regulators), the expressions of cyclin D1, and D2, cyclin-dependent kinases, and activated STAT3 were reduced by the therapy with agonist MR-409, but conditional inhibitor for cyclin-dependent kinase 4/6 was upregulated (1).

It has been shown that GHRH, produced locally in tumors, might function as an autocrine/paracrine growth factor in lung and other cancers (2, 9, 35, 53–55). *In vitro*, GHRH agonists promote cell growth (1). Induction of tumor formation or proliferation by parenteral therapy with GHRH agonists would nullify their planned use in a wide range of medical fields. Our findings of inhibitory action of the GHRH agonist on tumors *in vivo* dispel these fears (1). The hindering effects of repeated administration of MR-409 and other GHRH agonists on the proliferation of various human tumors exerted *in vivo* relieve our grave concerns about stimulating growth of cancers with this class of compounds in the course of

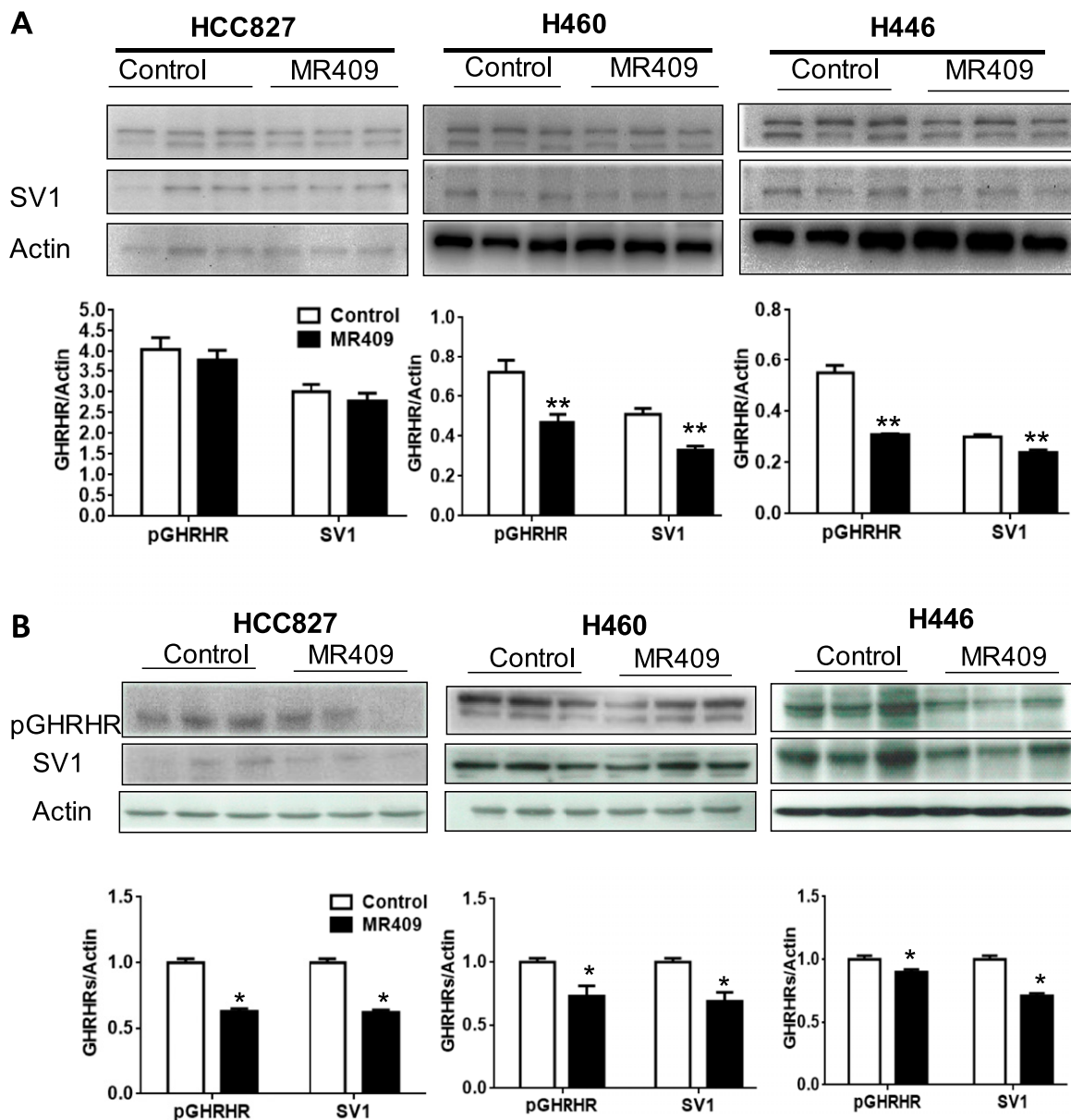


Figure 2. Downregulation of GHRH-Rs in the pituitary gland and tumor tissues after treatment with agonist MR-409. Representative images of Western blots of GHRH-Rs in (A) pituitary glands and (B) tumor specimen from animals xenografted with HCC827, H460, and H446 cells at termination of experiments. The relative expression of pGHRH-R and SV1 (means ± SEM) is presented, **P* < 0.05; ***P* < 0.01. A significant downregulation of pGHRH-R and SV1 was found in pituitary glands of mice bearing H460 and H446 tumors and of both types of GHRH-Rs in all three tumors. [Reproduced from Schally AV, Wang H, He JL, Cai R, Sha W, Popovics P, Perez R, Vidaurre I, and Zhang X. “Agonists of growth hormone-releasing hormone (GHRH) inhibit human experimental cancers *in vivo* by downregulating receptors for GHRH.” *Proc Natl Acad Sci*, November 2018;115(47):12028–12033.]

their clinical use in cardiology, diabetes, or other diseases and conditions. Moreover, in view of their suppressive action on tumor growth and the decline in IGF-1 level, GHRH agonists could find some applications in oncology, for instance, the potentiation of anticancer activity of traditional chemotherapeutic drugs, such as doxorubicin (56).

The inhibition of tumor growth *in vivo* by the GHRH agonist MR-409 seems similar to that induced by GHRH antagonists MIA-602 or MIA-690 (36). This blocking of tumor growth by agonists of GHRH, which at first may

seem paradoxical, appears to be caused mainly by the downregulation of the pGHRH-Rs and tumoral GHRH-Rs (1). These phenomena are thus analogous and correspond to the well-known downregulation of receptors for LHRH, induced by therapy with agonistic analogs of LHRH (57). It is thoroughly documented clinically that this event leads to suppression of the levels of LH, FSH, and sex steroids and inhibition of growth of prostatic and breast cancers [reviewed in Schally *et al.* (57)]. Thus, short-term activation of GHRH-Rs by GHRH agonists *in vitro* leads to stimulatory effects, but chronic, long-term, persistent

exposure to these ligands produces downregulation of receptors and inhibitory effects, as pointed out by Kiaris and Chatzistamou (58). The detailed mechanism of action of downregulation of GHRH-Rs by GHRH analogs must continue to be investigated, as other phenomena, such as cell differentiation, could be also involved in inhibition of tumor growth (23, 56).

Effects of GHRH Analogs on Diabetes

Diabetes currently affects more than 400 million people worldwide, with an incidence expected to rise dramatically (59). In both type 1 diabetes (T1D) and type 2 diabetes (T2D), the supply of insulin-producing tissue/cells is inadequate. Strategies aimed at the prevention of pancreatic β -cell loss and an increase in insulin production are among important approaches to treat diabetes.

The effects of GHRH on the stimulation of the secretion of the endocrine pancreas were demonstrated in the late 1980s (60, 61). Direct stimulatory action of human GHRH(1-44) NH_2 on the release of insulin and glucagon from perfused dog pancreas and isolated rat islets of Langerhans, as well as the effects on glucose homeostasis in diabetic mice and normal rats, was reported by Hermansen *et al.* (60) and Bailey *et al.* (61). The mechanism of action of the effect of GHRH on insulin release was also investigated (62). Following the demonstration that the GHRH-R and its SV1 were expressed in INS-1 cells, a rat β -cell line derived from rat insulinoma, and rat and human pancreatic islets, Ludwig *et al.* (32) reported that GHRH agonist JI-36, one of our early analogs, could function as a potential effector for survival and proliferation of pancreatic islets. This agonist significantly increased cell proliferation, reduced cell apoptosis, and promoted glucose-responsive insulin secretion *in vitro*. *In vivo*, JI-36 improved engraftment and metabolic function of rat islets following transplantation under the kidney capsule of streptozotocin (STZ)-induced diabetic nonobese diabetic–severe combined immunodeficiency (NOD/SCID) mice (32). Further studies showed that JI-36 facilitated the function of rat islets encapsulated in a bioartificial pancreas after implantation into rats with STZ-induced diabetes (44). Following the development of the MR series of GHRH analogs (24), the activity of several MR series compounds on β -cell proliferation was tested compared with that of JI-36 (33, 46). Analog MR-403 significantly increased cell viability and proliferation, reduced apoptosis on INS-1 cells, and promoted viability of rat islets when in coculture with rat adrenal cells in which the expression of the GHRH-R was demonstrated. Intra-adrenal transplantation of rat islets pretreated with MR-403 resulted in a rapid normoglycemia in STZ-induced

NOD/SCID mice (46). Furthermore, analogs MR-356, MR-409, and MR-502 displayed higher potency on cell viability and stimulated more efficiently the expression of cellular insulin, IGF-1, GHRH-Rs, and glucose-responsive insulin secretion in INS-1 cells (33). Agonist MR-409 also induced activation of phosphorylation of ERK and protein kinase B (AKT) and significantly increased the levels of cellular cAMP and the phosphorylation of cAMP response element-binding protein in INS-1 cells (33). Treatment of rat islets with agonist MR-409 significantly increased islet size and the expression of insulin. An *in vivo* study revealed that the maximal therapeutic benefits with respect to the efficiency of engraftment, ability to reach normoglycemia, gain in body weight, response to high glucose challenge, and induction of higher levels of serum insulin and IGF-1 were observed when STZ-induced NOD/SCID mice were transplanted with rat islets preconditioned with the GHRH agonist, MR-409, and received additional treatment with MR-409 post-transplantation (33). The study provides evidence for the beneficial action of synthetic GHRH agonists on pancreatic β -cells *in vitro* and *in vivo* (33). The results of a preliminary clinical trial with the GHRH agonist tesamorelin in patients with T2D support the safety of clinical use of GHRH agonists (52). Although the treatment with tesamorelin for 12 weeks did not alter insulin response or glycemic control, the levels of total cholesterol and non-high-density lipoprotein cholesterol decreased significantly (52). The use of GHRH agonists offers a physiologically and therapeutically simple and convenient approach to increase the viability of islet cells. Long-acting GHRH agonists may fulfill the requirements of effective drugs for improvement of engraftment of islets following transplantation. The synthetic GHRH agonists appear to allow the reduction of the islet mass needed to treat a diabetic status by transplantation (44). This effect would be a useful addition to the armamentarium for the β -cell replacement or other types of cell-based therapy for management of diabetes mellitus (63, 64) (Table 1).

Dyslipidemia, an elevation of triglyceride-rich lipoproteins in plasma, frequently accompanies diabetes mellitus and represents an important component of the disease, imposing cardiovascular risk and correlating with renal dysfunction. Romero *et al.* (43) reported that GHRH might play an important role in dyslipidemia associated with T1D. In STZ-induced rat T1D, GHRH-R expression was found to be upregulated in the distal small intestine, a tissue involved in chylomicron synthesis. Treatment of T1D rats with the GHRH antagonist, MIA-602, at a dose that did not affect plasma GH levels significantly reduced triglyceride-rich lipoprotein, as well as markers of renal injury, and improved

endothelial-dependent vasorelaxation. MIA-602 restored glucagon-like peptide 1 actions on hyperlipidemia and hyperglucagonemia in T1D rats. These findings revealed a previously unidentified pathway in T1D mediated by GHRH, associated with impaired glucagon-like peptide 1 signaling and hyperlipidemia. Inhibition of GHRH signaling by the use of GHRH antagonists might be a promising approach to improve the dyslipidemia, kidney damage and cardiovascular diseases associated with T1D and probably T2D (43). Whereas the loss of islets is predominant in T1D, T2D is a long-term metabolic disorder characterized by dysfunction of β -cells and/or insulin resistance occurring primarily within the muscles, liver, and fat tissue. The proportion between insulin resistance and dysfunction of β -cells varies among individuals with T2D. Dyslipidemia may play a pathological role in the early stages of T2D. Further studies to reveal the effects of GHRH analogs in the development of T2D and insulin resistance in appropriate animal models of T2D would therefore be particularly important and informative (Table 1).

Cardioprotective Effects of GHRH

In 1988, Hasegawa *et al.* (65) showed that GHRH—at that time, defined as GH-releasing factor—displayed a direct positive inotropic effect in guinea pig papillary muscles. A decade later, we started to investigate in detail the cardiovascular effects of GHRH, also considering evidence on the cardioprotective activities of other neuropeptides, such as corticotropin-releasing factor and urocortins, or GH secretagogues, such as ghrelin and its analogs (66–68). We first examined the potential cardioprotective effects of GHRH and demonstrated the ability of GHRH(1-44)NH₂ to prevent apoptosis induced by serum deprivation and isoproterenol in adult rat ventricular myocytes (ARVMs) and H9c2 rat cardiomyocytes (CMs), expressing pGHRH-R. These effects were abolished by the GHRH antagonist JV-1-36, indicating receptor-mediated mechanisms. Accordingly, underlying signaling included an increase in adenylyl cyclase/cAMP/protein kinase A (PKA) and activation of MAPK ERK1/2 and phosphatidylinositol 3-kinase/Akt pathways. GHRH also counteracted isoproterenol-induced elevation of the proapoptotic protein, inducible cAMP early repressor. In agreement with the *in vitro* findings, in isolated rat hearts subjected to ischemia/reperfusion, preischemic administration of GHRH improved heart function and strongly reduced infarct size after reperfusion through GHRH-R and phosphatidylinositol 3-kinase/Akt-mediated mechanisms (69). We later demonstrated that postischemic treatment with GHRH also reduced infarct size and contractile dysfunction in the

same model. The mechanisms included activation of the reperfusion injury salvage kinases and the survivor-activating factor-enhancement pathway (70). GHRH also promoted the phosphorylation of endothelial nitric oxide synthase and AMP-activated protein kinase and preserved postischemic nicotinamide adenine dinucleotide levels. Overall, these findings suggested potential therapeutic implication for GHRH and its analogs in myocardial infarction (MI) and heart failure (HF). Consistent with these findings, Ma *et al.* (31) recently showed that pretreatment of mesenchymal stem cells (MSCs) with GHRH agonist JI-34 enhanced viability and mobility of MSCs *in vitro*, along with increased STAT3 activity. *In vivo*, JI-34-pretreated MSCs showed improved engraftment into ischemic limbs and superior proangiogenic effects (31). Furthermore, Kiscsatári *et al.* (47) demonstrated that GHRH agonists JI-34 and MR-356 can alleviate radiation-induced damage in rat CMs. In addition, Shen *et al.* (48) reported that MR-409 alleviates *in vivo* smooth muscle cell-mediated aortic calcification in osteoprotegerin knockout mice through an increase in cAMP/PKA signaling and inhibition of inflammatory pathways.

In 2010, Kanashiro-Takeuchi *et al.* (26) performed a series of studies testing the hypothesis the GHRH agonists ameliorate myocardial injury as a result of acute infarction in the rat. Administration of agonist JI-38 to rats with MI, immediately following ligation of the left anterior descending coronary artery led to a reduction in infarct size and offset the decline in ejection fraction relative to placebo (Fig. 3). Importantly, administration of JI-38 for 4 weeks did not elevate either GH or IGF-1 (26). Subsequently, Kanashiro-Takeuchi *et al.* (25) tested whether JI-38 produced reversed remodeling post-MI in the rat model. In similar fashion to the previous study, animals were subjected to left anterior descending ligation, but administration of JI-38 was only begun 4 weeks following cardiac injury, at a time that ventricular remodeling had already matured. Importantly, even in the setting of cardiac enlargement, administration of JI-38 stimulated reverse remodeling. This was accompanied by a reduction in tissue fibrosis. Coadministration of a GHRH-R antagonist offset the impact of the agonist on the improvement in ventricular structure and function, demonstrating that this is a GHRH-R-mediated phenomenon. These two studies performed in the rat formed the basis for testing the impact of GHRH analogs in a large animal infarction model (29). In this study, female Yorkshire pigs were randomized to receive either placebo or the GHRH agonist MR-409. The dominant finding was that stimulation of the GHRH-R signaling pathway led to a 22% reduction in myocardial scar mass compared with an increase in the placebo group (Fig. 4). Accordingly, the results collectively revealed an

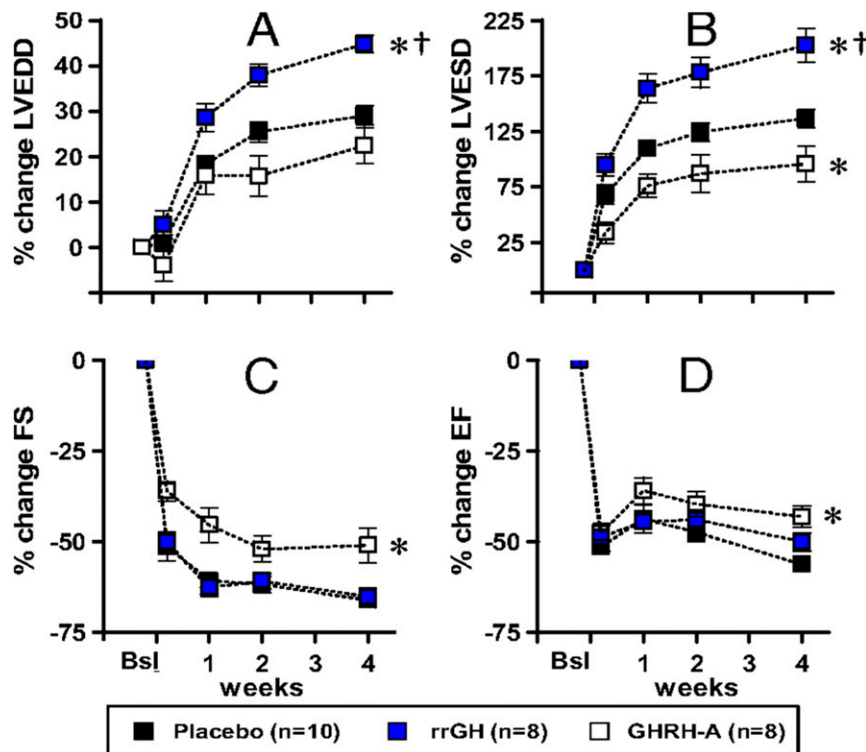


Figure 3. Impact of rat recombinant GH (rrGH) and GHRH-A on left-ventricle (LV) chamber size and function. Changes over time in (A) LV end-diastolic diameter (LVEDD; * $P < 0.05$ vs placebo; $^{\dagger}P < 0.01$ vs GHRH-A), (B) LV end-systolic diameter (LVESD; * $P < 0.01$ vs placebo; $^{\dagger}P < 0.001$ vs GHRH-A), (C) ejection fraction (EF; * $P < 0.05$ vs placebo), and (D) fraction shortening (FS; * $P < 0.05$ vs placebo vs GHRH-A). GHRH-A indicates GHRH agonist. Bsl, baseline. [Reproduced from Kanashiro-Takeuchi RM, Tziomalos K, Takeuchi LM, Treuer A, Laumiralt G, Dulce R, Hurtado M, Song Y, Block NL, Rick F, Hu Q, Varga JL, Schally AV, and Hare JM. "Cardioprotective effects of growth-hormone-releasing hormone (GHRH) agonist following myocardial infarction (MI)." *Proc Natl Acad Sci*, 2010;107(6):2604–2609.]

important and potentially clinically relevant improvement in parameters of left-ventricular (LV) function and remodeling in two mammalian species. These findings support the consideration of clinical testing of GHRH agonists for the treatment of myocardial injury in humans.

Pathological cardiac hypertrophy, in response to hypertension or MI, is one of the main risks for arrhythmias, dilated cardiomyopathy, and HF (71). The role of GHRH and its agonists was studied in different *in vitro* and *in vivo* models of cardiac hypertrophy. Gesmundo *et al.* (30) recently demonstrated that GHRH(1-44)NH₂ counteracts phenylephrine (PE)-induced hypertrophy in H9c2 cells, ARVMs, and CMs, derived from human induced pluripotent stem cell-CMs by inhibition of the expression of hypertrophic genes and regulation of hypertrophic pathways. In H9c2 cells, GHRH blocked the $\alpha 1$ -adrenergic receptor/Gq/phospholipase C β hypertrophic pathway, which was elevated by PE. GHRH also attenuated the phosphorylation of prohypertrophic phospholamban at Thr17, dependent on Ca²⁺/calmodulin-dependent protein kinase II. These effects were blocked by the

GHRH antagonist JV-1-36, suggesting GHRH-R-mediated mechanisms; accordingly, GHRH elevated G α_s /cAMP/PKA signaling, along with phosphorylation of anti-hypertrophic phospholamban at Ser16. Importantly, in H9c2 cells, ARVMs, and induced pluripotent stem cell-CMs, the anti-hypertrophic effects of GHRH included blockade of PE-induced expression of the exchange protein directly activated by cAMP1, a key player in hypertrophy and HF (72).

In vivo, the role of the GHRH agonistic analog MR-409 was studied in mice subjected to transverse aortic constriction (TAC), a model of pressure overload hypertrophy and HF. TAC mice treated for 2 weeks with MR-409 showed reduced hypertrophy and improved cardiac function compared with untreated mice. Furthermore, in agreement with the results *in vitro*, MR-409 reduced the size of CMs in TAC hearts; inhibited the expression of hypertrophic genes, including the exchange protein directly activated by cAMP1; and upregulated sarco/endoplasmic reticulum Ca²⁺ ATPase 2a protein that was reduced by TAC. Moreover, CMs isolated from TAC mice showed

normalization of contractile responses after chronic treatment with MR-409. Overall, the results of this study suggest therapeutic use of GHRH analogs in pathological hypertrophy and HF (30) (Table 1).

Effects of GHRH in Eye Diseases

GHRH and GHRH-Rs have been identified in several ocular tissues. In particular, Dubovy *et al.* (73) have analyzed postmortem human eyes and demonstrated the presence of GHRH and GHRH-Rs in different human ocular tissues, such as the following: cornea epithelium and endothelium, ciliary body, trabecular meshwork, optic nerve, and neuroretina. These studies validated the potential relevance of modulation of the GHRH–GHRH-Rs signaling in human ocular pathologies. Analogs of GHRH and GHRH-Rs have been studied in a number of eye diseases. Chu *et al.* (38) have shown that antagonists of GHRH promote apoptosis in retinoblastoma cells, and Qin *et al.* (39) determined that GHRH antagonists could mitigate ocular surface inflammation in experimental uveitis and in pterygium (40). Similar results were obtained

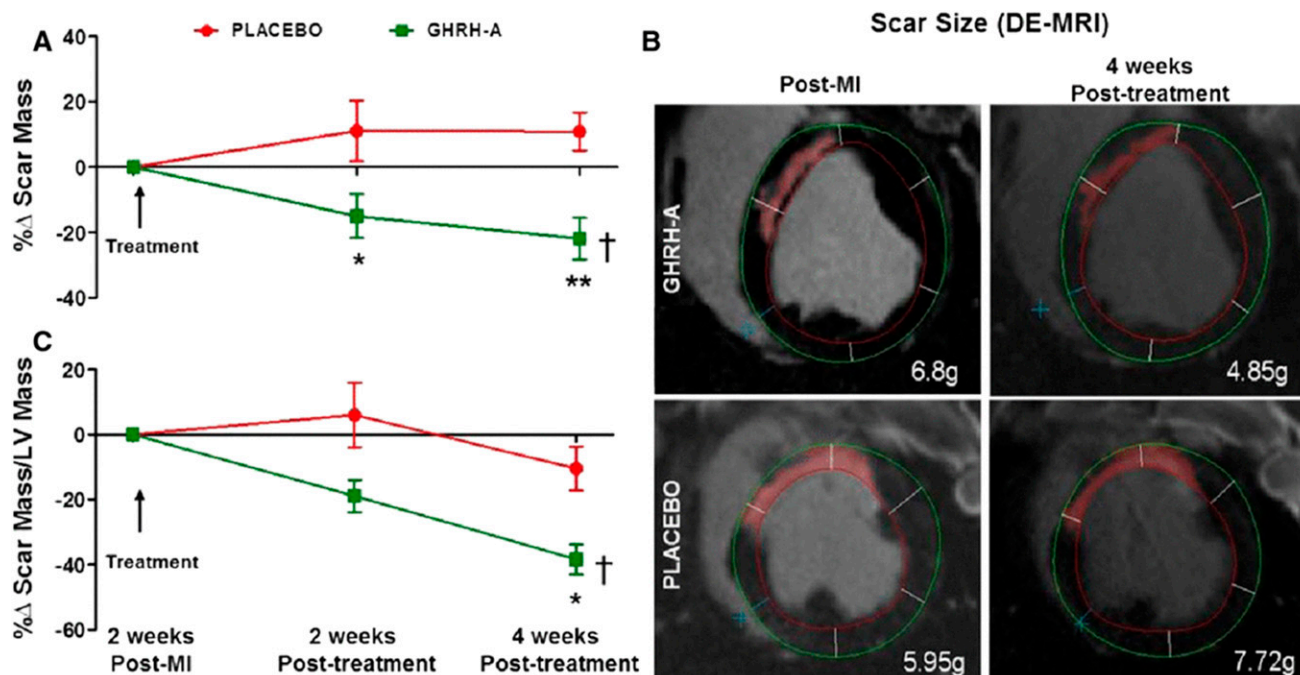


Figure 4. GHRH-A treatment reduces scar mass. (A) Percent change (% Δ) of scar mass in the GHRH-A group vs placebo group at 2 and 4 weeks postinitiation of treatment (two-way ANOVA, between group: $*P = 0.04$ and $**P = 0.003$ vs placebo, respectively; one-way ANOVA, $^{\dagger}P = 0.02$ for the GHRH-A group). (B) Images of hearts from an animal in each group before and 4 weeks after the initiation of treatment. (C) Percent change of scar as a percentage of LV mass in the GHRH-A group vs placebo group at 2 and 4 weeks postinitiation of treatment (two-way ANOVA, between group: $P = \text{NS}$ and $*P = 0.02$ vs placebo, respectively; one-way ANOVA, $^{\dagger}P = 0.0002$ for the GHRH-A group). GHRH-A indicates GHRH agonist. DE-MRI, delayed enhancement MRI. NS, not significant. [Reproduced from Bagno LL, Kanashiro-Takeuchi RM, Suncion VY, Golpanian S, Karantalis V, Wolf A, Wang B, Premer C, Balkan W, Rodriguez J, Valdes D, Rosado M, Block NL, Goldstein P, Morales A, Cai RZ, Sha W, Schally AV, Hare JM. Growth hormone–releasing hormone agonists reduce myocardial infarct scar in swine with subacute ischemic cardiomyopathy. *J Am Heart Assoc.* 2015;4 (4):e001464. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.]

by Ren *et al.* (41) in models of ocular inflammation. However, of major relevance for this review are the findings of Thounaojam *et al.* (50), revealing that the GHRH agonistic analog MR-409 exerted neuro- and vascular-protective effects in early experimental diabetic retinopathy through a mechanism involving antioxidative and anti-inflammatory activities. In these studies, the authors showed that the expression of GHRH-R was significantly downregulated in both human postmortem retinas of diabetic donors, as well as in a rat model of T1D. Accordingly, maintenance of retinal GHRH signaling by systemic administration of MR-409 in diabetic rats halted hyperglycemia-induced retinal vascular permeability and death of retinal neurons, the retinal ganglion cells (50). Interestingly, anti-permeability effects of GHRH agonists have been previously documented in response to bacterial toxin-induced pulmonary edema (74). The observed neuroprotective effects of GHRH on retinal ganglion cells are supported by historical studies demonstrating the presence and importance of the GHRH/GH axis for the maintenance of retinal ganglion cell survival (75, 76). Moreover, clinical studies have reported retinal neurovascular abnormalities in congenital GH deficiency (77). Taken together, these studies

underscore the importance of the GHRH-GHRH-R signaling in ocular diseases and warrant further studies to characterize/validate better the effects of GHRH agonistic analogs for retinal neuroprotection (Table 1).

Other Effects of GHRH and Its Analogs

Based on the demonstration that agonistic analogs of GHRH increase proliferation of human fibroblasts (45), we tested whether GHRH would promote wound healing and repair. Dioufa *et al.* (78) demonstrated that GHRH and GHRH agonist JI-38 can increase the migration of mouse embryonic fibroblasts and accelerate healing in skin wounds of mice. With the use of the new agonists MR-409 and MR-502, Cui *et al.* (34) demonstrated that *in vitro*, both analogs stimulated cell growth and increased cell survival under serum depletion-induced stress. *In vivo*, topical application of MR-409 speeded up wound healing in mice (34). Skin regeneration was likely related to an increase in migration of fibroblasts and activation of α -smooth muscle actin. These findings suggest that GHRH analogs may be considered for promotion of the healing of skin wounds.

Gallo *et al.* (79) showed that GHRH(1-44)NH₂ prevented apoptosis and atrophy in skeletal myotubes through inhibition of proteolytic pathways, indicating a role for GHRH agonists in muscle atrophy-associated diseases. With regard to the immune system, Khorram *et al.* (51, 80) reported that the administration of a GHRH agonist [Norleucine27]GHRH (1–29)-NH₂ to elderly subjects resulted in activation of both immune cells (51) and somatotrophic axis (80). Thus, GHRH agonists could be of therapeutic benefit for stimulation of compromised immune systems in aging persons (51). Moreover, it has been shown that GHRH(1-44)NH₂ can inhibit IL-6 and increase levels of IL-17 in peripheral blood mononuclear cells (81, 82).

Interestingly, a recent study reported that adipocytes from morbidly obese subjects showed a higher expression of GHRH and a lower expression of GHRH-R compared with nonobese subjects. *In vitro* exposure of GHRH inhibited the differentiation of human MSCs to adipocytes and promoted lipolysis in human differentiated adipocytes. These effects were blocked when the expression of mRNA for the GH receptor was silenced, suggesting effects mediated by the GH or GH receptor (83). Furthermore, it has been shown that the GHRH agonist tesamorelin reduces visceral adipose tissue in HIV-infected patients and improves liver enzymes (84, 85). These findings suggest therapeutic implications for GHRH agonists in obesity and metabolic dysfunctions.

Both the agonists and antagonists of GHRH show important effects on the central nervous system, and GHRH-Rs are present in the brain cortex and other brain areas (42). Thus, Baker *et al.* (86) showed that the GHRH agonist tesamorelin improves cognitive function in adults with mild cognitive impairment and in healthy older adults (Table 1). Furthermore, GHRH antagonists MIA-690 and MIA-602 exhibited the capability to inhibit amyloid aggregation and proteotoxicity and decreased the deterioration in cognitive performance in a transgenic mouse model of Alzheimer's disease (42).

Perspectives and Conclusions

The findings on GHRH analogs reviewed in this article appear to have profound implications. The antagonists of GHRH are being developed for therapy of cancers but should also be evaluated in further studies on dyslipidemia, ocular pathologies, and Alzheimer's disease. The agonists of GHRH warrant continued investigations in cardiology on their possible applications in myocardial injuries, HF, and pathological hypertrophy. GHRH agonists may also find possible uses in the field of diabetes for β -cell replacement and other types of cell-based therapy. Experimental research is also revealing possible beneficial effects

of agonists of GHRH in eye diseases. These various potential beneficial effects of GHRH analogs are possible because of the presence of GHRH-Rs in many cells and tissues. We can no longer ignore the many possible clinical applications of GHRH analogs, the list of which is continuously extending.

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References and Notes

- Schally AV, Wang H, He J, Cai R, Sha W, Popovics P, Perez R, Vidaurre I, Zhang X. Agonists of growth hormone-releasing hormone (GHRH) inhibit human experimental cancers in vivo by down-regulating receptors for GHRH. *Proc Natl Acad Sci USA*. 2018;115(47):12028–12033.
- Frohman LA, Szabo M. Ectopic production of growth hormone-releasing factor by carcinoid and pancreatic islet tumors associated with acromegaly. *Prog Clin Biol Res*. 1981;74:259–271.
- Guillemin R, Brazeau P, Böhlen P, Esch F, Ling N, Wehrenberg WB. Growth hormone-releasing factor from a human pancreatic tumor that caused acromegaly. *Science*. 1982;218(4572):585–587.
- Rivier J, Spiess J, Thorner M, Vale W. Characterization of a growth hormone-releasing factor from a human pancreatic islet tumour. *Nature*. 1982;300(5889):276–278.
- Thorner MO. The discovery of growth hormone-releasing hormone. *J Clin Endocrinol Metab*. 1999;84(12):4671–4676.
- Gaylinn BD, Harrison JK, Zysk JR, Lyons CE, Lynch KR, Thorner MO. Molecular cloning and expression of a human anterior pituitary receptor for growth hormone-releasing hormone. *Mol Endocrinol*. 1993;7(1):77–84.
- Mayo KE. Molecular cloning and expression of a pituitary-specific receptor for growth hormone-releasing hormone. *Mol Endocrinol*. 1992;6(10):1734–1744.
- Rekasi Z, Czompoly T, Schally AV, Halmos G. Isolation and sequencing of cDNAs for splice variants of growth hormone-releasing hormone receptors from human cancers. *Proc Natl Acad Sci USA*. 2000;97(19):10561–10566.
- Schally AV, Varga JL, Engel JB. Antagonists of growth-hormone-releasing hormone: an emerging new therapy for cancer. *Nat Clin Pract Endocrinol Metab*. 2008;4(1):33–43.
- Kiari H, Chatzistamou I, Schally AV, Halmos G, Varga JL, Koutselini H, Kalofoutis A. Ligand-dependent and -independent effects of splice variant 1 of growth hormone-releasing hormone receptor. *Proc Natl Acad Sci USA*. 2003;100(16):9512–9517.
- Thorner MO, Chapman IM, Gaylinn BD, Pezzoli SS, Hartman ML. Growth hormone-releasing hormone and growth hormone-releasing peptide as therapeutic agents to enhance growth hormone secretion in

- disease and aging. *Recent Prog Horm Res.* 1997;52:215–244, discussion 244–246.
12. Rogol AD, Blizzard RM, Johanson AJ, Furlanetto RW, Evans WS, Rivier J, Vale WW, Thorner MO. Growth hormone release in response to human pancreatic tumor growth hormone-releasing hormone-40 in children with short stature. *J Clin Endocrinol Metab.* 1984;59(4):580–586.
 13. Müller EE, Locatelli V, Cocchi D. Neuroendocrine control of growth hormone secretion. *Physiol Rev.* 1999;79(2):511–607.
 14. Gelato MC. Growth hormone-releasing hormone: clinical perspectives. *Endocrinologist.* 1994;4(1):64–68.
 15. Kahán Z, Arencibia JM, Csernus VJ, Groot K, Kineman RD, Robinson WR, Schally AV. Expression of growth hormone-releasing hormone (GHRH) messenger ribonucleic acid and the presence of biologically active GHRH in human breast, endometrial, and ovarian cancers. *J Clin Endocrinol Metab.* 1999;84(2):582–589.
 16. Bagnato A, Moretti C, Ohnishi J, Frajese G, Catt KJ. Expression of the growth hormone-releasing hormone gene and its peptide product in the rat ovary. *Endocrinology.* 1992;130(3):1097–1102.
 17. Khorram O, Garthwaite M, Grosen E, Golos T. Human uterine and ovarian expression of growth hormone-releasing hormone messenger RNA in benign and malignant gynecologic conditions. *Fertil Steril.* 2001;75(1):174–179.
 18. Izdebski J, Pinski J, Horvath JE, Halmos G, Groot K, Schally AV. Synthesis and biological evaluation of superactive agonists of growth hormone-releasing hormone. *Proc Natl Acad Sci USA.* 1995;92(11):4872–4876.
 19. Zarandi M, Csernus V, Bokser L, Bajusz S, Groot K, Schally AV. Synthesis and in vitro and in vivo activity of analogs of growth hormone-releasing hormone (GH-RH) with C-terminal agmatine. *Int J Pept Protein Res.* 1990;36(6):499–505.
 20. Robberecht P, Coy DH, Waelbroeck M, Heiman ML, de Neef P, Camus JC, Christophe J. Structural requirements for the activation of rat anterior pituitary adenylate cyclase by growth hormone-releasing factor (GRF): discovery of (N-Ac-Tyr1, D-Arg2)-GRF(1–29)-NH₂ as a GRF antagonist on membranes. *Endocrinology.* 1985;117(5):1759–1764.
 21. Zarandi M, Horvath JE, Halmos G, Pinski J, Nagy A, Groot K, Rekasi Z, Schally AV. Synthesis and biological activities of highly potent antagonists of growth hormone-releasing hormone. *Proc Natl Acad Sci USA.* 1994;91(25):12298–12302.
 22. Varga JL, Schally AV, Csernus VJ, Zarándi M, Halmos G, Groot K, Rékási Z. Synthesis and biological evaluation of antagonists of growth hormone-releasing hormone with high and protracted in vivo activities. *Proc Natl Acad Sci USA.* 1999;96(2):692–697.
 23. Zarandi M, Cai R, Kovacs M, Popovics P, Szalontay L, Cui T, Sha W, Jaszberenyi M, Varga J, Zhang X, Block NL, Rick FG, Halmos G, Schally AV. Synthesis and structure-activity studies on novel analogs of human growth hormone releasing hormone (GHRH) with enhanced inhibitory activities on tumor growth. *Peptides.* 2017;89:60–70.
 24. Cai R, Schally AV, Cui T, Szalontay L, Halmos G, Sha W, Kovacs M, Jaszberenyi M, He J, Rick FG, Popovics P, Kanashiro-Takeuchi R, Hare JM, Block NL, Zarandi M. Synthesis of new potent agonistic analogs of growth hormone-releasing hormone (GHRH) and evaluation of their endocrine and cardiac activities. *Peptides.* 2014;52:104–112.
 25. Kanashiro-Takeuchi RM, Takeuchi LM, Rick FG, Dulce R, Treuer AV, Florea V, Rodrigues CO, Paulino EC, Hatzistergos KE, Selem SM, Gonzalez DR, Block NL, Schally AV, Hare JM. Activation of growth hormone releasing hormone (GHRH) receptor stimulates cardiac reverse remodeling after myocardial infarction (MI). *Proc Natl Acad Sci USA.* 2012;109(2):559–563.
 26. Kanashiro-Takeuchi RM, Tziomalos K, Takeuchi LM, Treuer AV, Lamirault G, Dulce R, Hurtado M, Song Y, Block NL, Rick F, Klukovits A, Hu Q, Varga JL, Schally AV, Hare JM. Cardioprotective effects of growth hormone-releasing hormone agonist after myocardial infarction. *Proc Natl Acad Sci USA.* 2010;107(6):2604–2609.
 27. Kanashiro-Takeuchi RM, Szalontay L, Schally AV, Takeuchi LM, Popovics P, Jaszberenyi M, Vidaurre I, Zarandi M, Cai RZ, Block NL, Hare JM, Rick FG. New therapeutic approach to heart failure due to myocardial infarction based on targeting growth hormone-releasing hormone receptor. *Oncotarget.* 2015;6(12):9728–9739.
 28. Florea V, Majid SS, Kanashiro-Takeuchi RM, Cai RZ, Block NL, Schally AV, Hare JM, Rodrigues CO. Agonists of growth hormone-releasing hormone stimulate self-renewal of cardiac stem cells and promote their survival. *Proc Natl Acad Sci USA.* 2014;111(48):17260–17265.
 29. Bagno LL, Kanashiro-Takeuchi RM, Suncion VY, Golpanian S, Karantalis V, Wolf A, Wang B, Premer C, Balkan W, Rodriguez J, Valdes D, Rosado M, Block NL, Goldstein P, Morales A, Cai RZ, Sha W, Schally AV, Hare JM. Growth hormone-releasing hormone agonists reduce myocardial infarct scar in swine with subacute ischemic cardiomyopathy. *J Am Heart Assoc.* 2015;4(4):e001464.
 30. Gesmundo I, Miragoli M, Carullo P, Trovato L, Larcher V, Di Pasquale E, Brancaccio M, Mazzola M, Villanova T, Sorge M, Taliano M, Gallo MP, Alloati G, Penna C, Hare JM, Ghigo E, Schally AV, Condorelli G, Granata R. Growth hormone-releasing hormone attenuates cardiac hypertrophy and improves heart function in pressure overload-induced heart failure. *Proc Natl Acad Sci USA.* 2017;114(45):12033–12038.
 31. Ma Q, Xia X, Tao Q, Lu K, Shen J, Xu Q, Hu X, Tang Y, Block NL, Webster KA, Schally AV, Wang J, Yu H. Profound actions of an agonist of growth hormone-releasing hormone on angiogenic therapy by mesenchymal stem cells. *Arterioscler Thromb Vasc Biol.* 2016;36(4):663–672.
 32. Ludwig B, Ziegler CG, Schally AV, Richter C, Steffen A, Jabs N, Funk RH, Brendel MD, Block NL, Ehrhart-Bornstein M, Bornstein SR. Agonist of growth hormone-releasing hormone as a potential effector for survival and proliferation of pancreatic islets. *Proc Natl Acad Sci USA.* 2010;107(28):12623–12628.
 33. Zhang X, Cui T, He J, Wang H, Cai R, Popovics P, Vidaurre I, Sha W, Schmid J, Ludwig B, Block NL, Bornstein SR, Schally AV. Beneficial effects of growth hormone-releasing hormone agonists on rat INS-1 cells and on streptozotocin-induced NOD/SCID mice. *Proc Natl Acad Sci USA.* 2015;112(44):13651–13656.
 34. Cui T, Jimenez JJ, Block NL, Badiavas EV, Rodriguez-Menocal L, Vila Granda A, Cai R, Sha W, Zarandi M, Perez R, Schally AV. Agonistic analogs of growth hormone releasing hormone (GHRH) promote wound healing by stimulating the proliferation and survival of human dermal fibroblasts through ERK and AKT pathways. *Oncotarget.* 2016;7(33):52661–52672.
 35. Kiaris H, Schally AV, Varga JL, Groot K, Armatis P. Growth hormone-releasing hormone: an autocrine growth factor for small cell lung carcinoma. *Proc Natl Acad Sci USA.* 1999;96(26):14894–14898.
 36. Wang H, Zhang X, Vidaurre I, Cai R, Sha W, Schally AV. Inhibition of experimental small-cell and non-small-cell lung cancers by novel antagonists of growth hormone-releasing hormone. *Int J Cancer.* 2018;142(11):2394–2404.
 37. Gan J, Ke X, Jiang J, Dong H, Yao Z, Lin Y, Lin W, Wu X, Yan S, Zhuang Y, Chu WK, Cai R, Zhang X, Cheung HS, Block NL, Pang CP, Schally AV, Zhang H. Growth hormone-releasing hormone receptor antagonists inhibit human gastric cancer through downregulation of PAK1-STAT3/NF- κ B signaling. *Proc Natl Acad Sci USA.* 2016;113(51):14745–14750.
 38. Chu WK, Law KS, Chan SO, Yam JC, Chen LJ, Zhang H, Cheung HS, Block NL, Schally AV, Pang CP. Antagonists of growth hormone-releasing hormone receptor induce apoptosis specifically in retinoblastoma cells. *Proc Natl Acad Sci USA.* 2016;113(50):14396–14401.
 39. Qin YJ, Chan SO, Chong KK, Li BF, Ng TK, Yip YW, Chen H, Zhang M, Block NL, Cheung HS, Schally AV, Pang CP. Antagonist of GH-releasing hormone receptors alleviates experimental ocular inflammation. *Proc Natl Acad Sci USA.* 2014;111(51):18303–18308.

40. Qin YJ, Chu WK, Huang L, Ng CHY, Chan TCY, Cao D, Yang C, Zhang L, Huang SP, Li J, Lin HL, Li WQ, Chen L, Schally AV, Chan SO, Zhang HY, Pang CP. Induction of apoptosis in pterygium cells by antagonists of growth hormone-releasing hormone receptors. *Invest Ophthalmol Vis Sci.* 2018;**59**(12):5060–5066.
41. Ren JL, Yu QX, Ma D, Liang WC, Leung PY, Ng TK, Chu WK, Schally AV, Pang CP, Chan SO. Growth hormone-releasing hormone receptor mediates cytokine production in ciliary and iris epithelial cells during LPS-induced ocular inflammation. *Exp Eye Res.* 2019;**181**:277–284.
42. Jaszberenyi M, Rick FG, Szalontay L, Block NL, Zarandi M, Cai RZ, Schally AV. Beneficial effects of novel antagonists of GHRH in different models of Alzheimer's disease. *Aging (Albany NY).* 2012;**4**(11):755–767.
43. Romero MJ, Lucas R, Dou H, Sridhar S, Czikota I, Mosieri EM, Rick FG, Block NL, Sridhar S, Fulton D, Weintraub NL, Bagi Z, Schally AV. Role of growth hormone-releasing hormone in dyslipidemia associated with experimental type 1 diabetes. *Proc Natl Acad Sci USA.* 2016;**113**(7):1895–1900.
44. Ludwig B, Rotem A, Schmid J, Weir GC, Colton CK, Brendel MD, Neufeld T, Block NL, Yavriyants K, Steffen A, Ludwig S, Chavakis T, Reichel A, Azarov D, Zimmermann B, Maimon S, Balyura M, Rozenshtein T, Shabtay N, Vardi P, Bloch K, de Vos P, Schally AV, Bornstein SR, Barkai U. Improvement of islet function in a bio-artificial pancreas by enhanced oxygen supply and growth hormone releasing hormone agonist. *Proc Natl Acad Sci USA.* 2012;**109**(13):5022–5027.
45. Kiaris H, Schally AV, Armatas P. Direct action of growth hormone-releasing hormone agonist JI-38 on normal human fibroblasts: evidence from studies on cell proliferation and c-myc proto-oncogene expression. *Regul Pept.* 2001;**96**(3):119–124.
46. Schubert U, Schmid J, Lehmann S, Zhang XY, Morawietz H, Block NL, Kanczkowski W, Schally AV, Bornstein SR, Ludwig B. Transplantation of pancreatic islets to adrenal gland is promoted by agonists of growth-hormone-releasing hormone. *Proc Natl Acad Sci USA.* 2013;**110**(6):2288–2293.
47. Kiscsatári L, Varga Z, Schally AV, Gáspár R, Nagy CT, Giricz Z, Ferdinandy P, Fábrián G, Kahán Z, Görbe A. Protection of neonatal rat cardiac myocytes against radiation-induced damage with agonists of growth hormone-releasing hormone. *Pharmacol Res.* 2016;**111**:859–866.
48. Shen J, Zhang N, Lin YN, Xiang P, Liu XB, Shan PF, Hu XY, Zhu W, Tang YL, Webster KA, Cai R, Schally AV, Wang J, Yu H. Regulation of vascular calcification by growth hormone-releasing hormone and its agonists. *Circ Res.* 2018;**122**(10):1395–1408.
49. Cui T, Schally AV. Growth hormone-releasing hormone (GHRH) and its agonists inhibit hepatic and tumoral secretion of IGF-1. *Oncotarget.* 2018;**9**(47):28745–28756.
50. Thounaojam MC, Powell FL, Patel S, Gutsaeva DR, Tawfik A, Smith SB, Nussbaum J, Block NL, Martin PM, Schally AV, Bartoli M. Protective effects of agonists of growth hormone-releasing hormone (GHRH) in early experimental diabetic retinopathy. *Proc Natl Acad Sci USA.* 2017;**114**(50):13248–13253.
51. Khorram O, Yeung M, Vu L, Yen SS. Effects of [Norleucine²⁷] growth hormone-releasing hormone (GHRH) (1-29)-NH₂ administration on the immune system of aging men and women. *J Clin Endocrinol Metab.* 1997;**82**(11):3590–3596.
52. Clemmons DR, Miller S, Mamputu JC. Safety and metabolic effects of tesamorelin, a growth hormone-releasing factor analogue, in patients with type 2 diabetes: a randomized, placebo-controlled trial. *PLoS One.* 2017;**12**(6):e0179538.
53. Stępień T, Sacewicz M, Lawnicka H, Krupiński R, Komorowski J, Siejka A, Stępień H. Stimulatory effect of growth hormone-releasing hormone (GHRH(1-29)NH₂) on the proliferation, VEGF and chromogranin A secretion by human neuroendocrine tumor cell line NCI-H727 in vitro. *Neuropeptides.* 2009;**43**(5):397–400.
54. Barabutis N, Siejka A, Schally AV. Effects of growth hormone-releasing hormone and its agonistic and antagonistic analogs in cancer and non-cancerous cell lines. *Int J Oncol.* 2010;**36**(5):1285–1289.
55. Muñoz-Moreno L, Bajo AM, Prieto JC, Carmena MJ. Growth hormone-releasing hormone (GHRH) promotes metastatic phenotypes through EGFR/HER2 transactivation in prostate cancer cells. *Mol Cell Endocrinol.* 2017;**446**:59–69.
56. Jaszberenyi M, Rick FG, Popovics P, Block NL, Zarandi M, Cai RZ, Vidaurre I, Szalontay L, Jayakumar AR, Schally AV. Potentiation of cytotoxic chemotherapy by growth hormone-releasing hormone agonists. *Proc Natl Acad Sci USA.* 2014;**111**(2):781–786.
57. Schally AV, Block NL, Rick FG. Discovery of LHRH and development of LHRH analogs for prostate cancer treatment. *Prostate.* 2017;**77**(9):1036–1054.
58. Kiaris H, Chatzistamou I. Inhibition of tumor growth by agonists of growth hormone-releasing hormone. *Proc Natl Acad Sci USA.* 2018;**115**(47):11876–11878.
59. Ali MK, Siegel KR, Chandrasekar E, Tandon R, Montoya PA, Mbanya JC, Chan J, Zhang P, Narayan KM. Diabetes: an update on the pandemic and potential solutions. In: Prabhakaran D, Anand S, Gaziano TA, Mbanya JC, Wu Y, Nugent R, eds. *Cardiovascular, Respiratory, and Related Disorders.* Vol 5. 3rd ed. Washington, DC: The World Bank; 2017:209–234.
60. Hermansen K, Kappelgaard AM, Esmann J, Orskov H. Synthetic pancreatic growth hormone-releasing factor (GRF-40) stimulates the secretion of the endocrine pancreas. *Diabetes.* 1986;**35**(1):119–123.
61. Bailey CJ, Wilkes LC, Flatt PR, Conlon JM, Buchanan KD. Effects of growth hormone-releasing hormone on the secretion of islet hormones and on glucose homeostasis in lean and genetically obese-diabetic (ob/ob) mice and normal rats. *J Endocrinol.* 1989;**123**(1):19–24.
62. Green IC, Southern C, Ray K. Mechanism of action of growth-hormone-releasing hormone in stimulating insulin secretion in vitro from isolated rat islets and dispersed islet cells. *Horm Res.* 1990;**33**(5):199–204.
63. Zhang X, Block NL, Schally AV. Cell-based therapy for treatment of diabetes mellitus: can the agonists of growth hormone-releasing hormone make a contribution? *Int J Stem Cell Res Ther.* 2016;**3**(1):1–6.
64. Fridlyand LE, Tamarina NA, Schally AV, Philipson LH. Growth hormone-releasing hormone in diabetes. *Front Endocrinol (Lausanne).* 2016;**7**:129.
65. Hasegawa J, Saitoh M, Hirai S, Kinugawa T, Kotake H, Mashiba H. Positive inotropic effect of growth hormone releasing factor in the guinea-pig cardiac muscles in vitro. *Horm Metab Res.* 1988;**20**(2):130–131.
66. Chatzaki E, Kefala N, Drosos I, Lalidou F, Baritaki S. Do urocortins have a role in treating cardiovascular disease? *Drug Discov Today.* 2019;**24**(1):279–284.
67. Tokudome T, Otani K, Miyazato M, Kangawa K. Ghrelin and the heart. *Peptides.* 2019;**111**:42–46.
68. Granata R, Isgaard J, Alloati G, Ghigo E. Cardiovascular actions of the ghrelin gene-derived peptides and growth hormone-releasing hormone. *Exp Biol Med (Maywood).* 2011;**236**(5):505–514.
69. Granata R, Trovato L, Gallo MP, Destefanis S, Settanni F, Scarlatti F, Brero A, Ramella R, Volante M, Isgaard J, Levi R, Papotti M, Alloati G, Ghigo E. Growth hormone-releasing hormone promotes survival of cardiac myocytes in vitro and protects against ischaemia-reperfusion injury in rat heart. *Cardiovasc Res.* 2009;**83**(2):303–312.
70. Penna C, Settanni F, Tullio F, Trovato L, Pagliaro P, Alloati G, Ghigo E, Granata R. GH-releasing hormone induces cardioprotection in isolated male rat heart via activation of RISK and SAFE pathways. *Endocrinology.* 2013;**154**(4):1624–1635.
71. van Berlo JH, Maillet M, Molkentin JD. Signaling effectors underlying pathologic growth and remodeling of the heart. *J Clin Invest.* 2013;**123**(1):37–45.

72. Lezoualc'h F, Fazal L, Laudette M, Conte C. Cyclic AMP sensor EPAC proteins and their role in cardiovascular function and disease. *Circ Res*. 2016;**118**(5):881–897.
73. Dubovy SR, Fernandez MP, Echegaray JJ, Block NL, Unoki N, Perez R, Vidaurre I, Lee RK, Nadji M, Schally AV. Expression of hypothalamic neurohormones and their receptors in the human eye. *Oncotarget*. 2017;**8**(40):66796–66814.
74. Lucas R, Sridhar S, Rick FG, Gorshkov B, Umapathy NS, Yang G, Oseghale A, Verin AD, Chakraborty T, Matthay MA, Zemskov EA, White R, Block NL, Schally AV. Agonist of growth hormone-releasing hormone reduces pneumolysin-induced pulmonary permeability edema. *Proc Natl Acad Sci USA*. 2012;**109**(6):2084–2089.
75. Martínez-Moreno CG, Trudeau VL, Harvey S. Co-storage and secretion of growth hormone and secretoneurin in retinal ganglion cells. *Gen Comp Endocrinol*. 2015;**220**:124–132.
76. Martínez-Moreno CG, Giterman D, Henderson D, Harvey S. Secretagogue induction of GH release in QNR/D cells: prevention of cell death. *Gen Comp Endocrinol*. 2014;**203**:274–280.
77. Pereira-Gurgel VM, Faro AC, Salvatori R, Chagas TA, Carvalho-Junior JF, Oliveira CR, Costa UM, Melo GB, Hellström A, Aguiar-Oliveira MH. Abnormal vascular and neural retinal morphology in congenital lifetime isolated growth hormone deficiency. *Growth Horm IGF Res*. 2016;**30–31**:11–15.
78. Dioufa N, Schally AV, Chatzistamou I, Moustou E, Block NL, Owens GK, Papavassiliou AG, Kiaris H. Acceleration of wound healing by growth hormone-releasing hormone and its agonists. *Proc Natl Acad Sci USA*. 2010;**107**(43):18611–18615.
79. Gallo D, Gesmundo I, Trovato L, Pera G, Gargantini E, Minetto MA, Ghigo E, Granata R. GH-releasing hormone promotes survival and prevents TNF- α -induced apoptosis and atrophy in C2C12 myotubes. *Endocrinology*. 2015;**156**(9):3239–3252.
80. Khorram O, Laughlin GA, Yen SS. Endocrine and metabolic effects of long-term administration of [Nle²⁷]growth hormone-releasing hormone-(1-29)-NH₂ in age-advanced men and women. *J Clin Endocrinol Metab*. 1997;**82**(5):1472–1479.
81. Siejka A, Stepień T, Lawnicka H, Krupinski R, Komorowski J, Stepień H. Effect of the growth hormone-releasing hormone [GHRH(1-44)NH₂] on IL-6 and IL-8 secretion from human peripheral blood mononuclear cells in vitro. *Endocr Regul*. 2005;**39**(1):7–11.
82. Stepień T, Lawnicka H, Komorowski J, Stepień H, Siejka A. Growth hormone-releasing hormone stimulates the secretion of interleukin 17 from human peripheral blood mononuclear cells in vitro. *Neuroendocrinol Lett*. 2010;**31**(6):852–856.
83. Rodríguez-Pacheco F, Gutierrez-Repiso C, García-Serrano S, Ho-Plagaro A, Gómez-Zumaquero JM, Valdes S, Gonzalo M, Rivas-Becerra J, Montiel-Casado C, Rojo-Martínez G, García-Escobar E, García-Fuentes E. Growth hormone-releasing hormone is produced by adipocytes and regulates lipolysis through growth hormone receptor. *Int J Obes*. 2017;**41**(10):1547–1555.
84. Stanley TL, Feldpausch MN, Oh J, Branch KL, Lee H, Torriani M, Grinspoon SK. Effect of tesamorelin on visceral fat and liver fat in HIV-infected patients with abdominal fat accumulation: a randomized clinical trial. *JAMA*. 2014;**312**(4):380–389.
85. Fourman LT, Czerwonka N, Feldpausch MN, Weiss J, Mamputu JC, Falutz J, Morin J, Marsolais C, Stanley TL, Grinspoon SK. Visceral fat reduction with tesamorelin is associated with improved liver enzymes in HIV. *AIDS*. 2017;**31**(16):2253–2259.
86. Baker LD, Barsness SM, Borson S, Merriam GR, Friedman SD, Craft S, Vitiello MV. Effects of growth hormone–releasing hormone on cognitive function in adults with mild cognitive impairment and healthy older adults: results of a controlled trial. *Arch Neurol*. 2012;**69**(11):1420–1429.