COMMENTARY

Inhibition of tumor growth by agonists of growth hormone-releasing hormone

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In PNAS, Schally et al. (1) demonstrate the in vivo antitumor activity of the recently developed growth hormone-releasing hormone (GHRH) agonist MR409. Lung cancers were used as the primary tumor model, and the results were extended to colorectal, prostatic, stomach, bladder, breast, and pancreatic cancers (1).

GHRH was originally discovered in hypothalamic extracts more than 50 y ago; since then, it was thought that its only biologically and clinically relevant activity was to stimulate growth hormone (GH) production and release from the pituitary (2). GH, in turn, promotes growth either by acting directly in the periphery or by stimulating the hepatic production of insulin-like growth factor 1 (IGF-1), a major mitogen and survival factor for various cell types (3).

This GHRH–GH–IGF-1 neuroendocrine axis dominated the field of GHRH-related research for more than 30 y, and despite various demonstrations of extrapituitary production of GHRH (4–9), the regulation of growth through GH stimulation was considered the sole function of GHRH. It was subsequently shown, in the late 1990s, that GHRH also acts directly in peripheral tissues, operating as an autocrine growth factor in lung (10) and other cancers. In addition to the evolving prooncogenic activity (11), GHRH and its analogs were shown to interfere directly with various other processes such as metabolism (12), wound healing (13, 14), and survival of mesenchymal stromal cells (15). Various possibilities for therapeutic intervention soon emerged that spanned oncology (11), diabetes (12, 16), cardiology (17), and other applications involving mesenchymal stromal cell-based therapies (15) in which the activity of GHRH is manipulated by specific agonistic and antagonistic analogs to attain the desired outcome. In these cases, the general mode of action of GHRH was consistently viewed to be mitogenic and/or prosurvival; hence, the use of agonists was proposed when stimulation of cell proliferation was preferred as the therapeutic outcome, whereas the use of antagonists emerged as the strategy of choice in oncology. These activities are mediated by the specific action of GHRH analogs on the receptors for GHRH that can be either the receptors in the intact pituitary form or splice variants of the GHRH receptor (18).

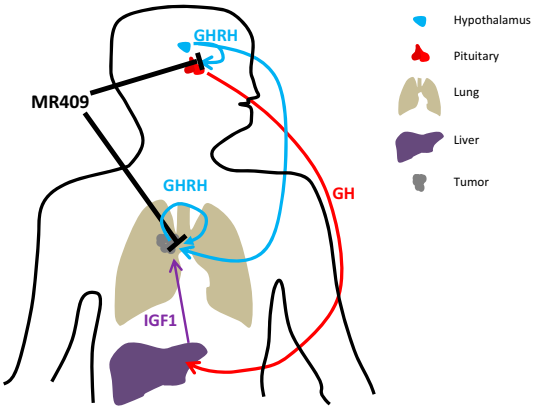

Fig. 1. Diagrammatic depiction of the endocrine and autocrine loops interrupted by the GHRH agonist MR409 through down-regulation of GHRH receptors.

Although the antineoplastic action of GHRH antagonists is well established in oncology, the consequences of stimulating GHRH activity in the context of tumorigenesis are elusive. Schally et al. (1) address this question by comparing the effects of GHRH agonists in human lung cancer cells cultured in vitro or in tumor-bearing mice in vivo. As expected, in vitro, the stimulation of GHRH activity promoted cell growth and shifted the profile of cell cycle and several associated cell cycle regulators (cyclins D1 and D2, CDK4, CDK6, and $p27^{kip1}$) toward a cycling mode (1). When these cells were implanted into nude mice, however, treatment with the GHRH agonist MR409 caused exactly the opposite effects: Tumor growth was inhibited, and the expression profile of cell cycle proteins, as well as of the mitogenic PAK1-STAT3 pathway, was shifted toward a cytostatic mode, as illustrated by the inhibition

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See companion article on page 12028.

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of cyclins D1 and D2, CDK4, and CDK6 and by the induction in expression of the cell cycle inhibitor $p27^{kip1}$.

Two major mechanisms may account for these dual opposing activities of GHRH agonists in vitro and in vivo, both of which are supported by the results of the study by Schally et al. (1): first, a mechanism associated with the direct action of the agonists on the cancer cells that operates both in vivo and in vitro, and second, the systemic (endocrine) action of GHRH that operates only in vivo and modulates tumor growth indirectly (Fig. 1).

The first mechanism that applies to the direct effects of MR409 in the cancer cells in vivo is linked to the down-regulation of the tumoral GHRH receptors. GHRH receptors are mitogenic (19), and the major splice variant (SV1) expressed in the lung cancer cells used in this study (1) also possesses ligand-independent activity (20). Thus, the down-regulation of GHRH receptors in the tumor can contribute to the antineoplastic action of MR409. Indeed, the down-regulation of receptors following persistent stimulation by a ligand agonist is a well-established phenomenon for pituitary receptors: The down-regulation of the pituitary receptors for luteinizing hormone-releasing hormone (LHRH) by chronic administration of agonists of LHRH is well known and has been applied for the management of LHRH-dependent cancers (21). To that end, the fact that GHRH receptors also respond in a similar manner provides a paradigm that generalizes the dual effects of agonistic analogs of hypothalamic hormones being stimulatory after acute, short-term activation, followed by inhibitory activity after persistent, long-term stimulation. It is likely, that these opposite effects of short-term versus long-term administration of GHRH agonists in GHRH receptors account for the contrasting effects of MR409 in vitro and in vivo. In vitro, exposure of cells to MR409 lasted for only 4 d and potentially mimicked the effects of acute, short-term activation, which stimulates GHRH receptor expression and promotes cell growth. In vivo, however, treatment was daily and lasted for more than 1 mo, a period that is long enough to unveil the consequences of persistent chronic stimulation and results in down-regulation of receptors and inhibition of tumor growth. Indeed, the down-regulation of GHRH receptors in vivo in the tumors was recorded (1).

The second endocrine mechanism is also supported by the experimental findings. Chronic administration of the GHRH agonist MR409 acted, as expected, not only in the periphery in the tumor but also in the pituitary. As shown in the study (1), somatotropic cells responded to MR409 administration by down-regulation of their receptors for GHRH, which eventually causes inhibition of the neuroendocrine GHRH–GH–IGF-1 axis, a modulation with established antioncogenic activity (22).

These notions not only raise considerations with regard to the therapeutic value of agonists of hypothalamic hormones but also contribute to our better understanding of the normal function and dysfunction of the hypothalamic–pituitary neuroendocrine axis in normal physiology and in disease. Furthermore, they imply that in cases of local expression of GHRH in the periphery, niches with high concentrations of GHRH may be sustained, exceeding those measured in the sera or whole-tissue extracts; thus, the neuropeptide may cause opposite effects from the anticipated mitogenic effects.

At the mechanistic level, some points remain elusive, dictating additional research to address them. First, what is the relative contribution of each of these mechanisms, systemic and direct, with regard to the anticancer activity produced? Is there a point at which a given amount of GHRH antagonist or even of endogenously produced GHRH simultaneously induces stimulatory effects at the level of the pituitary and inhibitory effects at the level of the periphery? Alternatively, do the peripheral and pituitary receptors for GHRH have similar sensitivity against GHRH agonists? If so, given their similar structure, are there different downstream effectors that operate as modulators between the pituitary and the periphery? We can expect that studies in the near future involving the independent ablation of each of these mechanisms—that is, by specific blockade of either the pituitary GHRH receptor or IGF-1 or by inhibition of GHRH receptors in the cancer cells—will illuminate these questions and predict which cancers will likely be more responsive to this strategy. Another topic that needs to be addressed is relevant to the specific signaling cascades activated by GHRH receptors in cancer cells and engaged during the production of the antiproliferative activity. While these signaling networks are adequately understood for the pituitary receptors, they remain obscure in peripheral tissues and especially cancer cells.

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Since GHRH ligand can be a tumor growth factor when secreted by a tumor in an autocrine/paracrine fashion (11), the findings of the inhibitory effect of GHRH agonists on tumor growth should alleviate serious concerns about the possible stimulatory effects on the growth of some cancers during therapy with growth factors. Major efforts are being made to develop GHRH agonists for clinical uses in cardiology, diabetes type 1 treatment, wound healing, ophthalmology, and other applications, and the revealing of antitumor activity of GHRH agonists, and not stimulation of tumor growth, is a welcome finding.

Collectively, the combined effect of GHRH agonists in the pituitary and in cancer cells by down-regulation of GHRH receptors was shown to produce significant tumor growth inhibition that, given the virtual absence of toxicity, implies promising application in cancer management and in other diseases and conditions. This notion is also reinforced by the fact that tackling two major relatively common and independently operating yet interconnected processes simultaneously—the IGF-1 dependency of cancers through the systemic action of GHRH and the direct inhibition of tumoral GHRH receptors—will permit minimal drug resistance and wide effectiveness in diverse cancers.

Acknowledgments

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