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Mini-Review

Role of IGF-I in the intracellular signaling pathway of growth hormone-stimulated hepatocyte proliferation

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ABSTRACT

Growth hormone (GH) is known to stimulate liver regeneration in 70% partially hepatectomized rats *in vivo*. Because complex involvement by multiple factors is unavoidable in *in vivo* experimental systems, clear interpretation becomes almost impossible when attempting to study the detailed mechanism of action. Therefore, we used a simpler, *in vitro* experimental system of primary cultured liver parenchymal cells to study the proliferation-stimulating effect of GH and its associated intracellular signal transduction mechanism from the GH receptor (GHR) to the nucleus. Many small molecular-targeted (specific signal transduction inhibitors) and large molecular-targeted agents (biological products and monoclonal antibodies) have now become available as pharmacological tools for research, and such agents enable a very detailed analysis of the mechanism underlying GH-induced stimulation of hepatic parenchymal cell proliferation. Consequently, we discovered that GH stimulates the proliferation of primary cultured mature rat liver parenchymal cells. We also found that the GH-induced stimulation of hepatic parenchymal cell proliferation is mediated by insulin-like growth factor-I (IGF-I), which is secreted by an autocrine mechanism.

KEYWORDS: growth hormone (GH), insulin-like growth factor (IGF-I), autocrine secretion, molecular targeted agent, hepatocyte proliferation, cultured hepatocytes.

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1. Introduction

The liver plays a central role in metabolizing substances, but its ability to regenerate is another characteristic function. Liver regeneration is a phenomenon that has been known since ancient times, even appearing in Greek mythology [1, 2]. Scientific research on the liver, however, did not begin until the 1930s. After a partial hepatectomy of approximately 70%, the remaining liver in anesthetized rats begins to divide and regenerate on its own in about 7 days. Moreover, it has been found that the hepatocyte proliferation response stops autonomously when the liver recovers its original volume [3]. *In vivo* studies of liver regeneration have increased the opportunities for *in vitro* research on the mechanism that stimulates hepatocyte proliferation. Techniques that enabled easy dispersion and isolation of hepatic parenchymal cells by *in situ* perfusion of the liver with a collagenase solution, as well as primary monolayer cell culturing techniques were generally established in the 1970s and 1980s [4-7]. The resulting cultured hepatocytes exhibit metabolic activity highly comparable to that of *in vivo* hepatocytes, and they also respond to stimulation by signaling substances such as neurotransmitters, hormones, and cytokines. Therefore, in the 1990s, our laboratory began using primary cultured liver parenchymal cells from mature rats to investigate the intracellular signaling pathways of various growth factors that trigger hepatic

parenchymal cell proliferation [8]. Here we present an overview of what we have learned about the growth hormone (GH) signal transduction pathway that stimulates the proliferation of hepatic parenchymal cells [9, 10].

2. Physiological functions of GH

Human GH is an anterior pituitary hormone in the form of a single-chain polypeptide of 191 amino acids. GH induces major functional changes in the metabolic capacities of various organs, such as the growth and differentiation of cells and tissues, bone mineralization, and the metabolism of carbohydrates, lipids, and proteins [11]. When GH binds to a GH receptor (GHR), it activates Janus kinase (JAK)2, which in turn activates various downstream intracellular signaling pathways that lead to gene expression in the nucleus [12-15]. GH also acts on the liver to stimulate the synthesis and secretion of insulin-like growth factor-I (IGF-I) [16].

3. Role of GH and IGF-I in liver regeneration in 70% partially hepatectomized rats

Several studies have been conducted on the *in vivo* roles of GH and IGF-I in 70% partially hepatectomized rats. When a 200-μg dose of human GH was administered twice daily to 70% partially hepatectomized rats, liver weight in the GH-treated group increased significantly compared with the control group (3.18 g vs. 2.68 g), and the number of mitotic cells in the liver also increased [17]. Another study used mice with an IGF-I receptor knockout to investigate whether IGF-I is involved in partial hepatectomy-induced liver regeneration, and the results showed a significant decrease in hepatocyte proliferation in males [18]. Regenerating livers also showed significantly less activated ERKs than controls [18]. An *in vivo* study has also been conducted on whether the effect of GH is mediated through IGF-I [16].

4. GH stimulates DNA synthesis and increases the number of nuclei in parenchymal hepatocytes: Effectiveness of molecular targeted agents

We isolated parenchymal hepatocytes by *in situ* collagenase perfusion, seeded the cells at low density, and cultured them in serum-supplemented medium for 3 hours to facilitate adhesion to the culture dish. We then switched to serum-free medium, added GH (100 ng/mL) and continued incubation. We found that in parenchymal hepatocytes, GH increased both time-dependent and dose-dependent DNA synthesis (a cell cycle S phase marker) and the number of nuclei (a cell cycle M phase marker) [9]. Then under the same culture conditions, we examined the effects of GH (100 ng/mL) in combination with small or large molecular targeted agents (specific signal transduction inhibitors). Molecular targeted agents can be broadly classified into small molecules and large molecules (biological products, monoclonal antibodies). Because small molecular targeted agents (small molecules) are relatively lipophilic, they can enter cells to exert their effects. High-molecular-weight monoclonal antibodies (large molecules), on the other hand, do not enter the cell, but act by neutralizing the responsible molecules on the cell surface (biomarkers) and extracellularly secreting growth factors (Table 1).

We discovered that among large molecular targeted agents, anti-GHR and anti-IGF-IR monoclonal antibodies inhibited the GH-stimulated increases in DNA synthesis and the number of nuclei in parenchymal hepatocytes in a dose-dependent manner. Therefore, our findings indicated that GHR and IGF-IR contribute to the hepatocyte proliferation-stimulating effects of GH. Moreover, the addition of an anti-IGF-I monoclonal antibody inhibited GH-induced stimulation of DNA synthesis and the increase in the number of nuclei, but an anti-transforming growth factor (TGF)-α monoclonal antibody had no effect [10]. Therefore, our results suggested that autocrine secretion of IGF-I is involved in the effects of GH.

On the other hand, among low-molecular-weight molecular targeted agents, TG101209 [19], LY294002 [20, 21], U73122 [22, 23], AG538 [24, 25], PD98059 [26], and rapamycin [27] inhibited the GH-stimulated DNA synthesis and the increase in the number of nuclei. This suggested that signal transduction factors such as JAK2, phosphoinositide 3-kinase (PI3K), phospholipase C (PLC), extracellular signal-regulated kinase (ERK), and mammalian target of rapamycin (mTOR) are also involved. Both the STAT inhibitor SH4-54 [28] and the PKC inhibitor GF109203X [29, 30] had no effect, suggesting that STAT and PKC are not involved.

Table 1. List of molecular targeted agents.

Target	Specific inhibitor	Molecular type	Effect
GH	anti-GH mAb	L	+++
GH receptor	anti-GHR mAb	L	+++
AC	2,4-dideoxyadenosine	S	-
PKA	H89	S	-
PLC	U7322	S	+++
PKC	GF109203	S	-
$[Ca^{2+}]_i$	BAPTA/AM	S	+++
Granule secretion	Somatostatin	L	+++
L-type calcium channel	Verapamil	S	+++
EGF/TGF-α-RTK	AG1478	S	+
IGF-I-RTK	AG538	S	+++
JAK2	TG101209	S	+++
STAT3/5	SH-5-54	S	-
PI3K	LY294002	S	+++
ERK2	PD98059	S	+++
mTOR	Rapamycin	S	+++
EGF receptor	anti-EGFR mAb	L	-
TGF-α	anti-TGF-α mAb	L	-
IGF-I	anti-IGF-I mAb	L	+++
IGF-I receptor	anti-IGF-IR mAb	L	+++
S: small molecular L: large molecular			

Abbreviations: GH: growth hormone, GHR: growth hormone receptor, JAK: Janus kinase, PLC: phospholipase C, IGF-IR: insulin-like growth factor-I receptor, RTK: receptor tyrosine kinase, PI3K: phosphoinositide 3-kinase, ERK: extracellular signal-regulated kinase, mTOR: mammalian target of rapamycin, P70 S6K: ribosomal p70 S6 kinase, PKC: protein kinase C, TGF-α: transforming growth factor-α, mAb: monoclonal antibody, +++: strong effect, +: weak effect, -: no effect.

5. GH stimulates signal transduction factor phosphorylation in parenchymal hepatocytes: Effectiveness of molecular targeted agents

In this study, we used western blot analysis to examine the time-course kinetics of the phosphorylation of several signal transduction factors suspected of contributing to the GH-stimulated proliferative effect on hepatocytes. As a result, we found that GH significantly increased phosphorylation of JAK2, IGF-I receptor tyrosine kinase (RTK), and ERK2 in parenchymal hepatocytes in a time-dependent manner (peak activation at 5, 30, and 45 minutes, respectively) [9]. Therefore, we suspected that signal transduction for promoting hepatocyte proliferation by GH transmits in this order from upstream to downstream. Meanwhile, the addition of somatostatin, which inhibits granule secretion, inhibited the GH-stimulated phosphorylation of IGF-IR RTK (maximum activity observed 30 minutes after GH addition). This indicated that GH acts through parenchymal hepatocyte secretion of some kinds of autocrine factor [31], and we considered IGF-I to be a candidate substance. Figure 1 schematically shows the changes in the various reactions over time. Taken together, these results supported the hypothesis that both the GHR/JAK2/PLC/Ca^{2+} pathway and IGF-IR/PI3K/ERK2/mTOR pathway are deeply involved in the GH proliferation-stimulating effect on parenchymal hepatocytes [9]. Thus, the interrelationship between the GHR/JAK2/PLC/Ca^{2+} pathway and the IGF-I-R/PI3K/ERK/mTOR pathway became a topic for further investigation.

6. Mechanism underlying GH-induced autocrine secretion of IGF-I

In the previous section, our findings indicated that the extracellular secretion of IGF-I is involved in the GH-induced stimulation of hepatocyte proliferation. Therefore, we measured the concentration of GH-stimulated IGF-I secretion in the culture medium. We found that GH stimulation resulted in a rapid increase in IGF-I concentration in the culture medium (5 to 10 minutes after GH addition). This secretion was inhibited by the addition of TG101209, U73122, the cell membrane-permeable Ca^{2+} chelator BAPTA/AM [32], and

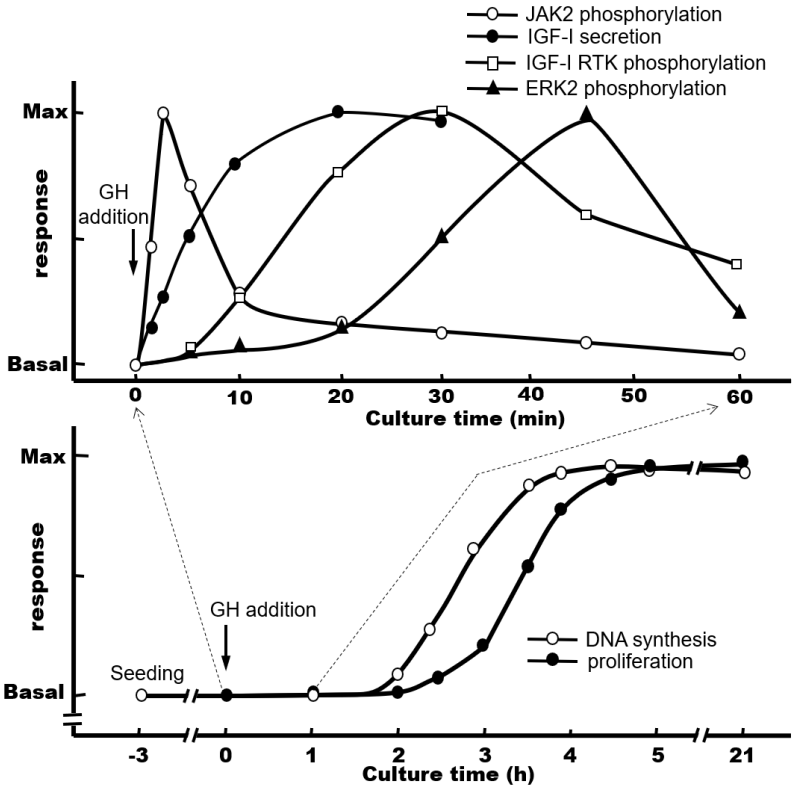

Figure 1. Time course of activation of signal transducing elements and proliferation of hepatocytes induced by growth hormone (GH).

somatostatin [10]. As a result, we found that the GH-stimulated autocrine secretion of IGF-I occurs *via* the GHR/JAK2/PLC/Ca^{2+} pathway. We also discovered that extracellularly secreted IGF-I binds to IGF-IR on parenchymal hepatocyte cell membranes and phosphorylates IGF-I RTK, and that the proliferation-stimulating signal then flows sequentially through PI3K, ERK2, and mTOR and eventually reaches the nucleus, where it stimulates cell proliferation [33-36]. We should also note that AG538, LY294002, PD98059, and rapamycin, which are low-molecular-weight molecular targeted agents that inhibit GH-stimulated hepatocyte proliferation, did not affect GH-induced autocrine secretion of IGF-I [10].

We found that GH stimulates parenchymal hepatocytes to secrete IGF-I rapidly, and that the extracellularly secreted IGF-I stimulates the proliferation of hepatic parenchymal cells. Therefore, we can say that GH is an indirect mitogen under the culture conditions used in our studies. On the other hand, bioactive substances that stimulate proliferation of parenchymal hepatocytes by a similar mechanism include prostaglandin E [37], prostacyclin [38], interleukin-1β [39], and serotonin [40]. These bioactive substances are known to use TGF-α as an autocrine mediator. The difference in the secretion of autocrine mediators in the two may be due to differences in the subtypes of the conjugated PLC.

CONCLUSION

We found that GH stimulates the GHR/JAK2/PLC/Ca^{2+} pathway to secrete IGF-I *via* an autocrine mechanism in primary cultured parenchymal hepatocytes *in vitro*, and that the secreted IGF-I stimulates hepatocyte proliferation *via* the IGF-IR/IGF-IR RTK/PI3K/ERK/mTOR pathway. Therefore, the inhibition of the GH/IGF-I axis by molecular targeted agents can be expected to contribute to the development of therapies for acromegaly and cancer [41].

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest with respect to this article.

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