Hepatocyte Growth Regulators

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Researchers have studied whether growth factors, cytokines, hormones, neurotransmitters, and local hormones (autacoids) promote the proliferation of hepatic parenchymal cells (i.e., hepatocytes) using in vitro primary cultured hepatocytes. The indicators used for this purpose include changes in DNA synthesis activity, nuclear number, cell number, cell cycle, and gene expression. In addition, the intracellular signaling pathways from the plasma membrane receptors to the nucleus have been examined in detail for representative growth-promoting factors that have been found to promote DNA synthesis and cell proliferation of hepatocytes.

cytokine growth factor hepatocyte proliferation direct mitogen indirect mitogen co-mitogen autocrine mechanism signaling pathway

1. Introduction

The liver is the central organ of metabolism and is responsible for the homeostasis of the body by metabolizing carbohydrates, proteins, lipids, and other substances. Furthermore, detoxification and regeneration are characteristic functions of the liver. These abilities are thought to have been acquired during the long evolutionary process to protect animals from catastrophic damage to liver tissues caused by food toxins (toxic chemicals) [1].

The mechanisms of liver regeneration have been studied for many years, and numerous reports have been published. There are also excellent review articles integrating those results [1][2][3][4][5][6]. The 70% partial hepatic resection of rodent livers (PHx) has been widely used as a research model for liver regeneration. For example, when approximately 70% of the liver of an anesthetized rat is surgically removed, the residual liver spontaneously and rapidly initiates cell division and proliferation, regenerating to the volume and weight of the original organ, and this response is automatically terminated [1][2]. In this animal model, the only signals for the initiation of cell proliferation are local wounding and tissue loss, making this a simpler experimental system than models of liver regeneration from viral infection or chemical-induced liver injury. Nevertheless, in vivo experimental systems have inherent difficulties in examining the detailed mechanisms of liver regeneration after PHx, because many factors are involved in a single event.

Therefore, researchers used a simpler in vitro primary cultured hepatocyte system to examine the actions of individual hepatocyte growth factors (and candidate substances) and their intracellular signaling pathways. Primary cultured hepatocytes retain metabolic activity comparable to that in vivo and express many kinds of receptors, each of which is known to be responsive to agonists [3][7]. It is also known that, among the parenchymal (i.e.,

hepatocytes) and nonparenchymal (i.e., Kupffer, pit, stellate, endothelial, etc.) cells that constitute liver tissues, the hepatocytes initiate proliferation at the earliest stage.

2. Classification and Characteristics of Hepatocyte Growth Regulators

Mitogens can be defined as factors that promote the proliferation of hepatocytes by themselves, such as EGF, TGF-α, HGF, platelet-derived growth factor (PDGF), and IGF-I. Co-mitogens, by themselves, do not promote hepatocyte proliferation, but, when used in combination with mitogens, the co-mitogens enhance the activity of the mitogens, and these include adrenergic α- and β-agonists and glucagon. In addition, there are many indirect mitogens, such as TNF-α, IL-1β, prostaglandin (PG) E_2, 5-HT, and GH, which indirectly promote hepatocyte proliferation by secreting autocrine factors. In contrast, some factors, such as transforming growth factor-β1 (TGF-β1) and glucocorticoids, strongly suppress mitogen-induced hepatocyte proliferation (i.e., they are inhibitory factors).

2.1 Mitogens

2.1.1 Direct Mitogens

EGF

EGF is a protein with a molecular weight of approximately 6 kDa, and it has three disulfide bonds. EGF binds to the EGF receptor (EGFR) and activates intracellular signaling pathways by phosphorylating the receptor tyrosine kinase (RTK); the EGF receptor is phosphorylated in vivo 30–60 min after PHx [3].

The proliferative effects of EGF on hepatocytes have been examined in culture. The results have shown that EGF alone promotes DNA synthesis and the proliferation of hepatocytes. It has been suggested that RTK, extracellular signal-regulated kinase (ERK), and the mammalian target of rapamycin (mTOR) are involved in the signaling [8]. Noradrenaline and an adrenergic β_2 receptor agonist, on their own, do not promote hepatocyte proliferation, but these agents enhance the growth-promoting effects of EGF. Thus, there is crosstalk between the adrenergic β_2 receptor-mediated signaling system and the EGF receptor-mediated signaling system (**Figure 1**).

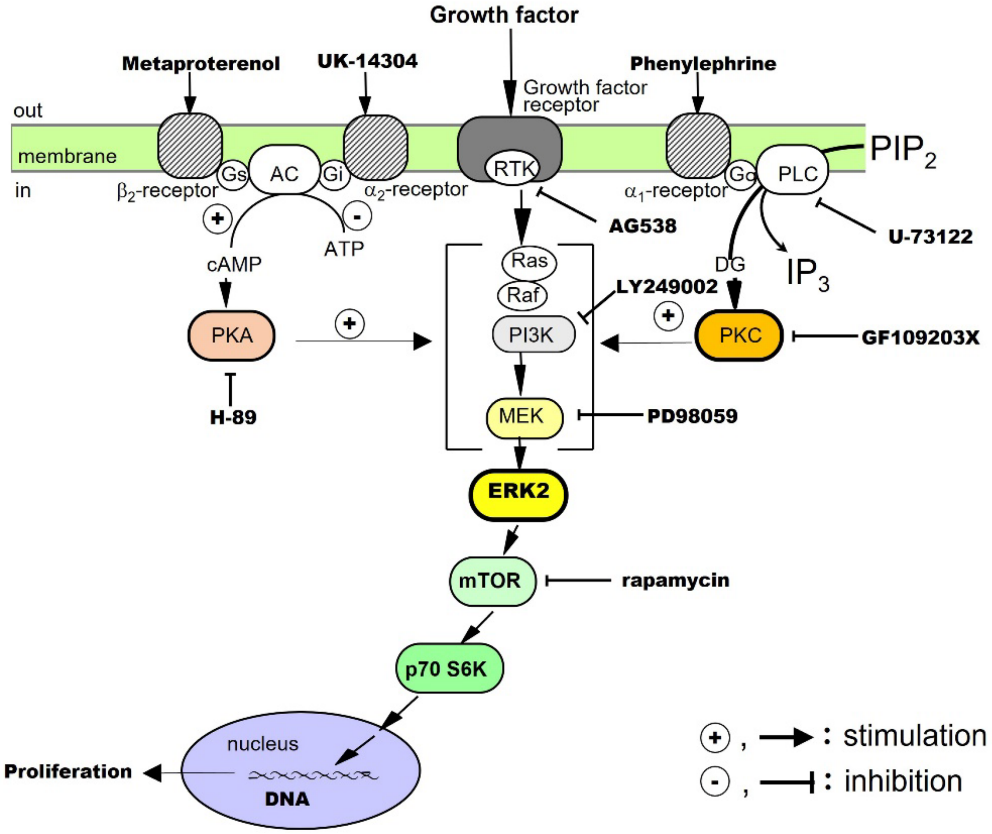

Figure 1. Co-mitogenic effects of α- and β-adrenergic receptor agonists on hepatocyte proliferation stimulated by growth factors.

TGF-α

TGF-α is a protein with a molecular weight of approximately 5.5 kDa, which has high amino acid homology with EGF and binds to EGFR/ErbB1. After receptor dimerization, TGF-α activates RTK, which, in turn, activates the mitogen-activated protein (MAP) kinase cascade via Smad, an adaptor protein, for intracellular signal transduction [9]. TGF-α, a cytokine produced by keratinocytes and various cancer cells, directly promotes DNA synthesis and the proliferation of hepatocytes alone in culture [10]. It is also an autocrine factor secreted by hepatocytes in response to several cytokines (see below).

The effects of TGF-α on hepatocytes have been investigated in culture. The results have shown that TGF-α alone promotes DNA synthesis and the proliferation of hepatocytes and that RTK, phosphatidylinositol-3 kinase (PI3K), ERK, and mTOR are involved in its signal transduction. In addition, adrenergic α1 receptor agonists enhance the effects of TGF-α, suggesting crosstalk with adrenergic α_1 receptor-mediated signaling pathways (**Figure 1**).

HGF

HGF is a protein consisting of 728 amino acids and a heterodimeric structure, with a heavy chain of approximately 60 kDa and a light chain of 3.5 kDa. It is a cytokine produced by stellate cells and endothelial cells in the liver. Its

receptor is cMet, which dimerizes and activates the RTK in the intracellular domain to mediate the action of the HGF [11][12][13].

The proliferative effect of HGF on hepatocytes has been examined in culture. The results have shown that HGF alone promotes DNA synthesis and the proliferation of hepatocytes and that RTK, PI3K, ERK, and mTOR are involved in its signaling [14]. Researchers have also found that both adrenergic α_1 and β_2 receptor agonists potentiate the growth-promoting effects of HGF by crosstalk (**Figure 1**).

PDGF

PDGF belongs to the PDGF/VEGF family. Its molecular weight is approximately 30 kDa, and its A and B chains form homo- and hetero-dimeric structures (PDGF-AA, PDGF-BB, and PDGF-AB), which activate RTK for intracellular signal transduction [15]. It was first isolated from platelets, but it is also produced by macrophages, vascular endothelial cells, smooth muscle cells, and cancer cells. In addition to PDGF, the platelets also contain TGF-β1, HGF, and 5-HT, which are released during the platelet adhesion and aggregation associated with tissue injury [3]. The released PDGF is thought to trigger a series of responses including inflammation and macrophage, neutrophil, and fibroblast migration.

Researchers investigated the proliferative effects of PDGF on hepatocytes in culture. The results showed that PDGF-BB alone promotes DNA synthesis and proliferation of hepatocytes and that RTK, PI3K, ERK, and mTOR are involved in its signal transduction [16]. In addition, an adrenergic α_1 receptor agonist enhances the effects of the PDGF-BB, suggesting crosstalk with adrenergic α_1 receptor-mediated signaling pathways (**Figure 1**).

Insulin

Human insulin is a heterodimer consisting of an A chain of 21 amino acids and a B chain of 30 amino acids, joined by two disulfide bonds. It is produced by pancreatic B cells and can always act on the liver via the portal bloodstream. The receptor for insulin is a built-in tyrosine kinase, and its activation phosphorylates its intracellular substrate, insulin receptor substrate-1 (IRS-1). Subsequently, signals are transmitted to PI3K and protein kinase B, and glucose transporter-4 is translocated to the cell surface. It has also been reported that blood levels of insulin increase soon after PHx [17].

The proliferative effect of insulin on hepatocytes has been said to be a co-mitogen that enhances the action of direct mitogens such as EGF. Therefore, researchers investigated the effects of insulin in culture. The results showed that insulin alone promotes DNA synthesis and hepatocyte proliferation and that RTK, PI3K, ERK, and mTOR are involved in its signaling [18][19]. In addition, an adrenergic α_1 receptor agonist potentiates the action of insulin, suggesting crosstalk with adrenergic α_1 receptor-mediated signaling pathways (**Figure 1**).

2.1.2. Co-Mitogens

Noradrenaline

Noradrenaline is a sympathetic neurotransmitter with a catecholamine structure. Noradrenaline receptors include adrenergic α and β types, each of which has subtypes. All the receptors for noradrenaline are G-protein-coupled ones. It has been reported that sympathetic hyperactivity due to post-PHx invasion increases the blood levels of noradrenaline in about 1 h [20]. It activates the duodenal Brunner's gland and stimulates EGF production and HGF expression [21][22].

In primary cultured hepatocytes, noradrenaline alone does not promote the proliferation of hepatocytes, but, through crosstalk, it can enhance the hepatocyte proliferation-promoting effects of EGF [8], TGF-α [10], HGF [14], PDGF [16], and insulin [19] (**Figure 1**). In addition, in combination with phenylephrine (a selective adrenergic α_1-receptor agonist), it enhances the hepatocyte-proliferative effects of EGF, HGF, TGF-α, PDGF, and insulin. Metaproterenol (a selective β_2-receptor agonist) enhances the proliferative action of EGF, IGF-I, and HGF. These results show that noradrenaline exhibits unique regulatory mechanisms for these growth factors.

2.1.3. Indirect Mitogens

IL-1β

IL-1β was the first inflammatory cytokine among the ILs to be identified, and there are two types, IL-1α and IL-1β [23]. IL-1β is a protein consisting of 110–140 amino acids. IL-1 receptors are highly homologous to toll-like receptors and activate serine/threonine kinases via adaptor proteins for intracellular signal transduction. IL-1 is rarely detected in normal tissues and is produced and secreted by macrophages and other immune cells activated by infiltrating inflammation. It has proliferative effects on monocytes and granulocytes.

The proliferative effects of IL-1β on hepatocytes have been examined in culture. The results have shown that IL-1β alone promotes DNA synthesis and the proliferation of hepatocytes and that the IL-1β effects are mediated via autocrine secretion of TGF-α from hepatocytes (**Figure 2**) [24]. IL-1α does not promote the proliferation of hepatocytes.

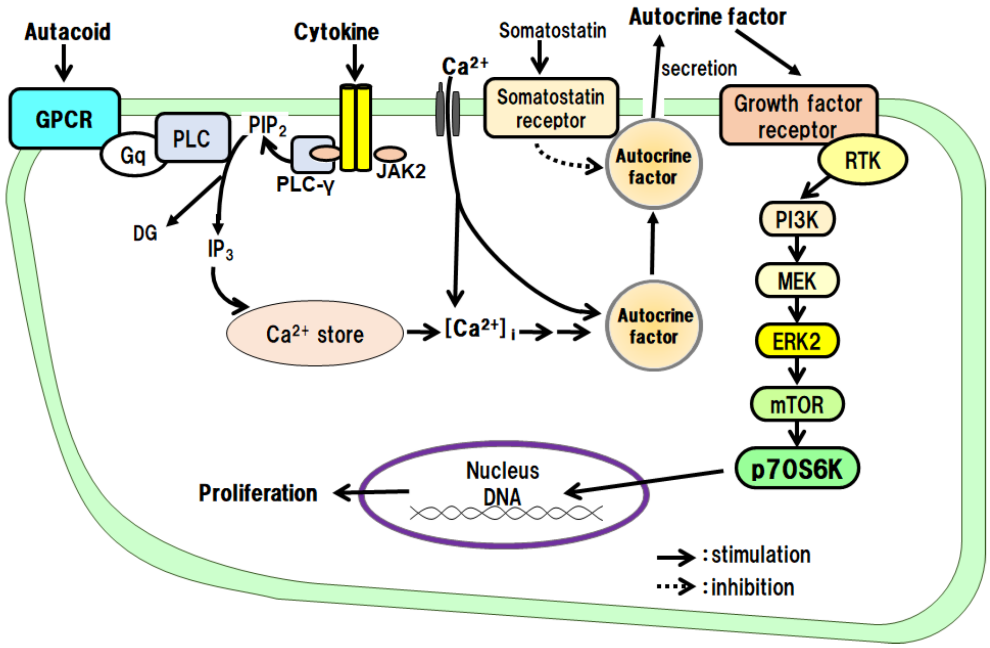

Figure 2. Hepatocyte proliferative effects of autacoids and growth hormone via an autocrine mechanism.

TNF-α

TNF-α is a protein consisting of 157 amino acids and is a potent proinflammatory cytokine similar to IL-1 and IL-6. TNF-α promotes the proliferation of hepatocytes via TNF-α type 1 receptors [25]. The major TNF-α-producing cells in peripheral tissues are macrophages (Kupffer cells in the liver), but it is also released from mast cells. It is involved in the pathogenesis of chronic inflammatory diseases such as Crohn's disease and rheumatoid arthritis.

Researchers investigated the proliferative effects of TNF-α on hepatocytes in culture. The results showed that TNF-α alone promotes DNA synthesis and the proliferation of hepatocytes and that its effects are mediated via its autocrine secretion of TGF-α from the hepatocytes (**Figure 2**) [26].

PGE_2 and Prostacyclin (PGI_2)

Prostaglandins (PGs) are low-molecular-weight local hormones. The receptors for PGs include EP, IP, and TP types, all of which are G protein-coupled. PGs are synthesized under the influence of cyclooxygenase (COX) from arachidonic acid and excised from the plasma membrane by phospholipase A_2. The physiological process involves constitutive COX-1 expression, while the inflammatory process involves inducible COX-2 expression. Biosynthesized PG products exhibit a variety of physiological effects, but inactivation is relatively rapid. PGs act on cells of the immune system to enhance inflammatory responses (redness, fever, swelling, pain, and dysfunction) by promoting the release of IL-1 and TNF-α. PGI_2 is produced by vascular endothelial cells, regulates blood flow, and inhibits platelet aggregation. It has been reported that PGs stimulate hepatocyte DNA synthesis in culture [27].

In primary cultured hepatocytes, researchers investigated the intracellular signaling pathways of PGE_2 and PGI_2, which promote DNA synthesis and the proliferation of hepatocytes. As a result, researchers found that both PGE_2

and PGI_2 indirectly promote DNA synthesis and the proliferation of hepatocytes via the secretion of the autocrine factor TGF-α from the hepatocytes (**Figure 2**) [15][17][28].

5-HT

5-HT is a low-molecular-weight substance with receptor subtypes designated $5\text{-}HT_1$ to $5\text{-}HT_7$. 5-HT acts as a neurotransmitter in the central nervous system and as a messenger molecule in the periphery. For example, it is released from enterochromaffin (EC) cells in the small intestinal epithelium and acts as a local hormone. 5-HT is stored in platelets and released upon stimulation and is involved in blood coagulation by promoting platelet aggregate formation (hemostatic thrombus). 5-HT has been reported to be involved in the promotion of liver regeneration [29]. That is, thrombocytopenic mice have impaired liver regeneration, and the administration of 5-HT restores liver regeneration. However, since platelets also contain other mediators (PDGF, HGF, etc.) that promote hepatocyte proliferation, it is necessary to examine whether this is a direct or indirect action of 5-HT.

When researchers examined the direct action of 5-HT on the proliferation of hepatocytes in culture, they found that 5-HT promotes the autocrine secretion of TGF-α from the hepatocytes via the $5\text{-}HT_{2B}$/Gq/phosphoinositide-specific phospholipase C (PLC)/Ca^{2+} pathway. Researchers then found that the secreted TGF-α directly promotes DNA synthesis and hepatocyte proliferation (**Figure 2**) [30][31][32].

GH

GH is a single-chain peptide consisting of 191 amino acids, which is secreted from the anterior pituitary gland. GH induces 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 [33]. Its receptor is a Janus kinase 2 (JAK2)-related type. In vivo, GH is known to promote liver regeneration in PHx rats [34].

Researchers examined the effects of GH on the proliferation of hepatocytes and intracellular signaling pathways in culture. GH promotes the autocrine secretion of IGF-I from the hepatocytes via the GH receptor/JAK2/PLC/Ca^{2+} pathway. Researchers then found that the secreted IGF-I directly promotes DNA synthesis and hepatocyte proliferation (**Figure 2**) [35][36][37].

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