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論　文　要　旨（英文）　（800語程度）

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（要　旨）

Hepatocyte growth factor (HGF) is a pleiotropic glycoprotein which is produced and secreted by mesenchymal cells and associated with heparin in a wide variety of tissues. Its high-affinity receptor c-Met is encoded by the c-*met* proto-oncogene and is widely expressed in epithelial cells. Binding of HGF to c-Met elevates its tyrosine kinase activity, leading to cell proliferation, cell scattering, and angiogenesis. However, HGF has an opposing effect on cell proliferation in different cell types: HGF accelerates the proliferation of some tumor cells while suppresses that of others. The opposing effect of HGF on different cell types is considered to depend on differences in the downstream signaling pathways. Hence, elucidation of the pathways responsible for these opposing effects is expected to lead to the development of therapeutics that suppresses the malignancy of cancer cells.

My laboratory has reported that HGF has an inhibitory effect on the proliferation of human HepG2 hepatoma cells. p16, a key cyclin-dependent kinase (CDK) inhibitor associated with this effect of HGF, is activated by the transcription factor Ets, which in turn is inhibited by the protein Id1 (inhibitor of DNA binding 1). Previous study showed that HGF downregulates the expression of Id1 and upregulates p16 expression in an ERK-dependent manner, leading to the inhibition of cell proliferation. However, the comprehensive mechanisms of p16 upregulation by HGF have remained to be elucidated.

Because another important CDK inhibitor p27 is also upregualted by HGF, I started this study aiming to discover the mechanism of p27 upregulation in HGF-treated cells. p27 is a major substrate of the ubiquitin ligase complex SCF^{Skp2} in many other cancer cells. I found that Skp2, a component of the SCF^{Skp2} complex and a potential prognosticator of some cancers, was downregulated by HGF in HepG2 cells. RT-PCR and immunoblot experiments in cells treated with HGF and an ERK pathway inhibitor PD98059 showed that the Skp2 downregulation occurred at mRNA and protein levels in an ERK-dependent manner. Ectopic overexpression of Skp2 restored cell proliferation inhibited by HGF, confirming that the inhibitory effect of HGF requires the downregulation of Skp2. However,

overexpression of Skp2 failed to inhibit the upregulation of p27 expression by HGF. This was consistent with the result that PD98059, which recovered both Skp2 expression and cell proliferation suppressed by HGF, failed to affect p27 upregualtion by HGF. These results suggested that p27 is not involved in Skp2-mediated regulation of proliferation of HGF-treated cells and therefore another downstream pathway is involved. HGF-induced transcriptional downregulation of Skp2 through the ERK pathway was also observed in HuH7, another human hepatoma cell line of which proliferation is also inhibited by HGF partially through the ERK pathway, suggesting that the Skp2-mediated regulation of cell proliferation by HGF is conserved in multiple hepatoma cell lines.

The transcription factor Myc, which has important roles in hepatocarcinogenesis, has been reported to be ubiquitinated and activated by Skp2 in other cancer cell lines. In a reporter assay performed with a reporter plasmid containing the Myc-responsive enhancer sequences, I observed that the Myc transcription factor activity was suppressed by HGF through the ERK pathway and that knockdown of Skp2 reduced the Myc activity in the absence of HGF. Importantly, overexpression of not only wild-type Skp2 but also its mutant unable to associate with the SCF complex restored the Myc activity. Collectively, these data showed that HGF reduces the Myc activity through downregulation of Skp2, and suggest that Skp2 functions as a Myc activator independently of its role in protein ubiquitination.

As previously reported, Id1 is downregulated by HGF through the ERK pathway. In other cancer cell lines, Id1 is reported to be a transcriptional target of Myc. To examine whether Id1 expression is regulated by Myc also in HepG2 cells, I detected Id1 expression in cells in which Myc was knocked down using RNA interference. I observed that Myc knockdown resulted in the downregulation of Id1 expression. Skp2 knockdown by RNA interference also downregulated Id1. Consistent with the results in Myc reporter assays, overexpression of wild-type Skp2 as well as its mutant unable to associate with the SCF complex recovered Id1 expression downregulated by HGF. These results suggested that Skp2 upregulates Id1 expression through the activation of Myc in a ubiquitin ligase activity-independent mode. Finally, I showed that overexpression of Skp2 reduced the activity of the p16 promoter which is unregulated by HGF and downregulated by Id1 in an Ets-dependent manner, suggesting that regulation of Skp2 is involved in the regulation of p16 promoter activity through regulation of Id1 expression in HGF-treated cells.

In summary, this study provides the first evidence that ERK-dependent downregulation of Skp2 by HGF reduces Myc activity, leading to the inhibition of cell proliferation through decreased Id1 expression and increased p16 expression in hepatoma cells.