Current Research Trends in the Exploration of Therapeutic Targets for Liver Disease

Peptidyl–Prolyl *cis/trans* Isomerase NIMA-Interacting 1 as a Therapeutic Target in Hepatocellular Carcinoma

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Received March 17, 2015

Phosphorylation of proteins on serine or threonine residues preceding proline is a pivotal signaling mechanism regulating cell proliferation. The recent identification and characterization of the enzyme peptidyl–prolyl *cis/trans* isomerase never in mitosis A (NIMA)-interacting 1 (PIN1) has led to the discovery of a new mechanism regulating phosphorylation in cell signaling. PIN1 specifically binds phosphorylated serine or threonine residues immediately preceding proline (pSer/Thr-Pro) and then regulates protein functions, including catalytic activity, phosphorylation status, protein interactions, subcellular location, and protein stability, by promoting *cis/trans* isomerization of the peptide bond. Recent results have indicated that such conformational changes following phosphorylation represent a novel signaling mechanism in the regulation of many cellular functions. Understanding this mechanism also provides new insight into the pathogenesis and treatment of human hepatocellular carcinoma. A better understanding of the role of PIN1 in the pathogenesis of hepatocellular carcinoma may lead to the identification of molecular targets for prevention and therapeutic intervention.

Key words hepatocellular carcinoma; β -catenin; never in mitosis A (NIMA)-related kinase 6; hepatitis B viral protein; peptidyl–prolyl *cis/trans* isomerase NIMA-interacting 1

1. INTRODUCTION

The increase in the global cancer burden, from 12.7 million new cases in 2008 to a predicted 22.2 million in 2030, is due to factors including population growth and an evolving age distribution, as well as other important changes in the prevalence and distribution of risk factors.¹⁾ Hepatocellular carcinoma (HCC) is the fifth most frequently diagnosed cancer worldwide and is the third leading cause of cancer death globally.²⁾ In most cases, HCC develops against a background of repetitive hepatic injury and cirrhosis. The association of chronic active hepatitis and cirrhosis with the development of HCC is well known, and major etiologic factors include chronic alcohol consumption, exposure to hepatotoxins (e.g., aflatoxins), genetic conditions (e.g., hemochromatosis), and chronic viral infection with hepatitis B virus (HBV) and/or hepatitis C virus.³⁾ Hepatocarcinogenesis is a complex process associated with the accumulation of genetic and epigenetic changes that occur during initiation, promotion, and progression of the disease. Cellular events are often accompanied by increased expression of several factors that influence the survival of cancerous cells by suppressing apoptosis and regulating the cell cycle.

Peptidyl–prolyl *cis/trans* isomerase, never in mitosis A (NIMA)-interacting 1 (PIN1) is a conserved eukaryotic protein with important roles in cellular regulation.⁴⁾ Due to its ability to switch the conformation of peptidyl–proline bonds in polypeptide chains, PIN1 operates as a binary switch, often

in fate-determining pathways.⁵⁾ PIN1 and its homologues contain an amino-terminal WW domain that specifically binds to phosphorylated serine or threonine residues immediately preceding proline (pSer/Thr-Pro) motifs, and a carboxy-terminal enzymatic domain that catalyzes the *cis/trans* isomerization of pSer/Thr-Pro bonds.⁴⁾ A substantial body of work has shown that PIN1-mediated conformational changes following phosphorylation can have profound effects on phosphorylation signaling, thereby regulating a spectrum of target activities, including catalysis, protein dephosphorylation, protein interaction, subcellular location, and protein turnover.⁴⁾ PIN1 is overexpressed in many cancers, and recent research has demonstrated a high prevalence of PIN1 overexpression in HCC.⁶⁾ Interestingly, PIN1 contributes to β -catenin and cyclin D1 overexpression in HCC cells, but PIN1 overexpression and β -catenin gene mutations are mutually exclusive events in HCC.⁷⁾ Two important risk factors for HCC are metabolic disease and viral infection, both of which can cause cirrhosis, often leading to HCC.⁸⁾ It is one of the hallmarks of cancer that many of the signaling pathways affected by genetic mutations and the tumor microenvironment have a profound effect on metabolism in cancer cells,⁹⁾ and the role of PIN1 in the regulation of cancer cell metabolism has attracted increasing attention in recent years.¹⁰⁾ Therefore, the role of PIN1 in promoting the disordered metabolic process leading to HCC is a potential avenue for the development of targeted therapies against the disease. In this review, we focus on the role of the PIN1 pathway in HCC, but the basic principles discussed here may also be applied to other cancers.

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2. MITOTIC REGULATION BY PIN1 IN HCC

Events of the eukaryotic cell cycle are regulated by an evolutionarily conserved set of protein kinases. The cyclin-dependent kinases (CDKs) are important for driving cells through different phases of the cell cycle, and their sequential activation and inactivation are tightly regulated.¹¹⁾ At the G2/M transition, activation of the mitotic kinase CDK1 requires multiple events: the synthesis and binding of cyclin B; phosphorylation of CDK1 by CDK-activating kinase; and activation of sites phosphorylated by WEE1 and MYT1 by CDC25-family phosphatases.¹¹⁾ Activation of cyclin B/CDK1 leads to the phosphorylation of many proteins, mainly at Ser/Thr-Pro motifs, altering their function and triggering the events of mitosis.¹²⁾ PIN1 interacts directly with a subset of mitotic phosphoproteins, including cell division cycle 25,¹³⁾ WEE1,¹⁴⁾ and NIMA,¹⁵⁾ at pSer/Thr-Pro motifs in a phosphorylation-dependent and mitosis-specific manner. NIMA was originally identified in *Aspergillus nidulans* as a serine/threonine kinase critical for cell cycle progression into mitosis.¹⁶⁾ Dominant-negative mutations in NIMA can adversely affect the progression of human cells into mitosis as they do in *A. nidulans*.¹⁷⁾ Human NIMA-related kinases (NEKs) have high homology to NIMA in their N-terminal catalytic domain sequences but diverge substantially from NIMA at their noncatalytic C-termini. Eleven NEKs have been identified in the human genome, including NEK6, which is upregulated in about 70% of HCC tumors.¹⁸⁾ NEK6 plays an important role in mitotic cell cycle progression and could arrest cells in the G2/M phase when its expression is blocked.¹⁹⁾ Several lines of evidence suggested that NEK6 is required for chromosome segregation at the metaphase-anaphase transition, robust mitotic spindle formation, and cytokinesis.¹⁹⁾ Recently, it has been reported that the enforced expression of NEK6 completely suppresses p53-induced senescence, suggested a possible role of NEK6 in tumorigenesis by suppressing p53-induced cellular senescence.²⁰⁾ Interestingly, it was shown that NEK6 interacts with PIN1, and its expression positively correlates with that of PIN1 in HCC, implying that PIN1 may regulate NEK6 function and contribute to the carcinogenesis leading to HCC.²¹⁾

3. PIN1-HBX PATHWAY IN HCC

The dual-function protein β -catenin plays an important role in cell-cell adhesion as well as regulating gene transcription.²²⁾ β -Catenin acts in the Wnt signaling pathway, activating the transcription of crucial target genes responsible for cellular proliferation and differentiation.²²⁾ Mutations in members of the Wnt- β -catenin pathway occur in approximately 90% of colorectal cancers and in other cancer types, including HCC and gastric cancers.^{23,24)} Furthermore, several reports revealed different mechanisms by which the hepatitis B viral protein HBx activates the β -catenin signaling pathway.²⁵⁾ HBx, working in concert with wingless-type MMTV integration site family member 1 (WNT1), activates Wnt/ β -catenin signaling by stabilizing cytoplasmic β -catenin in HCC cells.²⁶⁾ It was also reported that HBx may activate Wnt/ β -catenin signaling through the activation of extracellular signal-regulated kinase 1, which results in phosphorylation and hence inactivation of glycogen synthase kinase 3- β , thus stabilizing β -catenin.²⁷⁾ In addition to protein-protein interactions, HBx also transacti-

vates transcription factors and target genes such as activator protein-1 (AP-1), c-Myc, and cyclin D1 through the activation of signaling cascades involving the mitogen-activated protein kinase pathway.²⁸⁻³⁰⁾ HBx is therefore considered to be important in hepatocarcinogenesis related to chronic HBV infection. HBx contains two phosphorylated Ser-Pro motifs, which are potential targets of PIN1.³¹⁾ Recently, it has been shown that binding of PIN1 to phosphorylated HBx enhanced the transactivation activities of HBx.³²⁾ This effect of PIN1 was also observed for all HBx mutants with the putative PIN1-binding motif Ser41-Pro preserved, but not for mutants in which this motif was substituted to abrogate PIN1 binding. PIN1 with mutated WW and isomerase domains also failed to enhance the transactivation activity of HBx. Furthermore, PIN1 contributes to β -catenin and cyclin D1 overexpression in HCC cells, although PIN1 overexpression and β -catenin gene mutation are mutually exclusive events in HCC.⁷⁾ Collectively, these findings suggest that PIN1 and HBx are directed at common molecular targets that induce cell proliferation and enhance cell survival in HCC.

4. ANTIAPOPTOTIC ROLE OF PIN1 IN HCC

Apoptosis is tightly controlled through both extrinsic and intrinsic pathways. Extracellular signaling molecules, including Fas ligand or tumor necrosis factor- α , bind to their integral membrane receptors, leading to activation of caspase-8 and a cascade of downstream caspases.³³⁾ The release of apoptotic signals due to intracellular stress dissipates the mitochondrial membrane potential and induces the release of the second mitochondria-derived activator of caspase (SMAC) and cytochrome *c*.³³⁾ SMAC promotes caspase-9 activation through binding to the inhibitor of apoptosis proteins (IAPs) and removing their inhibitory activity.³⁴⁾ Caspase-9 in turn activates downstream caspases including caspase-3, -6, and -7, resulting in execution of apoptosis.³⁵⁾

Seven IAPs have been identified to date, including survivin, XIAP, cIAP-1, cIAP-2, apollon, DIAP-1, and DIAP-2.³⁶⁾ Most of the IAPs are expressed broadly in normal adult tissues, with the exception of survivin, which is undetectable in normal adult tissues but expressed abundantly in transformed cells and a variety of human cancers.³⁷⁾ Antisense knockdown of survivin expression and/or function has been found to cause aberrant mitotic progression and spontaneous apoptosis.³⁸⁾ According to recent studies, survivin forms complexes with hepatitis B X-interacting protein (HBXIP), a cellular protein recognized originally for its association with HBx.³⁹⁾ The survivin-HBXIP complex, but not survivin or HBXIP alone, binds procaspase-9, preventing its recruitment by apoptotic peptidase activating factor 1 and selectively suppressing apoptosis initiated *via* the mitochondria/cytochrome *c* pathway.³⁹⁾ Interestingly, a recent study has shown that PIN1 can interact with the phosphorylated Thr3-Pro35 motif of survivin, thereby increasing the binding of survivin to procaspase-9 *via* HBXIP, which inhibits caspase-9- and caspase-3-dependent apoptosis in HCC cells.⁴⁰⁾ PIN1 was also shown to bind myeloid cell leukemia 1 (MCL1), a B-cell CLL/lymphoma 2-like anti-apoptotic protein, and increase its protein stability, leading to inhibition of apoptosis in breast cancer and HCC.^{41,42)} Interestingly, MCL1 is overexpressed in half of HCCs and its down-regulation in HCC cells leads to significantly increased apo-

Fig. 1. PIN1 Functions as a Critical Catalyst for Inducing Multiple Oncogenic Signaling Pathways in HCC

PIN1 binds to and isomerizes phosphorylated NEK6, HBx, β -catenin, survivin, MCL-1, and SMAD2/3 to enhance their stability or activity in HCC. These enhanced signaling pathways eventually lead to mitotic regulation, cell proliferation, antiapoptosis, and metastasis involved in HCC development.

ptosis and chemosensitization to cisplatin by inducing BAK-mediated apoptosis.⁴²⁾ PIN1 overexpression was also found to inhibit the apoptotic response induced by hydrogen peroxide or by stimulatory Fas antibodies through the degradation of death domain-associated protein 6 in malignant tumor cells.⁴³⁾ The functional interaction between PIN1 and antiapoptotic proteins in HCC cells may be critical in hepatocarcinogenesis and may in part account for chemoresistance in tumors with PIN1 overexpression.

5. PIN1 TRIGGERS HCC INVASIVENESS

Current curative therapies for HCC are limited to hepatectomy and liver transplantation. However, postoperative recurrence and metastasis are common complications,⁴⁴⁾ caused by a complex process involving circulating tumor cells derived from the primary tumor.⁴⁵⁾ HCC cells interact with several different extracellular matrix (ECM) components to migrate to and invade surrounding tissues. Such interactions are mediated by integrins, a class of heterodimeric transmembrane receptors composed of one α and one β chain.⁴⁶⁾ Integrins are polarized at cellular surfaces and involved in a number of cell functions such as adhesion, migration, invasion, proliferation, and survival.⁴⁷⁾ Transforming growth factor- β 1 (TGF- β 1) is another multifunctional growth factor involved in tumor progression which is increased during the course of HCC.⁴⁸⁾ TGF- β 1 signal transduction is mediated by two types of cell surface serine/threonine kinase receptors ($T\beta$ 1RI and $T\beta$ 1RII) and their downstream effectors, the Sma- and Mad-related protein (SMAD) family proteins.⁴⁹⁾ TGF- β 1 binding induces the formation and activation of a receptor complex containing $T\beta$ 1RI and $T\beta$ 1RII. Activated $T\beta$ 1RI directly phosphorylates SMAD2 and SMAD3 at the SSXS motif in their C-termini. The phosphorylated SMAD2 and SMAD3 then form a complex with SMAD4 and together accumulate in the nucleus to regulate the transcription of a wide variety of target genes, leading to distinct biological effects in a cell context-dependent manner.⁵⁰⁾ Serum TGF- β 1 levels have been shown to be significantly higher in patients with alcoholic and viral liver cirrhosis and in HCC.⁵¹⁾ It was also reported that local induction by TGF- β 1, through autocrine or paracrine pathways, is crucial for the activation of hepatic stellate cells and the production of ECM proteins involved in the pathogenesis of liver cirrhosis and HCC.⁵²⁾ Interestingly, TGF- β 1 promotes

HCC cell invasion through effects on α 3 β 1-integrin expression at the transcriptional level.⁵³⁾

PIN1 overexpression is prevalent in several types of human cancer and is involved in the regulation of cancer cell proliferation, angiogenesis, and the epithelial-mesenchymal transition.⁵⁴⁾ A recent study by Nakano *et al.* reported that PIN1 enhances SMAD ubiquitination-related factor 2 (SMURF2) interaction with SMAD2/3 and leads to decreased levels of these proteins.⁵⁵⁾ Another study showed that SMAD2/3 interaction with PIN1 occurs specifically in response to TGF- β 1.⁵⁶⁾ Interestingly, a study showed that depletion of PIN1 reduced the TGF- β 1-induced epithelial-mesenchymal transition in PC3 prostate cancer cells, suggesting that PIN1 has a specific role in promoting migration and invasion in response to TGF- β 1. In addition, pulmonary fibrosis and TGF- β 1-stimulated SMAD3 signaling are abrogated in Pin1-null mice.⁵⁶⁾ PIN1 is also involved in the stability of TGF- β 1 mRNA in human eosinophils. Given the essential role of TGF- β 1 in the progression of HCC invasiveness, these results highlight the importance of PIN1 in cancer progression as well as metastasis, especially in HCC.

6. FUTURE STUDIES AND CONCLUSION

Future studies should focus on the regulation of metabolism by PIN1, especially in the reversible fatty liver stage. This will help to elucidate the central molecular mechanisms at this pre-HCC stage, with the aim of identifying new targets for the suppression of HCC in its vulnerable early stage. The effect of PIN1 on cancer progression is complex, and despite the numerous reports on this topic, it is clear that a better understanding of tumor cell-specific signal pathways that modulate PIN1 signaling is required for the improvement of clinical outcomes. In this review, we summarized the vital roles that PIN1 plays in the development of HCC and the specific mechanisms that are known to facilitate HCC development by altering the specified substrates, such as NEK6, HBx, β -catenin, survivin, MCL-1 and SMAD2/3, regulated by PIN1 (Fig. 1). By focusing on HCC, we hope to provide a detailed account of the molecular mechanisms affecting the PIN1 pathway and the important role that balanced PIN1 expression plays in HCC, which can be translated to other forms of cancer.

Acknowledgment This study was supported by a research

fund from Chosun University (2013).

Conflict of Interest The authors declare no conflict of interest.

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