REVIEW

Oncogenic role of p21 in hepatocarcinogenesis suggests a new treatment strategy

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Abstract

A well-known tumor suppressor, p21, acts paradoxically by promoting tumor growth in some cellular conditions. These conflicting functions have been demonstrated in association with the *HBx* gene and in hepatocarcinogenesis. The molecular behavior of p21 depends on its subcellular localization. Nuclear p21 may inhibit cell proliferation and be proapoptotic, while cytoplasmic p21 may have oncogenic and anti-apoptotic functions. Because most typical tumor suppressive proteins also have different effects according to subcellular localization, elucidating the regulatory mechanisms underlying nucleo-cytoplasmic transport of these proteins would be significant and may lead to a new strategy for anti-hepatocellular carcinoma (HCC) therapy. Chromosome region maintenance 1 (CRM1) is a major nuclear export receptor involved in transport of tumor suppressors from nucleus to cytoplasm. Expression of CRM1 is enhanced in a variety of malignancies and *in vitro* studies have shown the efficacy of specific inhibition of CRM1 against cancer cell lines. Interestingly, interferon may keep p21 in the nucleus; this is one of the mechanisms of its anti-hepatocarcinogenic function. Here we review the oncogenic property of p21, which depends on its subcellular localization, and discuss the rationale underlying a new strategy for HCC treatment and prevention.

Key words: p21; Tumor suppressors; Oncogene; Subcellular localization; Hepatocellular carcinoma; HBx; Nucleo-cytoplasmic export; Chromosome region maintenance 1; Selective inhibitors of nuclear export; Interferon

Core tip: A well-known tumor suppressor, p21, can act paradoxically by promoting tumor growth, depending on its subcellular localization. Nuclear p21 may inhibit cell proliferation while cytoplasmic p21 may be associated with anti-apoptotic and oncogenic functions.

These conflicting roles are reviewed in the context of the *HBx* gene and hepatocarcinogenesis. Because most tumor suppressors act in a similar manner to p21, regulation of their nucleo-cytoplasmic export, which is mainly effected *via* chromosome region maintenance 1, may be a basis for developing a new strategy for anti-hepatocellular carcinoma therapy.

INTRODUCTION

Primary liver cancer is the 5th most common cancer in men and the 7th in women, with high mortality worldwide; therapeutic options for cure are urgently needed[1,2]. Hepatocellular carcinoma (HCC) accounts for most primary liver cancer. Although there is a geographic difference in incidence of HCC caused by some etiological variation, the major etiological agents are hepatitis B (HBV) and hepatitis C virus (HCV) infection. Once these viruses infect liver, they ingeniously evade host immune surveillance and induce chronic necroinflammation, leading to fibrosis and, ultimately, liver cirrhosis. Hepatocytes, *via* their innate regenerative capacity, continue to proliferate in order to compensate for the necrotic tissue. Genetic alterations continuously accumulate during these processes, resulting in pathogenic liver changes such as cirrhosis from which HCC frequently arises[3]. Once HBV-related cirrhosis is established, HCC develops at an annual rate of about 4% in Japan, for example[4].

Several lines of evidence support the direct involvement of HBV in the transformation processes. HBV is like a retrovirus in that it integrates into the host genome, causing chromosomal abnormalities. In addition, the *HBx* gene acts like an oncogene by trans-activating many genes involved in cellular transformation.

No common molecular mechanisms that account for the extremely complex process of hepatocarcinogenesis have yet been elucidated. Genetic alterations reported have been heterogenous, involving abnormalities of many signal transduction pathways[2,3]. However, a fundamental abnormality in hepatocarcinogenesis, like other malignancies, is deregulation of the cell cycle. The main regulators of the cell cycle are cyclin-dependent kinase inhibitors (CDKI), such as p21, p27, and p16, widely known as tumor suppressors. However, it is noteworthy that these tumor suppressors can function in an oncogenic manner depending on their precise intracellular localization. In this review, we explore the relevance of the intracellular localization of p21, in particular, and its function, to highlight the possibility that regulating the intracellular localization of tumor suppressors may be a potential future anti-HCC strategy in the context of both directly-killing tumor cells and preventive role.

P21 AS AN ONCOGENE

First identified in 1993[5,6], p21 is a universal CDKI that causes G1 growth arrest downstream of p53[7,8]. p21 binds to CDKs and inhibits the kinase activity, leading to growth arrest at specific stages in the cell cycle[9,10]. p21 also induces cellular differentiation and senescence.

Although p21 is one of the major tumor suppressors, it also can promotes oncogenesis. High expression of p21 is associated with poor prognosis of cancer[11-13]. Although mutation of the *p21* gene has been reported in bladder cancer[14], most reported studies failed to show the loss-of-function mutations of p21[15-17]. These results suggest that p21 may not be a classical tumor suppressor.

Experimental results of using genetically-engineered mice also support conflicting functions of p21. Spontaneous tumors occurred in p21-deficient mice, providing evidence that p21 is a tumor suppressor[18]. p21 also causes genomic instability[19]. However, the timing of tumor formation in p21-deficient mice was later than p53-deficient mice[20]. Moreover, the occurrence of lymphoma was suppressed when p21-deficient mice were crossed with p53- or ATM-deficient mice[21,22]. This result indicates that p21 also acts in an oncogenic way in particular conditions, reflecting its versatile function[9]. In addition, mammary gland tumorigenesis was accelerated in mice in which p21 was overexpressed in cytoplasm[23], and the cyclin-binding motif of p21 has been reported to have a direct tumorigenic function[24].

HCC, HBX AND P21

There have been many reports regarding the expression of p21 in HCC tissues. p21 was found to be down-regulated in HCC tissues, demonstrating its tumor suppressive function[25-27]. Kao *et al*[28] also reported that p21 expression was observed in 37% of HCC tissues, regardless of p53 expression, and was an independent survival good prognosis factor. While most of the reports show that p21 acts as a tumor suppressor, expression levels of p21 in liver cirrhosis have been reported to be correlated with the cumulative incidence of the occurrence HCC[29] and to be dominant in cytoplasm when histology became more undifferentiated[30].

There are also some reported studies examining the relationship between HBV and p21. Some reports have shown that the *HBx* gene exerts oncogenic activity by suppressing p21 expression[31,32] and that HBx genes having core promoter mutations suppress p21 more effectively[33]. Inversely, HBx enhanced p21

in some reports[34,35]. Park *et al*[34] reported that when the cell cycle was prolonged by enhancement of p21 by HBx, cells had survival advantages and chances for gene mutations, eventually leading to preneoplastic hepatocytes. In addition, Yano *et al*[36] reported that HBx enhanced cytoplasmic p21 in protein kinase C (PKC)-dependent manner to induce cell proliferation. These conflicting results may partly come from differences in experimental conditions, but mostly reflect the conflictive function of p21.

ASSOCIATION BETWEEN MOLECULAR BEHAVIOR OF P21 AND ITS ONCOGENIC FUNCTION

What molecular behavior of p21 does correlate with its tumor-promoting function? It is well-known that p21 has not only inhibitory effects on cell cycle, but also has a promoting role. p21-associated CDKs exist in both active and inactive states[37]; p21 promotes the assembly of CDK4,6 and cyclin D and exerts oncogenic activity without inhibiting kinase activity[38]. Mantel *et al*[39] found a high level of induction of p21 in a myeloid cell line that was induced to proliferate by growth factors. p21 also induces nuclear retention of cyclin D1, inhibiting its cytoplasmic degradation[40]. p21 induces cell cycle progression in glioma[41] and in vascular smooth muscle cells[42] by promoting the formation of active cyclin-CDK complexes with PKC-alpha. Because the lymphoma observed in p21-deficient mice has high levels of apoptosis[21], the oncogenic activity of p21 may be closely associated with its anti-apoptotic function

P21 has a dual function with regard to apoptosis. p21 halts the cell cycle and prevents apoptosis induced by genotoxic agents. This anti-apoptotic function of p21 may be associated with its oncogenic property. However, p21 acts as a pro-apoptotic in some conditions. Forced expression of p21 induces the apoptotic response against cisplatin in glioma[43] and in ovarian cancer[44]. p21 is a modulator of apoptosis in a p53-dependent or -independent manner[10]. Masgras *et al*[45] reported that cell-specific sensitivity to oxidative stress determined whether the cell was fated to undergo p21-induced cell death.

ACTIONS OF P21 DEPEND ON SUBCELLULAR LOCALIZATION

The dual functions of p21, apoptotic or anti-apoptotic, depend on its subcellular localization[9,46]. Nuclear p21 is anti-proliferative and cytoplasmic p21, which is anti-apoptotic, may be associated with oncogenic function. Cytoplasmic p21 is associated with poor prognosis or the aggressiveness of human cancer[11,12,30,47]. Cytoplasmic localization of p21 is closely associated with the phosphorylation status. Phosphorylation at

Thr57 and Ser130 by extracellular signal-regulated kinase (ERK) inhibits nuclear localization of p21 and causes its cytoplasmic accumulation, inducing cell cycle progression[48]. Phosphorylated p21 that locates in cytoplasm has anti-apoptotic action by inhibiting the apoptotic proteins. Koster *et al*[49] reported that cytoplasmic p21 conferred resistance against cisplatin-induced apoptosis, while it became pro-apoptotic when it entered in the nucleus by the inhibition of AKT. Involvement in signal transduction of phosphorylated p21 differs depending on the site of the phosphorylated amino acid; Thr145 by AKT[50,51] or Ser130 by p38 and JNK[52]. Cytoplasmic p21 prevents apoptosis by inhibiting procaspase 3[53], and apoptosis signal-regulating kinases (ASK) 1[54].

As a summary, p21 as a tumor suppressor may be associated with nuclear location that may be associated with inhibition of cell proliferation and pro-apoptotic function, while oncogenic, anti-apoptotic p21 may require a cytoplasmic location. Thus, the shifting subcellular localization of p21 may be the clearest way to explain its functional versatility.

It is well known that not only p21 but most tumor suppressive proteins have different effects in different subcellular compartment. Cancer cells respond to what are typically tumor suppressors, such as p21, Rb, p53, p27, breast cancer susceptibility gene (*BRCA*) 1 and *FOXO* (forkhead box-containing, O subfamily) by proliferating when these molecules relocate from nucleus to cytoplasm[55,56]. Thus, based on the discussion above regarding p21, it is possible to extend this view to tumor suppressors in general. Regulating the subcellular localization of these proteins may become a core rationale for anti-cancer strategy[55,56].

REGULATION OF THE SUBCELLULAR LOCALIZATION OF TUMOR SUPPRESSORS AND ITS POTENTIAL APPLICATION TO ANTI-HCC TREATMENT

Transport of macromolecules across the nuclear envelope occurs through nuclear pore complexes (NPC). Karyopherins, such as exportins and importins, are nuclear transport receptors that recognize nuclear export signal (NES) and nuclear localization signal (NLS) sequences, respectively, and transport cargo proteins at the NPC sites[55,57]. Subcellular localization of tumor suppressors is regulated by this nucleo-cytoplasmic transport system[55,56,58,59].

CRM1 (Exportin-1/chromosome region maintenance 1) is a major nuclear export receptor that forms NPC with nucleoporins, such as NUP214 and NUP88, transporting nuclear proteins with NES sequence to cytoplasm[55-59]. CRM1 is deeply involved in the mechanisms of cell proliferation by regulating the subcellular localization of tumor suppressors which have NES sequences, such as p53 and p21. For example, p53
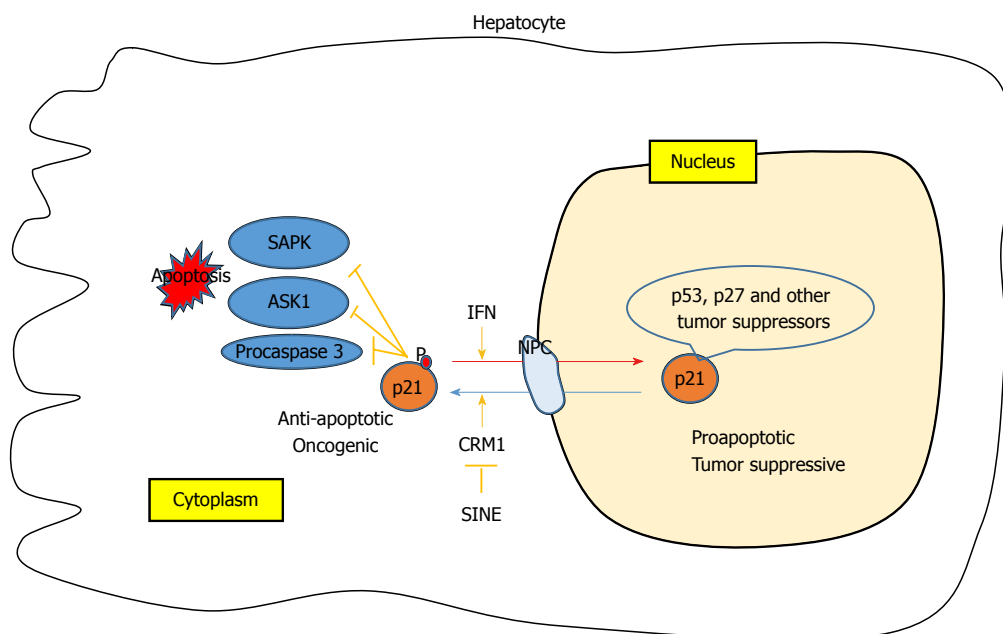
Figure 1 Outline of the overall aspects of this review. The subcellular localization of p21 and other tumor suppressors is regulated by CRM1. Inhibition of CRM1 by specific inhibitors and IFN may play a role in future anti-hepatocellular carcinoma strategies. CRM1: Chromosome region maintenance 1; IFN: Interferon; SINE: Selective inhibitors of nuclear export.

accumulates in the nucleus when poly (ADP-ribosyl)ation blocks its interaction with CRM1[60], while interaction with SUMO (small ubiquitin-like modifier) promotes its export to cytoplasm by CRM1[61]. p21 inhibits CRM1 by binding phosphorylated cyclin D, which promotes its nuclear accumulation[40].

Cancer cells use nucleo-cytoplasmic transport system for their proliferation and inhibition of apoptosis. CRM1 expression is enhanced in many cancer tissues. High expression of CRM1 in gastric, ovarian, and pancreatic cancers show poor prognosis[62-64]. The increase in CRM1 leads to cytoplasmic abundance of tumor suppressors and cell cycle regulators, which in turn results in their aberrant activation. Knock-down of CRM1 expression prevents nuclear export of p27, resulting in cell cycle arrest[65]. Specific suppression of CRM1 caused nuclear retention of p21 and induced apoptosis[66]. The cellular apoptosis susceptibility (CAS)/importin pathway was found to be enhanced in HCC, confirming the importance of the transport machinery[67].

Orally available selective inhibitors of nuclear export (SINEs) which specifically inhibit CRM1, have been developed in recent years[58,59]. SINEs specifically bind Cys528, located in NES-binding groove of CRM1, to promote nuclear retention of p53, p21, p27, Rb, and BRCA 1[68]. The effects on hematologic malignancies of KPT-330, the most effective SINE, have been reported[69-73]. KPT-330 had anti-proliferative effects and induced apoptosis of an HCC cell line[74] in which p53-upregulated-modulator of apoptosis was markedly up-regulated; and this was shown to be one of the similar mechanisms by which sorafenib exerts anti-HCC effects[75].

Interestingly, interferon (IFN)-beta was reported to

return cytoplasmic p21 to the nucleus and contributed to the prevention of hepatocarcinogenesis[36]. This was also true of p53 that was bound to cytoplasmic HBx and returned to the nucleus after IFN-treatment[76] (Figure 1). These observations suggest that natural substances such as IFN may be involved in an innate carcinogenesis prevention mechanisms, possibly by regulating CRM1. In fact, CRM1 is involved in the cytoplasmic localization of STAT2, which shifts to the nucleus by the action of IFN[77]. In addition, IFN inhibits beta-catenin signaling through the up-regulation of the nuclear RanBP3 which is a nuclear export factor[78].

FUTURE PERSPECTIVE

Sorafenib is a tyrosine kinase inhibitor widely used for the treatment of advanced HCC, and many other molecular-targeted drugs are now in development[79]. However its effect is still limited in many patients and the appearance of drug-resistance is a significant problem. Regulation of CRM1 involves many genes and specific multiple pathways associated with nuclear-cytoplasmic export; a new therapeutic strategy could be based on these developing concepts. Because such regulation would normalize molecular changes caused by multiple genes, its use might not cause drug resistance, or even being suggested to reverse drug resistance[55]. Thus, combination of such regulation with specific inhibitor use might maximize the impact of treatment. While expecting some promising results of clinical trials, taking the molecular approach to explore innate mechanisms regulating nuclear-cytoplasmic distribution of tumor suppressors will become an intriguing theme for development of cancer prevention strategies. In particular, IFN might

influence these mechanisms and play a role in anti-hepatocarcinogenesis. Future uses of this drug should be pursued in light of this functional biological aspect.

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