MPHOSPH1: A Potential Therapeutic Target for Hepatocellular Carcinoma

Xinran Liu¹,², Yafan Zhou¹, Xinyuan Liu³, Anlin Peng⁴, Hao Gong¹, Lizi Huang¹, Kaige Ji¹, Robert B. Petersen⁵, Ling Zheng⁶, and Kun Huang¹,²

Abstract

MPHOSPH1 is a critical kinesin protein that functions in cytokinesis. Here, we show that MPHOSPH1 is overexpressed in hepatocellular carcinoma (HCC) cells, where it is essential for proliferation. Attenuating MPHOSPH1 expression with a tumor-selective shRNA-expressing adenovirus (Ad-shMPP1) was sufficient to arrest HCC cell proliferation in a manner associated with an accumulation of multinucleated polyploid cells, induction of postmitotic apoptosis, and increased sensitivity to taxol cytotoxicity. Mechanistic investigations showed that attenuation of MPHOSPH1 stabilized p53, blocked STAT3 phosphorylation, and prolonged mitotic arrest. In a mouse subcutaneous xenograft model of HCC, tumoral injection of Ad-shMPP1 inhibited MPHOSPH1 expression and tumor growth in a manner correlated with induction of apoptosis. Combining Ad-shMPP1 injection with taxol administration enhanced antitumor efficacy relative to taxol alone. Furthermore, Ad-shMPP1 tail vein injection suppressed formation of orthotopic liver nodules and prevented hepatic dysfunction. Taken together, our results identify MPHOSPH1 as an oncogenic driver and candidate therapeutic target in HCC. Cancer Res 74(22); 6623–34. © 2014 AACR.

Introduction

Kinesin superfamily (KIF) proteins are a group of proteins with a highly conserved motor domain, some of which move toward the plus end of microtubules in an ATP-dependent process reliant on their adenosine triphosphatase (ATPase) activity (1). KIF proteins participate in many essential cellular biologic activities including mitosis, meiosis, and the transport of macromolecules (2). Recently, there has been increasing evidence of aberrant expression of KIF proteins in a variety of cancers, suggesting the oncogenic potential of KIFs in addition to their normal cellular physiologic functions (3, 4). Among these KIFs, M-phase phosphoprotein 1 (MPHOSPH1, also referred to as KIF20B) was reported to be a plus-end-directed kinesin protein, playing a critical role in completion of cytokinesis in late telophase of mitosis (5). Recently, an oncogenic role for MPHOSPH1 in bladder and colorectal cancer cells was reported (4, 6).

Hepatocellular carcinoma (HCC) accounts for 90% of human primary liver cancers, which is the fifth most common cancer and the third most common cause of cancer-related death, particularly in East Asia. Advanced HCC often has a poor prognosis and only few chemotherapeutic drugs, such as sorafenib, demonstrate efficacy in increasing overall survival in advanced or metastatic HCC (7). However, due to the rapid development of drug resistance, the effective course of chemotherapy for HCC often lasts for only a few months (8). Resistance to chemotherapeutic agents, therefore, remains a major challenge to HCC therapy. Recently, KIF proteins have been shown to play critical roles in the development of resistance to antimitotic drugs, such as taxanes (7, 9). Combined chemotherapy targeting kinesins may thus represent a promising anticancer strategy, especially in drug-resistant solid tumors.

Another challenge in treating HCC is reduced hepatic function. Poor hepatic function is common in HCC, resulting from a variety of causes, such as hepatic ascites and malnutrition. In many cases, hepatic dysfunction results in a bad prognosis for patients with HCC. Therefore, maintaining hepatic function is critical for HCC therapy. For this reason, improving liver function of patients with HCC is usually initiated before carrying out further treatment, especially surgery. Consequently, due to the cytotoxicity of chemotherapy, an improved, combined strategy that prevents hepatic injury is urgently needed.

In the present study, we found that MPHOSPH1 expression is increased in HCC tissues compared with nontumor tissues, and that knockdown of endogenous MPHOSPH1 using an adenoviral vector inhibited HCC cell proliferation by triggering mitotic arrest and apoptosis. We also
demonstrated that combining taxol treatment with MPHOSPH1 interference resulted in synergistic repression of HCC cell viability. In addition, we demonstrated that Ad-shMPP1 treatment not only suppressed HCC development, but also significantly reduced hepatic dysfunction caused by orthotopic HCC xenografts, suggesting that use of Ad-shMPP1 produced a dual effect; anti-HCC activity and protection of liver function in vivo.

Materials and Methods

Cell lines, animals, and tissue specimens
The human liver cell lines L02 and QSG7701 and the human HCC cell lines Hep3B, HepG2, and BEL-7404 were obtained from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) or China Center for Type Culture Collection (Wuhan, China) within 6 months of the experiments carried out. All cell lines were authenticated by short tandem repeat profiling and were confirmed to be mycoplasma-negative before use. Cell lines were grown in DMEM (GIBCO BRL) supplemented with 10% FBS. The human samples including ten nonmalignant liver diseases (cirrhosis, cholelithiasis, and cavernous hemangiomia) and 19 HCC and four adjacent tissues were obtained from Wuhan Union Hospital, Tongji Medical College of Huazhong University of Science and Technology (Wuhan, China). The Institutional Review Board of Wuhan Union Hospital approved acquisition of tissue specimens and collection of human samples was in accordance with the established guidelines.

IHC assays
Clinical human tissues or the HCC xenograft samples were fixed in 4% formaldehyde, dehydrated with an ethanol gradient, and embedded in paraffin. Tissue sections were dewaxed and rehydrated according to a standard protocol. The sections were washed with PBS, treated with 3% H2O2, and blocked with a blocking solution. These steps were followed by overnight incubation with primary antibody at the proper dilution for IHC or hematoxylin and eosin (H&E) staining assays as previously described (10). To avoid experimental bias, the clinical human samples were scored from 0 to 3, based on the IHC staining of MPHOSPH1, by two people using a double-blind procedure.

Recombinant adenoviruses
Recombinant adenoviruses (rec-Ads) were generated by respective homologous recombination between the shuttle plasmids and the packaging plasmid pBHGE3 in HEK293 cells. Generation, identification, production, purification, and titration of the recombinant adenovirus were performed as previously described (6).

RNA extraction and quantitative reverse transcriptase PCR
Total RNA was isolated from cells using TRIzol (Invitrogen). A total of 2 μg of RNA was used to synthesize the first single-strand cDNA using the RevertAid First Strand cDNA Synthesis Kit (Fermentas). The relative quantification of MPHOSPH1 cDNA and adenovirus gene E3 cDNA was performed using an ABI 7500 Fast System or a Bio-Rad CFX96 Real-Time PCR Detection System with Power SYBR Green PCR Master Mix (Applied Biosystems). GAPDH cDNA was used as an internal standard. The primers used are provided in Supplementary Materials.

Cytotoxicity assay
HCC cells and normal cells were seeded in 24-well plates at a density of 20,000 cells per well. Twenty-four hours later, cells were infected with Ads at various multiplicity of infections (MOI). Four days after infection, the medium was removed, and the cells were exposed to 2% crystal violet in 20% methanol for 20 minutes. The plates were then washed with water and photographed.

Cell proliferation assays
Hep3B and HepG2 cells were seeded in 96-well plates at a density of 2,000 cells per well. For the combined treatment (Ads and taxol), cells were infected with Ads at an MOI of 2. Twenty-four hours after infection, taxol (Sigma-Aldrich) at a final concentration of 10 μg/mL was added to each well. Of note, 20 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; 5 mg/mL) was added to each well at 48 hours later, and the plates were incubated at 37°C for 4 hours. The medium was removed and 150 μL of DMSO was added to each well. Absorbance at 490 nm was measured using a Microplate Reader (Thermo). Six replicate wells were counted for each assay.

In vitro combination studies
Synergism was determined using the software package CalcuSyn (Biosoft). The combination index (CI) was calculated by the Chou–Talalay equation (11). A CI less than 0.9 was defined as synergism. The detailed description on the combination is provided in the Supplementary Materials.

Cell nuclei and cytoskeleton staining assay
Cells were seeded on glass cover slips in 6-well plates. Twenty-four hours later, cells were infected with Ads at an MOI of 2. Cells were then fixed with 0.5 mL of 4% paraformaldehyde for 10 minutes, washed twice with PBS, stained with Phalloidin Alexa Fluor 555 (Molecular Probes), washed twice with PBS, stained with DAPI (Sigma-Aldrich), and washed twice with PBS. Cell staining was analyzed using a BX51 upright microscope (Olympus) or a Leica TCS SP2 confocal microscope (Leica Microsystems).

DNA fragmentation assays
HepG2 cells were seeded in 10 cm culture dishes and infected with various Ads at MOI = 5.72 hours postinfection, DNA was extracted and analyzed using agarose gel (12).

Flow-cytometric analyses
Cells fixed with 70% ethanol were incubated for 2 hours with an RNase A (40 μg/mL; Promega)/propidium iodide solution (50 μg/mL; Sigma-Aldrich). FACs was performed on a FACS Calibur flow cytometer (BD Biosciences).
SA-β-gal staining assay
β-galactosidase staining was performed using a senescence-associated β-Galactosidase Staining Kit (Beyotime). Cells were washed three times with PBS and fixed with 4% paraformaldehyde for 15 minutes at room temperature. Next, the cells were incubated overnight at 37°C in the dark with a solution containing 0.05 mg/mL X-gal.

Western blot assays
Cells were harvested and washed with PBS, and then lysed for analysis of proteins. Western blot analysis was performed as previously reported (6). Detailed antibody information is provided in the Supplementary Materials.

Animal experiments
Animal experiments were performed according to the Guide for the Care and Use of Laboratory Animals set forth by the Huazhong University of Science and Technology. Male BALB/c nude mice (4-week-old) were purchased from the Beijing HFK Bioscience Co. Ltd. Details of the experiments are provided in the Supplementary Materials.

Statistical analyses
All data were expressed as the mean ± SD and were analyzed using independent sample t tests and ANOVA using SPSS Base 10.0. Results were considered statistically significant when P < 0.05.

Results
Overexpression of MPHOSPH1 in HCC tissues
MHOSPH1 was previously classified as an oncogene based on studies of bladder cancer as well as in colorectal and lung cancer cell lines (4, 6). To determine whether MPHOSPH1 is upregulated in HCC, IHC detection of MPHOSPH1 was performed in samples from 19 patients with HCC (including four samples of normal adjacent tissues) and 10 nonmalignant tissues (Fig. 1A and Supplementary Table S1). Two people scored each IHC assay of all samples according to the criteria provided in the methods section.

Figure 1. Overexpression of MPHOSPH1 in HCC patient samples and human HCC cell lines. A, IHC was conducted on normal liver and HCC tissues for MPHOSPH1 protein expression. Magnification, ×20. B, intensity of MPHOSPH1 staining was scored on a scale of 0–3. C, MPHOSPH1 mRNA was measured by RT-PCR in three liver cancer cell lines and a normal liver cell line (***, P < 0.001).
samples on a 0–3 point scale based on the MPHOSPH1 staining ranges and color degrees as shown in Fig. 1B and Supplementary Table S1 (Fig. 1B shows examples of MPHOSPH1 scoring 0–3). An IHC score of ≥2 is considered overexpression of MPHOSPH1. Approximately 84% (16 of 19) of HCC tissues demonstrated overexpressed MPHOSPH1, whereas, in contrast, only 14% (3 of 14) of nonmalignant liver tissues showed overexpression of MPHOSPH1 (Supplementary Table S1). Further statistical analysis showed a significant difference in the MPHOSPH1 expression level between HCC tissues and non-HCC tissues (Fig. 1C). In addition, although the HCC samples exhibited a range of Edmondson–Steiner grading (an index reflecting the differentiation degrees of HCC cells), we found no statistically significant correlation between the differentiation state of the HCC cells and the MPHOSPH1 expression level (Supplementary Table S1).

The MPHOSPH1 mRNA levels in three HCC cell lines were also compared by qRT-PCR. The HCC cell lines HepG2, Hep3B, and BEL-7404 all showed elevated mRNA levels of MPHOSPH1 compared with that of the normal liver cell line L02 (Fig. 1D), suggesting that both MPHOSPH1 protein and mRNA levels may be used as potential biomarkers for HCC.

**HCC-specific replication and MPHOSPH1 knockdown effects of the oncolytic recombinant adenovirus**

We previously constructed a recombinant adenoviral vector based on the oncolytic Ad in which the viral E1B55KD gene was deleted (6). This Ad-vector has a tumor-specific survivin promoter controlling the expression of the adenoviral early gene E1A (Fig. 2A), thus restricting replication to tumor cells (6). To verify its ability to replicate in HCC cells, we infected HCC and normal liver cells with the Ad-vector at an MOI = 0.1. The ability to replicate was evaluated by assessing the adenoviral E3 gene mRNA level. Ad-vector replication in HepG2 cells was at least 100-fold higher than in the normal QSG7701 cells at 48 hours after infection (Fig. 2B), suggesting that it is a potent HCC-targeting oncolytic virus.

To construct shRNA-expressing Ad, an MPHOSPH1-targeted shRNA (shMPP1) expression plasmid was used (6). We
cloned the shMPP1 expression cassette into the oncolytic vector to generate Ad-shMPP1 (Fig. 2A). As expected, this tumor-specific oncolytic Ad-shMPP1 displayed potent interference ability against endogenous MPHOSPH1 expression in HCC cells. The relative mRNA level of MPHOSPH1 in HepG2 and Hep3B cells was significantly reduced following Ad-shMPP1 infection (95% and 60% downregulation at an MOI of 2 respectively; Fig. 2C).

Ad-shMPP1 inhibits HCC cell growth and potentiates the efficacy of taxol

To investigate the cytopathic effect (CPE) and the potential anti-HCC activity induced by Ad-shMPP1, different liver cancer cell lines and a normal liver cell line were infected with Ad-vector and Ad-shMPP1 at various MOIs. Ninety-six hours later, CPEs were determined by crystal violet staining. As shown in Fig. 3A, in normal liver L02 cells, infection with Ad-shMPP1 at an MOI of 5 did not cause a CPE, whereas in HepG2, Hep3B and BEL-7404 cells, treatment at the same MOI (in HepG2 cells, even at a lower MOI of 2, Fig. 3A) caused obvious CPE, demonstrating HCC cell-selective cytotoxicity and the relative safety of Ad-shMPP1.

Some KIF proteins seem to be involved in the development of drug resistance by HCC to some microtubule-targeted agents, such as taxol (13). Thus, to further clarify whether Ad-shMPP1 could enhance the anti-HCC effects of taxol, 24 hours before taxol treatment, we infected HepG2 and Hep3B cells with Ad-shMPP1 at an MOI of 2, and 48 hours later, the cell viability was determined using an MTT assay. Ad-shMPP1 produced a significant cell growth arrest effect on HepG2 and Hep3B cells compared with Ad-vector control groups (Fig. 3B). More importantly, compared with...
the Ad or taxol mono-treatment group, the combined treatment amplified the anti-HCC effect \((P<0.01)\), indicating significantly enhanced cytotoxicity of taxol (Fig. 3B). To further investigate whether the combination of Ad-shMP1 and taxol act synergistically, the CI value of Ad-shMP1/taxol was calculated both in HepG2 and Hep3B cells using CalcuSyn software, yielding CI values of 0.538 and 0.705, respectively (Fig. 3C), suggesting that an increased antitumor effect of taxol was achieved by its combination with Ad-shMP1 treatment.

**Ad-shMP1 knockdown induces apoptosis, polyploidy, and senescence in HCC cells**

To investigate whether apoptosis is promoted during the course of HCC cell growth arrest induced by MP1 downregulation, we infected HepG2 cells with Ad-vector and Ad-shMP1 at MOI = 2, and performed Hoechst staining 48 hours after infection. Nuclear fragmentation and chromatin condensation, which are typical apoptotic features, were clearly observed in the Ad-shMP1–infected cells (Fig. 4A). Apoptotic DNA fragmentation, a key feature of apoptosis, was also induced by Ad-shMP1 infection in HepG2 cells (Fig. 4B).

Another obvious morphologic alteration following Ad-shMP1 infection is the appearance of multinuclear cells due to the mitotic slippage from cell-cycle arrest without the formation of two daughter cells by cytokinesis discussed previously (6). In the current study, 24 hours after infection with Ad-shMP1 at a relatively low MOI (MOI = 1), multinuclear cells were clearly observed by confocal microscopy.
ploid cells 24 hours after infection (Fig. 4C). FACS analysis demonstrated accumulation of poly-

than apoptosis, was accompanied by the growth arrest of HCC cells, as shown by altered cell shape and expression of pH-dependent β-galactosidase activity (Fig. 4E). Taken together, these results suggest that loss of MPHOSPH1 has a cytostatic effect on HCC cells probably through triggering mitotic arrest, and subsequent senescence or apoptosis depending on the MOI.

The p53 signal pathway inhibits proliferation of HCC cells after MPPH1 knockdown

To study the mechanism underlying the MPPH1 down-

regulation-induced HCC cell growth arrest, we analyzed the proteins involved in the process of apoptosis on Western blot analyses. The tumor suppressor p53, which is normal in HepG2 cells (14), was significantly elevated both in protein and mRNA levels when MPPH1 was reduced (Fig. 5A and E). Phosphorylation of STAT3, downstream of p53, was suppressed in HepG2 cells after infection with Ad-shMPP1, whereas the base expression level of STAT3 was unaltered in comparison with the control (Fig. 5A-C), suggesting that STAT3 signal transduction was blocked in the process because STAT3 is activated by phosphorylation (15). Expression of the G1 phase cyclin-D1, a critical oncoprotein in several cancers as the driver for entering the cell cycle (16), was significantly reduced by the downregulation of MPPH1 (Fig. 5A), suggesting a potential mechanism for mitotic arrest. Intriguingly, although infection with an Ad vector upregulates cyclin-

B1 (Fig. 5A), the expression level of this M-phase cyclin was independent of MPPH1 expression (Fig. 5A). In addition, Mad2, a critical spindle assembly checkpoint (SAC) promoting protein (17), was increased following Ad-shMPP1 infection (Fig. 5A), which suggested that mitotic arrest was induced by SAC. However, the expression of the mitotic inhibitor p27, which is also downstream of p53 and represses cell-cycle progression, was downregulated by MPPH1 knockdown (Fig. 5A); this contradictory result indicates a complex molecular mechanism in the mitotic arrest process induced by reducing MPPH1.

In addition, p-STAT1, which is induced by IFNα and is a part of the ISGF3 transcription factor complex (18), was significantly upregulated after Ad-vector and Ad-shMPP1 treatment (Fig. 5B and C), suggesting that an IFN effect is triggered by Ad infection, which may explain why the Ad-vector also induces some cytotoxicity (Fig. 5A and B) because the IFN effect is capable of enhancing the antitumor effect of the oncolytic Ad (19). Interestingly, Ad-shMPP1 slightly downregulated the total expression level of STAT1 compared with Ad-vector (Fig. 5B and C), which shows a dual-effect of Ad-shMPP1 on the cells. First, it reduces the total level of STAT1, presumably due to reducing MPPH1 expression; and second, as an oncolytic Ad, it significantly induces the phosphorylation of STAT1. This result indicates that infection with Ad-shMPP1 initiates a complicated process.

Other proteins involved in the combined treatment with Ad-shMPP1 and taxol were also analyzed by Western blotting. KIF inhibitors have been reported to induce the antiapoptotic Hsp70 in myeloma cells (20), potentially revealing an underlying mechanism by which cancer cells resist the KIF-targeted drugs through the antiapoptotic pathway. In the present study, we did not detect upregulation of Hsp70 following infection of HCC cells with Ad-shMPP1 (Fig. 5D), which suggests that Hsp70 may be not involved in the taxol resistance of HCC cells. In addition, cyclin-D1 was downregulated, not only in Ad-shMPP1–infected HepG2 cells, but also after the combined treatment with taxol to an even greater extent (Fig. 5D), suggesting a critical role for mitotic arrest in the affect of the Ad-shMPP1/taxol combined treatment. In addition, the expression of CDKI (cyclin-dependent kinase inhibitor) p15, which is also an antimitotic protein, was not affected by MPPH1 knockdown (Fig. 5D), suggesting that the CDKI pathway may not be involved.

To minimize the interference of the Ad system (Fig. 5A and B), we also used a plasmid containing the shMPP1 cassette (p-shMPP1) to downregulate MPPH1 in HCC cells (6). MPPH1 was significantly downregulated by p-shMPP1 transfection in HepG2 cells (Fig. 5F), and, consistent with the Ad results (Fig. 5A), MPPH1 knockdown significantly induced p53 expression while reducing the expression of p27, p-STAT3, and cyclin-D1 (Fig. 5F). The MTT assay also demonstrated that the downregulation of MPPH1 by p-shMPP1 significantly attenuated HCC cell proliferation (Fig. 5G), in agreement with the MTT results from the Ad infection (Fig. 3B).

Knockdown of endogenous MPPH1 inhibits HCC xenografts growth in vivo

To evaluate the efficacy of an MPPH1-targeted strategy against HCC, a subcutaneous HepG2 cell xenograft model was used. Intratumoral injections of $2 \times 10^7$ plaque-forming unit (pfu) Ads or taxol were carried out after the mean tumor volume reached 80 to 120 mm$^3$. As shown in Fig. 6A, at the end of a 6-week experiment, the Ad-shMPP1–treated animals demonstrated significant suppression of tumor growth with an average tumor volume of about 250 mm$^3$, whereas the average tumor volumes of PBS or Ad-vector–injected animals were 960 and 770 mm$^3$, respectively, indicating approximately 74% suppression of the xenograft tumor growth by Ad-shMPP1 ($P < 0.05$). The efficacy of combined treatment was also investigated. The combined treatment with Ad-shMPP1 and taxol demonstrated enhanced anti-HCC activity, with the mean tumor volumes of 180 mm$^3$, whereas the groups of taxol and taxol/Ad-vector were 790 and 700 mm$^3$ respectively, in contrast ($P < 0.05$; Fig. 6B), indicating the potent anti-HCC activity of the MPPH1 knockdown in combination with taxol treatment.

To study whether MPPH1 knockdown also triggers apoptosis in HCC cells in vivo, pathologic examination...
was performed 7 days after the injection of Ads. IHC indicated significant reduction of the endogenous MPHOSPH1 in HCC cells induced by Ad-shMMP1 (Fig. 6C), demonstrating its ability to downregulate MPHOSPH1 in vivo.

Furthermore, H&E staining showed large areas of nuclear fragmentation in HCC cells from Ad-shMPP1–treated xenografts, while few apoptotic cells were detected in the control groups (Fig. 6C). All these results suggest that Ad-shMPP1
may exert anti-HCC effects in vivo by interfering with the expression of endogenous MPHOSPH1 and inducing apoptosis in HCC tissues.

Furthermore, an orthotopic xenograft model was also used to better evaluate the anti-HCC efficacy of Ad-shMPP1 in vivo through intravenous injection. At 7 days after intrahepatic implantation of HepG2 cells, a total of $2 \times 10^9$ pfu of Ads were injected through the tail vein. Three weeks after the injection, only 33.3% (2 of 6) of the Ad-shMPP1–treated mice developed liver tumor nodules, and in the other four mice receiving Ad-shMPP1 (66.6%, 4 of 6), no macroscopic or microscopic tumors were found in their livers (Supplementary Table S2 and Supplementary Fig. S1). In contrast, all animals (6 of 6) in the control groups (PBS and Ad-vector groups) developed visible liver tumor nodules (Supplementary Table S2 and Supplementary Fig. S1). Ad-shMPP1 also prevented the hepatic damage normally caused by liver cancer. Compared with the control groups, the aspartate transaminase (AST) value, which is often elevated in patients with HCC due to hepatic injury, was significantly lower after intravenous injection of Ad-shMPP1 in the in situ HCC xenograft implanted mice (Fig. 6D and Supplementary...
strategy that brings synergistic effects in cancer cells (9, 28, 29). Therefore, a combined chemotherapy (8). Notably, some KIF proteins, such as KIFC3, have demonstrated potent antitumor effects in preclinical models (22). However, the results from current phase II clinical trials for these agents are disappointing. For instance, ispinesib (SB-715992, Cytokinetics and GlaxoSmithKline), the first Eg5 inhibitor to enter clinical trials, has been closed for further trials as a monotherapy due to its lack of clinical efficacy for a number of solid tumors (23–25). An explanation for the clinical failure of mitosis-specific suppressors is the much smaller proportion of mitotic cells in human tumors compared with the animal xenograft models (26). Because of the insufficient antitumor effect of monotherapy, several ongoing clinical studies have incorporated KIF inhibitors with chemotherapeutic agents including carboplatin, capecitabine, and doxorubicin (30).

In searching for novel HCC targets that potentially increase chemotherapy efficacy, we focused on the kinesin protein MPHOSPH1, first identified through its in vivo interaction with the mitotic peptidyl-prolyl isomerase PIN1 (30). In the current study, we investigated the efficacy of the combined treatment of targeting MPHOSPH1 in combination with taxol in HCC xenograft models, and demonstrated that reducing endogenous MPHOSPH1 significantly enhanced the cytotoxic effect of taxol against HCC, which suggests that MPHOSPH1 is a potential target for HCC therapy. Because of the intrinsic cytotoxicity of the Ad-vector, however, the observed combined effect (Fig. 3C) may simply indicate the synergistic interaction of Ad-shMPHOSPH1 as a complex with taxol, which would not be interpreted as the interaction of taxol and the shRNA of MPHOSPH1.

We recently reported that depletion of MPHOSPH1 in colorectal cancer cells resulted in mitotic arrest due to failure of cytokinesis and subsequent apoptosis (6). Intriguingly, our previous study indicated that the observed multinucleate (polyplody) cells eventually underwent apoptosis (6). In the present study, we also found that significant mitotic arrest was induced by downregulation of endogenous MPHOSPH1 in HCC cells, leading to polyplody (the multinucleated cells in Fig. 4C and D), which suggested that the mitotic slippage was induced presumably by the prolonged mitotic arrest (6). Intriguingly, mitotic slippage that causes polyplody cells is considered to be a protective mechanism that cancer cells use against mitotic inhibition (31). However, the infected cells with polyplody observed here eventually underwent apoptosis (Fig. 4A and B), suggesting that the mitotic slippage process triggered postmitotic cell death instead.

SNP is a useful tool to study the potential pattern of gene expression. By applying "SNPinfo" tool to predict the SNPs in the MPHOSPH1 promoter region (32), we found three SNPs with Minor Allele Frequency > 0.05 (rs10881625, rs9325443, and rs9325444) to be located at the transcription factor binding sites, which may regulate gene expression by impacting transcription factors binding. However, this is only a prediction and experimental verification is still needed.

Moreover, p53, which promotes postmitotic apoptosis due to its accumulation following mitotic arrest (33), was significantly elevated in Ad-shMPHOSPH1–treated HepG2 cells (Fig. 5A). Expression of the oncogene cyclin-D1, as well as phosphorylation of STAT3, which were reported to be regulated by p53 (34, 35), were also markedly inhibited by MPHOSPH1 knockdown, suggesting that STAT3 activation was blocked through the p53 pathway. A probable mechanism was proposed previously by Blagosklonny (33), suggesting that during the mitotic arrest when the nuclear envelope is dissolved and chromosomes are condensed, transcription is absent, while the p53 mRNA is long-lived and accumulates (Fig. 5A and E). However, p53 cannot induce its targets because transcription is blocked. When the cell escapes from the mitotic arrest (mitotic slippage), transcription resumes, and the accumulated p53 suddenly induces its proapoptotic targets such as PUMA, Bax, and CD95, which can trigger apoptosis (Fig. 4A and B; refs. 6, 33).

This hypothesis is consistent with the present study and our previous work (6).

Interestingly, in addition to HCC with wild-type p53, we also demonstrated in Hep3B cells, which are p53-null, sensitivity to MPHOSPH1 interference, although to a lesser extent (Fig. 3). In a recent study, Forys and colleagues reported that loss of p53 leads to an increase in ARF protein levels, which function to limit the proliferation and tumorigenicity of p53-null cells, suggesting a complementary effect of ARF when p53 is deficient (36). It will be of great future interest to further investigate whether ARF or other tumor suppressors are involved in the p53-null cells to better understand the alternative regulation mechanism of MPHOSPH1 knockdown.

A carcinogenic link to MPHOSPH1 was previously found in bladder cancer (4), and we recently reported its upregulation in...
colorectal cancer cells (6). Here, we demonstrated that infection with Ad-shMP1 not only suppressed HCC development, but also protected hepatic function from HCC impairment (Fig. 6D). Significantly, improved AST and ALB were observed following Ad-shMP1 treatment. Because the estimation of the hepatic function reserve is critical in the prognosis of patients with primary liver cancer, improving poor hepatic function of patients is critical for facilitating proper treatment and reducing the risk of surgery. Another potential benefit of Ad-shMP1 treatment is the IFN effect, which can be induced by Ad infection (Fig. 5B), and might further enhance the antitumor effects of the oncolytic Ad (19), although the IFN itself may also impair the replication of oncolytic Ad (37). In summary, the observed HCC inhibition and the improved hepatic function following treatment with Ad-shMP1 may present an outstanding advance in clinical outcomes of HCC treatment.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

References

Authors' Contributions
Conception and design: X. Liu, Y. Zhou, X. Liu, K. Huang
Development of methodology: X. Liu, Y. Zhou, K. Huang
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): X. Liu, A. Peng, L. Huang, K. Ji, X. Liu
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): X. Liu, A. Peng, K. Huang
Writing, review, and/or revision of the manuscript: X. Liu, H. Gong, R. B. Petersen, L. Zheng, K. Huang
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): X. Liu, A. Peng
Study supervision: X. Liu, K. Huang

Grant Support
This work was supported by the Natural Science Foundation of China (Nos. 81202557, 31271370, 81100687, 81272911, 81220403, and 31472898), the Program for New Century Excellent Talents in University (NECT11–0170), the Municipal Key Technology Program of Wuhu (Wuhan Bureau of Science and Technology, No. 201205023174), the Health Bureau of Wuhu (WU21806), and the Natural Science Foundation of Hubei Province (2013CFB1599). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received April 28, 2014; revised August 22, 2014; accepted September 6, 2014; published OnlineFirst September 30, 2014.

www.aacrjournals.org Cancer Res; 74(22) November 15, 2014


MPHOSPH1: A Potential Therapeutic Target for Hepatocellular Carcinoma

Xinran Liu, Yafan Zhou, Xinyuan Liu, et al.


Updated version

Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-14-1279

Supplementary Material

Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2014/09/30/0008-5472.CAN-14-1279.DC1

Cited articles

This article cites 36 articles, 15 of which you can access for free at:
http://cancerres.aacrjournals.org/content/74/22/6623.full.html#ref-list-1

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.