SHORT COMMUNICATION

Proteomic Analysis Reveals GMP Synthetase as p53 Repression Target in Liver Cancer

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Disruption of the tumor-suppressive p53 network is a key event in human malignancies, including primary liver cancer. In response to different types of stress, p53 mediates several antiproliferative cellular outcomes, such as cell cycle arrest, apoptosis, and senescence, by activation or repression of its target genes. Metabolic alterations initiating or being part of the p53 response have become an actively studied research area in the p53 field, with several aspects that still remain to be elucidated. Herein, we identified GMP synthetase (GMPS), a key enzyme of de novo purine biosynthesis, as an important p53 repression target using a large-scale proteomics approach. This p53-mediated repression of GMPS could be validated by immunoblotting in Sk-Hep1, HepG2, and HuH6 cells. Moreover, we found GMPS transcriptionally repressed in a p21-dependent manner and its repression maintained in the context of p53-mediated cellular senescence. More important, direct knockdown of GMPS by RNA interference resulted in reduced cell viability and was sufficient to trigger cellular senescence. Finally, by comparing murine hepatocellular carcinomas, which developed in p53 wild-type (+/+) versus p53 null (-/-) mice, we observed higher GMPS expression in the latter, supporting the *in vivo* relevance of our findings. We conclude that repression of GMPS by p53 through p21 is a functionally relevant part of the p53-mediated senescence program limiting tumor cell growth in liver cancer. (*Am J Pathol* 2017, 187: 228–235; <http://dx.doi.org/10.1016/j.ajpath.2016.09.022>)

Hepatocellular carcinoma (HCC) is one of the most frequent malignancies worldwide, with an increasing incidence and poor prognosis.¹ Among pivotal players in (liver) cancer biology is the tumor-suppressor protein p53, serving as a major barrier against malignant transformation and progression.² As an integral part of the cellular stress response, p53 is induced by a variety of stress signals, including DNA damage, oncogene activation, telomere shortening, hypoxia, and nutrient deprivation.^{2,3} Extensive stress and irreversible damage is usually followed by induction of apoptosis [eg, via Puma, Bax, p53-inducible gene 3 (PIG3), Fas, and Killer/DR5] or senescence (eg, via p21 and plasminogen activator inhibitor) to eradicate the affected cells. Mild or transient stress initiates p53 responses, such as cell cycle arrest (eg, through p21, Gadd45, and 14-3-3 σ) and DNA repair (eg, p48 and p53R2) to allow restoration and ultimately further survival.^{2,3} In this context, alterations of

metabolic pathways gained increasing attention during the past decade because they can either induce or be part of p53-mediated cellular outcomes.³ For instance, p53 negatively regulates glycolysis by down-regulation of glucose transporters (eg, GLUT1 and GLUT4) and reduces intracellular fructose-2,6-bisphosphate concentration by inducing TP53-induced glycolysis and apoptosis regulator

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(TIGAR).^{3,4} At the same time, p53 promotes mitochondrial oxidative phosphorylation and preserves mitochondrial integrity.³ Furthermore, p53 controls the metabolism of lipids as it activates fatty acid oxidation and reduces fatty acid synthesis and is also involved in the regulation of reactive oxygen species.³ Notably, *de novo* purine biosynthesis is one of the few essential metabolic pathways for which a regulation by p53 is largely unknown. The synthesis of guanine nucleotides as a part of purine biosynthesis involves conversion of inosine monophosphate (IMP) to xanthosine monophosphate (XMP) by the inosine monophosphate dehydrogenase and further conversion of xanthosine monophosphate to GMP by the GMP synthetase (GMPS).⁵ Guanosine nucleotides provided by these and additional reactions are essential for processes such as DNA and RNA formation, microtubule assembly,⁶ energy storage,⁷ and nuclear transport,⁸ particularly in high proliferative cells.⁹ Herein, we identified GMPS by using quantitative mass spectrometry [liquid chromatography–tandem mass spectrometry (LC/MS-MS)] as a previously unrecognized and functionally relevant target of p53-mediated repression.

Materials and Methods

Proteomic Analysis

Lysates isolated from Sk-Hep1 cells were processed and analyzed in duplicate by liquid chromatography–tandem mass spectrometry, as previously described.¹⁰ Peptides were assessed using a nano-Acquity UPLC system (Waters, Eschborn, Germany) connected online to a LTQ-Orbitrap Velos Pro instrument (Thermo Fisher Scientific, Bremen, Germany).

Cell Culture and Drug Treatment

HLE, HuH7, and HuH6 cells were purchased from the Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan). PLC/PRF/5, HepG2, and Sk-Hep1 cells were purchased from ATCC (Manassas, VA). All mentioned cell lines have been validated using STR analyses [Leibniz-Institut, Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany]. HepG2 cells were maintained in RPMI 1640 medium, whereas HuH6, Sk-Hep1, HuH7, PLC/PRF/5, and HLE cells were cultured in Dulbecco's modified Eagle's medium (both media supplemented with 10% fetal calf serum and 1% penicillin/streptomycin) in an atmosphere containing 5% CO₂. RPMI 1640 medium, Dulbecco's modified Eagle's medium, and antibiotics were purchased from Sigma (Taufkirchen, Germany). Aqueous stock solutions of doxorubicin were obtained from the local pharmacy (University of Heidelberg, Heidelberg, Germany) and applied in a final concentration of 100 nmol/L, being renewed every second day for 7 days. Nutlin-3a was dissolved in dimethyl sulfoxide (Calbiochem/Merck Biosciences, Schwalbach, Germany) and used in a final concentration of 10 μ mol/L for

(depending on the experiment) 24 hours, 48 hours, or 7 days (renewed every second day).

Transfection of siRNAs

The gene-specific siRNAs were designed and synthesized by Qiagen (Hilden, Germany). The Qiagen All-Stars duplex was used as negative control for all knockdown experiments. The siRNA transfections were performed with Oligofectamine (Invitrogen, Karlsruhe, Germany), according to the manufacturer's instructions, and were used at a final concentration of 50 nmol/L. siRNA sequences were as follows: GMPS#1, 5'-ACCGTGCAGAACAGAGAACTT-3'; GMPS#2, 5'-AAGAGAGAGTAGGCACGTCAA-3'; p21#1, 5'-CAGUUUGUGUGUCUUAUUAUUTT-3'; p21#2, 5'-CUGGCAUUAGAUUUAUUUAAATT-3'; p53#1, 5'-UGUUCGAGAGCUGAAUGA-3'; and p53#2, 5'-AGACCUAUGGAAACUACUU-3'.

Cell Viability Assay

Cells were seeded into a 6-well plate and transfected with the indicated siRNAs. The number of viable cells was assessed using CellTiter-Blue Cell Viability Assay (Promega, Mannheim, Germany). CellTiter-Blue Reagent (100 μ L/well) was added, and cells were incubated for 1 hour at 37°C before the supernatant was transferred into a 96-well plate and metabolic activity was measured using the filter combination excitation: 560 nm/emission: 590 nm, using a Microplate reader (FLUOstar Omega, BMG, Labtech, Ortenberg, Germany) and normalized to the control siRNA condition.

Cellular Senescence Assay

Senescence was assayed using a β -galactosidase staining kit at pH 6.0, according to the manufacturer's protocol (Cell Signaling Technology, Danvers, MA).

Gel Electrophoresis and Immunoblotting

Harvesting of cells and processing of the cell lysates were performed as previously described.¹¹ The following antibodies were diluted in 5% milk/tris-buffered saline with Tween 20 (Milchpulver, Roth, Karlsruhe, Germany): anti-GMPS (sc-376163; Santa Cruz, Heidelberg, Germany) in a 1:500 dilution, anti-p53 (554293; Pharmingen/BD Transductional Laboratories, Heidelberg, Germany) in a 1:10,000 dilution, anti-p21 (sc-397; Santa Cruz, Heidelberg, Germany) in a 1:250 dilution, and anti-minichromosome maintenance protein 2 homolog (4007S; Cell Signaling, New England Biolabs, Frankfurt/Main, Germany) in a 1:500 dilution. Equal loading of each protein sample was verified by using a monoclonal mouse anti- β -actin antibody in a 1:10,000 dilution (8691391; MP Biomedicals, Santa Ana, CA) or by using a monoclonal mouse anti-vinculin antibody in a 1:5000 dilution (V9131; Sigma, Taufkirchen, Germany).

Figure 1 Proteomic analysis reveals GMPS as a p53 repression target. **A:** Sk-Hep1 cells were harvested 24 and 48 hours after Nutlin-3a treatment (10 $\mu\text{mol/L}$), and cell extracts were analyzed in duplicate by label-free shotgun proteomics. Pie chart depicts percentage of proteins with twofold or more changes after 24 and/or 48 hours of a total of 1721 proteins being measured. **B:** Bar diagrams show relative (Rel.) fold change of protein abundance of ferredoxin reductase (FDXR), TP53-induced glycolysis and apoptosis regulator (TIGAR), dUTPase, and minichromosome maintenance protein 2 homolog (MCM2), each normalized to the dimethyl sulfoxide (DMSO)-treated condition. Data are derived from two biological replicates. **C:** Bar diagram shows relative protein expression of GMPS normalized to the DMSO-treated condition (-). Data are derived from two biological replicates. Simplified schematic view of de novo GTP biosynthesis involving inosine monophosphate (IMP), xanthosine monophosphate (XMP), GMP, and GMPS. **D:** Sk-Hep1 cells, HepG2 cells, and HuH6 cells were treated with either DMSO or 10 $\mu\text{mol/L}$ Nutlin-3a for 24 and 48 hours, and cell extracts were immunoblotted using the indicated antibodies. Data are presented as means $\pm$ SD (**B** and **C**). LC/MS-MS liquid chromatography–tandem mass spectrometry.

Total RNA Isolation, cDNA Synthesis, and Quantitative Real-Time PCR

Total cellular RNA was isolated using the NucleoSpin RNA II kit (Macherey-Nagel, Düren, Germany). RNA (1 μg) was reverse transcribed using the Sigma-Aldrich RT-PCR kit (Steinheim, Germany), according to the manufacturer's instructions. Samples were analyzed in triplicate reactions on a StepOnePlus real-time PCR instrument (Applied Biosystems, Darmstadt, Germany) using the Absolute qPCR SYBR Green ROX Mix (Thermo Scientific, Offenbach, Germany). Expression levels were normalized to those of L32. Primers were designed with Primer-BLAST (National Center for Biotechnology Information, Bethesda, MD) and obtained from APARA Bioscience GmbH (Denzlingen, Germany) or from ThermoFisher Scientific (Offenbach, Germany) with the following sequences: GMPS, 5'-TCA-AGGTTGTGGCAGCTTCT-3' (forward) and 5'-GCC-AACTTCAGGGTGGAACT-3' (reverse); p21, 5'-GGCGG-CAGACCAGCATGACAGATT-3' (forward) and 5'-GCAGGGGGCGGCCAGGGTAT-3' (reverse); and L32,

5'-TTCCGGTCCACAACGTCAAG-3' (forward) and 5'-TGTGAGCGATCTCGGCAC-3' (reverse).

Human and Murine HCC Samples

Our study used a published Affymetrix U133A2.0 gene expression data set derived from 247 HCCs (90.7% hepatitis B virus positive), as described by Roessler et al.^{12,13} The *TP53* status was available for 178 patients (*TP53* mutated: $n = 46$; *TP53* wild type: $n = 132$). Patient samples of this data set were obtained with informed consent from patients at the Liver Cancer Institute and Zhongshan Hospital (Fudan University, Shanghai, China).

Murine HCC samples were kindly provided by the Zender group and generated as described previously.¹⁴

Statistical Analysis and Software

Data are presented as the mean of three independent experiments, including SD except otherwise specified. Expression differences between quantitative real-time PCR

Figure 2 Induction of wild-type p53 reduces GMPS in a p21-dependent manner on protein and mRNA level in Sk-Hep1 cells. **A:** Sk-Hep1 cells were treated with either a control siRNA (AS) or two different p53-specific siRNAs (p53#1 and #2). Forty-eight hours after siRNA transfection, cells were treated with Nutlin-3a (10 μ mol/L) for 24 hours. Cell extracts were immunoblotted using the indicated antibodies. **B:** Sk-Hep1 cells were treated with either dimethyl sulfoxide (DMSO) or 10 μ mol/L Nutlin-3a for 24 and 48 hours. Relative (Rel.) GMPS mRNA expression normalized to the DMSO condition (-) was measured by quantitative real-time PCR. Data are derived from three biological replicates. **C:** Sk-Hep1 cells were treated with either a control siRNA (AS) or two different p21-specific siRNAs (p21#1 and #2). Forty-eight hours after siRNA transfection, cells were treated with Nutlin-3a (10 μ mol/L) for 24 hours. Cell extracts were immunoblotted using the indicated antibodies. **D:** Sk-Hep1 cells were treated as described in **C**. Relative GMPS mRNA expression normalized to the control siRNA (AS) DMSO-treated condition was measured by quantitative real-time PCR. Data are derived from three biological replicates. GMPS protein expression is not affected by knockdown of p53 mutants. **E:** PLC, HuH7, and HLE cells were treated with either a control siRNA (AS) or two p53-specific siRNAs (p53#1 and p53#2), and cell extracts were immunoblotted with the indicated antibodies. Data are presented as means $\pm$ SD (**B** and **D**). * P < 0.05, ** P < 0.01.

results and cell viability assays were assessed by *U-test* using Microsoft Excel 2010 (Microsoft Corp., Redmond, WA).

Gene expression analysis of HCC patients was performed, as described previously,¹³ using Affymetrix U133A2.0 array data that are publically available through Gene Expression Omnibus accession number GSE14520 (<http://www.ncbi.nlm.nih.gov/geo>).¹³ Affymetrix gene expression arrays obtained from different platforms were combined with the matchprobes package in R version 2.6.2 (<http://www.r-project.org>, last accessed April 23, 2014). Raw gene expression data were normalized using the robust multiarray average method and global median centering. For genes with more than one probe set, the mean gene expression was calculated. Expression differences between HCC and non-tumorous liver samples were assessed by nonparametric

Mann-Whitney *U* test using GraphPad Prism version 6.07 (GraphPad Software, Inc., La Jolla, CA). The statistical significance was defined as P < 0.05.

Results

To identify novel aspects of p53-dependent regulation of metabolic pathways, we analyzed global protein fold-changes on p53 induction. To do so, we treated the cancer cell line Sk-Hep1 (containing wild-type p53) with Nutlin-3a (inducing p53 by disrupting the MDM2-p53 interaction) for 24 and 48 hours and performed quantitative mass spectrometry (LC/MS-MS). As shown in Figure 1A, we could quantify 1721 proteins (Supplemental Table S1), with 4.5%

Figure 3 GMPS repression is maintained in the context of p53-mediated cellular senescence. **A:** Sk-Hep1 cells were either treated with dimethyl sulfoxide (DMSO) or Nutlin-3a (10 μ mol/L) or untreated (untr.) or treated with low-dose doxorubicin (Doxo; 100 nmol/L) for 7 days. Senescence was assessed by β -galactosidase staining. Corresponding cell extracts were analyzed by immunoblotting with the indicated antibodies. GMPS knockdown is sufficient to induce cellular senescence and reduces cell viability. **B:** Sk-Hep1 cells were treated with either a control siRNA (AS) or GMPS-specific siRNAs (GMPS#1 and GMPS#2) for 6 days. Senescence induction was assessed by β -galactosidase staining. Corresponding cell extracts were analyzed by immunoblotting with the indicated antibodies. **C:** Sk-Hep1 cells were treated with either a control siRNA (AS) or GMPS-specific siRNAs (GMPS#1 and GMPS#2). Relative viability (%) normalized to the control siRNA condition at each time point was analyzed 2 and 4 days after transfection using CellTiter-Blue. Data are represented from three biological replicates. **D:** Murine *N-ras*^{G12V} and Myc-driven hepatocellular carcinomas (HCCs), which developed in a *Trp53*-deleted (*Trp53*^{-/-}) or *Trp53* wild-type (*Trp53*^{+/+}) background, were analyzed by immunoblotting with the indicated antibodies. **E:** GMPS expression in HCC tissue compared to nontumorous liver tissue. Data are represented as means $\pm$ SD (C). *n* = 247 (E, HCC tissue); *n* = 233 (E, nontumorous liver tissue). **P* < 0.05, ***P* < 0.01, and ****P* < 0.001.

of those being twofold or more up- or down-regulated 24 and 48 hours after drug treatment, whereas 0.8% were twofold or more up- or down-regulated only after 24 hours and 8.5% were twofold or more up- or down-regulated exclusively after 48 hours. Figure 1B illustrates examples of proteins (mostly related to metabolism) previously described as p53 activation or repression targets, including ferredoxin reductase (FDXR),¹⁵ TP53-induced glycolysis and apoptosis regulator (TIGAR),⁴ mitochondrial dUT-Pase,¹⁶ and minichromosome maintenance protein 2 homolog (MCM2),¹⁷ supporting the reliability of the approach. Among the most down-regulated metabolic enzymes, we found GMP synthetase (GMPS) (Figure 1C). GMPS converts xanthosine monophosphate to GMP as an essential part of the purine biosynthesis pathway (Figure 1C). Thus, on the basis of its striking fold-change upon p53 induction, its link to an important metabolic pathway, and (to the best of our knowledge) no previously reported regulation by p53, we chose GMPS for detailed analysis. First, we confirmed p53-mediated GMPS repression by immunoblotting in Sk-Hep1, HepG2, and HuH6 cells, indicating a gradual decrease of GMPS protein (and of minichromosome maintenance protein 2 homolog) along with p53 and p21 induction (Figure 1D) in all three cell lines.

To rule out an unspecific p53-independent effect of Nutlin-3a treatment on GMPS, we tested if p53 knockdown can rescue GMPS repression. Indeed, repression of GMPS on Nutlin-3a treatment was completely abrogated in the p53 siRNA-transfected conditions (Figure 2A). To acquire a more detailed insight into p53-mediated GMPS regulation, we performed quantitative real-time PCR, revealing decreased GMPS mRNA levels at 24 and 48 hours on Nutlin treatment (Figure 2B). This finding suggested a transcriptional repression of GMPS by p53. Because p53-mediated transcriptional repression frequently occurs in a p21-dependent manner,¹⁸ we tested if p21 is required for GMPS down-regulation in this setting. Supporting this hypothesis, knockdown of p21 by RNA interference largely rescued reduced GMPS protein (Figure 2C) and mRNA (Figure 2D) on p53 induction.

Repression targets of wild-type (wt) p53 can also represent gain-of-function targets of mutant p53.¹⁹ However, knockdown of the p53^{R249S} (the most frequent p53 mutation in HCC), p53^{Y220C}, and p53^{G244A} mutants endogenously expressed in PLC/PRF/5, HuH7, and HLE, respectively, had no impact on GMPS protein (Figure 2E). Taken together, we conclude that GMPS is repressed by wild-type p53 in a p21-dependent manner and does not represent a gain-of-function target of the above-mentioned p53 mutants.

We then tested if GMPS is not only transiently reduced but maintained at low levels in the context of a long-term, liver cancer—relevant p53 response, such as senescence.²⁰ As Figure 3A illustrates, treatment with Nutlin-3a or doxorubicin for 7 days resulted, as expected, in cellular senescence, reflected by typical morphological changes and a strong increase in β -galactosidase activity. Moreover, the corresponding immunoblots (Figure 3A) show that reduction of GMPS protein was maintained in the setting of p53-induced senescence.

On the basis of these findings, we assumed that GMPS depletion alone might be sufficient to evoke cellular senescence. Corroborating this assumption, in Sk-Hep1 cells depleted of GMPS by RNA interference for 6 days, a striking increase of senescent cells was detectable (Figure 3B). Notably, GMPS knockdown was paralleled by an induction of p21, presumably triggering the observed senescence phenotype (Figure 3B). An induction of p21 induction upon GMPS depletion also occurred in HuH6 cells (Supplemental Figure S1A). Interestingly, p21 up-regulation in this scenario appeared unrelated to p53, which was further confirmed by time course experiments. Supplemental Figure S1B illustrates that already 1 day after GMPS knockdown, p21 was up-regulated and further increased, with no obvious correlation to the corresponding p53 levels. Consistent with these findings, a significant decrease of cell viability on GMPS ablation in Sk-Hep1 and HuH6 cells was measurable by CellTiter-Blue assays (Figure 3C).

Finally, we determined the link between p53 and GMPS in an *in vivo* setting by using an established HCC mouse model.¹⁴ To do so, we analyzed GMPS expression by immunoblotting in murine HCCs generated by transposon-based gene transfer of *N-ras*^{G12V} and *Myc* into wild-type mice (*Trp53*^{+/+}) or into mice with homozygous deletion of *Trp53* (*Trp53*^{-/-}). Figure 3D shows that GMPS was more strongly expressed in *Trp53*^{-/-} HCCs compared to *Trp53*^{+/+} HCCs. Furthermore, we found in a large HCC patient cohort, highly significant up-regulation ($P < 0.0001$) of GMPS transcript levels in HCC samples ($n = 247$) compared to nontumorous liver tissue ($n = 233$) (Figure 3E). Interestingly, in HCC patients for whom the p53 status was available ($n = 178$), we did not detect a significant difference in GMPS transcript levels between wt p53 ($n = 132$) and mutant p53 ($n = 46$) tumors (Supplemental Figure S1C). Thus, we assume that not only mutational, but also functional, inactivation of wt p53, for instance by the HBx protein²¹ (because >90% of the patients were hepatitis B virus positive), is sufficient to inhibit p53-dependent GMPS repression.

As summarized in Figure 4, our data suggest that repression of GMPS by p53 through p21 is a functionally relevant part of the p53-mediated senescence program limiting tumor cell growth in liver cancer.

Discussion

We could identify GMPS as a previously unrecognized p53 repression target based on a proteome-wide approach.

Figure 4 Graphic summarizes the link between p53, p21, and GMPS. Activation of p53 is followed by p21 induction, resulting in GMPS repression. Representing a feed-forward loop, reduced levels of GMPS result in p21 up-regulation and are associated with senescence induction and decreased cell viability.

Interestingly, a study by Reddy et al²² demonstrated that GMPS is required for ubiquitin-specific protease 7 (USP7)—mediated p53 stabilization. This finding is in agreement with decreasing p53 levels we observed on GMPS knockdown (Figure 3B). However, the authors did not report a significant impact of p53 induction on GMPS protein itself using U2OS cells and etoposide treatment in their experiments.¹⁹ This apparently indicates a cell type and context dependency of p53-mediated GMPS repression.

We observed a striking induction of p21 on GMPS depletion associated with cellular senescence. A plausible explanation for this phenomenon could be that GMPS knockdown contributes to a reduced pool of nucleotides. Supporting this hypothesis, Aird et al²³ could demonstrate that oncogenic ras reduces the key enzyme of dNTP biogenesis ribonucleotide reductase subunit M2 in IMR90 cells preceding oncogene-induced senescence. In addition to this enzyme, Mannava et al²⁴ also observed reduced thymidylate synthase in WI-38 cells on oncogenic ras expression. In both studies, the senescence phenotype could be reverted by adding nucleosides/dNTPs. Although these studies focused on oncogene-induced senescence and at least the former study does show p53 induction in this context (in contrast to our work), our data are in line with the emerging concept that suppression of nucleotide metabolism can trigger cellular senescence.

We found that p53-mediated repression of GMPS requires p21 induction. Interestingly, the other part of the feed-back loop (p21 induction on GMPS knockdown) appears to be p53 independent (Figures 3B and 4), suggesting other factors to be involved. Besides p53, other transcription factors, such as Sp1 and Sp3, have been reported to control p21 in response to diverse stress signals/stimuli, such as

nerve growth factor, phorbol myristate acetate, butyrate, and transforming growth factor- β .²⁵ More important, Rb also induces p21 through Sp1/Sp3 binding sites²⁵ and was linked to nucleotide depletion—associated senescence before.²⁴ However, because p21 is also regulated on co-transcriptional and post-transcriptional as well as post-translational levels,^{25–27} elucidating the detailed mechanism by which GMPS directly or indirectly affects p21 represents a rewarding subject for future studies.

Cancer cells with increased metabolic demands rely on guanine nucleotides provided by the guanine biosynthesis pathway being essential for DNA and RNA formation, microtubule assembly, energy storage, and nuclear transport. Thus, repression of GMPS by p53 appears as a powerful strategy to inhibit many of these processes simultaneously in a tumor-suppressive response. Conversely, a repression relief on p53 inactivation, resulting in GMPS overexpression, may have multifunctional (protumorigenic) implications. Given that inactivation of p53 (either functionally or genetically) is a frequent event in carcinogenesis, overexpression of GMPS in HCC and in other carcinomas²⁸ could be attributed to a loss of p53's wild-type function in a significant fraction of tumors. The data derived from the murine HCCs provide clear evidence that complete genetic inactivation (deletion) of *Trp53* is associated with particularly high GMPS expression in the respective tumors. The situation in human HCCs with mutations in the *TP53* locus is slightly different, where usually one point-mutated allele and a loss of the second wild-type allele are found. Nevertheless, one could have expected a difference in GMPS expression in p53 wt versus p53 mutant tumors unless p53, despite being genetically wt, is functionally inactivated. Supporting this scenario is the fact that >90% of the HCC patients were positive for hepatitis B virus and the hepatitis B virus–related protein HBx is well documented for interfering with the wild-type function of p53.^{21,29,30} Specifically, HBx protein was shown to interact with p53 *in vitro* and *in vivo*, to inhibit sequence-specific DNA binding of and transcriptional activation by p53 as well as p53's nuclear import.^{21,29,30} Collectively, these data indicate that GMPS is a functionally relevant p53 repression target representing a previously unidentified aspect in p53's ability to control tumor cell growth in liver cancer.

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Supplemental Data

Supplemental material for this article can be found at <http://dx.doi.org/10.1016/j.ajpath.2016.09.022>.

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