Heterochromatin Protein HP1γ Promotes Colorectal Cancer Progression and Is Regulated by miR-30a

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Abstract

Colorectal cancer pathogenesis remains incompletely understood. Here, we report that the heterochromatin protein HP1γ is upregulated commonly in human colorectal cancer, where it promotes cell proliferation in vitro and in vivo. Gene-expression and promoter-binding experiments demonstrated that HP1γ directly regulated CDKN1A (p21Waf1/Cip1) in a manner associated with methylation of histone H3K9 on its promoter. We identified miR-30a as a tumor-suppressive microRNA that targets HP1γ in vitro and in vivo to specifically suppress the growth of colorectal cancer in mouse xenograft models. MiR-30a was widely downregulated in primary human colorectal cancer tissues, where its expression correlated inversely with high levels of HP1γ protein. Our results identify a new miR-30a/HP1γ/p21 regulatory axis controlling colorectal cancer development, which may offer prognostic and therapeutic opportunities.

Introduction

Colorectal cancer is the third most prevalent carcinoma and the second leading cause of cancer-related deaths in Western countries (1). Every year, more than 1 million new cases are diagnosed. Although much effort has centered on probing the pathogenesis of the disease, the molecular mechanisms underlying the process are still largely unknown (2).

The mammalian heterochromatin protein 1 (HP1) family contains three isoforms, HP1α (CBX5), HP1β (CBX1), and HP1γ (CBX3). Despite their high conservation in sequence, the HP1 paralogs harbort distinct functions in a variety of cellular processes. These include heterochromatin formation, DNA repair, RNA splicing, cellular senescence, centromere and telomere homeostasis, and transcriptional activation and elongation (3–6). HP1α and HP1β primarily associate with heterochromatin whereas HP1γ localizes to both heterochromatic and euchromatic regions (6, 7). Given their widespread genomic distribution and different roles in gene regulation, it is not surprising that HP1 is linked with cancer progression (3, 8–10). Although levels of different HP1 isoforms have been reported to correlate with some cancers, the roles of HP1 were promiscuous, and somewhat controversial (9–12). Moreover, the mechanisms of action of HP1 remain obscure in cancer, including colorectal cancer.

MicroRNAs (miRNA) are small noncoding RNAs that can modulate the expression of cognate target genes by binding to their mRNA 3′-untranslated region (3′-UTR), resulting in either translational inhibition or mRNA cleavage (13). Primarily, miRNAs function as crucial factors in diverse regulation pathways, including development, tumorigenesis, cell differentiation, proliferation, and apoptosis (13, 14). Accumulating evidence indicates that miRNAs can act in human carcinogenesis as novel types of tumor suppressors or oncogenes (14–16). Although miRNAs have been studied extensively in recent years, the molecular functions of miRNAs and their effects on cancer are not well understood.

In the present study, we show that HP1γ promotes colon cancer growth by directly repressing expression of the cyclin-dependent kinase inhibitor, p21Waf1/Cip1 (CDKN1A; refs. 17, 18). We found that HP1γ is specifically targeted by miR-30a, which controls colorectal cancer cell proliferation both in vitro and in vivo, indicating a tumor-suppressor role of miR-30a in colorectal cancer. The newly identified miR-30a/HP1γ/p21 axis functions as a key pathway regulating tumor cell proliferation during colorectal cancer development.

Materials and Methods

Clinical samples and immunohistochemical staining

Two tissue microarray (TMA) chips containing a total of 178 pairs of colorectal tissue samples and matched adjacent normal tissues (NAT) with follow-up data were obtained from Shanghai Biochip Co., Ltd. Fresh colorectal tissue samples and NAT were derived from patients undergoing surgical procedures at the Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, China. All patients in the study provided written consent, and the Ethics Committee from the Affiliated Hospital of...
Nanjing University of Chinese Medicine approved all aspects of this study. Immunohistochemical staining was performed using paraffin-embedded sections of biopsies from colorectal cancer patients and controls according to standard protocols by Cell Signaling Technology (see Supplementary Materials). All colorectal cancer tissue sections were reviewed by two experienced pathologists (Drs. X. Wu and J. Zhou), and staining of HP1γ in the tissue was scored independently by two pathologists blinded to the clinical data adopting the semiquantitative immunoreactive score (IRS) system (see Supplementary Materials; refs. 19, 20). On the basis of the IRS from NAT, a cutoff value for positivity of HP1γ expression in colorectal cancer was set to IRS 5.52. The clinical features of the patients are listed in Supplementary Table S1.

Cell culture, transfections, luciferase reporter assay, and siRNA interference assay

Two colon carcinoma cell lines (HCT116 and SW620) and HEK293T were purchased from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). Cells were maintained at 37°C in a humidified air atmosphere containing 5% carbon dioxide in McCoy’s 5A medium (HCT116), DMEM (HEK293T), and RPMI-1640 (SW620) supplemented with 10% FBS (Invitrogen). Synthetic RNA, miR-30a mimic, miR-30a inhibitor (anisensemir-30a), control mimics, and siRNA against HP1γ were synthesized by liana. The sequences used were: miR-30a mimic, 5'-UGUAAAACAUUCUCUCAGCU-3'; miR-30a inhibitor, 5'-CUUCCAGUUCAGAAGUUGU-3'; HP1γ siRNA-1, 5'-GAAGUGUCCUCUCAAUUGUA-3'; siRNA-2, 5'-AGAAGAUCUCAGUCAUAUCAA-3'. HCT116 and SW620 cells were transfected with oligonucleotides or indicated constructs using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions.

To test the direct binding of miR-30a to the target gene HP1γ, a luciferase reporter assay (Promega) was performed as previously described, and a β-galactosidase (β-gal) expression plasmid (Invitrogen) was used as a transfection efficiency control (see Supplementary Materials; ref. 21).

An shRNA sequence targeting human HP1γ cDNA was designed and synthesized by GenePharma. The target sequence of RNAsi for HP1γ was inserted into the Xhol/HpaI sites in the pLL3.7 lentiviral vector according to the manufacturer’s recommendations (ATCC). The targeting oligonucleotides were: 5'-AGAAGAUGUCCUCUCAAUUGUA-3'. A scrambled shRNA was included as a negative control. For overexpressing miR-30a, a similar strategy as the one described above was used in the pLL3.7 lentiviral vector. For overexpressing HP1γ, human HP1γ cDNA without the 5'-UTR was cloned into the retroviral vector plasmid MSCV-ires-GFP at unique XhoI and BamHI sites. Lentivirus or retrovirus production in 293T cells and infection of HCT116 cells were performed as described previously (22, 23). Transduced cells were selected for GFP expression by flow cytometry.

Protein extraction and Western blot analysis

Protein extraction and Western blot analysis were performed following standard procedures (see Supplementary Materials; ref. 23).

RNA isolation and quantitative RT-PCR

RNA isolation and quantitative RT-PCR were performed according to the manufacturer’s instructions. The primer sequences for RT-PCR are listed in Supplementary Table S2 (see Supplementary Materials).

Proliferation assay, EdU incorporation assay, colony formation assay, and cell-cycle analysis

The in vitro viability of colorectal cancer cells was assessed using the Cell Counting Kit-8 (CCK-8). Cell proliferation was determined by incorporation of 5-ethyl-2'-deoxuryridine (EdU) using an EdU Cell Proliferation Assay Kit (Ribobio). Colony formation was observed by staining cells with 0.1% crystal violet (Sangon Biotechnologies Inc.). Cell cycles were analyzed by flow cytometry (Becton Dickinson; see Supplementary Materials).

Tumor xenografts in nude mice

All animal care and handling procedures were performed in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Review Board of Nanjing University (Nanjing, China). For these studies, 5- to 6-week-old female nude mice were purchased from the Model Animal Research Center of Nanjing University (Nanjing, China), and maintained under specific pathogen-free conditions at Nanjing University. To initiate tumors, 5 × 106 cells in 100 μL of PBS:Matrigel (9:1, v/v) were injected s.c. into each flank of nude mice (8 mice/group). Tumor growth rate was monitored by measuring tumor diameters every 4 days. Both maximum (L) and minimum (W) length of the tumor were measured using a slide caliper, and the tumor volume was calculated as \( \frac{1}{2} \times LW^2 \). Mice were euthanized at indicated time points, and tumors were collected, weighed, and analyzed.

Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) assays were performed with HCT116 cells as described previously (22). The p21 promoter ChIP primers are listed in Supplementary Table S3 (see Supplementary Materials and Methods).

Statistical analysis

The Student t test was used to derive the significance of the differences between mean values (See Supplementary Materials and Methods).

Results

Upregulation of HP1γ correlates with poor prognosis in colorectal cancer

To investigate the clinical relevance of HP1γ expression in colorectal cancer, we first examined HP1γ expression in colon tissue specimens from a cohort of 178 colorectal cancer patients by immunohistochemistry using specific anti-HP1γ antibody. Significantly higher expression levels of HP1γ were observed in colorectal tissues compared with matched adjacent normal tissues from colorectal cancer patients, and HP1γ was predominantly localized in the nuclei of tumor epithelial cells (Fig. 1A and B). We also found that the expression level of HP1γ in colorectal cancer inversely correlated with the differentiation state of the cancer cells (Fig. 1A and C). Importantly, colorectal cancer patients with high HP1γ expression had shorter overall survival (Fig. 1D). Western blot analysis confirmed that the expression levels of HP1γ in the tumor tissues from 38 patients were significantly higher than in matched adjacent nontumor tissues (Fig. 1E and F). These results indicate that HP1γ protein levels were upregulated in...
Figure 1.
Upregulation of HP1γ in colorectal cancer is associated with poor prognosis. A, hematoxylin and eosin (H&E) staining and IHC staining of HP1γ protein in normal, well-differentiated, moderately differentiated, and poorly differentiated colorectal cancer tissues. Representative micrographs are shown in original magnification (×40 and ×200) as indicated. B, total IHC score of HP1γ in NATs and human colorectal tumor tissues (colorectal cancer; n = 178); *** P < 0.001 compared with NAT control. C, IHC score of HP1γ in normal, well-differentiated, moderately differentiated, and poorly differentiated colorectal cancer tissues. ** P < 0.01; * P < 0.05 compared with indicated control. D, Kaplan-Meier plot of overall survival of 178 patients with colorectal cancer, stratified by HP1γ expression. E, Western blot analysis of HP1γ in cell lysates from NATs and colorectal tumor tissues (colorectal cancer; n = 38). GAPDH served as a loading control. F, HP1γ protein expression levels by quantification of density of protein bands from Western blot in E in colorectal tumors relative to the NATs (n = 38, ** P < 0.01).
colorectal cancer tissues, and suggest that high levels of HP1γ expression may correlate with poor prognosis in colorectal cancer.

**HP1γ promotes colorectal cancer cell proliferation and tumorigenesis**

To assess the role of HP1γ in colorectal cancer development, we examined the effect of HP1γ on cell proliferation. We knocked down HP1γ in two colon cancer lines, HCT116 and SW620, by RNAi, which reduced HP1γ expression to less than 80% of the scrambled control (Fig. 3B and C, left). Knockdown of HP1γ significantly reduced the cell growth rate in both cell lines (Fig. 2A). These results were further confirmed by EdU staining to detect nucleotide analogue incorporation into replicated DNA (Fig. 2B). In addition, stable knockdown of HP1γ significantly reduced the numbers of HCT116 colonies formed after culture compared with controls (Fig. 2C). To verify that HP1γ had a growth-promoting effect on colon cancer cells, a xenograft tumor growth assay was performed in nude mice. Subcutaneous tumor growth of HCT116 cells with HP1γ shRNA-mediated stable knockdown or scrambled control was monitored (Fig. 2D). Tumor cells with HP1γ knockdown grow more slowly than the scrambled control in the same mouse (Fig. 2E). Tumor volume and weight in HP1γ knockdown mice were greatly reduced compared with control mice (Fig. 2F). These data indicate that HP1γ promotes colorectal cancer proliferation, and is essential for colorectal cancer development.

**HP1γ directly suppresses the expression of p21 in colorectal cancer**

We next investigated potential mechanisms by which HP1γ knockdown inhibited colorectal cancer proliferation. We analyzed the expression of a spectrum of key proliferation-related genes, including p21, p16, RBL2, SAMD7, ST7, p27, CCND2, and CDH1 in HCT116 cells by quantitative real-time PCR (Fig. 3A; ref. 24). We found that the expression level of p21, a known tumor suppressor (17, 18), was the most increased when HP1γ was knocked down (Fig. 3A).

To confirm the effect of HP1γ on p21 expression at the protein level, we performed Western blot experiments using cellular extracts from HP1γ knockdown or scrambled control HCT116 and SW620 cells. The p21 protein was expressed at low levels in the scrambled control cells but was greatly increased in HP1γ knockdown cells (Fig. 3B). Corresponding p21 mRNA levels were confirmed by quantitative RT-PCR in both HCT116 and SW620 cells (Fig. 3C, right). Western blot analysis of xenograft tumor tissues also demonstrated that HP1γ knockdown markedly activated expression of p21 (Fig. 3D).

We performed ChIP to analyze HP1γ binding to the p21 promoter. Equal amounts of sonicated HCT116 chromatin DNA were incubated with IgG control or HP1γ antibody. Protein G bead–captured chromatin DNA was amplified as a template, and five pairs of primers across the p21 promoter were used for quantitative real-time PCR (Fig. 3E, top). The ChIP results demonstrated that HP1γ was most enriched on the proximal promoter region at 297 to −191, but was not bound on the far promoter regions at −2596 to −2414 or −2069 to −1906 (Fig. 3E, bottom). When HP1γ was knocked down, enrichment of HP1γ was much reduced on the proximal promoter region (Fig. 3E). These results indicate that HP1γ binds the p21 promoter. The histone marks H3K9me2/3 on the p21 promoter were also significantly reduced when HP1γ was knocked down (Fig. 3F). These results indicate that regulation of p21 expression by HP1γ is associated with histone methylation.

**HP1γ is specifically targeted by miR-30a**

miRNAs have been found to play diverse, key biologic roles in cancer development and have been widely used for cancer diagnosis, prognosis, and as therapeutic targets (25). To identify potential miRNAs targeting HP1γ, we used a combination of three algorithms, TargetScan (26), PicTar (27), and miRanda (http://www.microrna.org/microrna/home.do). Among the identified candidate miRNAs, miR-30 family members were identified by all three programs as targeting HP1γ (Fig. 4A). In Western blot analysis of 293T cells, miR-30a knocked down HP1γ expression more efficiently than did other members of the miR-30 cluster (Fig. 4B). This finding was supported by results of expression assays using a luciferase reporter in both 293T cells (Supplementary Fig. S1A) and HCT116 colon cancer cells (Fig. 4C). In addition, the ability of miR-30a to knock down HP1γ protein expression exhibited a dose-dependent effect (Supplementary Fig. S1B). Therefore, miRNA-30a was chosen for further studies.

To determine whether miR-30a regulates HP1γ expression through binding to the 3′-UTR of HP1γ mRNA, the entire 3′-UTR of HP1γ mRNA containing the presumed miR-30a–binding sites was fused downstream of the firefly luciferase gene in a reporter plasmid. The resulting plasmid was transfected into HCT116 and SW620 cells along with either miR-NC (a scrambled control of miR-30a), miR-30a, anti-miR-30a (a miR-30a inhibitor), or anti-miR-NC (a scrambled control of anti-miR-30a) RNA oligonucleotides. As expected, luciferase reporter activity in cells transfected with miR-30a was reduced by about 50% compared with cells transfected with the scrambled control (Fig. 4D). In addition, inhibition of transfected miR-30a by cotransfection of anti-miR-30a showed no reduction of reporter activity whereas cotransfection of anti-miR-NC still kept a reduction of reporter activity by miR-30a (Fig. 4D). To test the specificity of targeting of miR-30a to the putative binding sequence on the HP1γ 3′-UTR, we engineered point mutations into the corresponding binding sites in the HP1γ 3′-UTR (Supplementary Fig. S1C). We then performed a similar luciferase reporter assay in both HCT116 cells and SW620 cells. We found that luciferase activity was further decreased in cells overexpressing miR-30a (Fig. 4E). HP1γ mRNA levels were unchanged after miR-30a transfection (Supplementary Fig. S1D), suggesting that miR-30a directly recognizes and binds to the 3′-UTR of the HP1γ mRNA, resulting in reduction of HP1γ protein. Thus, we conclude that miR-30a specifically regulates HP1γ protein expression posttranscriptionally.

**miR-30a inhibits colorectal cancer cell growth and tumor progression**

Next, to determine the effects of miR-30a on colon cancer cell growth, we monitored proliferation using the CCK-8 assay. The proliferation rate of HCT116 and SW620 cells transfected with
miR-30a was markedly decreased compared to cells transfected with scrambled control (Fig. 5A). To further confirm the biologic effect of miR-30a on the growth of colorectal cancer cells, EdU staining was performed. Consistent with the results from the CCK-8 assay, the percentage of EdU-positive cells was significantly less in cells transfected with miR-30a in both lines compared to the...
control cells (Fig. 5B). Moreover, treatment of HCT116 cells with miR-30a significantly reduced colony-forming efficiency compared with the scrambled control cells (Fig. 5C). In keeping with this, HCT116 cells exhibited a G0/G1 cell-cycle arrest after transfection with miR-30a (Supplementary Fig. S2A). Western blot analysis indicated that p21 was significantly increased when cells were transfected with miR-30a (Fig. 5D). The histone mark H3K9me3 on the promoter of p21 was also significantly reduced when miR-30a was transfected, although there was no change in the enrichment level of histone mark H3K9me2 (Fig. 5E). To test whether restoration of HP1γ would reverse miR-30a–mediated inhibition of proliferation of colorectal cancer cells, we performed rescue experiments in HCT116 and SW620 cells. We found that overexpression of HP1γ by transfection of a cDNA that lacked the miR-30a–binding sites in the 3’-UTR partially abrogated miR-30a–mediated suppression of proliferation in HCT116 and SW620 cells (Fig. 5F, left). Expression of HP1γ was verified by Western blot analysis (Fig. 5F, right). To confirm the growth-inhibitory effect of miR-30a on colon cancer cells in vivo, the subcutaneous growth of miR-30a or vector control transfected HCT116 tumors in nude mice was assessed (Fig. 6A). miR-30a transfected and control transfected tumors were implanted on
opposite sides of each mouse. In all mice, tumor cells stably overexpressing miR-30a grew more slowly than control transfected tumor cells (Fig. 6B). Tumor volumes and weights were greatly reduced in miR-30a transfected tumors compared with the control (Fig. 6C and D). The levels of miR-30a in tumors were confirmed by real-time RT-PCR (Fig. 6E). HP1γ and p21 levels were also determined. HP1γ levels were greatly reduced in miR-30a transfected tumors, and p21 levels were significantly increased (Fig. 6F). Taken together, these results demonstrate that miR-30a suppresses proliferation of colorectal cancer cells at least in part through downregulating HP1γ protein levels.

miR-30a is downregulated in human colorectal cancer tissues and inversely correlates with HP1γ protein levels.

To study the expression pattern of miR-30a in colorectal cancer cancers, we examined miR-30a expression in 38 colorectal cancer tissues and their pair-matched NATs using quantitative real-time RT-PCR. Levels of miR-30a were found to be significantly downregulated in colorectal cancer tissues compared with levels from paired adjacent nontumorous tissues (Fig. 7A and B). Levels of miR-30a and levels of HP1γ protein (shown in Fig. 1E) in colorectal cancer tissues exhibited a significant inverse correlation calculated by Pearson correlation (Fig. 7C), further indicating that HP1γ is a potential target of miR-30a in cancer cells. As expected for the presumed regulatory mechanisms of miRNAs, the levels of miR-30a and levels of HP1γ mRNA showed no relation (Supplementary Fig. S3).

Discussion
This study has revealed a pivotal role of HP1γ in colorectal cancer progression and has identified the potential involvement of a miRNA-mediated mechanism in modulating HP1γ expression in colorectal cancer. We found that HP1γ promoted
colorectal cancer cell proliferation by directly targeting p21 in both colorectal cancer cells and xenografted tumors in mice. In addition, we demonstrated that HP1γ could be posttranscriptionally regulated by miR-30a, which suppressed colorectal cancer growth in vitro and in vivo. We found that HP1γ protein levels were significantly increased in primary colorectal cancer tissues compared with adjacent nontumor tissues. We also found that miR-30a was downregulated in colorectal cancer tissues, and exhibited a significant inverse correlation with the levels of HP1γ protein. Together, our data demonstrate that HP1γ is critical for colorectal cancer cell proliferation and can be regulated by miR-30a specifically, which suggests that, with respect to colorectal cancer, miR-30a is a tumor suppressor (Fig. 7D).

HP1 family members were identified nearly 30 years ago, but the divergent functions of the three isoforms in cancer development are still elusive (3, 6, 28). In this study, we found that HP1γ was highly expressed in most colorectal cancer samples, and cancer correlation analysis indicated prognostic potential. Thus, HP1γ appears to be a promising biomarker for colorectal cancer.

In tumor cells from breast cancer, prostate cancer, pancreas cancer, or uterus cancer, HP1α but not HP1β or HP1γ is significantly overexpressed (12). HP1α but not HP1β or HP1γ also exhibited proliferation-dependent regulation (12). In prostate cancer, other reports have shown that HP1β and HP1γ, but not HP1α, play a tumor-promoting role by transactivation of androgen receptor signaling (10, 29). In granulocytes of acute myelogenous leukemia and chronic myelogenous leukemia during the acute phase,
all three types of HP1 protein were upregulated (30, 31). As well, however, there are examples in which HP1 might have beneficial effects in cancer. In colon cancer, HP1α upregulation correlates with reduced metastasis, whereas decreased levels of HP1α indicate poor prognosis (32). Also, downregulation of HP1β increases the invasiveness of human colon cancer cells (33). The basis for the discrepancies in expression of different HP1 isoforms in various cancers is uncertain but may be cell/tissue or stage dependent. There may be a balance between effects of HP1 on proliferation, invasiveness, and metastasis. On the basis of a reverse relationship between cyclin E expression and invasion, it has been suggested that proliferation and invasion are coordinated and regulated sequentially (34). Decreased expression of HP1 may be more related to invasive potential, possibly by promoting expression of invasive genes whereas elevation of HP1 is more related to proliferation. Nevertheless, proliferation is a prerequisite for metastasis, and our results suggest that proliferation potential of tumor cells may help determine prognosis.

These uncertainties might be resolved by delineating expression of HP1 and HP1 isoforms at different stages of various cancers. The mechanism by which HP1 promotes tumorigenesis is still largely unknown. In an analysis of a spectrum of key proliferation-related genes, we observed that HP1γ depletion was most effective in inducing p21 expression in HCT116 and SW620 cells. ChIP experiments revealed that p21 is a direct target of HP1γ. p21 is a cyclin-dependent kinase inhibitor that inhibits cyclin–cdk2 kinase complexes and interrupts cell-cycle progression resulting in cell cycle arrest (17, 18). Accordingly, knockdown of HP1γ increased p21 expression, leading to significantly slower proliferation of colorectal cancer cells. Targeting of p21 by HP1γ in colorectal cancer was further validated by xenograft tumor growth experiments in vivo, providing further evidence of a role of HP1γ in colorectal cancer. By knocking down HP1γ, we also found that other genes, including p53, p27, CDH1, and ST7, were modulated. The mechanisms and role of HP1γ in regulation of these genes in colorectal cancer remain to be determined. We are in the

Figure 6.
miR-30a inhibits colon cancer cell growth in vivo. A, growth of miR-30a–transfected or miR-NC control transfected HCT116 tumors in nude mice (n = 8). B, individual tumors excised from mice shown in A. Top, control transfected tumors; bottom, miR-30a transfected tumors; scale bar, 1 cm. C, mean volumes of xenograft tumors in miR-30a–transfected or miR-NC controls; **, P < 0.01 compared with miR-NC controls. D, average tumor weight of miR-30a–transfected or miR-NC–transfected tumors (P < 0.001). E, quantitation of miR-30a from xenograft tumor tissues by quantitative real-time PCR; **, P < 0.01 compared with miR-NC vector control. F, Western blot analysis with indicated antibodies of cell lysate from xenograft tumor tissues. GAPDH serves as a loading control.

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process of identifying other potential targets related to the HP1 network.

HP1 is an evolutionarily conserved chromosomal protein that binds methylated H3K9, and there is a well-documented interplay between HP1, DNA methylation, and heterochromatin formation, which affects centromere stability and telomere capping (4–6). HP1 has been reported to be recruited by the epigenetic mark H3K9me3, which is mediated by histone methyltransferase, Suv39h, to form heterochromatin (4, 5). In our study, we observed that knockdown of HP1γ reduced enrichment of H3K9me2/3 on the p21 promoter in colorectal cancer cells. Enrichment of H3K9me3 on the p21 promoter was also decreased when miR-30a was overexpressed. This result indicates that the recruitment of HP1γ and enrichment of H3K9me3 could be interdependent. This
is reminiscent of the HP1 homolog Swi6 in fission yeast, which is required for accumulation of the Suv39h homolog Clr4, and thus Clr4-mediated H3K9 methylation to form heterochromatin (35). HP1 proteins have been also found to interact with transcriptional regulators of key cell-cycle genes, such as Cyclin E, E2F1, and p53 (8, 36, 37). Whether HP1 and other regulatory factors or epigenetic modifiers can form a functional complex on the p21 promoter in colorectal cancer cells needs to be examined. Nevertheless, our current results suggest that HP1γ may provide an effective target for colorectal cancer therapy.

The development of colorectal cancer is regarded as a progressive event involving complicated networks of aberrant gene expression and environmental alterations, in which miRNAs play important roles (25). Not only can miRNAs serve as potential biomarkers, but they also may have promise as therapeutics (25). In a bioinformatics search for potential miRNAs targeting HP1γ, we identified miR-30a as the most promising. A specific miRNA targeting HP1 family members has not been previously identified. We found that miR-30a inhibited colorectal cancer cell proliferation by decreasing the level of HP1γ protein posttranscriptionally. The levels of HP1γ protein, but not those of HP1β mRNA, exhibited an inverse correlation with the levels of miRNA-30a in pathologic samples of colorectal cancer patients. miR-30a upregulated the expression of p21, resulting in cell-cycle arrest at G0/G1 in colorectal cancer cells. This result was further supported by the rescue experiments in which ectopic expression of HP1γ was sufficient to overcome cell growth inhibition by miR-30a. Although we and others observed that miR-30a could also induce colorectal cancer cell apoptosis (Supplementary Fig. S2B; ref. 38), our data indicated that miR-30a suppressed colorectal cancer development at least in part through decreasing the level of HP1γ protein posttranscriptionally. We cannot exclude the possibility that miR-30a may target other genes in a different context (38–42). Nevertheless, miR-30a appears to be a critical miRNA that is downregulated in colorectal cancer, and might be a potential prognostic marker of colorectal cancer.

In conclusion, this study provides evidence that HP1γ functions as an oncogenic molecule in human colorectal cancer development. Our data demonstrate a critical role for HP1γ in colorectal cancer, and suggest that HP1γ could be a potential target for therapy of human colorectal cancer. miR-30a appears to be a tumor suppressor through direct inhibition of expression of HP1γ protein. Our results suggest that the miR-30a/HP1γ/p21 axis may represent a potential therapeutic window for treatment of human colorectal cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: C.-Y. Zhang, Y.-G. Chen, Q. Zhao

Development of methodology: M. Liu, Y. Wang

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): D. Zhang, X.-B. Wu, Y. Wang, J.-Y. Zhou, B.-L. Yang, Y.-C. Chen

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Liu, F. Huang, J. Ju, R. Tan, Q. Zhao

Writing, review, and/or revision of the manuscript: M. Liu, X. Chen, K. Zen, Y.-G. Chen, Q. Zhao

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Y. Wu, M. Nie, Z. Li, C. Ma, J.-Y. Zhou, R. Tan

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