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Original Article

Pre-mRNA processing factor 19 functions in DNA damage repair and radioresistance by modulating cyclin D1 in hepatocellular carcinoma

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Pre-mRNA processing factor 19 (PRP19) is elevated in hepatocellular carcinoma (HCC); however, little is known about its function in DNA damage repair in HCC. In this study, analysis of The Cancer Genome Atlas data and our tumor models after ionizing radiation (IR) treatment indicated that increased expression of *PRP19* was positively correlated with DNA damage repair. Gain of PRP19 expression induced by plasmids resulted in decreases in apoptosis and double-strand breaks (DSBs), and an increase in cell survival after IR. Loss of PRP19 expression induced by small interfering RNAs resulted in the accumulation of apoptosis and DSBs, and a decrease in cell survival. Mechanistically, the effect of PRP19 on DNA damage repair was mediated by the modulation of cyclin D1 expression in HCC. PRP19 controlled the translation of cyclin D1 by modulating eukaryotic initiation factor 4E. PRP19 affected the DNA damage repair ability of cyclin D1 by interacting with the WD40 domain. The combination of PRP19 and cyclin D1 was more valuable than each single marker for predicting the prognosis of patients. Taken together, the present results demonstrate that PRP19 promotes DNA damage repair by modulating cyclin D1 expression and function, thereby contributing to the radioresistance in HCC.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a major type of liver cancer, with approximately 841,000 new cases and 782,000 deaths in 2018.[1] HCC is characterized by a dormant onset and difficulty in early diagnosis. Most patients have progressed to the middle or late stage at the time of diagnosis, making them ineligible for surgery.[2–4] Radiotherapy is an effective treatment for most tumors[5]; however, HCC is not sensitive to this therapy.[6] Therefore, it is urgent to explore the mechanisms underlying resistance to improve the sensitivity of HCC to this therapy.

Radiotherapy destroys tumor cells by inducing extensive DNA damage.[7,8] In response to DNA lesions, cells initiate DNA damage repair by activating a precisely regulated network of signaling pathways and repair mechanisms to maintain genomic stability.[9] Although dysregulation of the DNA damage repair pathway is correlated with the predisposition to cancer development, it can also contribute to tolerance or hypersensitivity of tumors to treatment and can be exploited to improve cancer therapy. For example, pY240-PTEN upregulation facilitates the recruitment of RAD51 to promote DNA repair, resulting in glioblastoma resistance to radiotherapy, whereas pY240-PTEN inhibition blocks DNA damage repair, conferring radiation sensitivity to tumors.[10]

In previous work, we showed that downregulation of pre-mRNA processing factor 19 (PRP19) increased the sensitivity of HCC to chemotherapeutic drugs, including cisplatin and doxorubicin.[11] PRP19 is a multifunctional protein with highly conserved properties across species. It is involved in spliceosome assembly, activation, and stability, as well as ubiquitination and DNA damage repair.[12] PRP19 plays a direct role in DNA damage repair by recruiting DNA repair proteins and modulating cell-cycle progression.[12,13] Suppression of PRP19 contributes to the induction of cell apoptosis, whereas upregulation of PRP19 exerts a protective effect on DNA damage in HeLa cells.[14,15] Recent studies demonstrated that PRP19 functions in DNA damage repair by modulating BRCA1 expression[16] and interacting with replication protein A.[17] Although these findings indicate that PRP19 is involved in the DNA damage repair pathway, its exact role in this process remains unclear in HCC.

In this report, we describe the role of PRP19 in HCC DNA damage repair and resistance to ionizing radiation (IR). The effect of PRP19 on DNA damage repair induced by γ radiation was studied. The mechanism underlying the role of PRP19 in DNA damage repair

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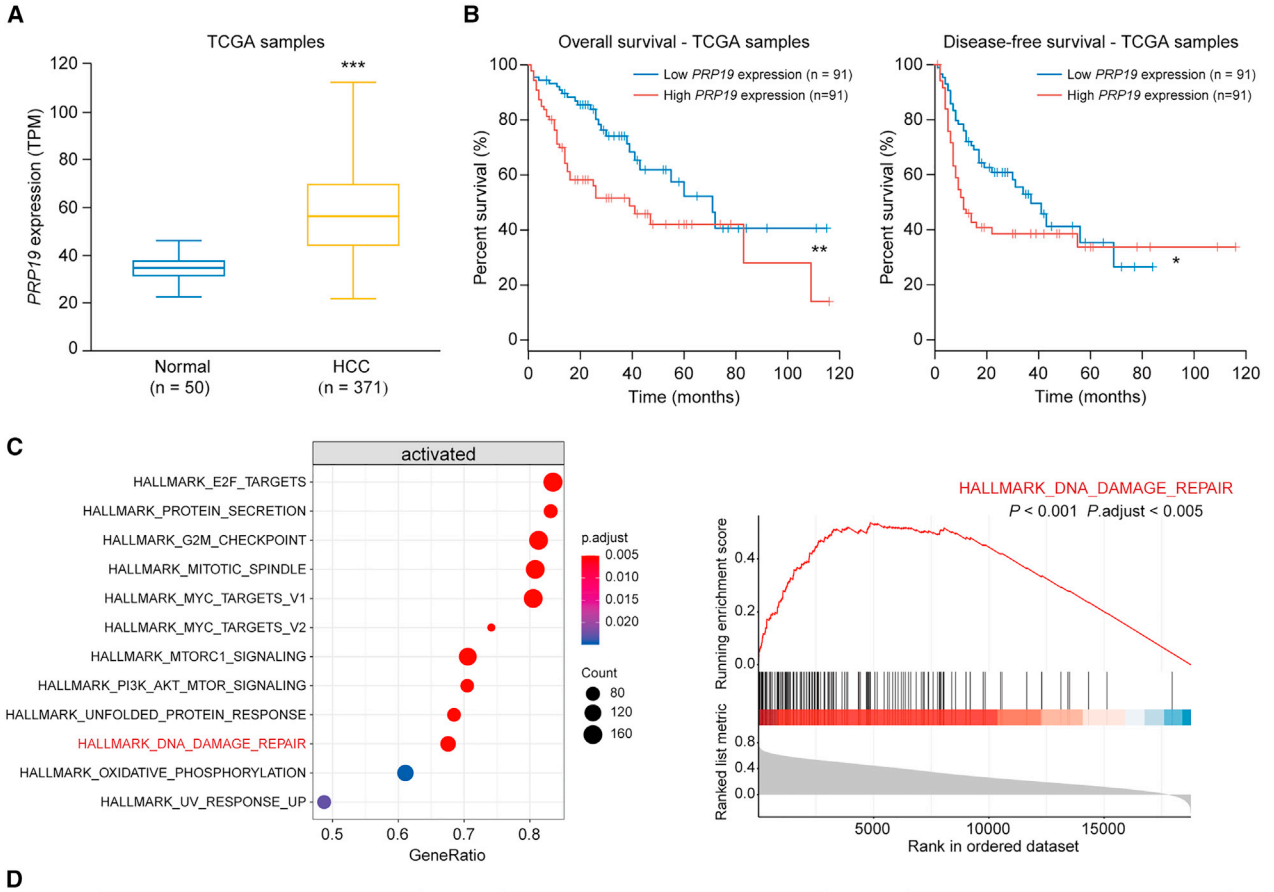

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was elucidated, and the correlation and clinical significance of PRP19 and related genes were analyzed in clinical samples.

RESULTS

Increased expression of PRP19 is positively correlated with DNA damage repair

To study the role of PRP19 in HCC, we first analyzed data from The Cancer Genome Atlas (TCGA). The results showed that *PRP19* was elevated in HCC (Figure 1A) and associated with poor prognosis in patients with HCC (Figure 1B). Biological functions of *PRP19* were explored with Gene Set Enrichment Analysis (GSEA) in HCC. The results demonstrated that *PRP19* had a positive role in DNA damage repair ($p < 0.05$, Normalized Enrichment Score [NES] = 1.89; Figure 1C). Spearman correlation analysis from TCGA database showed that *PRP19* was positively associated with DNA damage repair markers (including *RAD51*, checkpoint kinase 1 [*CHEK1*], and replication protein A2 [*RPA2*]) in HCC tissues (Figure 1D). We then established HCC subcutaneous tumor models treated with IR (Figure 1E). In comparison with that in control tumors, the expression of *PRP19*, *RAD51*, *CHEK1*, and *RPA2* was elevated in the tumor tissues after IR treatment (Figure 1F), thus indicating that IR induced PRP19 expression and PRP19 may participate in DNA damage repair. Collectively, these findings reveal that PRP19 upregulation is positively correlated with DNA damage repair in HCC.

PRP19 contributes to radioresistance through regulating DNA damage repair in HCC

PRP19 expression was first detected in normal hepatocytes (L02) and HCC lines. The results showed that PRP19 protein levels in six HCC cell lines were dramatically higher than those in L02 cells (Figure S1A). IR and cisplatin, classical methods to cause DNA damage,[9] were then used to further study the role of PRP19 in DNA damage repair. Colony formation assays and flow cytometry were performed to study the role of PRP19 in radiosensitivity. PRP19 upregulation increased colony numbers and decreased cell apoptosis, whereas PRP19 knockdown reduced colony numbers and increased cell apoptosis after IR ($p < 0.05$; Figures 2A and 2B). Overexpression of PRP19 decreased cisplatin-induced apoptosis, whereas silencing of PRP19 increased cisplatin-induced apoptosis ($p < 0.05$; Figure S1B). Because IR destroys tumor cells through inducing DNA damage,[7] γ-H2AX, a sensitive marker of DNA damage,[18,19] was detected to examine the effect of PRP19 on DNA damage repair. The treated cells were exposed to 10 Gy of IR and then allowed to recover for 0 h, 0.5 h, 1 h, 2 h, and 6 h. γ-H2AX-positive cells were less abundant in the PRP19 overexpression group, whereas γ-H2AX-positive cells were more abundant in the PRP19 knockdown group ($p < 0.05$, Figure 2C).

The effect of PRP19 on DNA damage repair was examined *in vivo* in subcutaneous tumors of nude mice treated with radiotherapy. Our results demonstrated that PRP19 expression had no effect on tumor growth in mice that did not receive radiotherapy (Figure S2). PRP19 overexpression promoted tumor growth, whereas PRP19 downregulation reduced tumor growth in mice receiving radiotherapy ($p < 0.05$; Figure 3A). The results of the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) test demonstrated that the number of apoptotic cells decreased after PRP19 overexpression and significantly increased after PRP19 knockdown ($p < 0.01$; Figure 3B). The number of γ-H2AX-positive cells was lower in PRP19-overexpressing cells and higher in PRP19-depleted cells ($p < 0.05$; Figures 3C and 3D). Taken together, these findings suggest that PRP19 promotes HCC resistance to radiotherapy by participating in DNA damage repair.

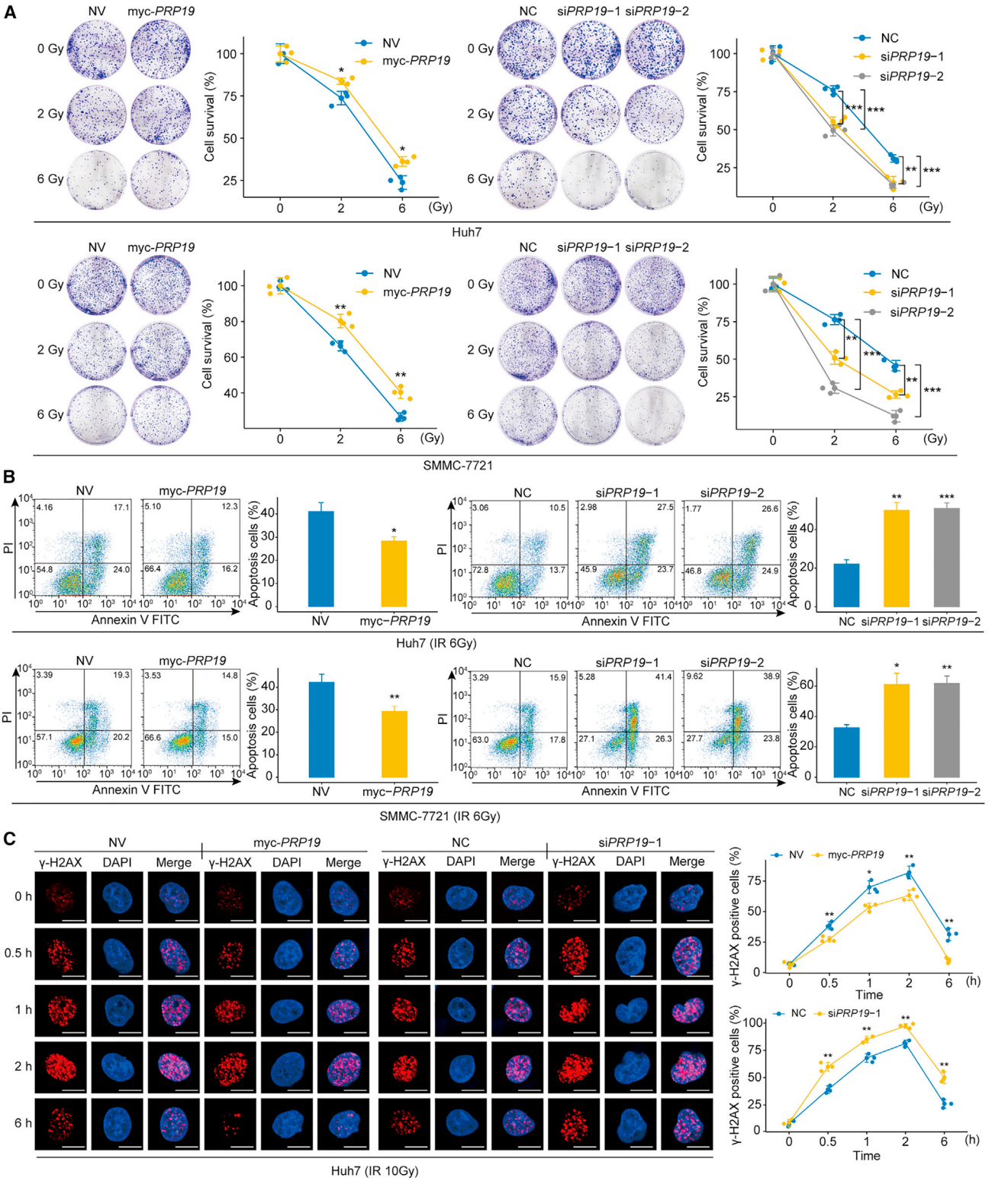
PRP19 functions in DNA damage repair by modulating cyclin D1 expression

Next, we examined the mechanism underlying the role of PRP19 in DNA damage repair. We found that cyclin D1 expression was also elevated in the tumor tissues after IR treatment (Figure 4A), which was proved to be involved in promoting DNA damage repair and radioresistance in multiple tumors.[20–22] Further examination indicated that PRP19 overexpression upregulated cyclin D1, whereas PRP19 knockdown downregulated cyclin D1 ($p < 0.01$; Figures 4B and S3A). Myc-*PRP19* plasmid was used as a bait to induce flag-cyclin D1 expression in the plasmid co-transfection assays in 293T cells. The results demonstrated that cyclin D1 expression increased in correlation with the increase of *PRP19* plasmid concentration, confirming that PRP19 regulates cyclin D1 expression (Figure 4C). The results of *in vivo* experiments were consistent with *in vitro* results ($p < 0.05$; Figures 4D and 4E).

To investigate whether PRP19 functions in DNA damage repair by regulating cyclin D1 expression, rescue assays were performed. Colony number was decreased after IR under PRP19 overexpression and cyclin D1 knockdown conditions, whereas colony number was increased after IR under PRP19 knockdown and cyclin D1 upregulation (Figures 4F and S3B). Cyclin D1 knockdown promoted cell

Figure 1. Increased expression of PRP19 is positively correlated with DNA damage repair

(A) Human mRNA expression of *PRP19* in normal liver (n = 50) and HCC tissues (n = 371) from TCGA database. The results were analyzed with Student's t test. Adapted from UALCAN: http://ualcan.path.uab.edu/index.html. TPM, transcripts per million. (B) OS and DFS curves for HCC patients with high and low *PRP19* expression from TCGA database using the Kaplan-Meier method. Adapted from GEPIA: http://gepia.cancer-pku.cn/. Cutoff-high = 75%, cutoff-low = 25%. (C) The relationship between *PRP19* and DNA damage repair was assessed in TCGA by Spearman correlation analysis and GSEA. (D) Correlations of *PRP19* and *RAD51*, *CHEK1*, or *RPA2* in HCC tissues by Spearman correlation analysis from TCGA database. Adapted from GEPIA: http://gepia.cancer-pku.cn/. TPM, transcripts per million. (E) HCC subcutaneous tumor models were established with Huh7 cell lines (n = 8). Tumors in IR group received 6 Gy of radiation on days 5, 8, 11, 14, and 17 after tumor cell injection using Cs137 as a source of IR (the remaining mice were shielded from radiation). The tumor tissues were resected at day 20 after tumor cell injection. The results were analyzed with Student's t test. (F) Immunohistochemical assays and qRT-PCR were conducted to examine the expression of *PRP19*, *RAD51*, *CHEK1*, and *RPA2* in the tumor tissues after IR treatment (n = 8; scale bar, 50 μm). The results were analyzed with Student's t test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.



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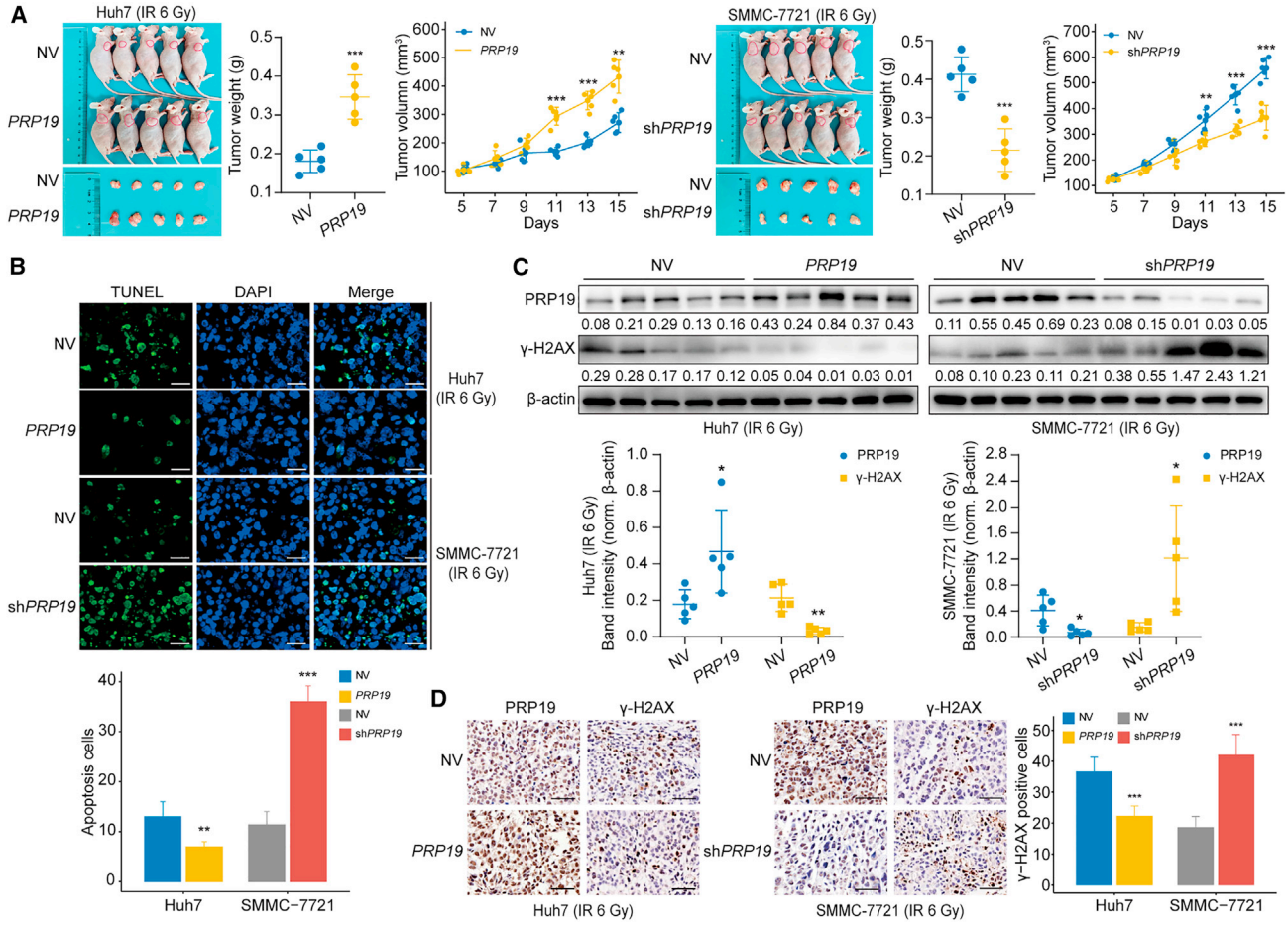


Figure 3. PRP19 functions in DNA damage repair in HCC *in vivo*

(A) Effects of PRP19 overexpression and depletion on tumor growth after radiotherapy (n = 5). (B) TUNEL assays showing the effects of PRP19 overexpression and depletion on cell apoptosis driven by radiotherapy (n = 5; scale bar, 25 μm). Western blot assays (C) and IHC (D) indicating the effects of PRP19 overexpression and depletion on the expression of γ-H2AX under radiotherapy (n = 5; scale bar, 50 μm). The results were analyzed with Student's t test. NC, negative control. **p < 0.01, ***p < 0.001.

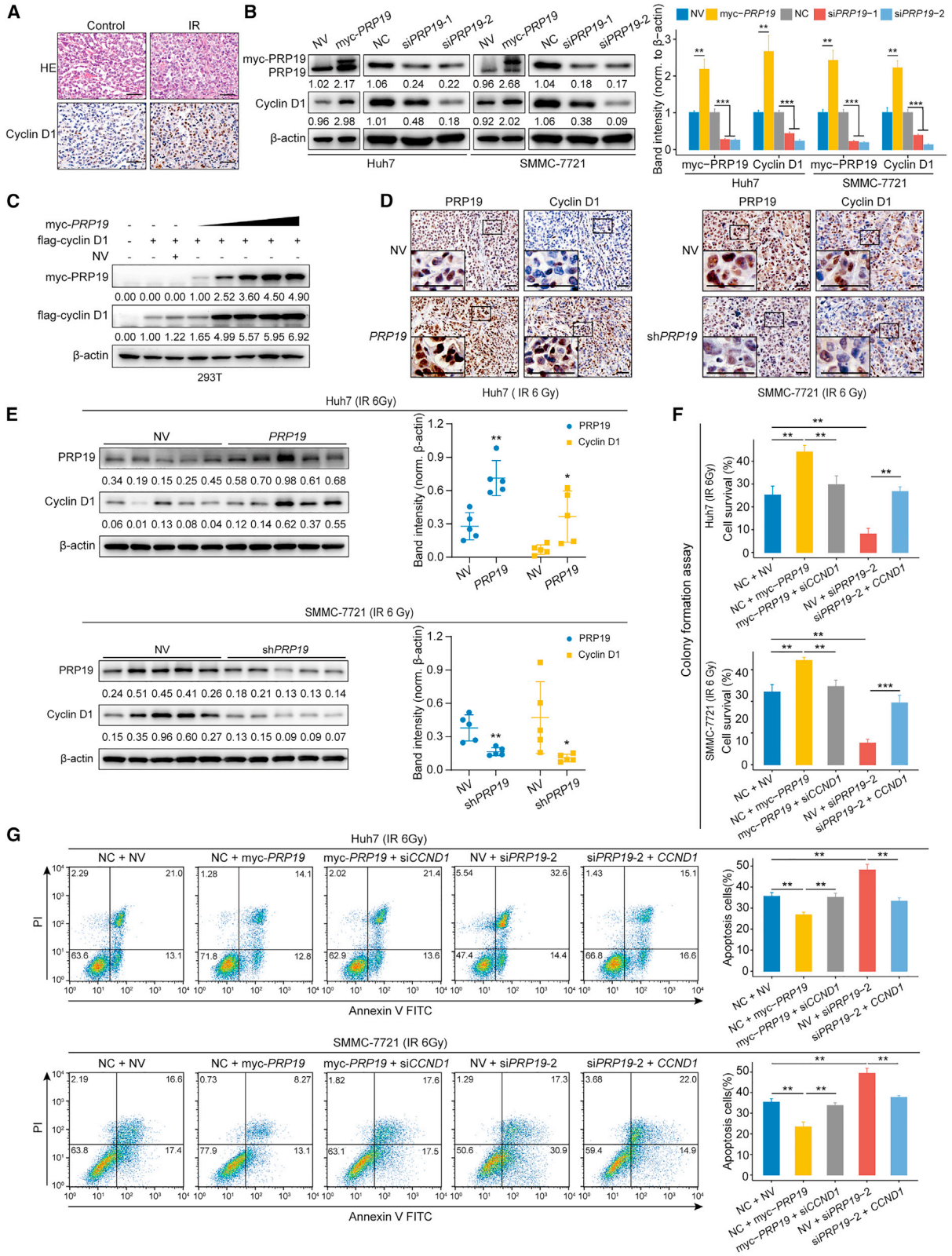
apoptosis induced by IR in cells overexpressing PRP19, whereas cyclin D1 overexpression reduced cell apoptosis induced by IR in cells with PRP19 knockdown (Figures 4G and S3C). Taken together, these results indicate that PRP19 promotes DNA damage repair by upregulating cyclin D1 expression.

PRP19 controls cyclin D1 translation through modulating eIF4E expression

The potential mechanism by which PRP19 regulates cyclin D1 expression was explored. PRP19 had no significant effect on the transcription, splicing, and nucleoplasm distribution of cyclin D1 mRNA (*CCND1*; Figures S4A–S4C). In addition, PRP19 had no obvious effect on the stability of the cyclin D1 protein (Figures S4D and S4E). Considering that both PRP19 and cyclin D1 are cyclin proteins in HCC,[23,24] we examined whether the underlying mechanism involved the cell cycle. The results showed that the regulation of cyclin D1 by PRP19 was independent of the cell cycle (Figure S4F). Dual-luciferase reporter assays demonstrated that overexpression of PRP19 increased the luciferase activity of the *CCND1* 5′ UTR, whereas downregulation of PRP19 decreased luciferase activity ($p < 0.01$; Figure 5A), indicating that PRP19 affected cyclin D1 expression by controlling its translation.

Figure 2. PRP19 functions in DNA damage repair in HCC *in vitro*

Colony formation assays (A) and flow cytometry (B) showing the effects of PRP19 overexpression and depletion on cell viability or apoptosis in HCC cells after IR (n = 3). The results were analyzed with Student's t test. (C) Immunofluorescence assays showing the effects of PRP19 overexpression and depletion on the expression of γ-H2AX after exposure to IR (n = 3; scale bar, 10 μm). At 48 h after transfection with the indicated plasmids or siRNAs, cells were treated with 10 Gy of IR (source: Cs137). At 0 h, 0.5 h, 1 h, 2 h, and 6 h after IR, cells were fixed and processed for immunofluorescence confocal microscopy by staining with an antibody against γ-H2AX; the γ-H2AX-positive cells were then quantified. For each set of conditions, a total of ≥500 cells were counted. NC, negative control. *p < 0.05, **p < 0.01, ***p < 0.001.



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Previous studies reported that translation of the 5′ UTR of CCND1 is regulated by eukaryotic initiation factor 4E (eIF4E) and eukaryotic initiation factor 4A1 (eIF4A1).[25–27] Therefore, we explored whether PRP19 affected CCND1 translation by modulating eIF4E and eIF4A1. eIF4E was upregulated after PRP19 overexpression and downregulated after PRP19 downregulation in Huh7 and SMMC-7721 cells, whereas eIF4A1 expression was not affected (Figures 5B and 5C). eIF4E downregulation suppressed cyclin D1 expression in cells overexpressing PRP19 compared with PRP19 overexpression alone, whereas eIF4E overexpression increased cyclin D1 expression in cells with PRP19 downregulation compared with PRP19 knockdown alone (Figures 5D and 5E). A dual-luciferase reporter assay was performed to investigate whether PRP19 affected the luciferase activity of the *CCND1* 5′ UTR by modulating eIF4E. The luciferase activity of the *CCND1* 5′ UTR was decreased in response to PRP19 upregulation and eIF4E reduction and increased in response to PRP19 downregulation and eIF4E overexpression (Figure 5F). Collectively, these findings suggest that PRP19 controls cyclin D1 translation by modulating eIF4E expression.

PRP19 is involved in DNA damage repair through affecting cyclin D1 function

PRP19 is a recruiting platform for the exchange of DNA repair proteins to promote repair.[13,28] We investigated whether PRP19 interacts with cyclin D1 to facilitate DNA damage repair. Immunofluorescence assays showed that PRP19 and cyclin D1 co-localized in the nucleus and cytoplasm (Figure 6A). The results of the co-immunoprecipitation (coIP) assays showed that PRP19 interacted with cyclin D1 (Figure 6B). *PRP19* depletion mutant plasmids were constructed to identify the binding sites according to previous reports.[29–31] The results indicated that PRP19 binds to cyclin D1 through the WD40 domain (Figure 6C). Next, we examined the effect of the WD40 domain on DNA damage repair mediated by cyclin D1. To exclude the influence of PRP19 background levels on the experimental results, PRP19 was depleted in SMMC-7721 cells. The treated cells were exposed to 10 Gy of IR and then allowed to recover for 8 h. The number of γ-H2AX-positive cells was lower in the sh*PRP19* + *CCND1* group than in the sh*PRP19* group (Figures 6D and 6E), indicating that cyclin D1 promoted DNA damage repair. γ-H2AX-positive cells were dramatically lower in the sh*PRP19* + *CCND1* + *PRP19* group than in the sh*PRP19* + *CCND1* group, whereas the number of γ-H2AX-positive cells did not change in sh*PRP19* + *CCND1* + *PRP19* WD40 depletion (WD) group (Figures 6D and 6E). These findings indicate that PRP19 may affect DNA damage repair mediated by cyclin D1 through the WD40 domain.

Correlation between the expression of PRP19 and cyclin D1 and their prognostic significance in HCC

After clarifying the role of PRP19 in the regulation of cyclin D1 expression and function, we examined the potential correlation between PRP19 and cyclin D1 in clinical HCC samples. Immunohistochemistry (IHC) results indicated that PRP19 was overexpressed in HCC compared with non-tumor tissues (5.187 ± 2.286 versus 3.647 ± 1.667, $p < 0.001$; Figure 7A). High levels of PRP19 were detected in 58 out of 139 (41.73%) HCC samples, whereas high levels were only observed in 21 out of 139 (15.11%) non-tumor samples (Figure 7A). Cyclin D1 expression was higher in HCC than in non-tumor tissues (4.799 ± 1.814 versus 3.633 ± 1.280, $p < 0.001$; Figure 7B). High levels of cyclin D1 were detected in 51 out of 139 (37.41%) HCC samples, whereas high levels were only observed in 15 out of 139 (10.79%) non-tumor samples (Figure 7B). Cyclin D1 expression was positively correlated with PRP19 expression (Figure 7C), which was confirmed by western blotting (Figure 7D). The high expression of PRP19 and cyclin D1 was correlated with tumor size, vascular invasion, and tumor-node-metastasis (TNM) stage (Tables 1 and S1). Furthermore, the combination of PRP19 and cyclin D1 was more valuable than a single marker for predicting overall survival (OS) and disease-free survival (DFS) in HCC patients (Figures 7E, S5, S6; Table S2). These findings suggest that PRP19 expression is positively correlated with cyclin D1 expression and their combination predicts a poor prognosis in HCC patients.

DISCUSSION

Radiotherapy, which destroys tumor cells by inducing DNA damage,[7] is not effective for HCC. Tumor cells are characterized by elevated DNA damage repair capacities, which accounts for the resistance to this therapy.[5,7,9] Although PRP19 is involved in DNA damage repair,[13,16,17] its precise role in HCC remains unknown. In this study, we showed that PRP19 is involved in DNA damage repair by modulating the expression and function of cyclin D1 in HCC.

PRP19 was originally studied for its pre-mRNA splicing function. In the past decade, several reports have described the role of PRP19 in tumors including larynx, lung, and colon cancer.[32,33] In our previous study, we explored the effect of PRP19 in HCC. We found that PRP19 is upregulated in HCC, promotes metastasis by preventing Twist1 degradation,[31] and promotes cell-cycle progression by modulating cell division cycle 5-like (Cdc5L) expression.[23] We also provided evidence that PRP19 affects the sensitivity of HCC to cisplatin.[11] Cisplatin, which has been used as a treatment for solid tumor for decades, exerts its antitumor activity by interacting with chromosomal DNA to form DNA intra- and inter-strand cross-links.[7] IR, a common modality for malignancies, can directly cause DNA damage

Figure 4. PRP19 is involved in DNA damage repair through the modulation of cyclin D1 expression

(A) Immunohistochemical assays were conducted to examine the expression of cyclin D1 in tumor tissues after IR treatment (n = 5; scale bar, 50 μm). Western blot analysis of the effects of PRP19 overexpression and depletion on cyclin D1 expression in HCC cell lines (B; n = 3) and 293T cell lines (C). IHC assays (D) and western blotting (E) evaluating the effects of PRP19 overexpression and depletion on cyclin D1 expression *in vivo* (n = 5; scale bar, 50 μm). (F) Colony formation assays evaluating cell viability in response to IR in Huh7 and SMMC-7721 cells under different treatments (n = 3). (G) Flow cytometry evaluating cell apoptosis induced by IR in Huh7 and SMMC-7721 cells under different treatments (n = 3). The results were analyzed with Student's t test. NC, negative control. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

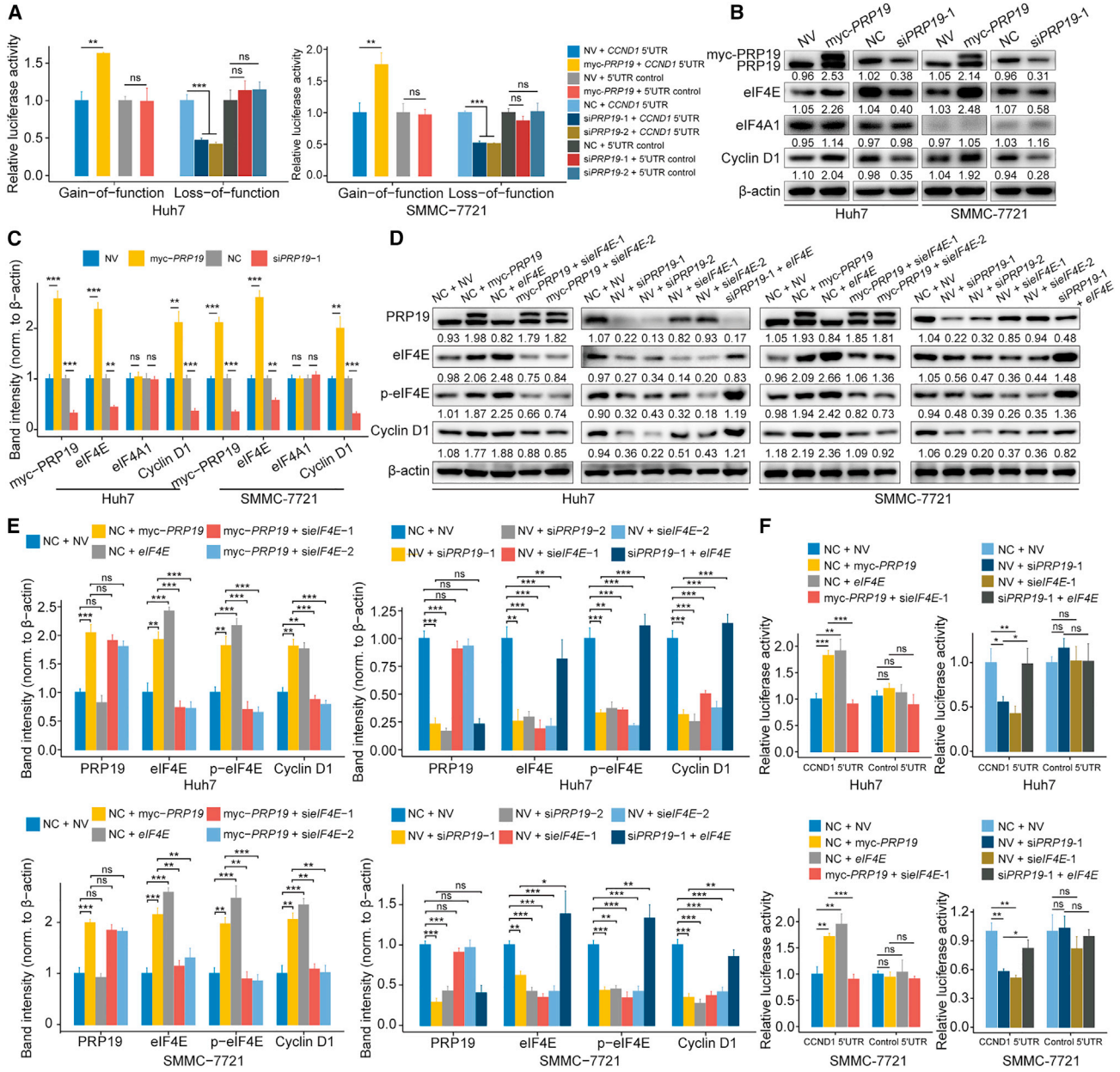

Figure 5. PRP19 controls cyclin D1 translation through the modulation of eIF4E expression and consequently affects cyclin D1 expression

(A) Dual-luciferase reporter assays showing the effects of PRP19 overexpression and depletion on the luciferase activity of the cyclin D1 5′ UTR (n = 3). (B and C) Western blot analysis of the effects of PRP19 overexpression and depletion on *eIF4E* expression in HCC cell lines (n = 3). (D and E) Western blot analysis of the effects of PRP19 on cyclin D1 and eIF4E expression (n = 3). (F) Dual-luciferase reporter assays evaluating the effect of PRP19 on the luciferase activity of cyclin D1 5′ UTR through the modulation of eIF4E expression (n = 3). The results were analyzed with Student's t test. NC, negative control. ns, not significant. *p < 0.05, **p < 0.01, ***p < 0.001.

and indirectly induce DNA lesions by generating reactive oxygen species (ROS).[9] In response to DNA damage, cells can repair the lesions and resume cell-cycle progression or undergo cell death by apoptosis when DNA repair fails.[9] In the present study, the analysis of TCGA data demonstrated that *PRP19* was elevated and positively associated with DNA damage repair in HCC. RAD51, CHEK1, and RPA2 have been reported to play decisive roles in DNA damage repair,[34,35] and RAD51 is considered to be a marker of DNA repair capability.[36] *PRP19* was positively associated with *RAD51*, *CHEK1*, and *RPA2* in TCGA database. Furthermore, *PRP19*, *RAD51*, *CHEK1*, and *RPA2* were increased in the residue tumor tissues after IR treatment. PRP19 overexpression promoted HCC resistance to IR and cisplatin,

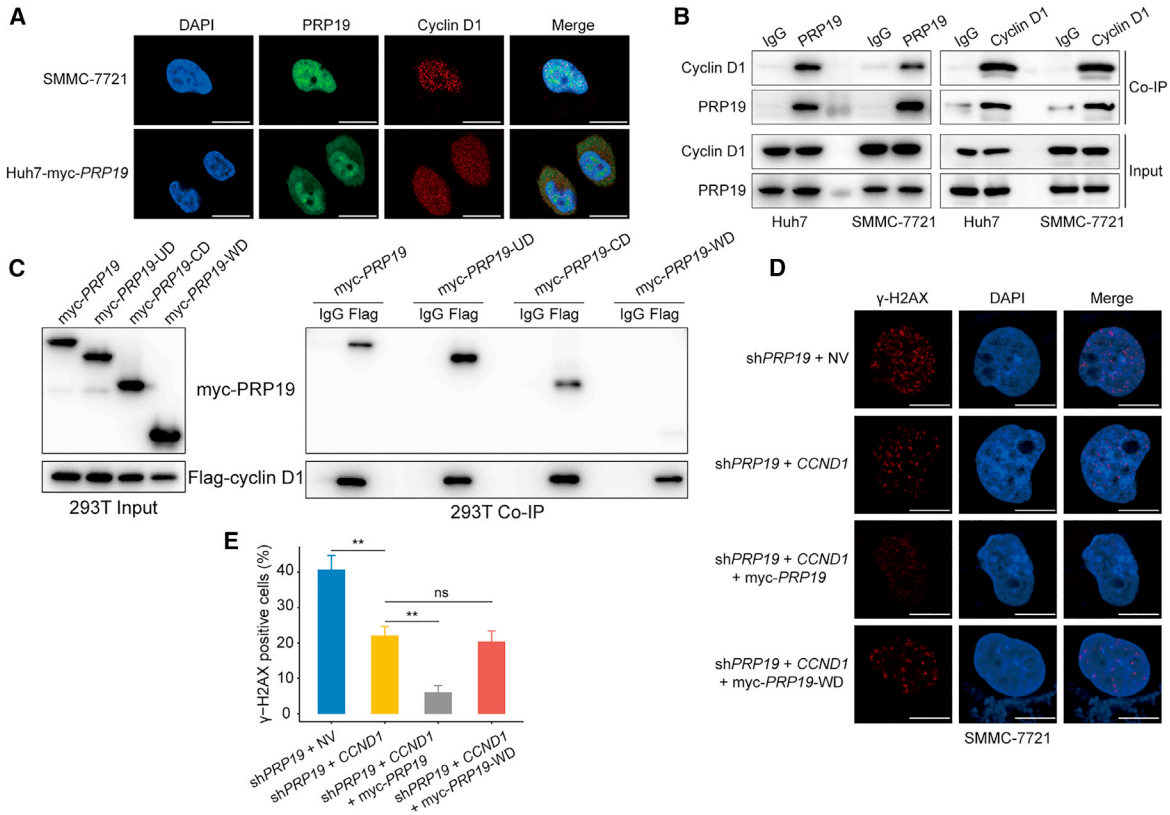

Figure 6. PRP19 is involved in DNA damage repair by affecting cyclin D1 function

(A) Nuclear and cytoplasmic expression of PRP19 and cyclin D1 was analyzed by immunofluorescence in Huh7 and SMMC-7721 cells (scale bar, 10 μm). (B) The physical interaction between PRP19 and cyclin D1 was assessed using coIP assays in Huh7 and SMMC-7721 cells. Immunoglobulin G (IgG) was used as a negative control. (C) The physical interaction between PRP19 (or its domain-depletion mutants) and cyclin D1 was evaluated by coIP in 293T cells. (D and E) Immunofluorescence assays showing the effect of PRP19 WD on the expression of γ-H2AX in cells treated with IR (n = 3, scale bar, 10 μm). The treated cells were exposed to 10 Gy of IR and then allowed to recover for 8 h. UD, U-box depletion; CD, coiled-coil domain depletion. The results were analyzed with Student's t test. ns, not significant. NC, negative control. **p < 0.01.

whereas its depletion improved the sensitivity to IR and cisplatin. Immunofluorescence assays and IHC showed that PRP19 promoted DNA damage repair in HCC after IR, which was consistent with the results of a study evaluating the effect of PRP19 on hydroxyurea-induced DNA damage.[16] Therefore, PRP19 contributes to resistance to IR by facilitating DNA damage repair in HCC.

We showed that PRP19 functions in DNA damage repair by modulating cyclin D1 expression in HCC. Cyclin D1 is involved in cell-cycle regulation by forming an active complex with cyclin-dependent kinase 4 (CDK4) or CDK6 to induce cell-cycle transition from G1 to the S phase.[37,38] Amplification of *CCND1*, which encodes cyclin D1, and overexpression of cyclin D1 are observed in human cancers, including HCC.[24,38,39] Cyclin D1 is involved in multiple cellular functions, such as cell proliferation, cell-cycle progression, and apoptosis, and is a predictor of poor prognosis in HCC.[40,41] Additionally, cyclin D1 promotes DNA damage repair in malignancies.[22,42,43] However, the role and precise mechanism underlying the role of cyclin D1 in DNA damage repair in HCC remain unknown. In the present study, we showed that the role of cyclin D1 in DNA damage repair in HCC is mediated by PRP19. PRP19 overexpression upregulated cyclin D1 and activated DNA damage repair, whereas PRP19 knockdown downregulated cyclin D1 and decreased DNA damage repair. Cyclin D1 was previously shown to participate in DNA damage repair by interacting with RAD51,[22] suggesting that the role of PRP19 in DNA damage repair in HCC is mediated by the cyclin D1-RAD51 interaction.

PRP19 regulates downstream target gene expression by modulating pre-mRNA splicing, mRNA translation, or protein ubiquitination.[23,31,44] In the present study, the effect of PRP19 on cyclin D1 expression was not mediated by the modulation of transcription, splicing, or the nucleoplasm distribution of *CCND1*, and the protein stability of cyclin D1 was not affected. Dual-luciferase reporter assays showed that PRP19 affected cyclin D1 expression by regulating its mRNA translation. The translation of the 5′ UTR of *CCND1* is regulated by eIF4E and eIF4A1.[25–27] This study showed that PRP19 regulated *CCND1* translation by inducing eIF4E expression rather than eIF4A1 expression, thus revealing the precise mechanism by which PRP19 regulates cyclin D1 expression in HCC.

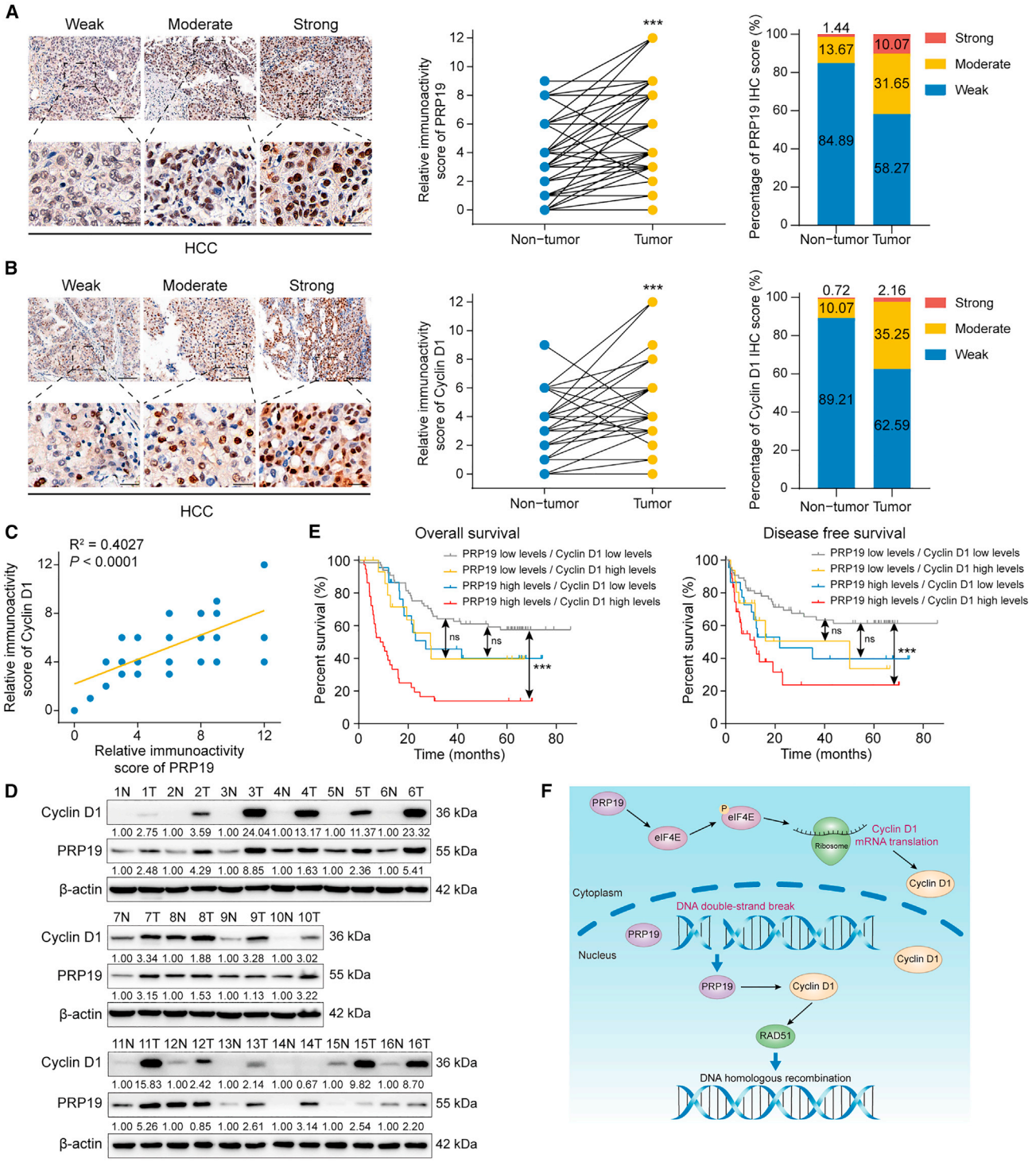

Figure 7. Correlation between the expression of PRP19 and cyclin D1 and their prognostic significance in HCC

Representative immunohistochemical staining and relative immunoactivity score of PRP19 (A) and cyclin D1 (B) in HCC and non-tumor tissues (n = 139); scale bars, 100 μm (upper), 25 μm (lower). The results were analyzed with Student's t test. (C) The correlation between PRP19 and cyclin D1 levels in HCC tissues using Spearman correlation analysis. (D) Western blot detection of PRP19 and cyclin D1 levels in HCC tissues. (E) OS and DFS curves for HCC cases with high and low PRP19 and cyclin D1 expression using the Kaplan-Meier method. (F) Schematic illustration of the role of PRP19 in DNA damage repair in HCC. ns, not significant. ***p < 0.001.

Table 1. Correlations between overexpression of PRP19 and cyclin D1 and clinicopathological features in HCC patients

Characteristics	Total (n = 139)	PRP19 + cyclin D1		Chi square	p value[a]
		High (n = 36)	Low (n = 103)		
Gender				0.063	0.802[b]
Male	128	34	94		
Female	11	2	9		
Age (years)				0.227	0.634
≤55	96	26	70		
>55	43	10	33		
AFP (ng/mL)				2.630	0.105
≤20	26	10	16		
>20	113	26	87		
HBsAg				0.077	0.782[b]
Negative	7	1	6		
Positive	132	35	97		
Tumor size (cm)				5.653	0.017
≤5	54	8	46		
>5	85	28	57		
Tumor number				1.135	0.287
Solitary	80	18	62		
Multiple	59	18	41		
Encapsulation				0.887	0.346
Complete	64	19	45		
Incomplete	75	17	58		
Cirrhosis				1.172	0.279[b]
Negative	19	3	16		
Positive	120	33	87		
Differentiation				0.031	0.860
I–II	91	24	67		
III–IV	48	12	36		
Vessel invasion				3.964	0.046
Negative	109	24	85		
Positive	30	12	18		
Lymph node metastasis				0.000	1.000[b]
Negative	133	34	99		
Positive	6	2	4		
TNM stage				7.443	0.006
I–II	88	16	72		
III–IV	51	20	31		

[a]Chi square test.
[b]Continuity correction.

PRP19 contains three main domains: an N-terminal U-box domain, a central coiled-coil domain, and a C-terminal WD40 repeat domain.[12] The function of *PRP19* is mediated by these domains, which can serve as interaction platforms for binding proteins.[12,13] The U-box domain of PRP19 exhibits ubiquitin ligase activity, and the central coiled-coil region is sufficient for PRP19 function in the context of the spliceosome.[12] However, the C-terminal WD40 repeat domain is the least understood. In the present study, we showed that PRP19 interacts with cyclin D1 through the WD40 domain. Furthermore, the DNA damage repair capacity of cyclin D1 was modulated by PRP19 through the WD40 domain. However, further study is needed to determine whether PRP19 recruits cyclin D1 to DNA damage sites through the WD40 domain.

There are currently no effective biomarkers for predicting the prognosis of HCC patients.[2,3] Therefore, identifying useful prognostic indicators is a challenging task. The present study demonstrated that both PRP19 and cyclin D1 are upregulated in HCC. There was a positive correlation between PRP19 and cyclin D1 expression in clinical samples. Furthermore, the combination of PRP19 and cyclin D1 was more valuable than each single marker for predicting OS and DFS of HCC patients. These findings indicate that the combination of PRP19 and cyclin D1 may be useful as a predictive indicator for HCC.

Taken together, the present results indicate that PRP19 plays an important role in DNA damage repair in HCC (Figure 7F). PRP19 functioned in DNA damage repair by modulating cyclin D1 expression and function, contributing to HCC resistance to radiotherapy. PRP19 controlled the translation of cyclin D1 by modulating the expression of eIF4E to affect the expression of cyclin D1. PRP19 and cyclin D1 expression was positively correlated in HCC patients, and their combination was more effective than each single marker for predicting the prognosis of patients. The present findings suggest that PRP19 silencing could be a promising therapeutic approach to improve HCC sensitivity to radiotherapy.

MATERIALS AND METHODS

Patient tissues and cell lines

A total of 139 pairs of HCC tissues and adjacent non-tumor samples were obtained from patients who underwent surgery at our institution. Informed consent was obtained from all participants, and the study was approved by the Institutional Ethics Committee of Zhongshan Hospital Fudan University (no. B2020-130R) and conducted following the Declaration of Helsinki. The inclusion criteria were as follows: (1) HCC diagnosed by pathology; (2) no previous treatment for cancer; (3) complete resection of all tumor lesions with the cut surface being free of cancer by histologic examination; and (4) availability of complete clinicopathologic and follow-up data.[45,46]

Huh7, PLC/PRF/5, SMMC-7721, Hep3B, L02, and 293T cell lines were obtained from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). MHCC97-H and HCCLM3 were established in the liver cancer institute of our institution.[47] All cell lines except Hep3B were cultured in Dulbecco's modified Eagle's medium (Wisent, Nanjing, China), whereas Hep3B cells were cultured in minimum essential medium (Wisent). All culture media were supplemented with 10% fetal bovine serum (Corning, NY) and 1% penicillin/streptomycin (YEASEN, Shanghai, China) in an atmosphere

containing 5% CO_2 at 37°C. Other cultivation conditions were as documented in our previous study.[37,48]

qRT-PCR

Total RNA was extracted from cells or tissues using RNAiso Plus (TaKaRa, Beijing, China). Cytoplasmic and nuclear RNA extraction was performed using a Cytoplasmic & Nuclear RNA Purification Kit (NORGEN, ON, Canada) according to the protocol. cDNA was synthesized using the PrimeScript RT reagent kit with gDNA Eraser, and PCR analyses were performed using TB Green Premix Ex Taq (TaKaRa). The conditions for the reverse transcription reaction were as follows: 37°C for 15 min and then 85°C for 5 s. The conditions for PCR reaction were as follows: 95°C for 30 s for one cycle, and then 95°C for 5 s, 60°C for 34 s for 40 cycles. β-Actin was used as the internal control. Expression of every mRNA was examined with the $2^{-\Delta\Delta Ct}$ method. The primers used for *Actin*, *PRP19*, *CCND1*, *CCND1b*, *eIF4E*, *LMNB1*, *RAD51*, *CHEK1*, and *RPA2* are listed in Table S3.

Plasmids, small interfering RNAs, lentivirus particles and cell transfection

PRP19 and its depletion mutant plasmids were established in the pcDNA3.1/myc-His (−) vector according to our previous study,[31] and the primers for the construction of these plasmids are shown in Table S4. The *CCND1* plasmid was constructed by inserting a full-length *CCND1* coding sequence into the pEZ-M35 vector (GeneCopoeia, Guangzhou, China). The *eIF4E* plasmid was generated by inserting a full-length *eIF4E* coding sequence into the pEX-2 vector (GenePharma, Shanghai, China). Negative vector (NV) was an empty control plasmid. Small interfering RNAs (siRNAs) against *PRP19*, *CCND1*, and *eIF4E* were acquired from GenePharma and the sequences are provided in Table S5. The transfection of plasmid vectors and siRNAs was carried out using Lipofectamine 3000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The transfected cells were treated with cycloheximide (CHX; 50 μg/mL; Sigma-Aldrich, St Louis, MO) or MG132 (20 μmol/L; Sigma-Aldrich) for indicated times to measure cyclin D1 stability. Subsequently, these cells were collected for western blotting analysis.

PRP19 overexpression lentivirus particles were purchase from Hanyin (Shanghai, China). *PRP19* short hairpin RNA (shRNA) lentivirus particles were obtained from Santa Cruz (Dallas, TX), which is a pool of three different shRNA plasmids. The sequences of shRNAs are provided in Table S6. With the help of polybrene (5 μg/mL; Santa Cruz), lentivirus particles were used to transfect cell lines according to the manufacturer's protocols. 48 h after transfection, the cells were selected in puromycin (2 μg/mL; Beyotime, Shanghai, China) for 7 days.

Animal studies

Animal care and experimental procedures were approved by the Animal Ethics Committee of Zhongshan Hospital Fudan University (nos. 2019-074 and 2021-057). All animals received humane care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals. BALB/c nude mice (5 weeks old, male) were acquired from Shanghai SLAC Laboratory Animal Company (Shanghai, China). Approximately 10^7 stable HCC cells suspended in 200 μL of phosphate-buffered saline were subcutaneously injected into the right dorsal flank of each mouse. To establish IR tumor models, we administered 6 Gy of radiation to tumors on days 5, 8, 11, 14, and 17 after tumor cell injection by using Cs137 as a source of IR (the remaining mice were shielded from radiation). To study the effect of *PRP19* expression on DNA damage repair, we administered 6 Gy of radiation to tumors on days 5 and 10 after tumor cell injection. Tumor growth was evaluated every other day, and tumor volumes were calculated using the following formula: tumor volume (mm^3) = (1/2) × (tumor length) × (tumor width)^2. Tumor weight was assessed at the end of studies.

Statistical analysis

Statistical analysis was performed using R software (R 3.5.3) or SPSS 22 for Windows (SPSS, Chicago, IL). Data are presented as the mean ± standard deviation (SD) from at least three independent studies. The Student's t test was used to analyze the differences between two groups, and one-way analysis of variance (ANOVA) was used to evaluate the differences between more than two groups. The chi square test was used to analyze the correlations between the target protein levels and clinical characteristics. The Kaplan-Meier method, univariate and multivariate Cox proportional hazards regression analyses, and a nomogram were used to assess the prognostic factors for predicting OS and DFS. Statistical significance was set at $p < 0.05$ (two sided).

A complete description of other materials and methods, including IHC and IHC score criteria, western blotting and coIP), terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) assays, flow cytometry and colony formation assays, immunofluorescence assays, dual-luciferase reporter assays, and identification of *PRP19*-related functions, can be found in the supplemental information.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.omtn.2021.12.002.

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AUTHOR CONTRIBUTIONS

X.-N.Y., G.-C.Z., H.-N.L., J.Y., and X.-Z.S. designed the experiment. X.-N.Y., G.-C.Z., and H.-N.L. conducted the experiments. T.-T.L., J.-M.Z., G.-Q.S., L.D., J.Y., and X.-Z.S. examined and interpreted the data. X.-N.Y., G.-C.Z., and H.-N.L. drafted the paper. J.Y. and X.-Z.S. revised the manuscript. All authors have read and approved the final submitted manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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