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Research Article

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Inhibitory Effect of TRIM22 Overexpression on the Proliferation of Hepatocellular Carcinoma through CREB1 Degradation

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Abstract

Tripartite motif-containing 22 (TRIM22) belongs to the extensive tripartite motif (TRIM) protein family, hypothesized to act as a tumor suppressor among various malignancies. However, its definitive role as well as clinical implications in hepatocellular carcinoma (HCC) have remained unclear. Our preliminary findings suggest frequent downregulation of TRIM22 expression in primary HCC samples. Conversely, we observed a favorable prognosis associated with overexpression of TRIM22. Rigorous experimentation revealed that elevated levels of TRIM22 significantly restricted the proliferation potential of HCC. Upon delving into the mechanistic intricacies, we identified a direct interaction between TRIM22 and cAMP response element-binding protein 1 (CREB1), a pivotal player in cancer growth regulation. In the presence of TRIM22, CREB1 undergoes ubiquitination and subsequent degradation, inhibiting the expression of CREB1-related genes in HCC. Importantly, the inhibitory effect of TRIM22 on HCC proliferation was dependent on CREB1 degradation. Therefore, for the first time, the present study proved the crucial anti-tumor role of TRIM22 in HCC tumorigenesis and highlighted its potential as a prognostic and therapeutic target in the treatment of HCC.

Keywords: HCC, TRIM22, CREB1, Proliferation, Ubiquitination.

1. Introduction

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related deaths worldwide [1, 2]. Establishing accurate prognostication and prediction strategies for HCC is challenging due to its extensive heterogeneity and high malignancy, resulting in delayed diagnosis and limited efficacy of treatments [3, 4]. Current treatment options, such as surgical resection, percutaneous ablation, and systemic therapies, offer only marginal improvement for advanced HCC patients, who often face a poor prognosis with a five-year survival rate of 15 % [5, 6]. Hence, a comprehensive exploration of the molecular mechanisms driving HCC progression becomes crucial in order to enhance therapeutic strategies and patient outcomes.

Tripartite motif-containing 22 (TRIM22) belongs to the tripartite motif (TRIM) protein family, which comprises nearly 75 proteins[7]. Most TRIM proteins possess distinctive domains, including the RING finger, B-box, and coiled-coil domains [8]. These domains confer upon TRIM proteins diverse capabilities ranging from regulating innate immunity to influencing cellular proliferation, autophagy, and tumorigenesis [9]. TRIM22 is known as an Immune interferon -γ inducible E3 ubiquitin ligase that exerts important influence on antipathogenic mechanisms, such as inhibiting the replication of HBV, HIV, and Encephalomyocarditis virus (EMCV) [10-13]. Additionally, TRIM22 plays a multifaceted role in various cancers. In osteosarcoma, TRIM22 destabilizes nuclear factor erythroid 2-related factor 2, thereby activating the reactive oxygen species (ROS) / AMP-activated protein kinase (AMPK) signaling pathway and suppressing tumor growth [14]. Conversely, it accelerates IκBα degradation to stimulate the pro-tumorigenic nuclear factor kappa B signaling pathway in glioblastoma [15]. A contrasting role is observed in gastric cancer, where increased TRIM22 expression inhibits tumor growth and migration by attenuating Smad2 phosphorylation [16]. These studies collectively

demonstrate that the role of TRIM22 in cancer regulation is contingent on the specific tumor context. Nevertheless, the impact of TRIM22 in HCC remains unclear.

In this study, our findings revealed a significantly positive correlation between TRIM22 expression and the progression and outcome of HCC patients. Importantly, we determined that TRIM22 impedes HCC proliferation. Furthermore, we elucidated the underlying mechanism by identifying a direct interaction between TRIM22 and cAMP response element-binding protein 1 (CREB1), a pivotal transcription factor involved in cellular growth and associated with key oncogenes such as MYC, E2F1, and Cyclin A1. The engagement of TRIM22 with CREB1 led to the ubiquitination and subsequent degradation of CREB1, shedding light on its tumor-suppressive attributes. Essentially, this research uncovered the potential of TRIM22 as a formidable tumor suppressor in HCC. By unraveling its intricate molecular interactions and functional outcomes, we propose TRIM22 as a promising therapeutic target, providing hope for patients grappling with this aggressive malignancy.

2. Methods

2.1. Patients and Specimen Collection

HCC specimens and their corresponding non-tumorous counterparts were obtained from 12 patients who underwent surgical resection at the Department of Hepatobiliary Surgery, The Affiliated Hospital of the Affiliated Hospital of North Sichuan Medical College, in 2022. To ensure the preservation of cellular and molecular integrity, tissues were extracted and immediately frozen in liquid nitrogen for subsequent analyses. This study was conducted in accordance with the ethical standards and protocols established by the Ethics and Research Committees of the Affiliated Hospital of North Sichuan Medical College, situated in Nanchong, China. All patients involved were provided with detailed information about the research objectives and nature, and informed consent was obtained to protect patients' rights and autonomy.

2.2. Antibodies and Reagents

A variety of primary antibodies were utilized for this study, including Anti-MYC, Anti-E2F1, Anti-Cyclin A1, and Anti-TRIM22 (Abcam, Shanghai, China), as well as Anti-GAPDH, Anti-Ub, Anti-MYC-Tag, and Anti-Flag-Tag (Proteintech Group, Inc., Wuhan, China). Cycloheximide (CHX) was obtained from MCE (MedChemExpress, Shanghai, China).

2.3. Cell Lines and Transfection

HCC cell lines (HepG2, Huh7, QGY-7703, MHCC97, Hep3B, and SMMC-7721) and HEK293T cells were acquired from the American Type Culture Collection (Rockville, MD, USA). These cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Hyclone, Logan, UT) supplemented with 10% fetal bovine serum (FBS) (Sciencell, Carlsbad, CA). and maintained at 37°C with 5% CO2. Lipofectamine3000 (Thermofisher, Shanghai, China) was used for genetic manipulations according to the provided protocols. After 48 hours of transfection, the cells were subjected to further experimental procedures.

2.4. Plasmid Construction

Full-length human TRIM22 cDNA was amplified and cloned into the pLenti-CMV-GFP-puro vector (addgene, Plasmid #6585). In addition, full-length Flag-TRIM22 cDNA and myc-CREB1 cDNAs were amplified and then cloned into the 3xFlag-cmv-vector and pcDNA3.1/myc-His vector, respectively. The primer sequences for TRIM22 and CREB1 can be found in Supplementary Table S1. The shRNA oligonucleotides were annealed and integrated into the pLKO.1-TRC vector (addgene, Plasmid #10878) using T4 DNA ligase (Takara, Beijing, China). The primer sequences for shRNA are detailed in Supplementary Table S2. All constructs were verified through DNA sequencing.

2.5. RNA Extraction And Real-Time Quantitative Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from tissues and cells using Trizol reagent (Takara, Beijing, China). RNA was reverse-transcribed using murine leukemia virus (MLV) reverse transcriptase (Vazyme, Nanjing, China) along with a cDNA cycle kit (Vazyme), following the provided protocols. The synthesized cDNA was analyzed on a StepOne Plus quantitative Real-time PCR system (Takara) with a standard SYBR Green PCR kit (Vazyme). The mRNA expression levels of individual genes were quantified utilizing the 2-□□CT method. Primer pair sequences for qRT-PCR can be found in Supplementary Table S1.

2.6. Western Blot Analysis

Protein lysates from cells and tissues were obtained using RIPA lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China) following the recommended protocol. The lysates were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes (BioRad, Shanghai, China). After blocking with 5% skim milk, membranes were incubated with specific antibodies. Signal detection was performed using a chemiluminescence system (Odyssey, Wisconsin, USA).

2.7. Cell Viability Assay

For the cell growth curve analysis, 2,000 cells were seeded per well in a 96-well plate with DMEM containing 10% FBS. Cell viability assessments were conducted daily utilizing the cell counting kit-8 (CCK-8) assay kit (MedChemExpress) complying with the guidelines of the manufacturer.

2.8. Cell Colony Formation Assay

For the colony formation assay, 1,000 cells were seeded in each well of a 6-well plate and cultured in DMEM with 10% FBS. After two weeks, colonies were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet.

2.9. Mass Spectrometry (MS)

HEK293T cells transfected with Flag-TRIM22 were treated with MG132 (10 μM, 4 h). The cell lysates were immunoprecipitated

using Flag antibody and control IgG. These antibodies were captured with protein A/G magnetic beads (Selleck, Shanghai, China). Bound proteins were eluted by boiling in SDS sample buffer, separated by SDS-PAGE, and analyzed using MS.

2.10. Co-Immunoprecipitation (Co-IP) and Ubiquitination Assay

Protein extracts underwent overnight IP with antibodies, followed by the supplementation of protein A/G magnetic beads (Selleck). Following incubation for 4 hours at the temperature of 4°C, the beads were washed, resuspended in SDS sample buffer, and boiled. In the in vivo ubiquitination assay, HCC cells overexpressing TRIM22 or TRIM22 knockdown received the treatment of MG132 before collection. These lysates were immunoprecipitated using anti-CREB1 antibody, and CREB1's ubiquitination was evaluated with an anti-Ub antibody.

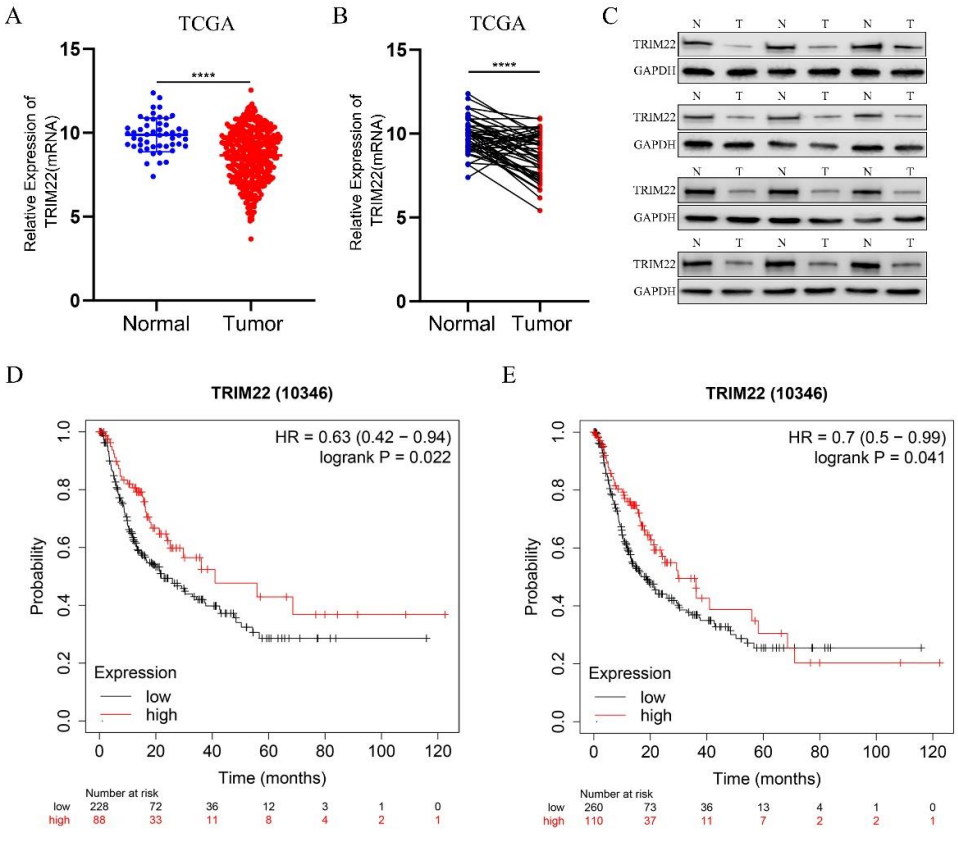
2.11. Statistical Analysis

The statistical analysis was carried out utilizing GraphPad Prism 8.0. The results were presented as means ± standard error of the mean (SEM). Unpaired Student's t-tests and one-way analysis of variance (ANOVA) were used to determine statistical significance. Survival rates were analyzed using the Kaplan-Meier (K-M) method. Cell culture experiments were performed at least three times independently, with each experiment being done in triplicate. A p-value of less than 0.05 indicated to be statistically significant.

3. Results

3.1. Downregulation of TRIM22 among HCC Correlates with Poor Prognosis for Patients

To investigate the role of TRIM22 in HCC progression, we assessed its expression in HCC tissues from the TCGA database. TRIM22 mRNA expression was notably decreased in HCC tissues compared to adjacent non-tumor samples (Fig. 1A). This finding was consistent with the analysis of paired HCC and non-tumor tissues (n=50), where normal tissues exhibited higher levels of TRIM22 (Fig. 1B). Western blot analysis of 12 pairs of HCC tissues further confirmed this trend (Fig. 1C). K-M analysis revealed that lower TRIM22 expression in HCC patients correlated with worse overall survival (Fig. 1D). Additionally, individuals with TRIM22 overexpression demonstrated improved relapse-free and disease-free survival (Fig. 1E-F). Notably, low TRIM22 expression was specifically associated with poor overall survival in advanced-stage HCC (stage III) (Fig. 1G). In summary, decreased TRIM22 levels in HCC tissues indicate a significant poor prognostic indicator for HCC.



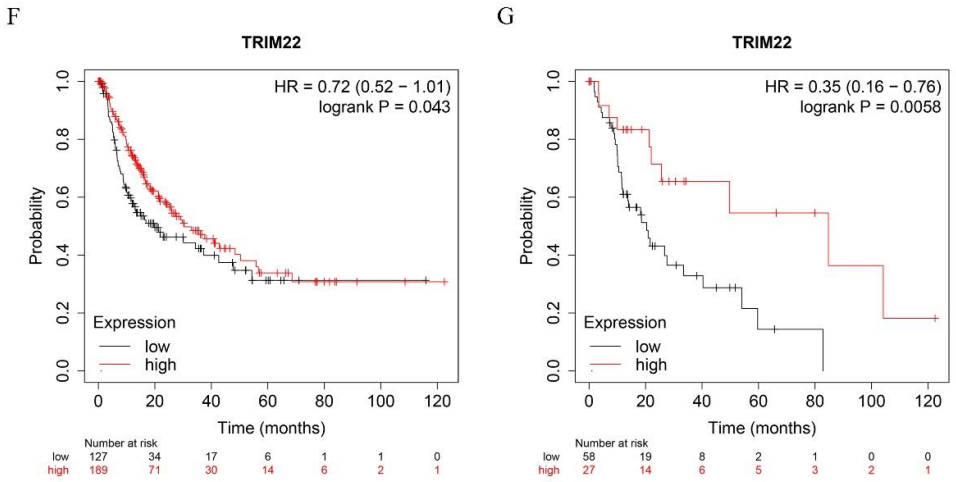
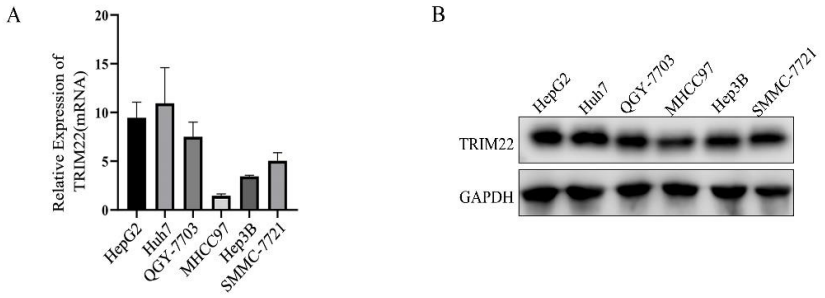


Figure 1: Downregulation of TRIM22 expression in HCC tissues and its association with better survival.
(A) Relative mRNA levels of TRIM22 in HCC tissues and corresponding noncancerous tissues in TCGA database. ****p < 0.0001. (B) Relative mRNA levels of TRIM22 in 50 pairs of HCC tissues and adjacent normal tissues in TCGA database. ****, p < 0.0001. (C) Western blot analysis of TRIM22 expression in 6 pairs of hepatocellular tissues and corresponding normal tissues. (D) Kaplan-Meier (K-M) survival analysis showing overall survival based on TRIM22 expression in liver cancer (https://kmplot.com/). (E) and (F) K-M survival analysis showing relapse-free survival and disease-free survival based on TRIM22 expression in liver cancer (https://kmplot.com/). (G) K-M survival analysis showing overall survival based on TRIM22 expression in stage III liver cancer (https://kmplot.com/).

3.2. Overexpression of TRIM22 Suppressed HCC Cell Proliferation

We examined TRIM22 expression in six HCC cell lines. qRT-PCR and western blot analyses showed elevated TRIM22 expression in HepG2 and Huh7 cells, while MHCC97 and Hep3B cells exhibited lower levels (Fig. 2A-B). To determine the impact of TRIM22 on HCC cell proliferation, we stably overexpressed TRIM22 in MHCC97 and Hep3B cells (Fig. 2C-D). Colony formation assays demonstrated a significant reduction in colony numbers in TRIM22-overexpressing cells compared to the control group (Fig. 2E-G). CCK8 assays consistently showed decreased cell viability in TRIM22-overexpressing cells (Fig. 2I-J). Overall, elevated levels of TRIM22 effectively impeded HCC cell proliferation.



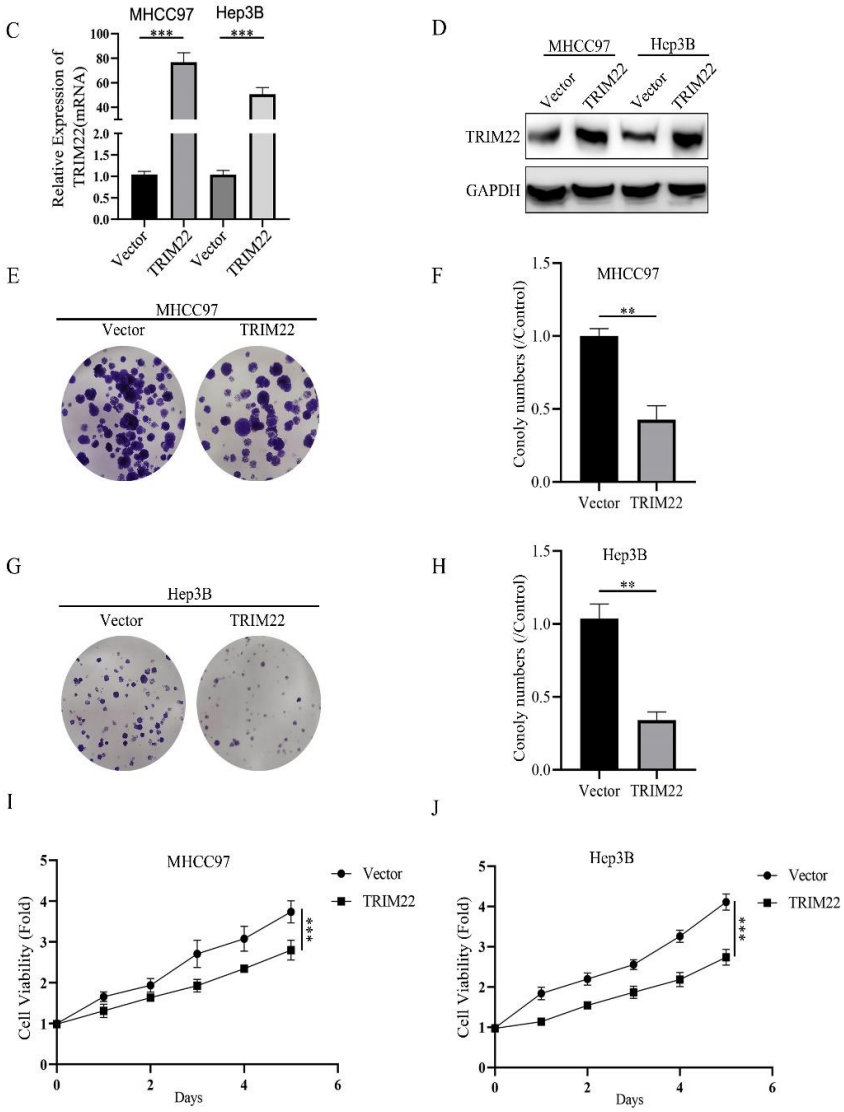


Figure 2: Inhibition of the proliferation of liver cancer cells by TRIM22 overexpression.
(A) and (B) Analysis of TRIM22 expression in liver cancer cell lines by qRT-PCR and western blot. (C) and (D) Analysis of TRIM22 overexpression in MHCC97 and Hep3B cells by qRT-PCR and western blot. ***, $p < 0.001$. (E) and (F) Colony formation assays of TRIM22- overexpressing and control MHCC97 cells, with colony numbers analyzed using Image J. **, $p < 0.01$. (G) and (H) Colony formation assays of TRIM22- overexpressing and control Hep3B cells, with colony numbers analyzed using Image J. **, $p < 0.01$. (I) and (J) CCK8 assays of TRIM22-overexpressing and control MHCC97 and HepG3B cells, with cell viability measured at a wavelength of 450 nm using a Microplate reader. ***, $p < 0.001$.

3.3. Silencing TRIM22 Promoted the Proliferation of HCC Cells

To further investigate the role of TRIM22 in HCC progression, we stably knocked down TRIM22 in HepG2 and Huh7 cells (Fig. 3A, 3D) to assess the impact on cell proliferation. The colony-forming ability of TRIM22-silenced cells was significantly upregulated (Fig. 3B-C, 3E-F). CCK8 assays were further performed and confirmed enhanced proliferation in TRIM22-silenced cells (Fig. 3G-H). These findings highlighted the pivotal role of TRIM22 in suppressing the proliferation of HCC cells.

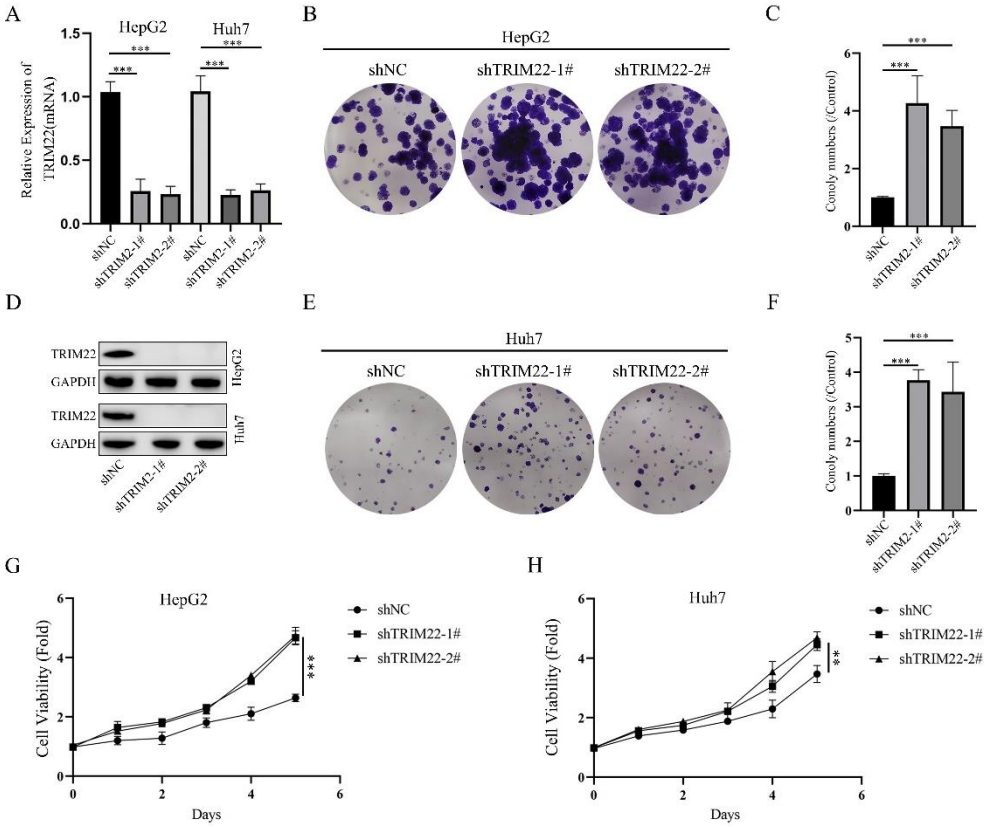


Figure 3: of Promotion of liver cancer cell growth by TRIM22 knockdown.
(A) and (D) Analysis of stable knockdown of TRIM22 in HepG2 and Huh7 cells by qPCR and western blot. ***, $p < 0.001$. (B) and (C) Colony formation assays of TRIM22 stable knockdown and control HepG2 cells, with colony numbers analyzed using Image J. ***, $p < 0.001$. (E) and (F) Colony formation assays of TRIM22 stable knockdown and shNC Huh7 cells, with colony numbers analyzed using Image J. ***, $p < 0.001$. (G) and (H) CCK8 assays of TRIM22-knockdown and shNC in HepG2 and Huh7 cells, with cell viability measured at a wavelength of 450 nm using a Microplate reader. **, $p < 0.01$; ***, $p < 0.001$.

3.4. TRIM22 Interacted with CREB1 at the Molecular Level

To gain insights into the underlying molecular mechanism by which TRIM22 modulated HCC progression, we used MS to delineate its potential interactions. The MS results indicated that CREB1, a versatile transcription factor, could be an interactor of TRIM22 (Fig. 3A). Subsequently, co-IP assays were conducted to validate their interaction (Fig. 3B). qRT-PCR analysis showed that TRIM22 did not affect the mRNA levels of CREB1 (Fig. 3C-D). However, TRIM22 overexpression in MHCC97 and Hep3B cells resulted in decreased CREB1 protein expression, while its depletion in HepG2 and Huh7 cells led to increased CREB1 protein levels (Fig. 5E, 5F). Given the significance of CREB1 as a transcription factor in HCC, we examined the impact of TRIM22 modulation on CREB1-associated signaling pathways. TRIM22 significantly influenced key proliferation-related effectors, including MYC, E2F1, and Cyclin A1. Elevating TRIM22 expression suppressed these effectors in MHCC97 and Hep3B cells (Fig. 5E-G), whereas its depletion increased their expression in HepG2 and Huh7 cells (Fig. 5F-J). In conclusion, these findings provided valuable insights into an essential interaction whereby TRIM22 functioned as a negative regulator of CREB1 and its subsequent downstream effectors within the cellular context of HCC.

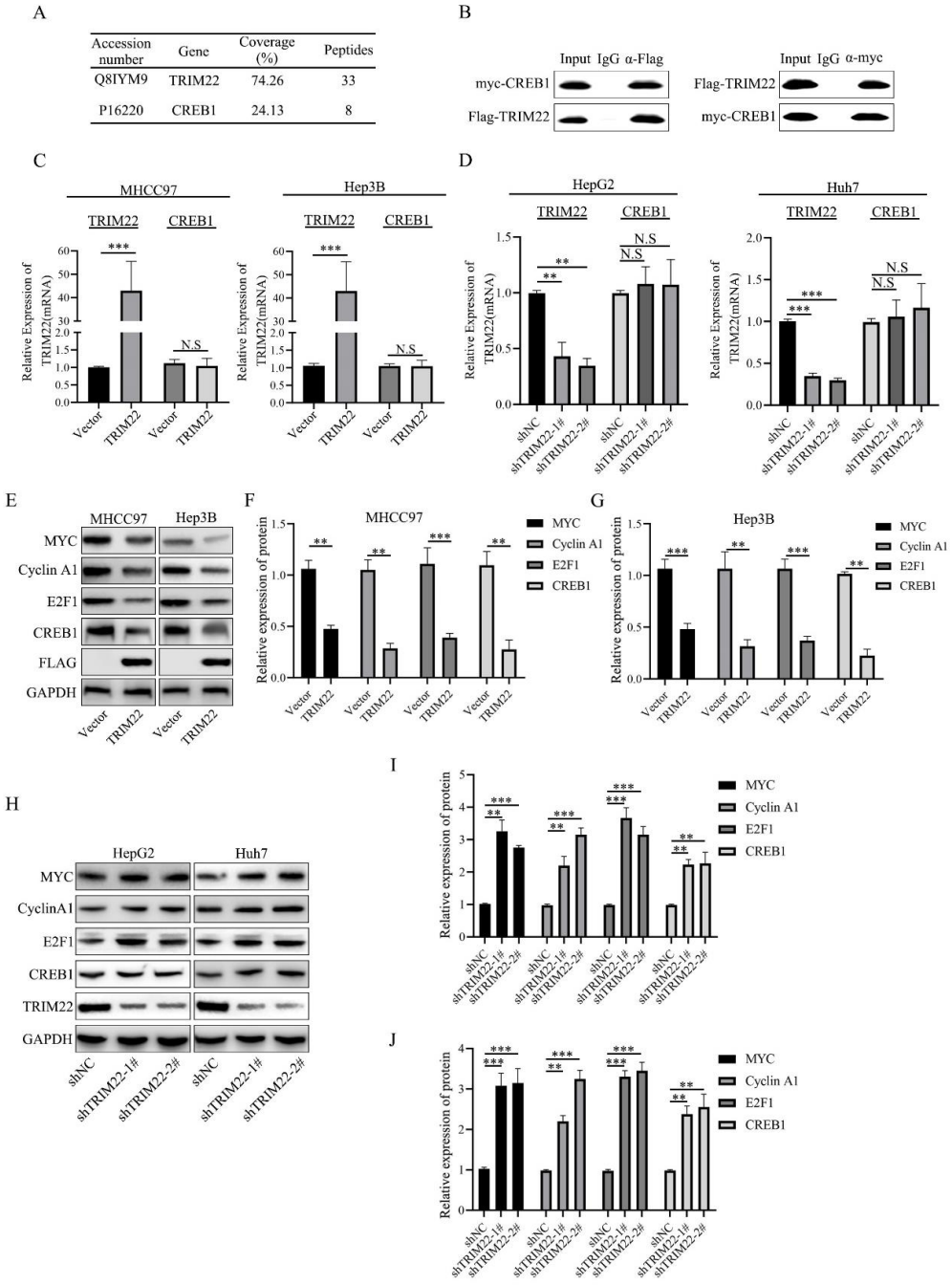
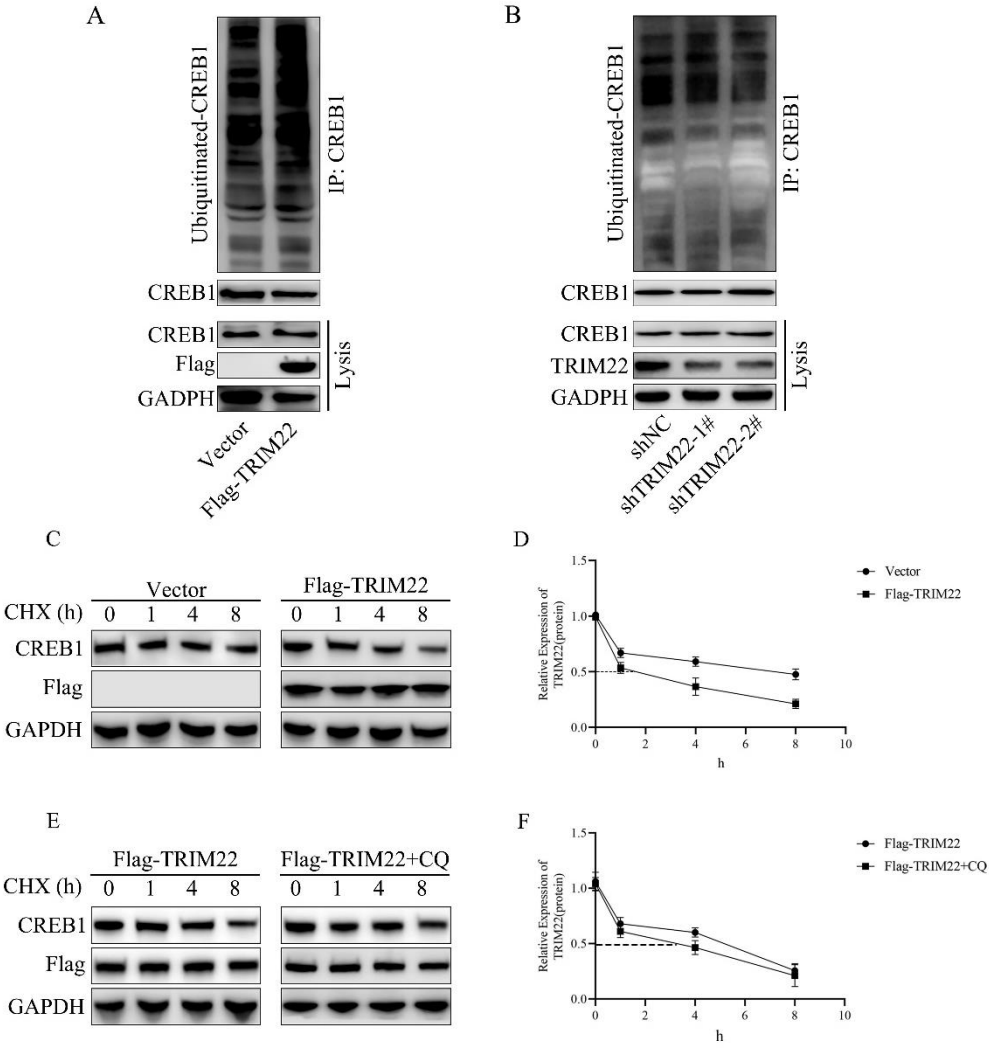


Figure 4: CREB1 as a binding partner of TRIM22.

(A) Transfection of cells using Flag-TRIM22. Total cell lysates were subjected to immunoprecipitation with anti-Flag and control IgG antibody, and CREB1 was identified via mass spectrometry. (B) Co-immunoprecipitation of cells transfected with Flag-TRIM22 and myc-CREB1 using anti-Flag, anti-myc, and control IgG antibody, followed by western blot analysis of myc-CREB1 and Flag-TRIM22. (C) Relative expression of CREB1 in TRIM22-overexpressing and control MHCC97 and Hep3B cells measured by qRT-PCR. ***, $p < 0.001$; N.S., non-significant. (D) Relative expression of CREB1 in TRIM22 stable knockdown and shNC HepG2 and Huh7 cells analyzed by qRT-PCR. **, $p < 0.01$; ***, $p < 0.001$; N.S., non-significant. (E) Western blot analysis of the expression of CREB1, MYC, E2F1, and Cyclin A1 in MHCC97 and Hep3B cells with stable TRIM22 overexpression. (F) and (G) Relative expression of CREB1-related molecules in MHCC97 and Hep3B stable overexpressing and control cells calculated using Image J. **, $p < 0.01$; ***, $p < 0.001$. (H) Western blot analysis of the expression of CREB1-related proteins in HepG2 and Huh7 cells with TRIM22 stable knockdown and shNC cells. (I) and (J) Relative expression of CREB1-related molecules in HepG2 and Huh7 cells with TRIM22 stable knockdown calculated using Image J. **, $p < 0.01$; ***, $p < 0.001$.

3.5. TRIM22 Regulated CREB1 Proteostasis via the Ubiquitin-Proteasome System in HCC

We investigated whether TRIM22 affects the protein stability of CREB1 in HCC. Ubiquitination assays revealed that TRIM22 augmentation in Hep3B cells promoted the ubiquitination of CREB1, while silencing TRIM22 in HepG2 cells inhibited CREB1 ubiquitination (Fig. 5A-B). To determine the temporal dynamics of CREB1 stability influenced by TRIM22, we treated TRIM22-overexpressing Hep3B cells with CHX and monitored CREB1 levels over specific time points. The results demonstrated that elevated TRIM22 levels expedited the proteolytic turnover of CREB1, indicating the destabilizing influence of TRIM22 on CREB1 (Fig. 5C-D). Intracellular protein turnover has been mainly governed by the ubiquitin-proteasome pathway as well as the autophagy-lysosome pathway [17]. In Hep3B cells, we investigated which of these systems TRIM22 leveraged to modulate CREB1 stability. We treated TRIM22-overexpressing Hep3B cells with proteasomal inhibitor (MG132) and lysosomal inhibitor (chloroquine, CQ). Western blot analyses showed that the proteasomal blockade with MG132 restored the destabilized state of CREB1 caused by TRIM22, while the lysosomal inhibitor CQ had no compensatory effect (Fig. 5E-H). These observations established that TRIM22 primarily utilized the ubiquitin-proteasome pathway to facilitate CREB1 degradation.



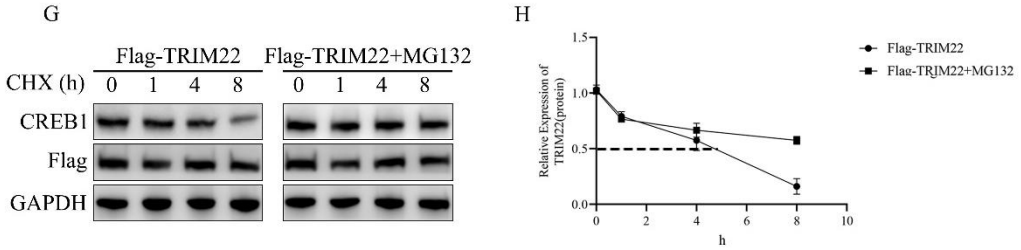
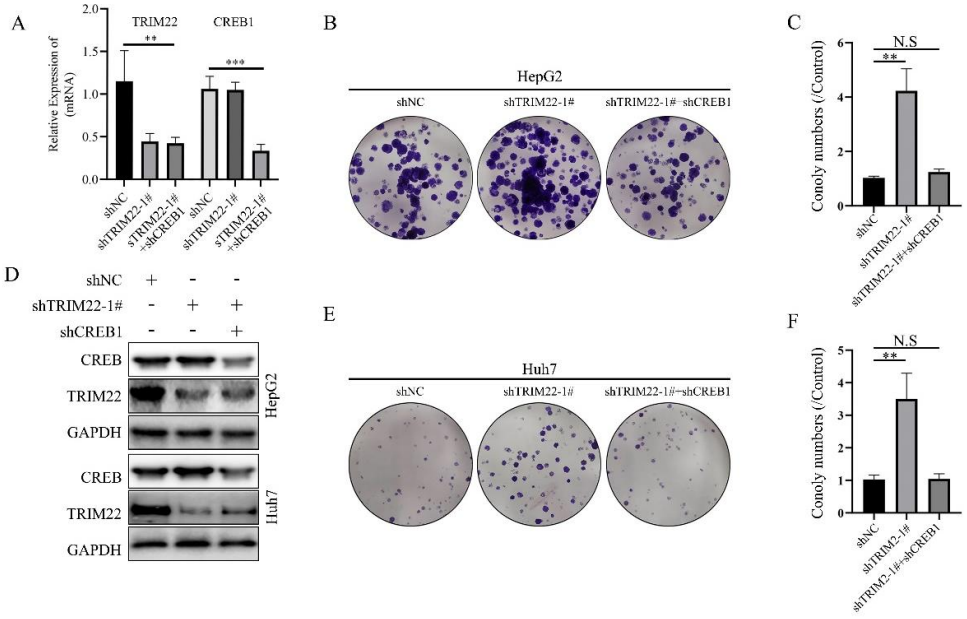


Figure 5: TRIM22 overexpression promoted the degradation of CREB1.
(A) Cells with TRIM22 stable overexpression and control cells treated with MG132 (10 μM) were subjected to immunoprecipitation with TRIM22 and immunoblotted with Ub. (B) Cells with TRIM22 stable knockdown and shNC cells treated with MG132 (10 μM) were subjected to immunoprecipitation with TRIM22 and immunoblotted with Ub. (C) and (D) Hep3B cells with Flag-TRIM22 stable overexpression treated with cycloheximide (CHX, 50 mg/ml) for indicated times were subjected to immunoblot analysis. The relative expression of CREB1 was calculated using Image J. (E) and (F) Hep3B cells with Flag-TRIM22 stable overexpression treated with CHX (50 mg/ml) and CQ (50 μM) for indicated times were subjected to immunoblot analysis, and the quantitative expression of CREB1 was measured using Image J. (G) and (H) Hep3B cells with Flag-TRIM22 stable overexpression treated with CHX (50 mg/ml) and MG132 (10 μM) for indicated times were subjected to western blot analysis, and the relative expression of CREB1 was analyzed with Image J.

3.6. TRIM22 Exerted Its Anti-Tumorigenic Effects in HCC via CREB1 Modulation

We subsequently investigated whether the anti-proliferative effects of TRIM22 in HCC are dependent on the regulation of CREB1. Cells with dual knockdown of TRIM22 and CREB1 were engineered to confirm the knockdown effect of CREB1 on TRIM22 (Fig. 6A, D). To explore the functional interplay, the proliferative propensity of HepG2 and Huh7 induced by TRIM22 ablation was assessed in the context of CREB1 inhibition. Strikingly, colony formation assays demonstrated that the pro-growth effect caused by TRIM22 silencing was mitigated after CREB1 knockdown (Fig. 6B, C, E, F). This indicated that the increased proliferation resulting from TRIM22 attenuation was counteracted by concurrent CREB1 inhibition. Similarly, CCK8 assays were employed to further elucidate the CREB1-mediated regulation of TRIM22's anti-proliferative actions (Fig. 6G-H), revealing that the enhanced cell viability due to TRIM22 knockdown was neutralized in the presence of CREB1 inhibition. Furthermore, the expression of CREB1-related genes was detected in stable TRIM22 knockdown HepG2 and Huh7 cells with or without CREB1 knockdown. Western blot analysis highlighted that the upregulated expression of proliferative markers induced by TRIM22 silencing was nullified upon simultaneous downregulation of CREB1 (Fig. 6I-J). In summary, our findings shed light on TRIM22 as a pivotal modulator with anti-tumorigenic properties in HCC, primarily exerted through its influence on CREB1 and its downstream effectors.



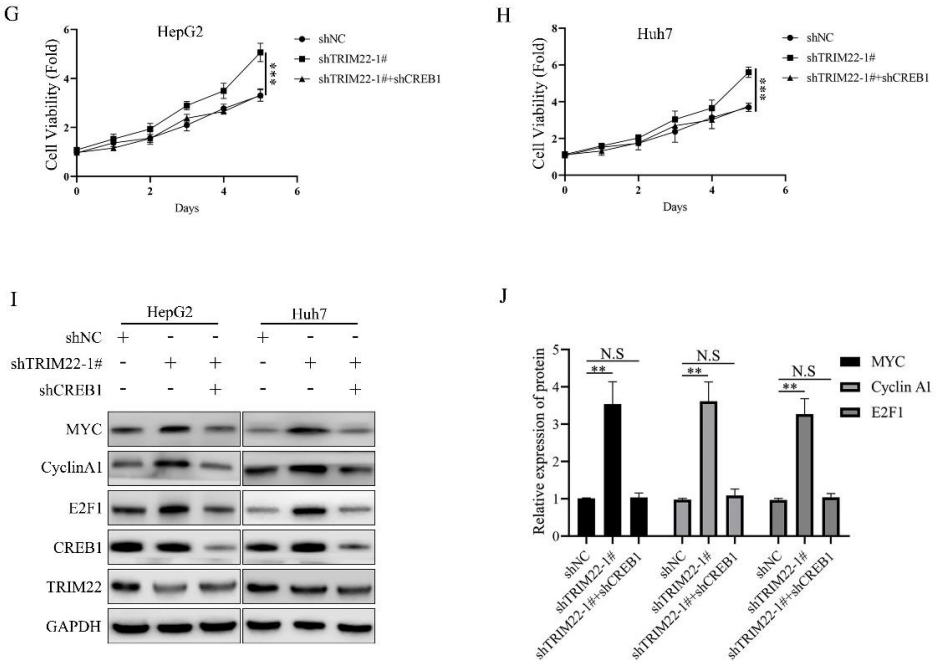


Figure 6: Dependence of TRIM22 - mediated anti-cancer effect in HCC on CREB1degradation.
(A) and (B) Colony formation assays to determine the proliferation capacity of HepG2 cells with TRIM22 stable silencing with or without CREB1 knockdown, and the number of colonies calculated with Image J. **, $p < 0.01$; N.S., non-significant. (C) and (D) Colony formation assays to evaluate the proliferation capacity of Huh7 cells with TRIM22 stable knockdown with or without CREB1 silencing, and the number of colonies calculated with Image J. **, $p < 0.01$; N.S., non-significant. (E) and (F) CCK8 assays to evaluate the proliferation capacity of Huh7 cells with TRIM22 stable knockdown with or without CREB1 silencing, and cell viability calculated. **, $p < 0.01$; N.S., non-significant. (G) and (H) Western blot analysis of the expression of CREB1, MYC, E2F1, and Cyclin A1 in HepG2 and Huh7 cells with TRIM22 knockdown with or without the silencing of CREB1. The relative expression of CREB1 relative molecules were measured with Image J. **, $p < 0.01$; N.S., non-significant.

4. Discussion

Previous research has shed light on the diverse roles of TRIM22 in immune defense, particularly its ability to recognize viral agents, suppress their replication, activate immune responses, and participate in ubiquitination processes [18-20]. Recent studies have reported the involvement of TRIM22 in various cancers, including osteosarcoma, gastric cancer, glioblastoma, and breast carcinoma attributing tumor suppressor properties to it [14, 16, 21, 22]. This highlighted the potential widespread influence of TRIM22 in oncogenesis. In our study, we were aimed to elucidate the crucial role of TRIM22 in HCC through its interaction with CREB1. Clinically, a noticeable decrease in TRIM22 expression was observed in HCC tissues compared to non-malignant counterparts. K-M analysis also revealed a positive correlation between elevated TRIM22 expression and favorable clinical outcomes, emphasizing the tumor-restrictive nature of TRIM22 in HCC. Our empirical findings shed light on the abnormally reduced expression of TRIM22 in HCC, suggesting that its overexpression may lead to improved survival and reduced relapse rates among HCC patients. Our cellular assays further supported the notion that TRIM22 acted as an intrinsic tumor suppressor, playing a pivotal role in HCC development. However, it is important to note that TRIM22's role in cancer development was context-dependent, as evidenced by its oncogenic tendencies in certain cancers like lung cancer and glioblastoma [20, 23].

To delve deeper into the molecular intricacies underlying the anti-proliferative actions of TRIM22, we employed a MS-based approach and screened several differentially expressed genes. Among them, CREB1 emerged as dominant, demonstrating its interaction with TRIM22. cAMP response element-binding protein 1 (CREB1), a member of the transcription factor family, is activated by increased intracellular cAMP levels and orchestrates cellular resilience against various injuries and stressors [24, 25]. CREB1's functionality has been linked to several proto-oncogenes such as Cyclin A1, MYC, E2F1, and EGR-1 [26-28]. These genes play crucial roles in processes including cell proliferation, differentiation, apoptosis, vascular genesis, inflammation, and tumorigenesis [29-32]. Additionally, CREB1 manipulation often intersects with pivotal tumor-related signaling pathways, notably extracellular signal-regulated kinase 1 and 2 (ERK1/2), protein kinase A (PKA), protein kinase C (PKC), and calcium/calmodulin-dependent kinase

II (CaMKII). A recent study even highlighted CREB1's ability to enhance the anti-cancer effects in bladder cancer through modulation of key signaling cascades [33-36]. Our results demonstrated a significant decrease in CREB1 protein levels in cells overexpressing TRIM22, while mRNA levels remained unchanged, suggesting TRIM22's involvement in the post-transcriptional regulation of CREB1. Concordantly, our subsequent experiments using colony formation and CCK-8 assays revealed that silencing CREB1 could counteract the observed anti-proliferative effects upon TRIM22 knockdown.

Collectively, our data proposed a potential mechanism: TRIM22 may mediate the degradation of CREB1, thereby influencing the molecular machinery regulated by CREB1. Our experimental findings highlighted TRIM22's regulatory dominance over CREB1 at the post-transcriptional level. Moreover, functional assays validated that CREB1's modulatory influence was central to the proliferative shifts induced by TRIM22 perturbations. In summary, our findings portray TRIM22 as a powerful determinant of HCC cell fate, executing its tumor-restrictive role through the ubiquitination and subsequent degradation of CREB1. This discovery of the TRIM22-CREB1 axis presented a promising avenue for HCC therapeutics, underscoring its potential as a therapeutic target for the benefit of patients.

Acknowledgements

We thank all the participants in this study.

Conflict of interest

All authors declare no conflict of interest concerning the presented analysis.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval and Consent to participate

This study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Affiliated Hospital of North Sichuan Medical College. All methods were performed

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