

MAD2L1 overexpression promotes breast cancer progression and confers resistance to paclitaxel via proteasomal stabilization

SHIH-HO WANG^{1*}, CHENG-HSI YEH^{1*}, CHIA-WEI WU², CHIA-YI HSU³, EING-MEI TSAI³,
CHAO-MING HUNG⁴, MING-WEI LIN² and TSUNG-HUA HSIEH²

¹Division of General Surgery, Kaohsiung Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Kaohsiung 83301, Taiwan, R.O.C.; ²Department of Medical Research, E-Da Cancer Hospital, I-Shou University, Kaohsiung 82445, Taiwan, R.O.C.;

³Department of Obstetrics and Gynecology, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 80756, Taiwan, R.O.C.; ⁴Department of Surgery, E-Da Cancer Hospital, I-Shou University, Kaohsiung 82445, Taiwan, R.O.C.

Received February 10, 2026; Accepted August 28, 2026

DOI: 10.3892/ol.2026.15877

Abstract. Mitotic arrest deficiency protein 2-like 1 (MAD2L1) is a notable component of the spindle assembly checkpoint that ensures proper chromosome segregation during mitosis. Analysis of The Cancer Genome Atlas breast cancer datasets revealed that *MAD2L1* expression was markedly upregulated in tumor tissues and was associated with advanced clinical stages, lymph node metastasis and poor overall survival. To investigate these findings, *in vitro* functional assays, flow cytometry, western blotting, protein stability assays and paclitaxel sensitivity treatments were performed. Functional experiments demonstrated that MAD2L1 overexpression promoted breast cancer cell proliferation and colony formation, reduced G₂/M phase accumulation and increased the protein levels of cyclin A and CDK1. MAD2L1 exerted these effects through inhibition of proteasomal degradation rather than through transcriptional regulation, suggesting that MAD2L1 stabilized key cell cycle regulators to sustain proliferation. Furthermore, MAD2L1 attenuated the cytotoxic effects of paclitaxel by counteracting drug-induced G₂/M phase arrest and apoptosis, thereby diminishing the chemotherapeutic response. Collectively, the present findings indicated that MAD2L1 drove breast cancer progression and contributed to paclitaxel resistance via proteasomal stabilization of cyclin A and CDK1, underscoring its potential as a prognostic biomarker and therapeutic target in breast cancer.

Introduction

Breast cancer is the most prevalent malignancy among women in Taiwan, accounting for a notable proportion of cancer-related morbidity and mortality. According to data from the Taiwan Cancer Registry (2000-2021), the incidence of breast cancer has steadily increased over the past decades (1). Epidemiological studies have indicated that the majority of patients with breast cancer in Taiwan have luminal subtypes (estrogen receptor-positive/HER2-negative), followed by HER2-positive and triple-negative subtypes, with the latter being associated with more aggressive clinical behavior and poorer prognosis (2,3). Despite advances in early detection and surgical management, metastatic and recurrent disease remain major clinical challenges. Paclitaxel, a widely used chemotherapeutic agent, primarily exerts its antitumor effects by targeting microtubules (4). By stabilizing microtubules and preventing their depolymerization, paclitaxel disrupts normal mitotic spindle formation, leading to G₂/M phase cell cycle arrest and activation of the mitotic checkpoint (5,6). This mitotic disruption subsequently induces apoptosis in rapidly proliferating cancer cells (7,8). In breast cancer, paclitaxel has been shown to inhibit cell proliferation, reduce the clonogenic potential and modulate the expression of key cell cycle regulators (9,10), including cyclin A and cyclin-dependent kinase 1 (CDK1) (11,12), which are essential for G₂/M transition. However, the development of chemoresistance (13), through mechanisms such as alterations in spindle assembly checkpoint proteins (14) or dysregulation of mitotic regulators (15), remains a major limitation to its long-term efficacy of paclitaxel.

Among the proteins regulating specific mitotic stages of the spindle assembly checkpoint, mitotic arrest deficiency protein 2-like 1 (MAD2L1) serves a notable role in ensuring accurate chromosome segregation during mitosis (16,17). By recruiting to unattached kinetochores to monitor kinetochore-microtubule attachments, MAD2L1 prevents premature anaphase onset, thereby maintaining genomic stability. Dysregulation or overexpression of MAD2L1 has been associated with aberrant cell cycle progression (18), increased proliferation (19) and enhanced tumorigenic potential in breast cancer (20,21).

Correspondence to: Dr Tsung-Hua Hsieh, Department of Medical Research, E-Da Cancer Hospital, I-Shou University, 6 Yi-Da Road, Yan-Chao, Kaohsiung 82445, Taiwan, R.O.C.
E-mail: pelagice@yahoo.com.tw

*Contributed equally

Key words: MAD2L1, breast cancer, paclitaxel

Experimental evidence has indicated that elevated MAD2L1 levels can promote cell proliferation, enhance the clonogenic capacity and stabilize key cell cycle regulators such as cyclin A and CDK1 (22). Furthermore, MAD2L1 overexpression may attenuate the cytotoxic effects of chemotherapeutic agents such as paclitaxel, suggesting its pivotal role in modulating both tumor growth and drug responses (23). Given its central function in mitotic regulation and potential involvement in chemoresistance, it is essential to elucidate the role of MAD2L1 in breast cancer progression and its interaction with paclitaxel. To this end, the specific aims of the present study were to investigate the functional effects of MAD2L1 on tumor cell proliferation, unveil its post-translational regulation of key cell cycle targets and characterize its contribution to paclitaxel resistance.

By identifying a previously unrecognized post-translational mechanism wherein MAD2L1 uncouples cyclin A and CDK1 protein stability from their mRNA transcription via ubiquitin inhibition, the present study demonstrates how MAD2L1 drives breast cancer progression and paclitaxel resistance, underscoring its potential as both a prognostic biomarker and a therapeutic target.

Materials and methods

Analysis of data from The University of Alabama at Birmingham Cancer data analysis portal (UALCAN) and the Human Protein Atlas (HPA). MAD2L1 expression in breast cancer was analyzed using the UALCAN web portal (<http://ualcan.path.uab.edu>), which provides access to publicly available, de-identified RNA-sequencing data from The Cancer Genome Atlas (TCGA). MAD2L1 expression levels were compared between breast cancer tissues (n=1,097) and normal breast tissues (n=114). Subgroup analyses were conducted according to pathological stage, lymph node metastasis status and molecular subtype, including luminal, HER2-positive and triple-negative breast cancer. The statistical significance of differential expression was calculated by the UALCAN platform using a two-tailed, paired Student's t-test, and $P < 0.05$ was considered to indicate a statistically significant difference. The expression profile of MAD2L1 across breast cancer cell lines was further evaluated using the HPA database (<https://www.proteinatlas.org>). Expression data from 62 breast cancer cell lines were analyzed to assess relative MAD2L1 expression levels. In addition, Gene Effect scores from CRISPR knockout screens (Broad's Achilles and Sanger's SCORE projects) were analyzed to assess genetic dependencies (<http://ualcan.path.uab.edu>). Chronos-computed scores were used, where a median score of 0 represents non-essential genes and -1 represents common essential genes; lower negative scores indicate stronger growth inhibition or cell death upon knockout (24,25). All analyses were performed using datasets generated by the respective platforms, and no additional normalization or transformation was applied beyond the default processing pipelines provided by UALCAN and HPA.

Cell lines and transfection. Human MDA-MB-231 and MCF-7 breast cancer cell lines were obtained from American Type Culture Collection. Cells were maintained in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin

(all from Gibco; Thermo Fisher Scientific, Inc.) at 37°C in a humidified incubator with 5% CO₂. Cell lines were authenticated by short tandem repeat profiling and routinely tested to confirm the absence of mycoplasma contamination. For transient overexpression, cells were transfected with 1, 2 or 4 µg of MAD2L1 expression plasmid or an empty vector control using TurboFect Transfection Reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. After 48 h transfection at 37°C, cells were harvested and subjected to subsequent cell proliferation and colony formation assays.

BrdU cell proliferation assay. Cell proliferation was evaluated using a BrdU Cell Proliferation ELISA Kit (cat. no. ab126556; Abcam) according to the manufacturer's instructions. Briefly, cells were seeded into 96-well plates and cultured for 24 h at 37°C. Following the indicated treatments, BrdU reagent was added to each well at a final concentration of 10 µM and incubated with the cells for 24 h at 37°C. Absorbance was measured at 450 nm using an INNO microplate reader (LTEK Co., Ltd.). All experiments were performed in triplicate and data are presented as the mean ± SD.

Colony formation assay. For colony formation analysis, 500 transfected cells were seeded into 10-cm culture dishes and cultured under standard conditions for 10-14 days to allow colony development. Colonies were fixed with 4% paraformaldehyde for 1 h at 37°C and stained with 0.1% crystal violet for 30 min at 37°C. Colonies were counted manually, and those containing >50 cells were defined as viable colonies. Colonies were visualized and imaged using a CKX53 microscope (Olympus Corporation). Three independent biological replicates were performed for each experiment.

Cell cycle analysis. Cell cycle distribution was analyzed using flow cytometry. After transfection, cells were harvested, washed with PBS and fixed in 70% ethanol at 4°C overnight. Fixed cells were washed with PBS and stained with PI (MilliporeSigma) solution containing RNase A (Thermo Fisher Scientific, Inc.) for 30 min at room temperature in the dark. DNA content was measured using a Guava flow cytometer (Luminex Corporation), and the data were analyzed using GuavaSoft software (version 3.3; Luminex Corporation). Cell cycle distribution, including G₀/G₁, S and G₂/M phases, was calculated based on PI fluorescence intensity. Doublets were excluded using appropriate gating strategies. Three independent biological replicates were performed for all experiments.

Immunoprecipitation and ubiquitination assays. To evaluate the ubiquitination levels of cyclin A and CDK1, cells were treated with 10 µM of the proteasome inhibitor MG132 (Sigma-Aldrich; Merck KGaA) at 37°C for 4 h prior to cell harvesting to inhibit proteasomal degradation and allow the accumulation of ubiquitinated proteins. Equal amounts of protein lysates (input) were incubated with specific primary antibodies against cyclin A or CDK1 overnight at 4°C with gentle rotation. Normal IgG was used as a negative control. Immune complexes were then captured by incubation with Protein G magnetic beads for 2 h at 4°C. The beads were washed thoroughly with lysis buffer to remove non-specifically

bound proteins, and the isolated proteins were subjected to subsequent western blot analysis.

Western blotting. Total cellular proteins were extracted using RIPA lysis buffer supplemented with a protease inhibitor cocktail (Roche Diagnostics). Protein concentrations were determined using a BCA protein assay (Thermo Fisher Scientific, Inc.). Equal amounts of protein (30 μ g per well) were separated by 10% SDS-PAGE and transferred onto PVDF membranes (MilliporeSigma). The membranes were blocked with 5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween-20 and incubated overnight at 4°C with the following primary antibodies: Anti-MAD2L1 (1:1,000; cat. no. 4636; Cell Signaling Technology, Inc.), anti-cyclin A (1:200; cat. no. ab185619; Abcam), anti-CDK1 (1:200; cat. no. ab131450; Abcam), anti-ubiquitin (1:1,000; cat. no. 10201-2-AP; Proteintech Group, Inc.) and anti- β -actin (1:1,000; cat. no. A5441; Sigma-Aldrich; Merck KGaA). After washing, membranes were incubated with appropriate HRP-conjugated secondary antibodies (1:1,000; cat. no. #58802 and cat. no. #93702; Cell Signaling Technology, Inc.) for 1 h at room temperature. Protein signals were detected using an enhanced chemiluminescence detection system (Thermo Fisher Scientific, Inc.) and visualized using a chemiluminescence imaging system. Three independent biological replicates were performed for all experiments.

Reverse transcription-quantitative PCR (RT-qPCR) analysis. Total RNA was extracted from cells using TRIzol[®] reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Complementary DNA was synthesized using the PrimeScript RT Reagent Kit (Takara Bio, Inc.) at 37°C for 15 min, followed by a reverse transcriptase inactivation step at 85°C for 5 sec. RT-qPCR was performed using SYBR Green Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) with the following thermal protocol: 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min, and a final melting curve stage and a real-time PCR detection system.

The mRNA expression levels of cyclin A (forward, 5'-CTC TACACAGTCACGGGACAAAG-3'; reverse, 5'-CTGTGG TGCTTTGAGGTAGGTC-3') and CDK1 (forward, 5'-AAA TGTGTGTAGGTCTCAC-3'; reverse, 5'-ATGATTAA GCCAACTCAAA-3') were normalized to those of GAPDH (forward, 5'-GGAGCGAGATCCCTCCAAAAT-3'; reverse, 5'-GGCTGTTGTCTACTTCTCATGG-3') as an internal control. Relative gene expression was calculated using the $2^{-\Delta\Delta C_q}$ method (26). Three independent biological replicates were performed for all experiments, with each reaction run in technical triplicates.

Apoptosis assay. Apoptosis was assessed using an annexin V-FITC/PI apoptosis detection kit (BD Biosciences). Following treatment with paclitaxel for 48 h, cells were harvested, washed with cold PBS and stained with annexin V-FITC and PI according to the manufacturer's instructions. Apoptotic cells were analyzed using an INNO microplate reader (LTEK, Co., Ltd.). Three independent biological replicates were performed for all experiments.

Statistical analysis. Statistical analyses and graphing were performed using GraphPad Prism 8.0 (Dotmatics). Data are presented as mean $\pm$ SD from three independent experiments (n=3). Differences between two independent groups were analyzed using the two-tailed unpaired Student's t-test. For comparisons among multiple groups, one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was performed. P<0.05 was considered to indicate a statistically significant difference. ImageJ software (National Institutes of Health) was used for the quantification of western blot proteins from three independent experiments (n=3).

Results

Elevated MAD2L1 expression is associated with poor prognosis and aggressive breast cancer phenotypes. Analysis of TCGA breast cancer data using the UALCAN database revealed that *MAD2L1* expression was significantly upregulated in breast cancer tissues (n=1,097) compared with adjacent normal tissues (n=114) (Fig. 1A). Higher *MAD2L1* levels were observed in advanced-stage tumors (Fig. 1B) and tumors with a high nodal metastasis stage (Fig. 1C), indicating its association with disease progression. Subtype analysis further demonstrated elevated *MAD2L1* expression across luminal, HER2-positive and triple-negative breast cancer (Fig. 1D). Kaplan-Meier survival analysis demonstrated that patients with high *MAD2L1* expression had a significantly worse overall survival compared with patients with low expression (P=0.015; Fig. 1E). Functional CRISPR knockout experiments (24,25) confirmed the oncogenic role of *MAD2L1*, as its knockout markedly reduced breast cancer cell proliferation, with the most pronounced effect observed in MDA-MB-231 triple-negative breast cancer cells and the mildest effect observed in MCF-7 cells (Fig. 1F). Consequently, these two cell lines were selected for further investigation. *MAD2L1* overexpression was closely associated with aggressive tumor behavior and poor clinical outcomes in breast cancer, suggesting it may serve as a potential biomarker and therapeutic target.

***MAD2L1* overexpression promotes proliferation and colony formation in breast cancer cells.** To further investigate the functional impact of *MAD2L1* in breast cancer, 62 breast cancer cell lines (Fig. 2A), including MCF-7 and MDA-MB-231 cells (Fig. 2B), were analyzed using the HPA database. This analysis aimed to characterize the endogenous expression landscape of *MAD2L1* across diverse breast cancer cell lines by quantifying baseline mRNA levels using normalized transcripts per million (nTPM) values. The biological effects of *MAD2L1* overexpression were subsequently assessed by transfecting 1, 2 and 4 μ g of *MAD2L1* plasmid into these cells, which led to a dose-dependent increase in *MAD2L1* expression levels (Figs. 2C and S1A and B). The results further revealed that cell proliferation increased proportionally with the amount of *MAD2L1* plasmid transfected, indicating a dose-dependent proliferative effect (Fig. 2D and E). In addition, colony formation assays demonstrated that transfection of 2 μ g of *MAD2L1* plasmid significantly enhanced colony formation in both MCF-7 (Fig. 2F and G)

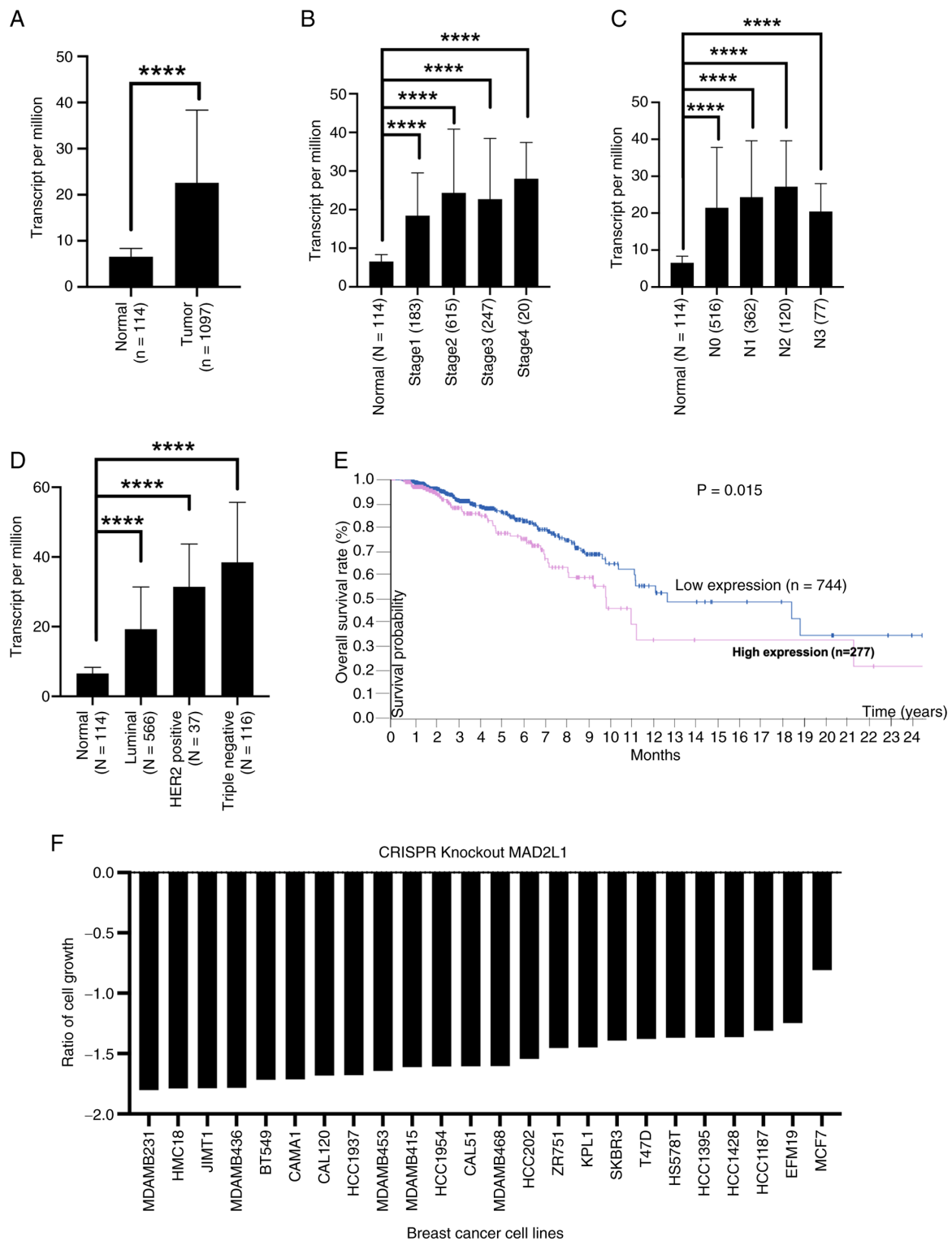


Figure 1. MAD2L1 expression is upregulated in breast cancer and associated with poor prognosis. (A) Expression levels of MAD2L1 in breast cancer tissues (n=1,097) and normal breast tissues (n=114) based on The Cancer Genome Atlas data analyzed using The University of Alabama at Birmingham Cancer Data Analysis Portal database. (B) MAD2L1 expression according to tumor stage. (C) MAD2L1 expression according to nodal metastasis status. (D) Subtype-specific expression of MAD2L1 in luminal, HER2-positive and triple-negative breast cancer. (E) Kaplan-Meier survival analysis showing that patients with high MAD2L1 expression exhibited significantly worse overall survival (P=0.015). (F) CRISPR-mediated knockout of MAD2L1 reduced proliferation in breast cancer cell lines. ****P<0.0001. MAD2L1, mitotic arrest deficiency protein 2-like 1.

and MDA-MB-231 (Fig. 2H and I) cells. Overexpression of MAD2L1 enhanced breast cancer cell proliferation and the clonogenic potential, supporting its role as a promoter of tumor cell proliferation.

MAD2L1 overexpression decreases G₂/M phase accumulation by stabilizing cyclin A and CDK1 through inhibition of ubiquitination. To determine the impact of MAD2L1 on the cell cycle, 2 μ g of MAD2L1 plasmid was transfected into

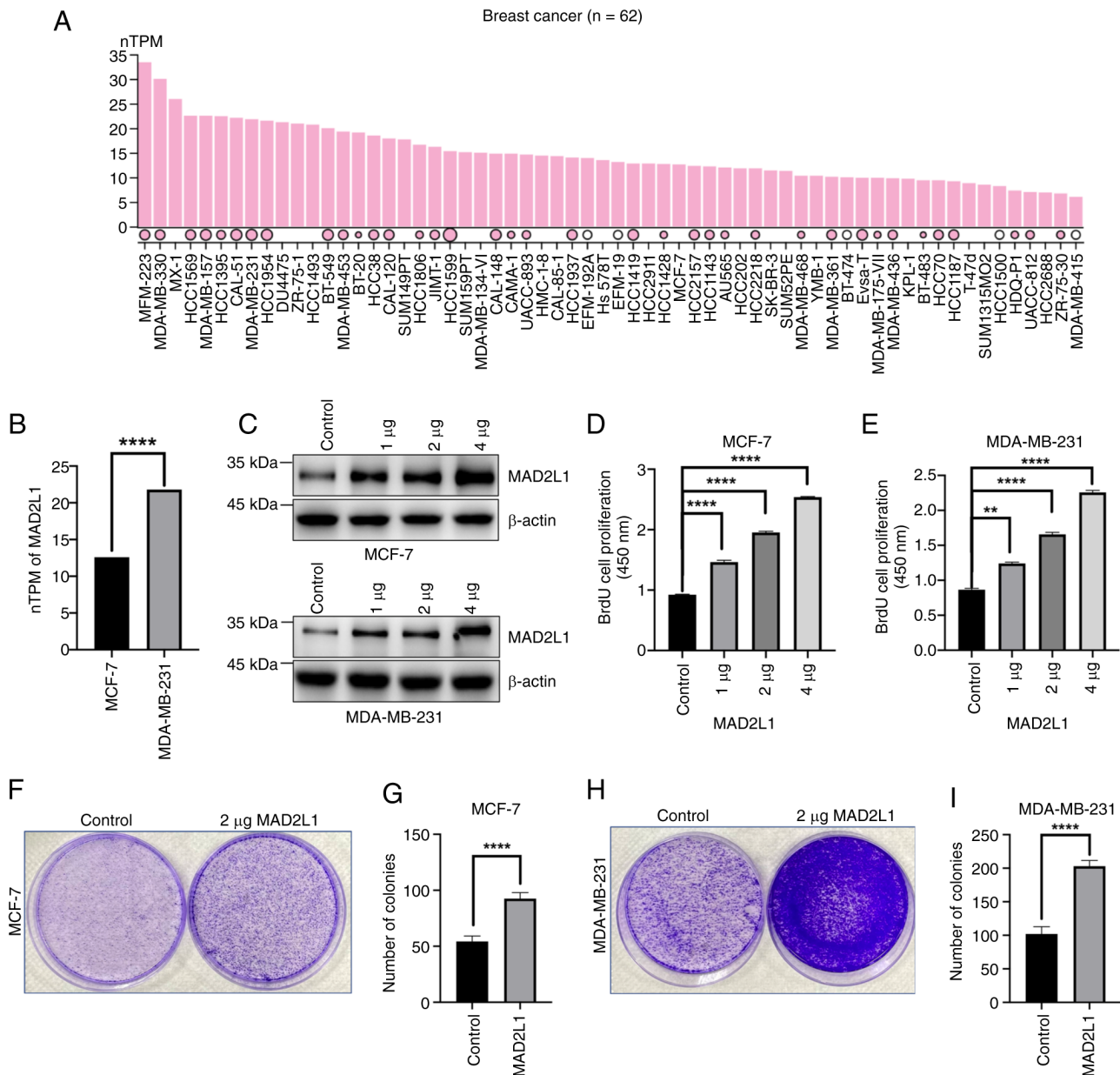


Figure 2. MAD2L1 overexpression promotes breast cancer cell proliferation and colony formation. (A) Expression levels of MAD2L1 across 62 breast cancer cell lines based on data obtained from the Human Protein Atlas database. (B) Representative expression profiles of MAD2L1 in MCF-7 and MDA-MB-231 cells. (C) Western blotting and (D and E) BrdU assays showing that cell proliferation was increased in a dose-dependent manner following transfection with 1, 2 or 4 µg of MAD2L1 plasmid in MCF-7 and MDA-MB-231 cells. Colony formation assay images and quantification of (F and G) MCF-7 and (H and I) MDA-MB-231 cells treated with 2 µg of MAD2L1. Data are presented as the mean ± SD of three independent experiments. **P<0.01; ****P<0.0001. MAD2L1, mitotic arrest deficiency protein 2-like 1; nTPM, number of transcripts per million.

MCF-7 and MDA-MB-231 cells, followed by cell cycle analysis using flow cytometry. The results demonstrated that MAD2L1 overexpression led to a marked decrease in the G₂/M phase population in both cell lines (Fig. 3A and B). Western blot analysis further revealed that MAD2L1 overexpression enhanced the protein levels of cyclin A and CDK1 (Fig. 3C), whereas their mRNA levels remained unchanged, as determined by RT-qPCR (Fig. 3D). These findings suggested that MAD2L1 may regulate cyclin A and CDK1 at the post-translational level. Consistently, treatment with the proteasome inhibitor MG132 elevated cyclin A and CDK1 expression, and co-treatment with MG132 and MAD2L1 overexpression resulted in the highest protein levels (Fig. 3E and F). To further investigate

whether MAD2L1 regulated cyclin A and CDK1 through the ubiquitin-proteasome pathway, *in vitro* ubiquitination assays were performed. As shown in Fig. 3G and H, immunoprecipitation of cyclin A and CDK1 followed by western blotting for ubiquitin revealed that MAD2L1 overexpression markedly reduced the ubiquitination levels of both proteins. These findings demonstrated that MAD2L1 stabilizes cyclin A and CDK1 protein levels by inhibiting their ubiquitination rather than directly altering proteasomal activity.

MAD2L1 attenuates the antitumor effects of paclitaxel by modulating the cell cycle and apoptosis. To explore the interaction between MAD2L1 and paclitaxel, a

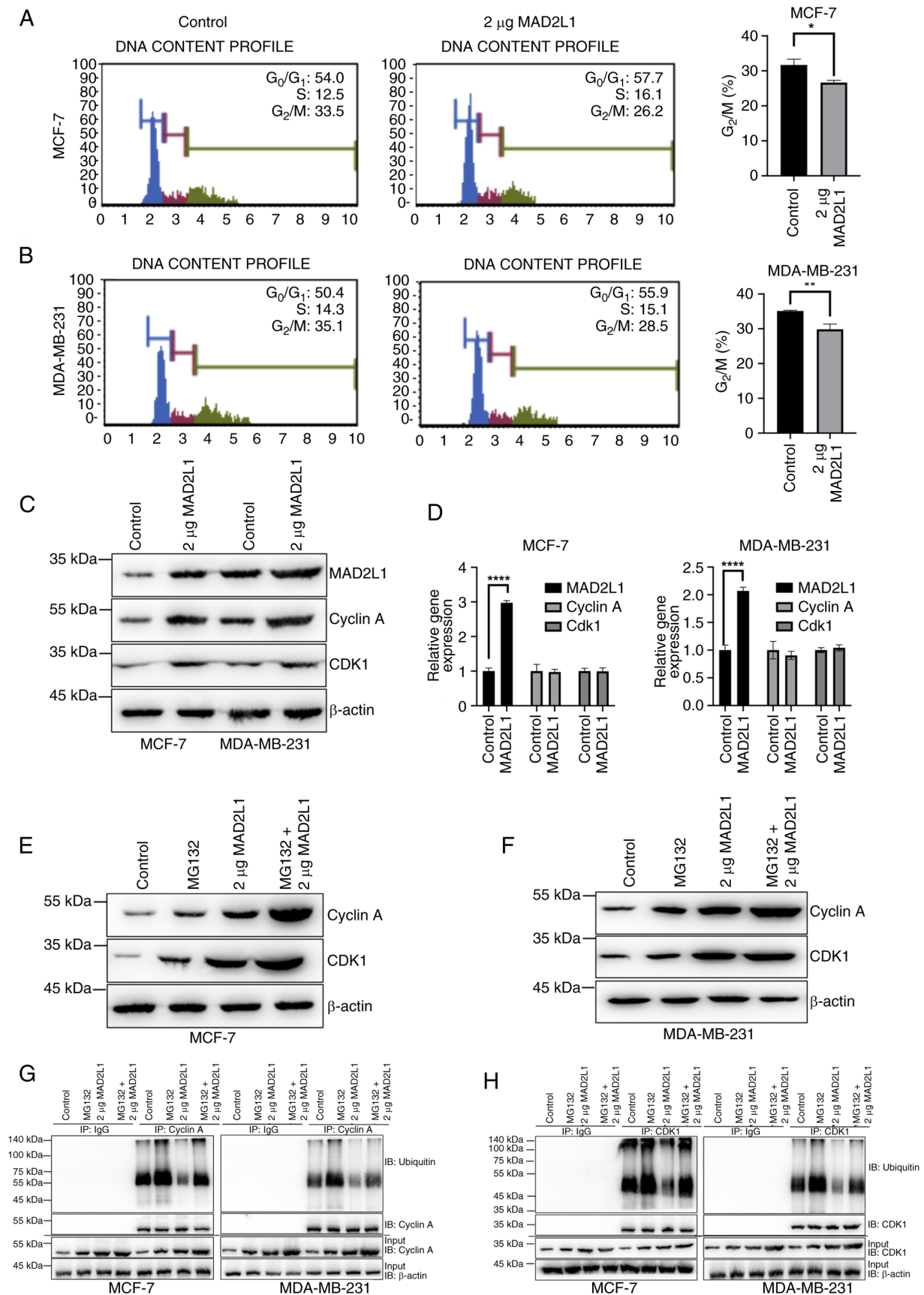


Figure 3. MAD2L1 overexpression reduces G_2/M phase accumulation and stabilizes cyclin A and CDK1 proteins. Flow cytometry analysis showing that MAD2L1 overexpression decreased the G_2/M phase population in (A) MCF-7 and (B) MDA-MB-231 cells. (C) Western blot analysis revealing elevated cyclin A and CDK1 protein expression following MAD2L1 overexpression. (D) Reverse transcription-quantitative PCR analysis demonstrating the mRNA levels of cyclin A and CDK1 in MCF-7 and MDA-MB-231 cells. (E and F) Western blotting results revealing the protein levels of cyclin A and CDK1 following co-treatment with MG132 and MAD2L1 overexpression. (G and H) Following immunoprecipitation, western blotting demonstrated that ubiquitination of cyclin A and CDK1 was decreased following MAD2L1 overexpression. Data are presented as the mean $\pm$ SD of three independent experiments. * $P < 0.05$; ** $P < 0.01$; **** $P < 0.0001$. MAD2L1, mitotic arrest deficiency protein 2-like 1; CDK1, Cyclin-dependent kinase 1.

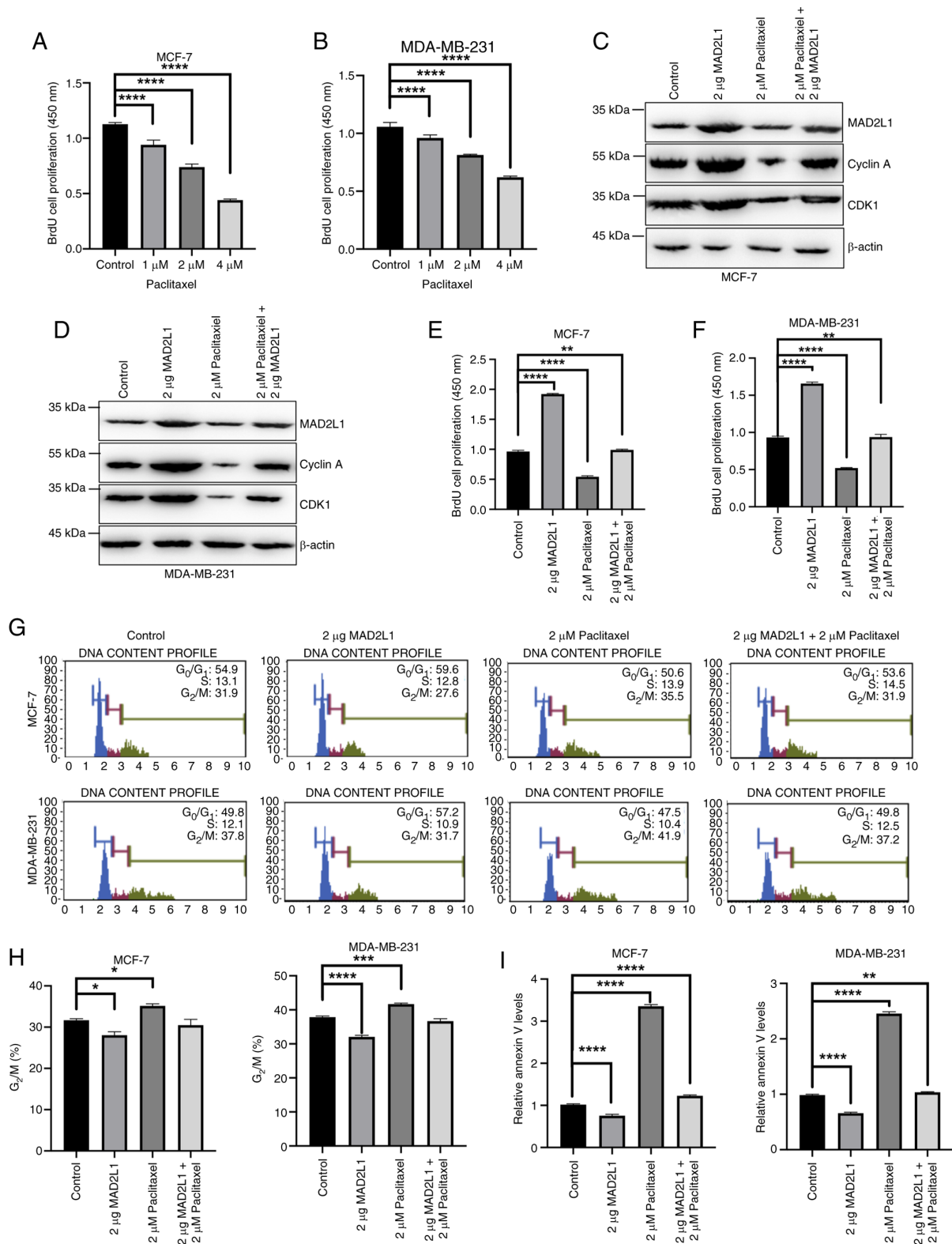


Figure 4. MAD2L1 attenuates the inhibitory effects of paclitaxel on breast cancer cells. BrdU assays showing the dose-dependent inhibitory effect of paclitaxel (1, 2 and 4 μ M) on the proliferation of (A) MCF-7 and (B) MDA-MB-231 cells. (C and D) Western blot analysis of MAD2L1, cyclin A and CDK1 expression following paclitaxel treatment, with or without MAD2L1 overexpression. (E and F) BrdU analysis showing that MAD2L1 overexpression mitigated paclitaxel-induced inhibition of cell proliferation. (G and H) Flow cytometry analysis of the cell cycle distribution demonstrating that MAD2L1 overexpression reduced paclitaxel-induced G₂/M phase arrest in MCF-7 and MDA-MB-231 cells. (I) Annexin V-FITC/PI apoptosis assay showing that MAD2L1 decreased paclitaxel-induced apoptosis. Data are presented as the mean $\pm$ SD of three independent experiments. *P<0.05; **P<0.01; ***P<0.001, ****P<0.0001. BrdU, bromodeoxyuridine; MAD2L1, mitotic arrest deficiency protein 2-like 1; CDK1, Cyclin-dependent kinase 1.

chemotherapeutic drug known to affect cell cycle progression, MCF-7 and MDA-MB-231 cells were treated with

increasing concentrations of paclitaxel (1, 2 and 4 μ M). BrdU assays demonstrated dose-dependent inhibition of

proliferation by paclitaxel in both cell lines (Fig. 4A and B). Western blot analysis revealed that paclitaxel suppressed the protein expression levels of MAD2L1, cyclin A and CDK1, consistent with its role in inducing G₂/M arrest. However, overexpression of MAD2L1 mitigated the inhibitory effects of paclitaxel, maintaining higher levels of cyclin A and CDK1 proteins (Figs. 4C and D and S1C and D). Functionally, MAD2L1 overexpression reduced paclitaxel-induced growth inhibition (Fig. 4E and F) and attenuated G₂/M phase arrest in both cell lines (Fig. 4G and H). Furthermore, annexin V staining indicated that MAD2L1 overexpression decreased paclitaxel-induced apoptosis (Fig. 4I), suggesting a cytoprotective effect against paclitaxel-mediated cytotoxicity. Taken together, these results indicated that MAD2L1 overexpression diminished the anticancer efficacy of paclitaxel by counteracting its effects on cell cycle arrest and apoptosis.

Discussion

The present findings unveil a novel post-translational mechanism wherein MAD2L1 dictates breast cancer progression by stabilizing the cell cycle machinery. Notably, MAD2L1 uncouples cyclin A and CDK1 protein abundance from their mRNA transcription. By suppressing ubiquitin-mediated proteasomal degradation, MAD2L1 extends the half-life of these core G₂/M regulators, shifting the conventional paradigm from transcriptional wiring to post-translational stabilization in mitotic control. Clinically, this biochemical shield directly translates into paclitaxel resistance. While paclitaxel normally triggers apoptosis by downregulating MAD2L1, cyclin A and CDK1, forced MAD2L1 expression overrides this suppression. By maintaining these cell cycle engines under drug pressure, MAD2L1-overexpressing cells successfully evade G₂/M arrest and apoptotic cascades. Collectively, these insights establish MAD2L1 as an active driver of chemoresistance and a promising therapeutic target to sensitize refractory breast cancers.

The present study demonstrated that MAD2L1 expression was upregulated in breast cancer and associated with aggressive tumor features, including higher stage, nodal metastasis and poor overall survival. Functionally, MAD2L1 overexpression promoted breast cancer cell proliferation, enhanced the clonogenic capacity and modulated G₂/M phase progression by stabilizing key cell cycle regulators such as cyclin A and CDK1. Notably, MAD2L1 attenuated the cytotoxic effects of paclitaxel, reducing drug-induced growth inhibition, G₂/M arrest and apoptosis in both MCF-7 and MDA-MB-231 cells. These findings collectively suggested that MAD2L1 serves a dual role in driving tumor progression and mediating chemoresistance, highlighting its potential as both a prognostic biomarker and a therapeutic target in breast cancer. Previous studies have demonstrated that MAD2L1 functions as a pro-tumorigenic regulator, and its overexpression suppressed G₂/M phase accumulation, whereas MAD2L1 inhibition induced G₂/M arrest, which was accompanied by decreased expression levels of cyclin A and cyclin E1 (22,27). These reports are consistent with the present observations.

The present results indicated that MAD2L1 overexpression maintained high levels of cyclin A and CDK1 proteins through post-translational regulation involving proteasomal degradation

pathways. Cyclin A and CDK1 are tightly regulated during the cell cycle, and their degradation is primarily mediated by the ubiquitin-proteasome system, in which E3 ubiquitin ligases tag proteins for proteasomal degradation during specific cell cycle phases (28,29). In the present study, MAD2L1 overexpression did not alter cyclin A or CDK1 mRNA levels, indicating that the increased protein abundance resulted from inhibition of proteasome-mediated degradation rather than transcriptional upregulation. Treatment with the proteasome inhibitor MG132 further increased cyclin A and CDK1 levels (30), and the combination of MAD2L1 overexpression with MG132 treatment resulted in the highest protein accumulation, suggesting that MAD2L1 may interfere with ubiquitination or recognition by the proteasome. Mechanistically, MAD2L1, as a core spindle assembly checkpoint (SAC) protein, could influence the activity of E3 ubiquitin ligases (31), which are responsible for targeting cyclin A and CDK1 for degradation at the metaphase-anaphase transition (32). Previous studies have also revealed that MAD2L1 possesses the capability to modulate oligoubiquitination or short-chain polyubiquitination. The research demonstrated that APC/C-mediated ubiquitination operates via a hierarchical two-step process: Initial chain nucleation or priming driven by Ube2C (or Ube2D) to conjugate the first few ubiquitin molecules onto the substrate, followed by processive chain elongation mediated by Ube2S, which specifically extends K11-linked polyubiquitin long chains to assemble high-molecular-weight complexes (33). Crucially, when MAD2L1 is activated as a core SAC component, it suppresses the E3 ligase complex by selectively blocking the recruitment and elongation capacity of Ube2S, while leaving the upstream, Ube2C-driven priming nucleation partially permissible (34). Consequently, the catalytic activity of the APC/C complex becomes restricted exclusively to the synthesis of short-chain ubiquitin conjugates, strategically placing a molecular cap on polyubiquitin chain topography and leading to the characteristic accumulation of these short conjugates. The present experimental findings align with this enzymatic paradigm. Notably, because MAD2L1 acts at the furthest upstream level to physically constrain chain extension, this characteristic low-molecular-weight topology remains consistent even when downstream proteasomal clearance is thoroughly blocked by MG132. Therefore, rather than reflecting a technical artifact, this uniform band distribution provides direct evidence that MAD2L1 structurally caps polyubiquitin chain topology, capturing these core cell cycle components as functional, short-chain intermediates to delay their degradation. By delaying E3 ubiquitin ligase activation, MAD2L1 may extend the half-life of cyclin A and CDK1, thereby sustaining their protein levels during G₂/M progression. This stabilization ensures continuous CDK1/cyclin A kinase activity, promoting mitotic entry and progression even under conditions of stress or chemotherapeutic challenge, such as paclitaxel treatment.

Paclitaxel is one of the most widely used chemotherapeutic agents for breast cancer treatment in both adjuvant and metastatic settings (35) due to its potent ability to disrupt mitotic spindle formation (36) and induce G₂/M cell cycle arrest (37). Clinically, paclitaxel exhibits marked benefits, including improved overall response rates, enhanced progression-free survival and efficacy across multiple breast cancer subtypes

compared to the control group, particularly in combination regimens with anthracyclines or targeted therapies (38,39). Its predictable mechanism of action and established dosing schedules have made it a cornerstone of breast cancer chemotherapy. However, the development of chemoresistance is a major clinical challenge, often leading to relapse or disease progression despite initial responsiveness. Resistance mechanisms include alterations in microtubule dynamics (40), overexpression of drug efflux pumps (41) and dysregulation of spindle assembly checkpoint proteins (14), which can attenuate paclitaxel-induced mitotic arrest and apoptosis. In addition, some breast cancer subtypes, including triple-negative tumors, may exhibit intrinsic resistance, limiting the therapeutic benefit of paclitaxel as a monotherapy (42,43). These clinical benefits and limitations underscore the need for an improved understanding of molecular determinants of the response to paclitaxel. Targeting regulators of the mitotic checkpoint or protein stability pathways, such as MAD2L1, could enhance paclitaxel efficacy, reduce chemoresistance and potentially expand its clinical utility.

The present findings revealed a functional interaction between MAD2L1 and paclitaxel in regulating G₂/M phase dynamics. While paclitaxel is well known to induce G₂/M arrest by stabilizing microtubules and preventing mitotic progression (44), overexpression of MAD2L1 had the opposite effect, reducing the proportion of cells in the G₂/M population. This reduction suggested that excessive MAD2L1 could override the checkpoint-mediated arrest normally triggered by paclitaxel. Mechanistically, MAD2L1 overexpression stabilized cyclin A and CDK1, which are two essential regulators that drive G₂/M transition, thereby promoting continued cell cycle progression despite paclitaxel treatment. As a result, cells were able to partially escape microtubule damage-induced cell cycle arrest, diminishing the cytostatic and cytotoxic impact of paclitaxel. This phenomenon offered a mechanistic explanation for why MAD2L1-overexpressing cells displayed attenuated G₂/M arrest and reduced apoptosis following paclitaxel exposure. When cyclin A/CDK1 levels remain elevated, cells may bypass the canonical spindle-assembly checkpoint, enter mitosis prematurely or undergo aberrant mitotic exit (45), all of which lessen the efficacy of paclitaxel-induced cell cycle arrest. Consequently, MAD2L1 functions as a resistance factor that counteracts the mechanism of action of paclitaxel, enabling sustained proliferation even under chemotherapeutic pressure.

The expression level of MAD2L1 may serve as a key determinant of paclitaxel responsiveness. Understanding how MAD2L1 orchestrates the balance between mitotic arrest, proteasomal degradation and apoptotic signaling could provide valuable insights for overcoming paclitaxel resistance. Furthermore, targeting of MAD2L1-mediated regulatory pathways may represent a promising strategy to enhance the therapeutic efficacy of taxane-based chemotherapy in breast cancer. These findings suggested that MAD2L1 links mitotic checkpoint control to the proteasomal degradation machinery, enabling breast cancer cells to maintain the levels of G₂/M regulatory proteins and enhancing the proliferative capacity. Targeting of the MAD2L1-proteasome axis may therefore represent a potential strategy to sensitize tumor cells to mitotic inhibitors or chemotherapy.

Acknowledgements

Not applicable.

Funding

The present study was funded by the Ministry of Science and Technology of Taiwan (grant nos. NSTC 112-2314-B-650-004-MY3 and 115-2314-B-650-003) and by E-Da Hospital/E-Da Cancer Hospital (grant nos. EDCHP115009, EDCHS115004 and EDCHJ114001).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

SHW, CHY and THH conceived and designed the experiments. CWW, MWL and CYH performed the experiments. EMT, CMH, MWL and THH analyzed the data. SHW and THH wrote the manuscript. CWW and THH confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Liu PC, Huang TH, Wu YC, Wang Y, Chiang CJ, Lee WC, Lin HH and Lo WC: Disease Burden of 30 cancer groups in Taiwan from 2000 to 2021. *J Epidemiol Glob Health* 15: 62, 2025.
2. Yin L, Duan JJ, Bian XW and Yu SC: Triple-negative breast cancer molecular subtyping and treatment progress. *Breast Cancer Res* 22: 61, 2020.
3. Rakha EA, El-Sayed ME, Green AR, Lee AH, Robertson JF and Ellis IO: Prognostic markers in triple-negative breast cancer. *Cancer* 109: 25-32, 2007.
4. Horwitz SB: Taxol (paclitaxel): Mechanisms of action. *Ann Oncol* 5 (Suppl 6): S3-S6, 1994.
5. Abal M, Andreu JM and Barasoain I: Taxanes: Microtubule and centrosome targets, and cell cycle dependent mechanisms of action. *Curr Cancer Drug Targets* 3: 193-203, 2003.
6. Giannakakou P, Robey R, Fojo T and Blagosklonny MV: Low concentrations of paclitaxel induce cell type-dependent p53, p21 and G1/G2 arrest instead of mitotic arrest: Molecular determinants of paclitaxel-induced cytotoxicity. *Oncogene* 20: 3806-3813, 2001.
7. Milross CG, Mason KA, Hunter NR, Chung WK, Peters LJ and Milas L: Relationship of mitotic arrest and apoptosis to antitumor effect of paclitaxel. *J Natl Cancer Inst* 88: 1308-1314, 1996.
8. Wang TH, Wang HS and Soong YK: Paclitaxel-induced cell death: Where the cell cycle and apoptosis come together. *Cancer* 88: 2619-2628, 2000.

9. Li G, Xu D, Sun J, Zhao S and Zheng D: Paclitaxel inhibits proliferation and invasion and promotes apoptosis of breast cancer cells by blocking activation of the PI3K/AKT signaling pathway. *Adv Clin Exp Med* 29: 1337-1345, 2020.
10. Fu S, Chen X, Lo HW and Lin J: Combined bazedoxifene and paclitaxel treatments inhibit cell viability, cell migration, colony formation, and tumor growth and induce apoptosis in breast cancer. *Cancer Lett* 448: 11-19, 2019.
11. Nakayama S, Torikoshi Y, Takahashi T, Yoshida T, Sudo T, Matsushima T, Kawasaki Y, Katayama A, Gohda K, Hortobagyi GN, *et al*: Prediction of paclitaxel sensitivity by CDK1 and CDK2 activity in human breast cancer cells. *Breast Cancer Res* 11: R12, 2009.
12. Takahashi T, Yamasaki F, Sudo T, Itamochi H, Adachi S, Tamamori-Adachi M and Ueno NT: Cyclin A-associated kinase activity is needed for paclitaxel sensitivity. *Mol Cancer Ther* 4: 1039-1046, 2005.
13. Barbuti AM and Chen ZS: Paclitaxel through the ages of anticancer therapy: Exploring its role in chemoresistance and radiation therapy. *Cancers (Basel)* 7: 2360-2371, 2015.
14. Chong T, Sarac A, Yao CQ, Liao L, Lyttle N, Boutros PC, Bartlett JMS and Spears M: Deregulation of the spindle assembly checkpoint is associated with paclitaxel resistance in ovarian cancer. *J Ovarian Res* 11: 27, 2018.
15. Swanton C, Marani M, Pardo O, Warne PH, Kelly G, Sahai E, Elustondo F, Chang J, Temple J, Ahmed AA, *et al*: Regulators of mitotic arrest and ceramide metabolism are determinants of sensitivity to paclitaxel and other chemotherapeutic drugs. *Cancer Cell* 11: 498-512, 2007.
16. Chan Y, Liu Y, Kong Y, Xu W, Zeng X, Li H, Guo Y, Tang X, Zhang J and Zhu B: Maternal genetic polymorphisms in the major mitotic checkpoint genes MAD1L1 and MAD2L1 associated with the risk of survival in abnormal chromosomal fetuses. *Front Genet* 14: 1105184, 2023.
17. Ko YH, Roh JH, Son YI, Chung MK, Jang JY, Byun H, Baek CH and Jeong HS: Expression of mitotic checkpoint proteins BUB1B and MAD2L1 in salivary duct carcinomas. *J Oral Pathol Med* 39: 349-355, 2010.
18. Ding X, Fu Q, Chen W, Chen L, Zeng Q, Zhang S and He L: Targeting of MAD2L1 by miR-515-5p involves the regulation of cell cycle arrest and apoptosis of colorectal cancer cells. *Cell Biol Int* 46: 840-848, 2022.
19. Li Q, Tong D, Jing X, Ma P, Li F, Jiang Q, Zhang J, Wen H, Cui M, Huang C and Zhang M: MAD2L1 is transcriptionally regulated by TEAD4 and promotes cell proliferation and migration in colorectal cancer. *Cancer Gene Ther* 30: 727-737, 2023.
20. Percy MJ, Myrie KA, Neeley CK, Azim JN, Ethier SP and Petty EM: Expression and mutational analyses of the human MAD2L1 gene in breast cancer cells. *Genes Chromosomes Cancer* 29: 356-362, 2000.
21. Wang Z, Katsaros D, Shen Y, Fu Y, Canuto EM, Benedetto C, Lu L, Chu WM, Risch HA and Yu H: Biological and clinical significance of MAD2L1 and BUB1, genes frequently appearing in expression signatures for breast cancer prognosis. *PLoS One* 10: e0136246, 2015.
22. Zhu L, Li X, Liu D, Bai W, Yang H, Cheng Q, Xu L and Fang J: The positive feedback loop of MAD2L1/TYK2/STAT3 induces progression in B-cell acute lymphoblastic leukaemia. *J Cancer Res Clin Oncol* 149: 6527-6540, 2023.
23. Bates M, Spillane CD, Gallagher MF, McCann A, Martin C, Blackshields G, Keegan H, Gubbins L, Brooks R, Brooks D, *et al*: The role of the MAD2-TLR4-MyD88 axis in paclitaxel resistance in ovarian cancer. *PLoS One* 15: e0243715, 2020.
24. Pacini C, Dempster JM, Boyle I, Gonçalves E, Najgebauer H, Karakoc E, van der Meer D, Barthorpe A, Lightfoot H, Jaaks P, *et al*: Integrated cross-study datasets of genetic dependencies in cancer. *Nat Commun* 12: 1661, 2021.
25. Dempster JM, Boyle I, Vazquez F, Root DE, Boehm JS, Hahn WC, Tsherniak A and McFarland JM: Chronos: A cell population dynamics model of CRISPR experiments that improves inference of gene fitness effects. *Genome Biol* 22: 343, 2021.
26. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25: 402-408, 2001.
27. He Y, Hao X, Hu B, Xia N, Wang C, Chen X, Zhang H, Duan Y, Ying Q and Dong Q: Identification of MAD2L1 as a novel biomarker for hepatoblastoma through bioinformatics and machine learning approaches. *Front Oncol* 15: 1524714, 2025.
28. Zou T and Lin Z: The involvement of ubiquitination machinery in cell cycle regulation and cancer progression. *Int J Mol Sci* 22: 5754, 2021.
29. Teixeira LK and Reed SI: Ubiquitin ligases and cell cycle control. *Annu Rev Biochem* 82: 387-414, 2013.
30. Gavilan E, Giraldez S, Sanchez-Aguayo I, Romero F, Ruano D and Daza P: Breast cancer cell line MCF7 escapes from G1/S arrest induced by proteasome inhibition through a GSK-3 β dependent mechanism. *Sci Rep* 5: 10027, 2015.
31. Jiang W, Yang X, Shi K, Zhang Y, Shi X, Wang J, Wang Y, Chenyan A, Shan J, Wang Y, *et al*: MAD2 activates IGF1R/PI3K/AKT pathway and promotes cholangiocarcinoma progression by interfering USP44/LIMA1 complex. *Oncogene* 42: 3344-3357, 2023.
32. Golan A, Yudkovsky Y and Hershko A: The cyclin-ubiquitin ligase activity of cyclosome/APC is jointly activated by protein kinases Cdk1-cyclin B and Plk. *J Biol Chem* 277: 15552-15557, 2002.
33. Garnett MJ, Mansfield J, Godwin C, Matsusaka T, Wu J, Russell P, Pines J and Venkitaraman AR: UBE2S elongates ubiquitin chains on APC/C substrates to promote mitotic exit. *Nat Cell Biol* 11: 1363-1369, 2009.
34. Craney A, Kelly A, Jia L, Fedrigo I, Yu H and Rape M: Control of APC/C-dependent ubiquitin chain elongation by reversible phosphorylation. *Proc Natl Acad Sci USA* 113: 1540-1545, 2016.
35. Miller K, Wang M, Gralow J, Dickler M, Cobleigh M, Perez EA, Shenker T, Cella D and Davidson NE: Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. *N Engl J Med* 357: 2666-2676, 2007.
36. Jordan MA, Wendell K, Gardiner S, Derry WB, Copp H and Wilson L: Mitotic block induced in HeLa cells by low concentrations of paclitaxel (Taxol) results in abnormal mitotic exit and apoptotic cell death. *Cancer Res* 56: 816-825, 1996.
37. Shu CH, Yang WK, Shih YL, Kuo ML and Huang TS: Cell cycle G2/M arrest and activation of cyclin-dependent kinases associated with low-dose paclitaxel-induced sub-G1 apoptosis. *Apoptosis* 2: 463-470, 1997.
38. Albain KS, Nag SM, Calderillo-Ruiz G, Jordaan JP, Llombart AC, Pluzanska A, Rolski J, Melemed AS, Reyes-Vidal JM, Sekhon JS, *et al*: Gemcitabine plus Paclitaxel versus Paclitaxel monotherapy in patients with metastatic breast cancer and prior anthracycline treatment. *J Clin Oncol* 26: 3950-3957, 2008.
39. Safavy A, Raisch KP, Khazaeli MB, Buchsbaum DJ and Bonner JA: Paclitaxel derivatives for targeted therapy of cancer: toward the development of smart taxanes. *J Med Chem* 42: 4919-4924, 1999.
40. Shi X and Sun X: Regulation of paclitaxel activity by microtubule-associated proteins in cancer chemotherapy. *Cancer Chemother Pharmacol* 80: 909-917, 2017.
41. Eckford PD and Sharom FJ: ABC efflux pump-based resistance to chemotherapy drugs. *Chem Rev* 109: 2989-3011, 2009.
42. O'Reilly EA, Gubbins L, Sharma S, Tully R, Guang MH, Weiner-Gorzel K, McCaffrey J, Harrison M, Furlong F, Kell M and McCann A: The fate of chemoresistance in triple negative breast cancer (TNBC). *BBA Clin* 3: 257-275, 2015.
43. Liu P, Kumar IS, Brown S, Kannappan V, Tawari PE, Tang JZ, Jiang W, Armesilla AL, Darling JL and Wang W: Disulfiram targets cancer stem-like cells and reverses resistance and cross-resistance in acquired paclitaxel-resistant triple-negative breast cancer cells. *Br J Cancer* 109: 1876-1885, 2013.
44. Dziadyk JM, Sui M, Zhu X and Fan W: Paclitaxel-induced apoptosis may occur without a prior G2/M-phase arrest. *Anticancer Res* 24: 27-36, 2004.
45. Hegarat N, Crncec A, Suarez Peredo Rodriguez MF, Echegaray Iturra F, Gu Y, Busby O, Lang PF, Barr AR, Bakal C, Kanemaki MT, *et al*: Cyclin A triggers Mitosis either via the Greatwall kinase pathway or Cyclin B. *EMBO J* 39: e104419, 2020.



Copyright © 2026 Wang et al. This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.