Biomedicine & Healthcare Research

Biomedicine & Healthcare Research 2026 July;7:10-18

https://doi.org/10.71599/bhr.v7i1.199

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Original article

Mps1 Inhibition Preferentially Induces DNA Damage in Tetraploid Cancer Cells

Yesmine Lahlaoui [1], Ranim Khemiri [1], Ghofrane Kitar [1], Tasnim Gassem [1] and Mohamed Jemaà [1-3]

1 Human Genetics Laboratory LR99ES1, Faculty of Medicine of Tunis, Tunis El Manar University, Tunis, Tunisia.
2 Neurophysiology, Cellular Physiopathology and Valorisation of Biomolecules Laboratory LR18ES03, Faculty of Sciences of Tunis, Tunis El Manar University, Tunis, Tunisia.
3 Department of Biology, Faculty of Sciences of Tunis, Tunis El Manar University, Tunis, Tunisia.

Abstract

Background: Tetraploidy, a state of whole-genome duplication, is frequently observed in human cancers and confers resistance to conventional DNA-damaging therapies. The spindle assembly checkpoint (SAC) kinase Mps1 (TTK) is essential for mitotic fidelity and has emerged as a promising anticancer target. However, whether Mps1 inhibition differentially induces DNA damage in tetraploid versus diploid cancer cells, and whether this response engages the p53 pathway, remains unknown.

Methods: Using isogenic diploid and tetraploid RKO colon carcinoma cells, we assessed the effects of pharmacological (Reversine) and genetic (siRNA) Mps1 inhibition on cell viability, clonogenic potential, and DNA damage induction. DNA double-strand breaks (DSBs) were quantified by γH2AX immunofluorescence and western blot analysis of γH2AX and phosphorylated CHK2 (T68). The involvement of the p53/p21 axis was examined using immunoblot and functional validation in p53-proficient and p53-deficient isogenic HCT116 cells.

Results: Tetraploid RKO cells exhibited significantly greater resistance to bleomycin, cisplatin, etoposide, and camptothecin compared to diploid cells ($p < 0.001$). Conversely, Mps1 inhibition or depletion preferentially compromised tetraploid cell viability and clonogenic potential. Mechanistically, Mps1 knockdown induced a marked increase in γH2AX foci and pCHK2 (T68) specifically in tetraploid cells, indicative of a robust double-strand break (DSB) response. Notably, this occurred without significant changes in p53 or p21 expression, and p53-deficient tetraploid cells remained sensitive to Mps1 targeting, suggesting a p53-independent mechanism.

Conclusion: Mps1 inhibition preferentially induces enhanced DNA damage in tetraploid cancer cells via a p53-independent pathway. These new findings position Mps1 as a promising therapeutic target for tetraploid-rich tumors and potentially including those with p53 mutations.

Received: June 12, 2026; Accepted: July 10, 2026

1. Introduction

Tetraploidy, a state characterized by the presence of four complete sets of chromosomes (4N), is frequently observed in human cancers. While once considered a mere byproduct of aberrant cell division, accumulating evidence indicates that tetraploidization can serve as a driver of tumorigenesis [1, 2].

Whole-genome duplication (WGD) events, occur in approximately 30-40% of human cancers, including colorectal, gastric, breast, and ovarian carcinomas [3, 4]. Tetraploid cells exhibit enhanced chromosomal instability (CIN), and an elevated propensity for aneuploidy, fueling tumor heterogeneity, progression, and therapeutic resistance [5, 6].

The relationship between tetraploidy and DNA damage is complex. Stable tetraploid cells often exhibit enhanced baseline activation of the p53 pathway, which can paradoxically increase their resistance to exogenous DNA-damaging agents such as irradiation and chemotherapy [7, 8].

Correspondence: Dr. Mohamed Jemaà: mohamed.jemaa@fmt.utm.tn

However, tetraploid cells harbor extra centrosomes and a doubled DNA content, predisposing them to mitotic errors, replication stress, and spontaneous DNA double-strand breaks (DSBs) during subsequent divisions. Consequently, their inherent genomic instability renders them acutely vulnerable to perturbations that exacerbate DNA breakage [9-12].

To maintain genomic fidelity during mitosis, eukaryotic cells rely on the spindle assembly checkpoint (SAC), a surveillance mechanism that delays anaphase until all chromosomes achieve proper bipolar attachment to the mitotic spindle [13].

The SAC is particularly critical for tetraploid cells, which frequently exhibit multipolar spindle formation, merotelic attachments, and lagging chromosomes due to supernumerary centrosomes [9, 12]. Tetraploid cells are disproportionately dependent on an intact SAC to avoid catastrophic mitotic errors. Abrogation of SAC signaling in this context is predicted to precipitate rapid genomic disarray and mitotic catastrophe [14]. Monopolar spindle 1 (Mps1), also known as TTK, is an essential dual-specificity kinase that serves as the master regulator of the SAC.

Beyond its SAC function, Mps1 also coordinates centrosome duplication, kinetochore-microtubule attachment correction, and the recruitment of checkpoint proteins [15, 16]. Given its central role, Mps1 activity is stringently regulated; its overexpression is frequently observed in aneuploid and tetraploid tumors, correlating with poor prognosis and chemoresistance [17]. Accordingly, several potent and selective small-molecule Mps1 inhibitors (e.g., reversine, AZ3146, NMS-P715) have been developed and evaluated preclinically [16]. By ablating SAC activity, Mps1 inhibition forces mitotic progression irrespective of chromosome alignment, resulting in severe aneuploidy, lagging chromosomes, micronuclei formation, and mitotic catastrophe [17]. Previous work has shown that Mps1 inhibition induces DNA damage in cancer cells [18, 19], and our group has demonstrated that Mps1 targeting preferentially kills tetraploid cancer cells across multiple models via p53-independent apoptosis [20, 21].

Despite these advances, a critical gap remains: whether Mps1 inhibition differentially induces DNA damage in tetraploid versus diploid cancer cells has not been directly established. In particular, the mechanistic link between SAC abrogation, mitotic catastrophe, and DNA fragmentation in tetraploid cells remains unexplored. Given that tetraploid cells harbor extra centrosomes and operate under chronic mitotic stress [11], we hypothesized that Mps1 inhibition would precipitate greater genomic fragmentation in tetraploid cells compared to their diploid counterparts. To test this, we quantified DNA double-strand breaks (γH2AX foci and phosphorylated CHK2) following Mps1 inhibitor treatment in isogenic diploid and tetraploid RKO colon carcinoma cells, and assessed the involvement of the p53/p21 axis using p53-proficient and p53-deficient isogenic HCT116 cells.

2. Materials and Methods

Cell lines, chemicals and culture conditions

RKO and HCT116 human colon carcinoma cells previously generated [18, 19] (both diploid and tetraploid clones) were grown in DMEM with 10% FCS and 100 U/mL penicillin G (Thermo Fisher Scientific-Gibco). Cells were tested for mycoplasma routinely. Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich (St. Louis, MO). All compounds used in this study (Reversine, Bleomycin, Cisplatin, Etoposide and Camptothecin) were purchased from Sigma-Aldrich (St. Louis, MO) and stocked as a 10 mM solution in DMSO and used at appropriate doses as described in figures legends. 24 hours prior to each experiment, cells were seeded into 6-, 12-, 24-, or 96-well plates.

RNA interference

Diploid and tetraploid cells were seeded at low confluence in 6-well plates. Following a 24-hour attachment period, cells were subjected to transfection using oligofectamine RNAiMAX reagent (Thermo Fisher Scientific-Invitrogen) in accordance with the manufacturer's specifications. Experimental conditions included transfection with a non-targeting control siRNA (siUNR) or with a validated siRNA directed against Mps1 mRNA; all oligonucleotides were procured from Eurogentec (Liege, Belgium). These are the sequences of the used siRNAs:
5′-GCCGGUAUGCCGGUUAAGUdTdT-3′(siUNR),
5′-UGGUUGAGUUUGUUGCUCAUUdTdT-3′(siMps1)

Crystal Violet Toxicity Assay

Cells were seeded at 2000 cells/well in 96-well plates and cultured for up to 3 days. At each time point, cells were PBS-washed, fixed with 4% PFA for 15 min, and then stained with 0.1% (w/v) crystal violet aqueous solution for 30 min at room temperature. After three distilled water washes, 200 µL/well of 10% acetic acid was added with pipette agitation. Absorbance was read at 595 nm using a Synergy 2 microplate reader (Biotek, Germany).

Cytofluorometric studies

Membrane permeabilization was measured by DAPI staining (Sigma-Aldrich) using a FACSCalibur cytofluorometer (BD Biosciences), with data analysis performed via CellQuestTM software (BD Biosciences). Statistical analysis included only events with normal forward and side scatter (FSC/SSC) parameters.

Immunoblotting

To assess protein expression levels, cells were collected, rinsed with PBS, and subjected to lysis on ice for 30 minutes using a buffer composed of 50 mM Tris (pH 7.4) supplemented with 250 mM NaCl, 0.1% NP-40, 0.1 mM PMSF, 10 mg/mL aprotinin, 10 mg/mL leupeptin, and 100 mM NaF. The resulting lysates were centrifuged at 13,000 rpm for 10 minutes, and the soluble protein concentration in the supernatant was determined via the Bradford method. Equivalent protein amounts (30 µg per sample) were separated by SDS-PAGE and transferred electrophoretically onto nitrocellulose membranes. Membranes were then incubated overnight with the appropriate primary antibody. Subsequently, they were incubated for 1 hour at room temperature with the corresponding DyLight 800-conjugated secondary antibody (Thermo Fisher Scientific-Pierce Antibodies) and visualized using a LI-COR Odyssey® scanner and associated software (LI-COR Biosciences, Lincoln, NE). The following primary antibodies were used: α-tubulin (#T9026; Sigma-Aldrich), Mps1 (#ab11108; Abcam, Cambridge, UK), p53 (#sc-126), p21 (#sc-6246), γH2AX (#sc-517348) and pCHK2 (#sc-5278) from Santa Cruz Biotechnology, Dallas, Texas, USA.

Immunofluorescence microscopy

To monitor DNA double-strand breaks via γH2AX foci, cells underwent fixation with 4% PFA (in PBS) followed by permeabilization in 0.1% Triton X-100 (in PBS). Immunofluorescence staining was performed using a mouse monoclonal antibody recognizing γH2AX phosphorylated at Ser139 (clone JWB301, 1:100 dilution; catalog #05-636, Millipore). After incubation with Alexa Fluor-conjugated secondary antibodies (Thermo Fisher Scientific-Invitrogen), cell nuclei were counterstained with Hoechst 33342 (1 µg/mL; BD Biosciences). Fluorescence images were captured on a Zeiss Axioimager Z1 motorized microscope (Zeiss, Oberkochen, Germany) using Axiovision acquisition software (Zeiss). Foci quantification and image analysis were carried out using ImageJ, an open-source software

distributed by the National Institutes of Health (http://rsb.info.nih.gov/ij/).

Statistical procedures

Data are expressed as arithmetic means ± SEM. At least 3 independent biological replicates were performed for every experimentation. As indicated in the figure legends, statistical analysis was made with GrapPpad Prism using ANOVA with Tukey's test as post-test. n denotes the number of different experiments or cells.

3. Results

Tetraploid Cells Exhibit Enhanced Resistance to DNA-Damaging Chemotherapeutic Agents

To characterize the baseline chemosensitivity of tetraploid cancer cells relative to their diploid counterparts, we exposed isogenic diploid and tetraploid RKO cells to a panel of DNA-damaging agents commonly used in clinical oncology: bleomycin (a radiomimetic that induces DNA double-strand breaks), cisplatin (a crosslinking agent), etoposide (a topoisomerase II inhibitor), and camptothecin (a topoisomerase I inhibitor). Following 72 hours of treatment, we assessed cell death by multiparametric flow cytometry using DiOC6(3) to monitor mitochondrial transmembrane potential (Δψm) and propidium iodide (PI) to evaluate plasma membrane integrity. Representative density plots for vehicle control and bleomycin-treated cells are shown in Figure 1A. Quantitative analysis revealed that tetraploid cells were significantly more resistant to all four DNA-damaging agents compared to their diploid counterparts (Figure 1B). Notably, basal cell death levels in vehicle-treated controls were comparable between diploid and tetraploid cells, indicating that the observed resistance is not due to intrinsic viability differences but rather reflects an acquired tolerance to genotoxic stress. These data demonstrate that tetraploid RKO cells are inherently more resistant to conventional DNA damage-inducing chemotherapy than their diploid counterparts. These findings are consistent with previous reports demonstrating that tetraploid cancer cells, including RKO and HCT116 clones, exhibit selective resistance to DNA-damaging agents such as cisplatin, oxaliplatin, camptothecin, and ionizing radiation, while remaining sensitive to non-genotoxic cytotoxic agents [20, 21]. This resistance phenotype highlights the rationale for evaluating alternative therapeutic strategies that selectively target tetraploid cells, such as Mps1 inhibition.

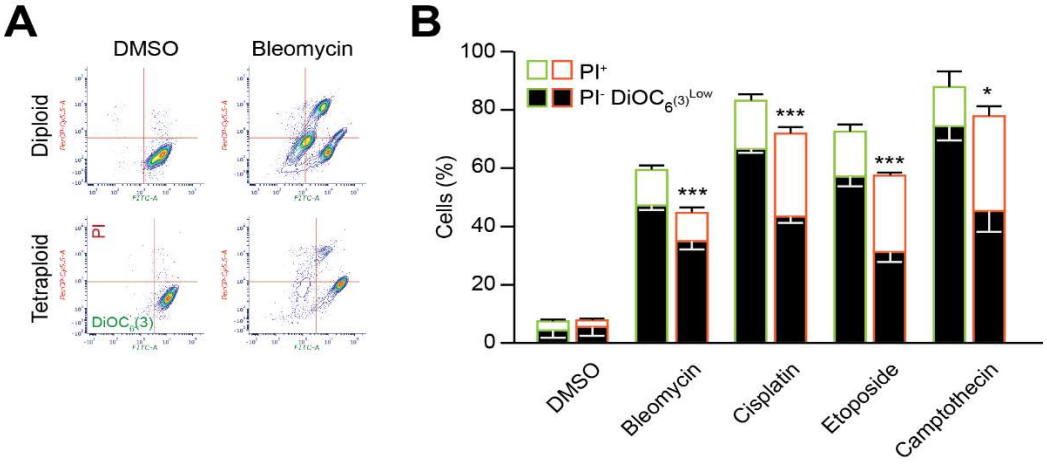

Fig 1. Tetraploid cells exhibit increased resistance to DNA damage-inducing chemotherapeutic agents compared to diploid counterparts.
(A) Representative flow cytometry density plots of diploid (top panels) and tetraploid (bottom panels) cells following a 72 hour treatment with either vehicle control (DMSO) or the DNA-damaging agent Bleomycin (20 □M). Cells were co-stained with DiOC6(3) (FITC channel, x-axis) to evaluate mitochondrial transmembrane potential (Δψm) and Propidium Iodide (PI; PerCP-Cy5.5 channel, y-axis) to assess plasma membrane integrity. Quadrants distinguish viable cells (DiOC6(3) high and PI low, the early dying/apoptotic cells DiOC6(3) low PI low, the late dying/apoptotic cells DiOC6(3) low PI high, and dead/necrotic cells DiOC6(3) high PI high).
(B) Quantitative analysis of cell death induction in diploid (green-bordered bars) and tetraploid (orange-bordered bars) cells after exposure to DMSO, (Bleomycin 20 □M), Cisplatin (20 □M), Etoposide (20 □M), or Camptothecin (1 □M). The stacked bar graph represents the cumulative percentage of dying/early and late apoptotic cells. Data are reported as means ± SEM ($n \geq 3$). ***$p < 0.001$ and *$p < 0.05$ (Mann–Whitney test), as compared with diploid.

Mps1 Inhibition or Depletion Preferentially Compromises the Viability and Clonogenic Potential of Tetraploid Cells

Given the relative resistance of tetraploid cells to conventional DNA-damaging agents, we next investigated whether targeting the spindle assembly checkpoint (SAC) via Mps1 inhibition would preferentially compromise tetraploid cell survival. We employed two complementary approaches: pharmacological inhibition with the small-molecule Mps1 inhibitor Reversine and genetic knockdown using Mps1-specific siRNA. We first assessed cell death by flow cytometry using DAPI uptake as a marker of plasma membrane permeabilization. Following 72 hours of treatment with Reversine, tetraploid cells exhibited a marked increase in the percentage of DAPI-high (dead) cells compared to their diploid counterparts (Figure 2A,B). To confirm the specificity of this effect, we performed siRNA-mediated knockdown of Mps1. Western blot analysis confirmed efficient Mps1 protein depletion in both diploid and tetraploid cells 72 hours post-transfection (Figure 2C). Under these conditions, crystal violet time-course assays revealed that tetraploid cells displayed a progressive and significant reduction in survival fraction over 72 hours compared to diploid cells, which remained relatively less affected (Figure 2D). Flow cytometric quantification of DAPI uptake at 72 hours post-transfection confirmed that

Mps1 knockdown induced significantly higher cell death in tetraploid versus diploid cells (Figure 2E).

To evaluate the long-term functional consequences of Mps1 inhibition, we performed clonogenic survival assays. Cells were treated with Reversine or transfected with Mps1 siRNA, then allowed to recover and form colonies over 10-15 days. Under basal conditions (DMSO or siUNR control), tetraploid cells formed significantly more colonies than diploid cells (Figure 2F,G), consistent with their enhanced proliferative capacity and resistance to spontaneous cell death [20]. Importantly, following Mps1 inhibition or depletion, the colony-forming ability of tetraploid cells was dramatically reduced, whereas diploid cells remained largely clonogenic (Figure 2F,G). Normalized survival fractions revealed that tetraploid cells were significantly more sensitive to Mps1 targeting than diploid cells under both pharmacological and genetic conditions (Figure 2G).

Collectively, these data demonstrate that Mps1 inhibition or depletion preferentially compromises the viability and long-term clonogenic potential of tetraploid RKO cells. This finding extends our prior observations in HCT116 and MFH152 cells [22], suggesting that preferential killing of tetraploid cells may extend beyond RKO cells, and further aligns with our demonstration that other mitotic perturbators such as SP600125 similarly induce preferential tetraploid cell death [23].

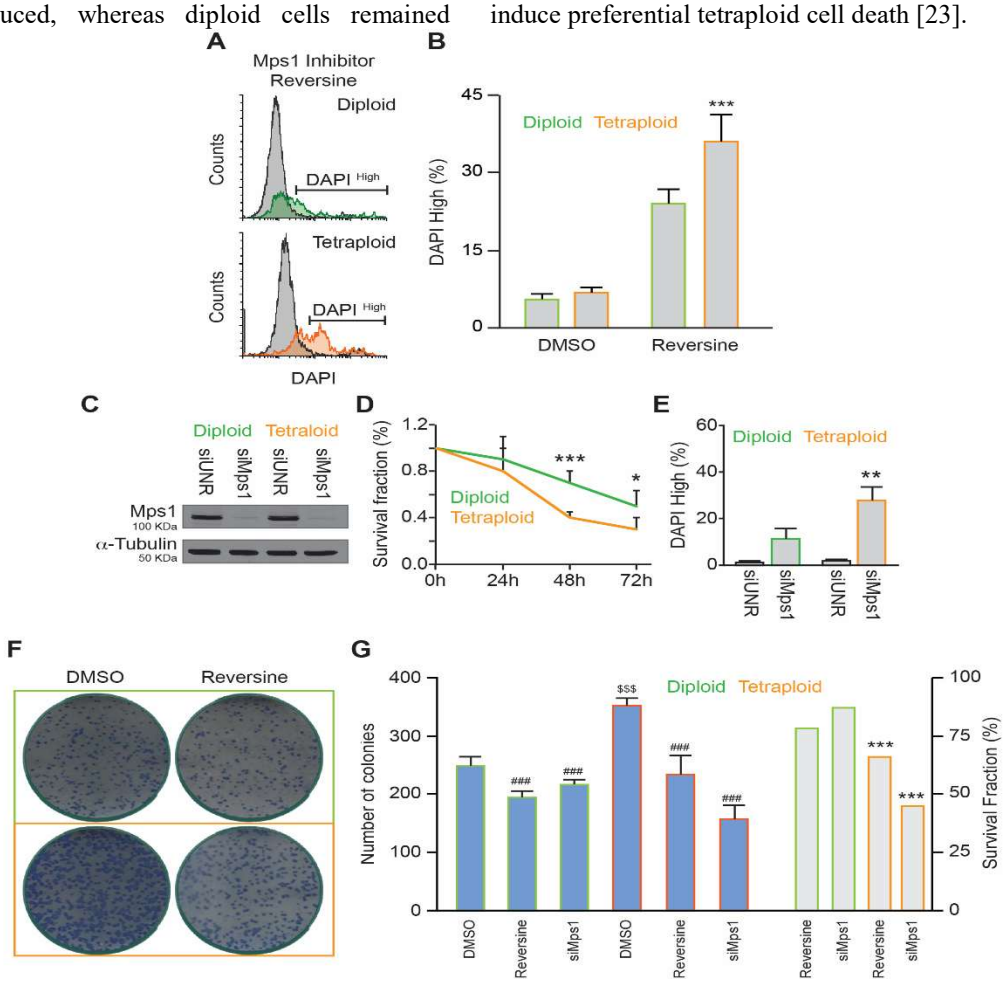

Fig 2. Inhibition or depletion of Mps1 preferentially compromises the viability and clonogenic potential of tetraploid cells.

(A, B) Flow cytometric assessment of cell death following pharmacological Mps1 inhibition. (A) Representative DAPI uptake histograms of diploid (top) and tetraploid (bottom) cells treated with vehicle control (DMSO) or the Mps1 inhibitor Reversine. The dead cells population is gated as DAPI High. (B) Quantification of the percentage of DAPI High for dead cells. Diploid cells are represented by green borders, tetraploid cells by orange borders. (C–E) Genetic knockdown of Mps1 via RNA interference. (C) Western blot analysis confirming the knockdown efficiency of Mps1 (100 kDa) using specific small interfering RNA (siMps1) compared to non-targeting control siRNA (siUNR) in both diploid and tetraploid cells. α-Tubulin (50 kDa) was used as the loading control. (D) Crystal violet time-course survival fraction (%) of diploid (green line) and tetraploid (orange line) cells over 72 hours post-transfection with siMps1. (E) Quantification of the percentage of DAPI High dead cells 72 hours post-transfection with siUNR or siMps1. (F, G) Long-term clonogenic survival assay. (F) Representative images of crystal violet-stained colonies formed by diploid (top, green outlines) and tetraploid (bottom, orange outlines) cells after treatment with DMSO or Reversine. (G) Quantitative analysis of clonogenicity. The left y-axis and solid blue bars indicate the absolute number of colonies formed under basal or Mps1-compromised (Reversine or siMps1) conditions. The right y-axis and open bars represent the normalized survival fraction (%) relative to respective DMSO/control conditions. Data are reported as means ± SEM ($n \geq 3$). ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$ (Mann–Whitney test), as compared with diploid. *** $p < 0.001$ comparing basal tetraploid vs. diploid colony numbers.### $p < 0.001$ compared to respective cell line DMSO controls.

Mps1 Depletion Induces DNA Double-Strand Breaks Specifically in Tetraploid Cells

To examine the impact of Mps1 silencing or inhibition on DNA damage, we knocked down Mps1 in diploid and tetraploid RKO cells using specific siRNA or treated with Reversine. 48-hours post-treatment, cells were fixed for immunofluorescence analysis of nuclei size and γH2AX foci, a well-established marker of DNA double-strand breaks (DSBs). Representative images revealed that nuclei were bigger in treated cells consistent with polyploidization [24]. In addition, γH2AX foci were significantly more

abundant in tetraploid nuclei compared to their diploid counterparts (Figure 3A-C). Quantification confirmed that tetraploid cells accumulated approximately 4- to 5-fold more γH2AX foci per nucleus than diploid cells at 48 hours.

To characterize the molecular nature of the DNA damage response, we performed immunoblot analysis of key DNA damage signalling proteins over a time course of 24 to 72 hours following Mps1 knockdown (Figure 3D). Consistent with the immunofluorescence data, tetraploid cells exhibited a strong and progressive accumulation of γH2AX beginning at 48 hours and peaking at 72 hours post-transfection. Concomitantly, we observed a marked increase in phosphorylated CHK2 at threonine 68 (pCHK2 T68), a canonical downstream effector of the DSB response, specifically in tetraploid cells. Of note, while pCHK2 T68 levels remained elevated in diploid cells at 72 hours, they appeared to decline in tetraploid cells at this time point, likely reflecting that the majority of tetraploid cells had already undergone cell death, whereas diploid cells were still in the process of dying.

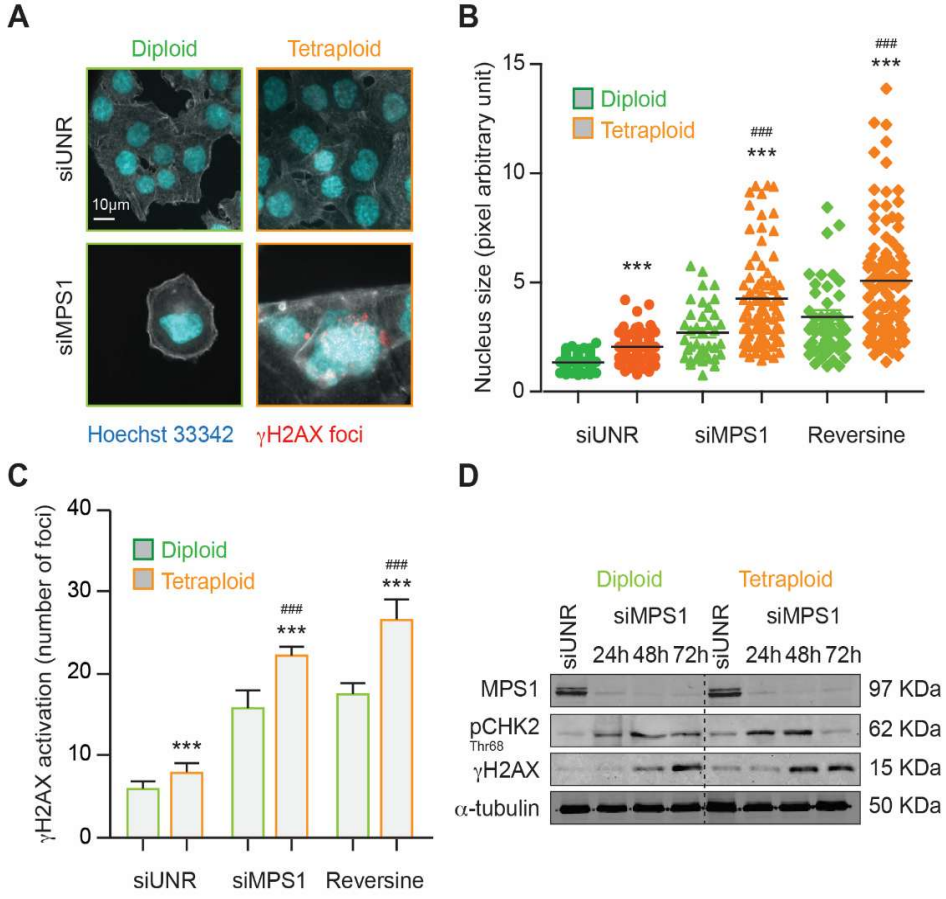

Fig 3. Mps1 depletion induces DNA double-strand breaks specifically in tetraploid cells.

(A-C) Immunofluorescence detection of γH2AX foci as a marker of DNA double-strand breaks (DSBs). **(A)** Representative fluorescence microscopy images of diploid and tetraploid cells transfected with non-targeting control siRNA (siUNR) or Mps1-specific siRNA (siMps1) for 48 hours. γH2AX foci (red) were visualized using an anti-γH2AX antibody; nuclei were counterstained with Hoechst 33342 (blue). Scale bar = 10 µm. **(B)** Quantification of nuclei size after siRNAs transfection or treatment with the Mps1 inhibitor Reversine. At least 100 nuclei were counted per condition per experiment. **(C)** Quantification of γH2AX foci per nucleus. At least 100 nuclei were counted per condition per experiment. **(D)** Immunoblot analysis of DNA damage response markers following Mps1 knockdown. Representative Western blots showing protein levels of γH2AX (15 kDa) and phosphorylated CHK2 at threonine 68 (pCHK2 T68, 62 kDa) in diploid and tetraploid cells. Cells were transfected with siUNR or siMps1 and harvested at the indicated time points (24, 48, and 72 hours). α-Tubulin (50 kDa) served as the loading control. Data are presented as mean ± SEM (n = 3 independent experiments). ***p < 0.001 (Mann–Whitney test), as compared with respective control cells. ###p < 0.001 (Mann–Whitney test), as compared with diploid cells.

The Hypersensitivity of Tetraploid Cells to Mps1 Depletion Is Independent of the p53/p21 Signaling Pathway

Having established that Mps1 inhibition induces preferential DNA damage and cell death in tetraploid cells, we next investigated whether this differential sensitivity involves the canonical p53/p21 DNA damage response axis. Given that tetraploid cells have been reported to exhibit constitutive p53 activation and elevated expression of p53 target genes [25], we reasoned that p53 might contribute to their resistance to conventional genotoxic agents (Figure 1) but could also potentially modulate their response to Mps1 targeting.

To address this, we performed immunoblot analysis of p53 and p21 protein levels following Mps1 siRNA knockdown over a time course of 24 to 72 hours. As shown in Figure 4A, Mps1 was efficiently depleted in both diploid and tetraploid cells. Notably, despite the robust induction of DNA damage documented in tetraploid cells (Figure 3), we observed no significant difference in p53 or p21 protein levels in either ploidy state at any time point examined. Densitometric quantification confirmed that p53 and p21 expression across the time course showed no differential activation between diploid and tetraploid conditions (Figure

4B). These data indicate that the DNA damage response associated with Mps1 depletion does not engage the canonical p53/p21 transcriptional axis.

To functionally confirm that the preferential killing of tetraploid cells is p53-independent, we took advantage of isogenic p53-proficient (p53^(+/+)) and p53-deficient (p53^(-/-)) diploid and tetraploid HCT116 cells. Following Mps1 siRNA transfection, cell death was assessed by DiOC6(3)/PI co-staining to quantify mitochondrial membrane potential dissipation (dying cells) and plasma membrane permeabilization (dead cells). As shown in Figure 4C, Mps1 depletion induced significant cell death in tetraploid cells regardless of p53 status, whereas diploid cells remained largely resistant. Importantly, there was no significant difference in the extent of cell death between p53^(+/+) and p53^(-/-) tetraploid cells, suggesting that the absence of p53 does not rescue tetraploid cells from Mps1 inhibition-mediated cytotoxicity.

Collectively, these findings demonstrate that the hypersensitivity of tetraploid cells to Mps1 depletion operates through a p53/p21-independent mechanism. This aligns with the observation that p53-deficient tetraploid cells rewire their signaling networks to rely on alternative pathways such as p38α for mitotic progression [26]. Thus, the vulnerability of tetraploid cells to Mps1 targeting is not contingent on an intact p53 pathway, making this strategy potentially applicable to tumors with diverse p53 functional statuses.

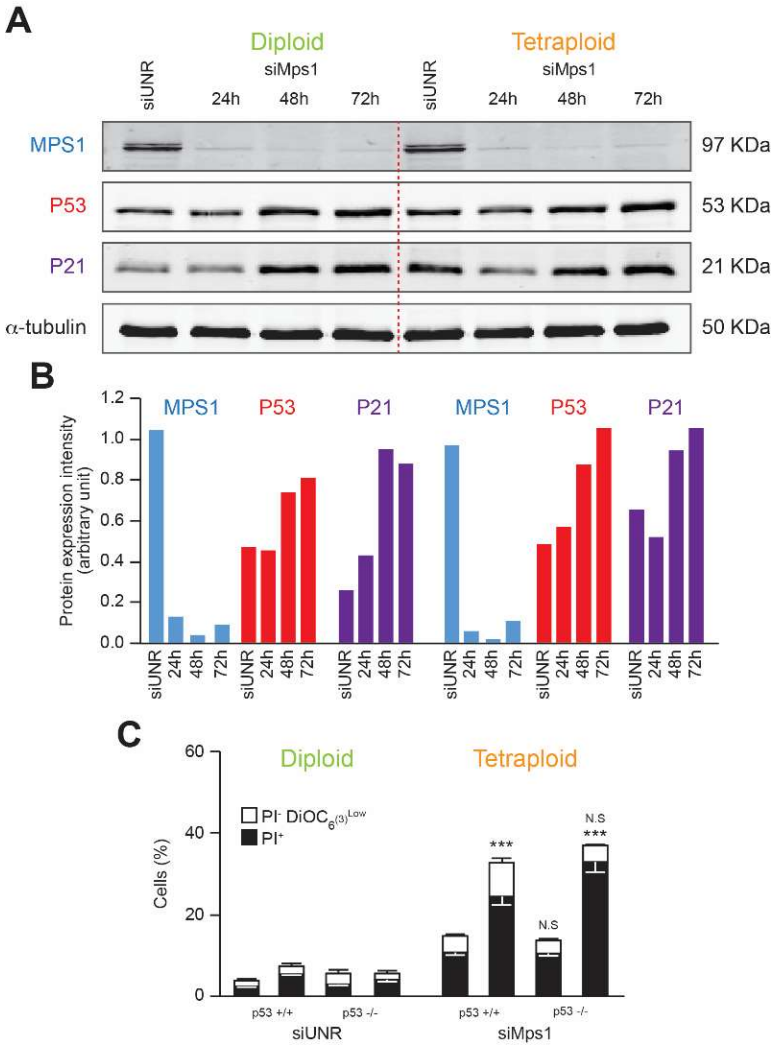

Fig4. The hypersensitivity of tetraploid cells to Mps1 depletion is independent of the p53/p21 signaling pathway.
(A, B) Immunoblot analysis of p53 and p21 expression kinetics following Mps1 knockdown. (A) Representative Western blots showing protein levels of MPS1 (97 kDa), p53 (53 kDa), and p21 (21 kDa) in diploid and tetraploid cells. Cells were transfected with non-targeting control siRNA (siUNR) or specific Mps1 siRNA (siMps1) and harvested at the indicated time points (24, 48, and 72 h). α-Tubulin (50 kDa) served as the loading control. (B) Densitometric quantification of MPS1 (blue), P53 (red), and P21 (purple) protein expression levels expressed in arbitrary units. No significant differential activation of the p53/p21 axis is observed between diploid and tetraploid conditions over time. (C) Genetic validation of p53-independent cell death induction. Functional evaluation of cell death in wild-type (p53+/+) and p53-deficient (p53-/-) diploid and tetraploid cells 72 hours post-transfection with siUNR or siMps1. Cell death was assessed by flow cytometry using DiOC6(3)-PI co-staining. The stacked bar graph indicates the cumulative percentage of dying and dead cells. Data are reported as means ± SEM ($n \geq 3$). $p < 0.001$ (Mann–Whitney test), as compared with diploid. NS. non-significant difference between corresponding p53+/+ and p53-/- phenotypes, confirming that the loss of p53 does not rescue tetraploid cells from siMps1-mediated cytotoxicity.

4. Discussion

In the present study, we observed that Mps1 depletion preferentially compromises the survival of tetraploid cancer cells compared to their diploid counterparts, this effect correlates with increased markers of DNA damage. Using siRNA-mediated knockdown and pharmacological inhibition with Reversine, we show that tetraploid RKO cells exhibit greater membrane permeabilization and

reduced viability following Mps1 abrogation. Mps1 silencing was associated with increased γH2AX foci formation and elevated pCHK2 (T68) specifically in tetraploid cells, consistent with a DNA damage response, without significant changes in p53 or p21 expression.

Several prior studies from our group and others have examined the vulnerabilities of tetraploid cells. Four prior studies establish the foundation for our current findings. It has been shown that p53-deficient tetraploid cells exhibit frequent multipolar mitoses driven by supernumerary centrosomes, with Mos upregulation preventing centrosome coalescence [12]. Also, it was discovered that p53-deficient tetraploid cells hyperphosphorylate p38α, which localizes to centrosomes and midbodies and is required for mitotic progression [26]. In addition, it has been demonstrated that SP600125 preferentially kills tetraploid cells via mitotic catastrophe [23]. Moreover, it has been shown that Mps1 inhibition (Reversine, AZ3146, or siRNA) preferentially kills tetraploid cells across multiple models (HCT116, RKO, MFH152) via intrinsic apoptosis, independently of p53 [22].

The present study provides additional observations. Indeed, one possible interpretation of our findings is that Mps1 inhibition leads to mitotic errors in tetraploid cells, which in turn may cause DNA damage. We demonstrate that the lethal mitotic catastrophe specifically in tetraploid cells and documented in previous studies is accompanied by, and likely driven by, the accumulation of frank DSBs. The structural basis for this enhanced DNA damage lies in centrosome biology. Tetraploid cells harbor extra centrosomes that remain dispersed due to Mos upregulation. Under normal conditions, the SAC, orchestrated by Mps1, delays anaphase until errors are corrected. When Mps1 is inhibited, tetraploid cells are forced through multipolar mitosis, and lagging chromosomes are physically damaged during cytokinesis, resulting in DSBs.

Beyond SAC regulation, Mps1 plays direct roles in DNA damage signaling and repair. Wei and colleagues (2005) demonstrated that Mps1 directly phosphorylates CHK2 on T68, positioning Mps1 as an upstream DDR activator [27]. Yeh and colleagues (2009) revealed a reciprocal loop wherein CHK2 stabilizes Mps1, amplifying checkpoint signaling [28]. Maachani and colleagues (2015) showed that Mps1 inhibition impairs both NHEJ and HR repair pathways, leading to persistent DNA damage [29]. The persistence of γH2AX foci we observed is consistent with defective DSB repair. Furthermore, aneuploidy generated from chromosome missegregation can induce replication stress-mediated DSBs (Ohashi et al., 2015), explaining the progressive damage accumulation in tetraploid daughter cells [30].

The absence of p53/p21 induction aligns with a prior work showing p53-independent killing [22] and with the observation that p53-deficient tetraploids rewire signaling to rely on p38α [26]. While Bhonde and colleagues (2006) [31] and Huang and colleagues (2009) [32] described p53-Mps1 cross-talk, our system engages p53-independent apoptotic pathways, likely because Mps1 inhibition in tetraploid cells induces catastrophic, irreparable damage that bypasses p53-mediated arrest.

Integrating these findings, we propose a refined model. Upon Mps1 inhibition, two synergistic mechanisms converge: (1) SAC abrogation forces tetraploid cells through multipolar mitosis, causing lagging chromosomes to be damaged during cytokinesis [33]; and (2) the direct roles of Mps1 in DDR signaling [27] and repair [34] are lost, preventing DSB resolution. The resulting DNA fragmentation triggers p53-independent apoptosis. The inherent genomic instability of tetraploid cells [20] amplifies this effect.

Taken together, our work across four prior studies and the current report positions Mps1 as a promising therapeutic target in tetraploid-rich tumors. We propose a refined model in which Mps1 loss-of-function precipitates lethal mitotic catastrophe in tetraploid cells specifically because it converts pre-existing chromosomal instability, rooted in centrosome amplification and Mos-dependent dispersal, into irreparable DNA double-strand breaks, while simultaneously crippling the DNA repair machinery required to resolve such lesions. The p53-independent nature of this cell death suggests that Mps1 inhibitors may be effective against a broad range of tetraploid tumors, regardless of p53 mutational status. Future studies should explore whether combining Mps1 inhibitors with agents that further compromise DNA repair (e.g., PARP inhibitors) or enhance centrosome clustering (e.g., HSET inhibitors) produces synergistic killing of tetraploid cancer cells.

In this study, several limitations should be acknowledged. Our conclusions regarding DNA damage rely on surrogate markers (γH2AX and pCHK2) rather than direct measures of DNA breakage. In addition, the majority of experiments were performed only in RKO and HCT116 cells, and our inference that Mps1 inhibition impairs DNA repair is indirect, based on persistent damage markers rather than direct repair assays. Finally, this study was conducted entirely in vitro, and in vivo validation will be necessary to assess the translational relevance of our findings.

Conclusion

In conclusion, our findings demonstrate that Mps1 inhibition is associated with preferential induction of DNA damage markers in tetraploid cancer cells, an effect that appears independent of the canonical p53/p21 signaling axis.

These observations suggest that tetraploid cells may be particularly sensitive to Mps1 depletion, possibly due to their inherent genomic instability. While the precise mechanisms remain to be fully elucidated, our results provide a rationale for further investigation of Mps1 as a candidate therapeutic target for tetraploid-rich tumors, including those with p53 mutations. Future studies employing direct measures of DNA damage and repair, along with in vivo models, will be necessary to validate these findings and establish the clinical relevance of this approach.

Acknowledgments

This work was supported by the Foundation Abdelwaheb Ben Ayed (FABA), Tunisia (FABA2025-MJ) and by funding from the Tunisian Ministry of Higher Education and Scientific Research MES (PEJC2025-D1P12).

Declaration of interests

The authors declare no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used DeepSeek for English editing. The authors used Gemini to create the graphical abstract. After using these tool/service, the authors reviewed and edited the content as needed, and the authors take full responsibility for the content of the published article.

Author Contributions

YL and RK: Experimentation, data analysis, reviewing & editing the manuscript. GK and TG: Reviewing & editing the manuscript. MJ: Conceptualization and writing the original draft, visualization, project administration, funding acquisition and supervision. All authors have read and agreed to the published version of the manuscript.

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Cite this article as : Lahlaoui Y, Khemiri R, Kitar G, Gassem T, Jemaà M. Mps1 inhibition preferentially induces DNA damage in tetraploid cancer cells. Biomedicine Healthcare Res. 2026;7:10-18. https://doi.org/10.71599/bhr.v7i1.199