Title

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Journal

Endocrinology, 160(8)

ISSN

0013-7227

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Publication Date

2019-08-01

DOI

10.1210/en.2019-00274

Peer reviewed

Menin Associates With the Mitotic Spindle and Is Important for Cell Division

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Menin is the protein mutated in patients with multiple endocrine neoplasia type 1 (MEN1) syndrome and their corresponding sporadic tumor counterparts. We have found that menin functions in promoting proper cell division. Here, we show that menin localizes to the mitotic spindle poles and the mitotic spindle during early mitosis and to the intercellular bridge microtubules during cytokinesis in HeLa cells. In our study, menin depletion led to defects in spindle assembly and chromosome congression during early mitosis, lagging chromosomes during anaphase, defective cytokinesis, multinucleated interphase cells, and cell death. In addition, pharmacological inhibition of the menin-MLL1 interaction also led to similar cell division defects. These results indicate that menin and the menin-MLL1 interaction are important for proper cell division. These results highlight a function for menin in cell division and aid our understanding of how mutation and misregulation of menin promotes tumorigenesis. (***Endocrinology*** **160: 1926–1936, 2019)**

Menin is the protein mutated in patients with the multiple endocrine neoplasia type 1 (MEN1) tumor syndrome and also their sporadic tumor counterparts (neuroendocrine pancreas, parathyroid, and pituitary tumors) (1). Menin is rarely mutated in other tumor types (2, 3). Although it functions as a tumor suppressor in MEN1, menin is unexpectedly pro-oncogenic in other tumors such as mixed-lineage leukemia (MLL)–associated leukemia and hepatocellular carcinoma (4–6). Thus, menin's oncogenic potential is context specific.

Most studies to understand menin function have focused on its role in cell signaling and gene transcription either through direct interaction with specific transcription factors such as c-myc (7) or through integration with large chromatin modifier complexes (8, 9). In either case, menin's specific activity in these protein interactions is unknown and often ascribed to a scaffolding role (10, 11). The most thoroughly studied of these is menin's interaction with the complex associated with Set1 (COMPASS)-like family proteins (11–13). Menin functions within two of the six known human Su(var)3-9, Enhancer-of-zeste and Trithorax (SET1)–based protein complexes that epigenetically activate gene transcription through histone-H3 lysine-K4 (H3K4) methylation (14). Menin specifically binds the *N*-terminal subunits of MLL1/KMT2A and MLL2/KMT2B proteins, but not the other SET1 family proteins (namely, MLL3/KMT2C, MLL4/KMT2D, SETD1A/KMT2F, and SETD1B/KMT2G) and cooperatively recruits MLL1/2 to chromatin for H3K4 methylation (15–17).

Studies of menin biological function have chiefly focused on its role in cell signaling and, particularly, in downstream events in transcription leading to changes in cell proliferation. But a direct role for menin during cell division is unclear (18). Menin has a bookmarking role during mitosis, but its expression is reduced during mitosis compared with interphase (19). Interestingly, a nontranscriptional role for MLL in mitosis was recently described (20, 21) and we hypothesize that menin will

ISSN Online 1945-7170

Received 4 April 2019. Accepted 11 June 2019.
First Published Online 18 June 2019

Abbreviations: DMSO, dimethyl sulfoxide; GFP, green fluorescent protein; MEN1, multiple endocrine neoplasia type 1; MLL, mixed-lineage leukemia; SET1, Su(var)3-9, Enhancer-of-zeste and Trithorax; siCont, control small interfering RNA; siMEN, small interfering RNA targeting *MEN1* expression; siRNA, small interfering RNA.

also have a nontranscriptional activity during cell division.

To better understand how menin misregulation can promote tumorigenesis, we studied menin specifically during cell division. Depletion of menin in HeLa cells and HCT116 cells led to defects in spindle assembly, chromosome congression, lagging chromosomes during anaphase, cytokinetic defects, multinucleated interphase cells, and cell death. In addition, pharmacological inhibition of the menin-MLL1 interaction revealed similar cell division defects. These data define a function for menin in ensuring proper mitotic spindle assembly and cell division. Furthermore, they advance our understanding of how mutation of *MEN1* that is likely to lead to misregulation of cell division promotes the downstream disease pathology associated with endocrine tumors that harbor *MEN1* mutations.

Materials and Methods

Cell culture and cell cycle synchronization

HeLa [CCL2; RRID:CVCL_0030 (22); ATCC] cell line growth and small interfering RNA (siRNA) treatments with OriGene control nontargeting siRNA (SR30004) and siRNA targeting *MEN1* (SR302867A and SR302867B) were used as described previously (23, 24). HCT116-GFP-H2B cells for live time-lapse microscopy were established and maintained as previously described (23). For G_1/S arrest and release experiments, cells were arrested with 2 mM thymidine for 18 hours, washed three times with PBS and two times with complete media and released into fresh media. For inhibition of the menin-MLL1 interaction, cells were treated with 10 μM MI-2 (catalog no. S7618; Selleckchem) or dimethyl sulfoxide (DMSO) for the indicated times.

Immunofluorescence and live-cell time-lapse microscopy

Immunofluorescence microscopy was performed as described previously (24) with the following modifications. A DMI6000 Leica microscope (Leica DFC360 FX Camera, 63×/1.40-0.60 NA oil objective; Leica AF6000 software) was used to acquire the immunofluorescence images. The Leica Application Suite 3D Deconvolution software was then used to deconvolve the images and they were subsequently exported as tagged image file format (TIFF) files. For quantifying spindle and cytokinetic defects, 100 cells from three independent experiments were counted and the data are presented as the mean ± SD. For live-cell time-lapse microscopy, HeLa cells were transfected with indicated siRNAs for 24 hours, arrested in G_1/S with 2 mM thymidine for 18 hours, washed, and released into the cell cycle. Cells were imaged live 6 hours after release for 24 hours using the microscope as used for immunofluorescence microscopy, except that a 20×/0.4 NA air objective was used and cells were kept at 37°C. Images were then converted to Audio Video Interleave movies. For MI-2–treated, live-cell time-lapse microscopy, HCT116-GFP-H2B cells were treated with 10 μM MI-2 2 hours before mitotic entry and imaged as indicated previously in this section and previously reported (23). Each frame represents a 10-minute interval.

Gene expression constructs

To create the green fluorescent protein (GFP)-menin expression plasmid, the full-length open reading frame of human wild-type menin from pCR2.1-menin previously described (16) was subcloned into pEGFP-N3 (Clontech) and fully sequenced to confirm fidelity. Construction of the *MEN1* wild-type cDNA expression plasmid (pCMV-Sport-menin) was previously described (25).

Antibodies and Western blotting

Immunofluorescence and immunoblotting were carried out using the following antibodies: menin from Bethyl (26), α-tubulin clone mca77g from AbD Serotec (27), cyclin B1 from Santa Cruz (28), MLL1-N from Millipore (29), MLL1-C from Millipore (30), and Kif2A from Abcam (31). Secondary antibodies conjugated to either fluorescein isothiocyanate (FITC; donkey anti-rabbit) (32), FITC (donkey anti-mouse) (33), FITC (donkey anti-rat) (34), Cy3 (donkey anti-rabbit) (35), Cy3 (donkey anti-mouse) (36), Cy3 (donkey anti-rat) (37), and Cy5 (donkey anti-rat) (38) were from Jackson ImmunoResearch and those conjugated to IRDye 680 (goat anti-rat) (39), and IRDye 680 (donkey anti-mouse) (40), and IRDye 800 (donkey anti-mouse) (41) were from LI-COR Biosciences. Western blotting was performed as previously reported (23).

Supplemental data

Supplemental figures and video can be found in an online repository (42).

Results

MLL1/KMT2A is a known interactor of menin that localizes to mitotic spindle poles and spindle microtubules during mitosis and functions in a noncanonical role during cell division (20). To better understand the function of menin and its misregulation in tumorigenesis, we began by asking if menin localized to microtubule structures similar to MLL1 during cell division. MLL1 is proteolytically processed into two fragments (MLL1-N and MLL1-C) that self-associate within the cell to form a functional heterodimer (43). Subcellular localization of both subunits was determined in this study. HeLa cells were fixed and stained for DNA, α–tubulin, and either menin, MLL1-N, or MLL1-C. During interphase, menin localized predominantly within the nucleus, as reported previously (24), similar to MLL1-N and MLL1-C) [Fig. 1A and 1B; (42)]. During mitosis, menin localized robustly to the spindle poles during prophase and prometaphase and to a lesser extent during metaphase and postmetaphase, similar to MLL1-N and MLL1-C [Fig. 1A and 1B; (42)]. Interestingly, menin, MLL1-N, and MLL1-C also localized to intercellular bridge microtubules during cytokinesis [Fig. 1A and 1B; (42)].

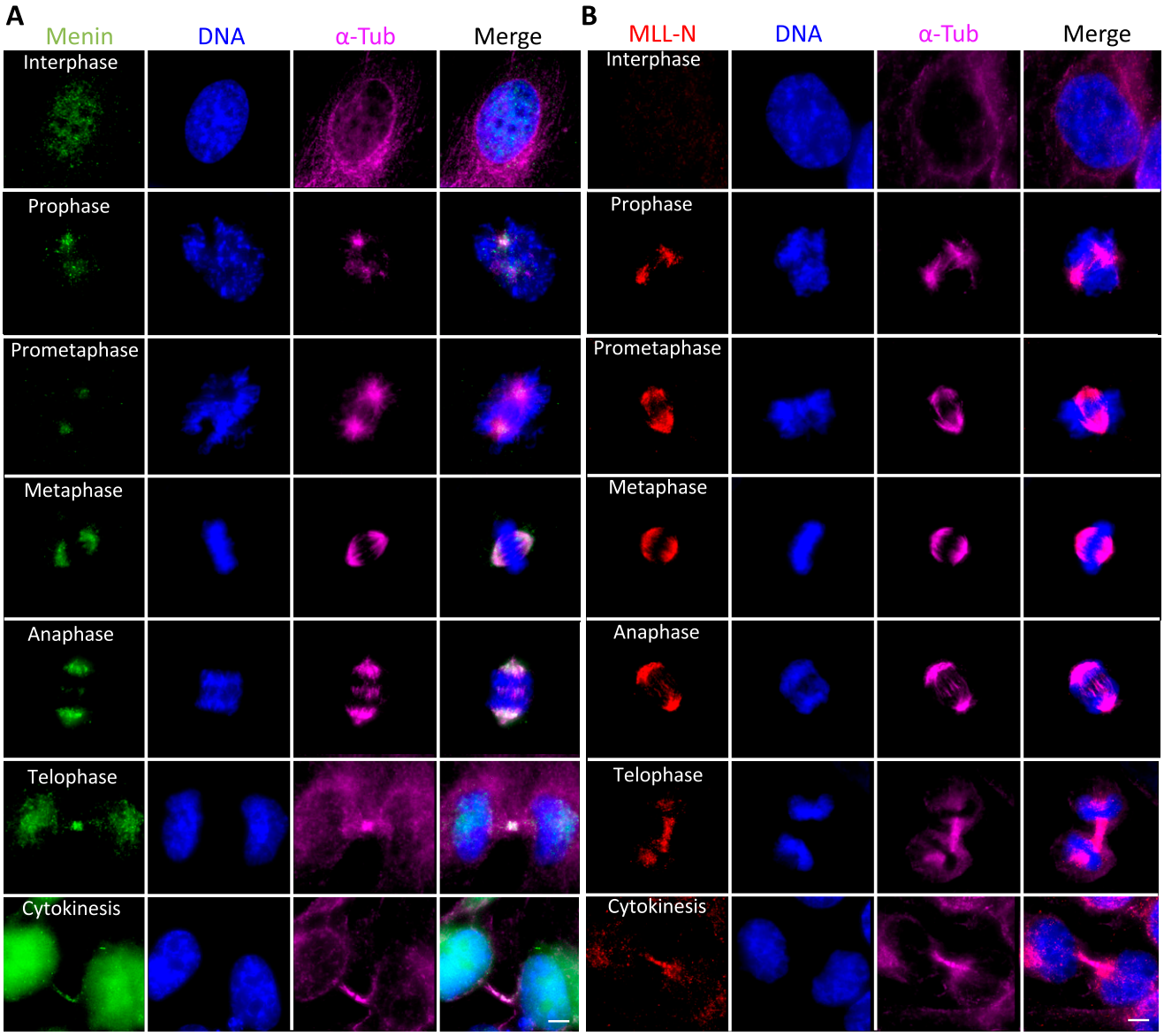

Figure 1. Cell cycle subcellular localization of menin and MLL1-N. Immunofluorescence microscopy of HeLa cells stained for DNA, α-tubulin (α-Tub) and either (A) menin or (B) MLL1-N. Note that menin localizes to the mitotic spindle poles and mitotic spindle during early mitosis and to intercellular bridge microtubules during cytokinesis, similar to MLL1-N. Scale bars, 5 μm.

That previous studies could not demonstrate menin localization to the mitotic apparatus could be due to different immunofluorescence microscopy conditions or different lots of the antibody (20). To address the specificity of the menin antibody used in this study, menin immunofluorescence was evaluated in HeLa cells treated with siRNA targeting *MEN1* expression (siMEN) and compared with control siRNA (siCont)–treated cells. As expected, the siMEN-treated cells showed a decrease in menin protein levels by immunoblot analysis and menin was not observed at the mitotic apparatus (Fig. 2A and 2B). To further address this issue, we visualized overexpressed GFP-tagged menin. The overexpressed GFP-tagged version of menin colocalized with MLL1-N and MLL1-C to the spindle poles during metaphase and to a lesser extent to intercellular bridge microtubules during cytokinesis (Fig. 2C and 2D). Together, these data indicated that menin was localizing to microtubule-based structures during mitosis, spindle poles in early mitosis, and intercellular bridge microtubules during cytokinesis, similar to MLL1. Importantly, to our knowledge, MLL1 had not been previously shown to localize to intercellular bridge microtubules.

Next, we asked if menin had a role in cell division by characterizing mitotic defects in HeLa cells deficient in menin expression. HeLa cells were transfected with siCont or siMEN, validated to decrease menin protein levels by immunofluorescence and immunoblot analyses (Fig. 2A and 2B), targeting siRNAs for 48 hours; fixed; and stained for DNA and α-tubulin. Although siMEN

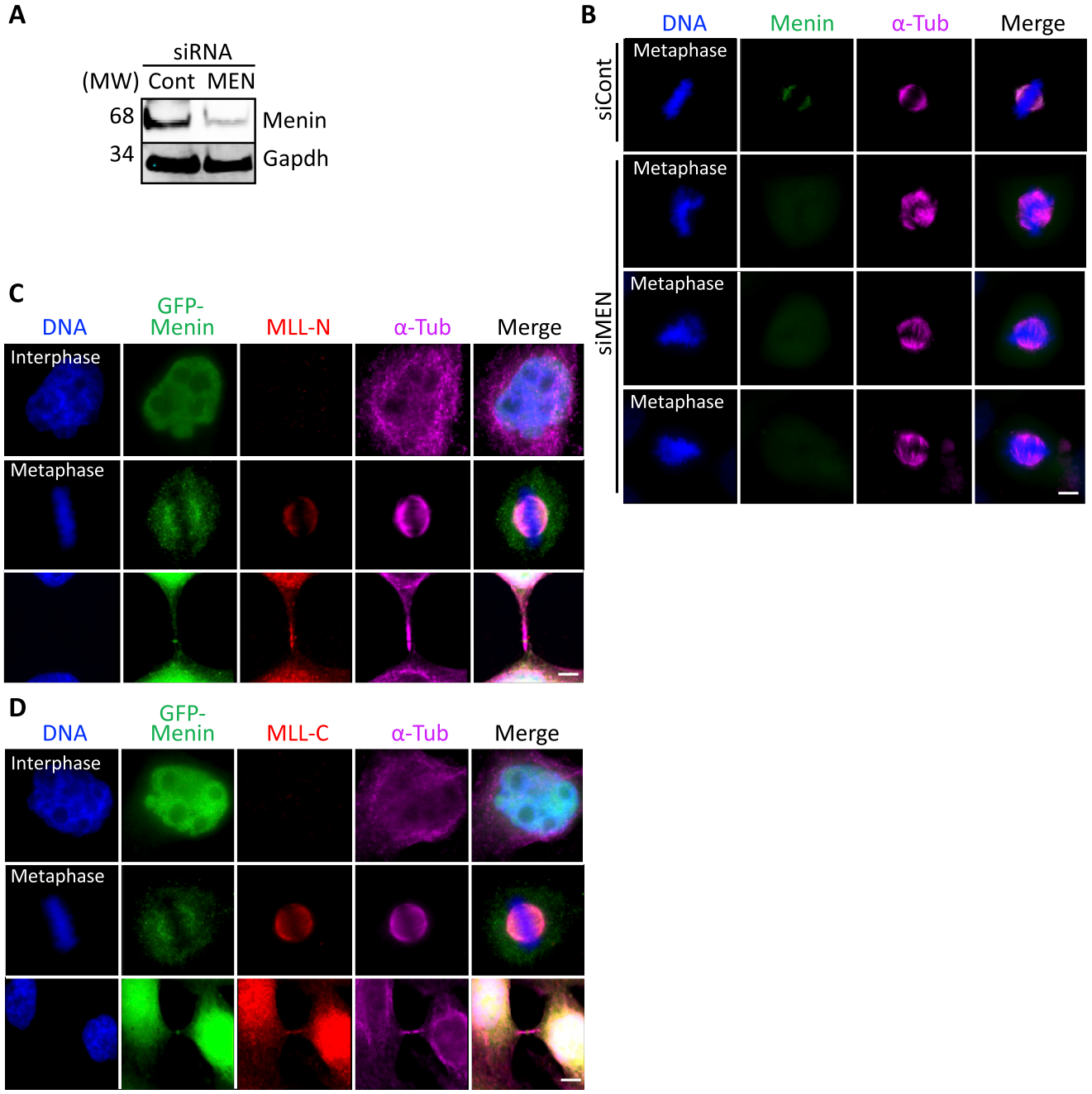

Figure 2. Validation of menin's localization to the mitotic spindle. (A) Immunoblot analysis showing that siMEN deplete menin protein levels compared with siCont. (B) Immunofluorescence microscopy of HeLa cells treated with siCont or siMEN for 48 h and stained for DNA, α-tubulin (α-Tub), and menin. Note that menin's localization to the mitotic spindle is depleted in siMEN-treated cells. (C, D) Immunofluorescence microscopy of HeLa cells transfected with the overexpressed GFP-tagged version of menin (GFP-menin) and stained for (C) DNA, α-Tub, and MLL1-N, or (D) MLL1-C. Scale bars, 5 μm. Cont, control; MEN, menin; MW, molecular weight (kDa).

cells showed no apparent perturbation in the localization of MLL1-N and MLL1-C to the mitotic spindle and intercellular bridge microtubules, they did show an increase in the percentage of mitotic cells with defective spindles (multipolar and unfocused; siMEN: 36.3% ± 5.1%; $P <$ 0.001 compared with siCont: 4.3% ± 1.2%) and metaphase cells with uncongressed chromosomes, where all but a few (n = 1 to 3) chromosomes were aligned at the metaphase plate [siMEN: 39.3% ± 5.1%; $P <$ 0.001 compared with siCont: 3.7% ± 1.2%; Fig. 3A and 3B; (42)]. siMEN cells also showed a pronounced increase in the percentage of cytokinetic cells undergoing a defective cytokinesis (siMEN: 45.0% ± 5.0%; $P <$ 0.001 compared with siCont: 11.7% ± 3.5%) and interphase cells with more than one nucleus (siMEN: 53.3% ± 6.0%; $P <$ 0.001 compared with siCont: 5.7% ± 2.1%; Fig. 3A and 3B). Similar results were obtained with a second siRNA (SR302867B; data not shown). In

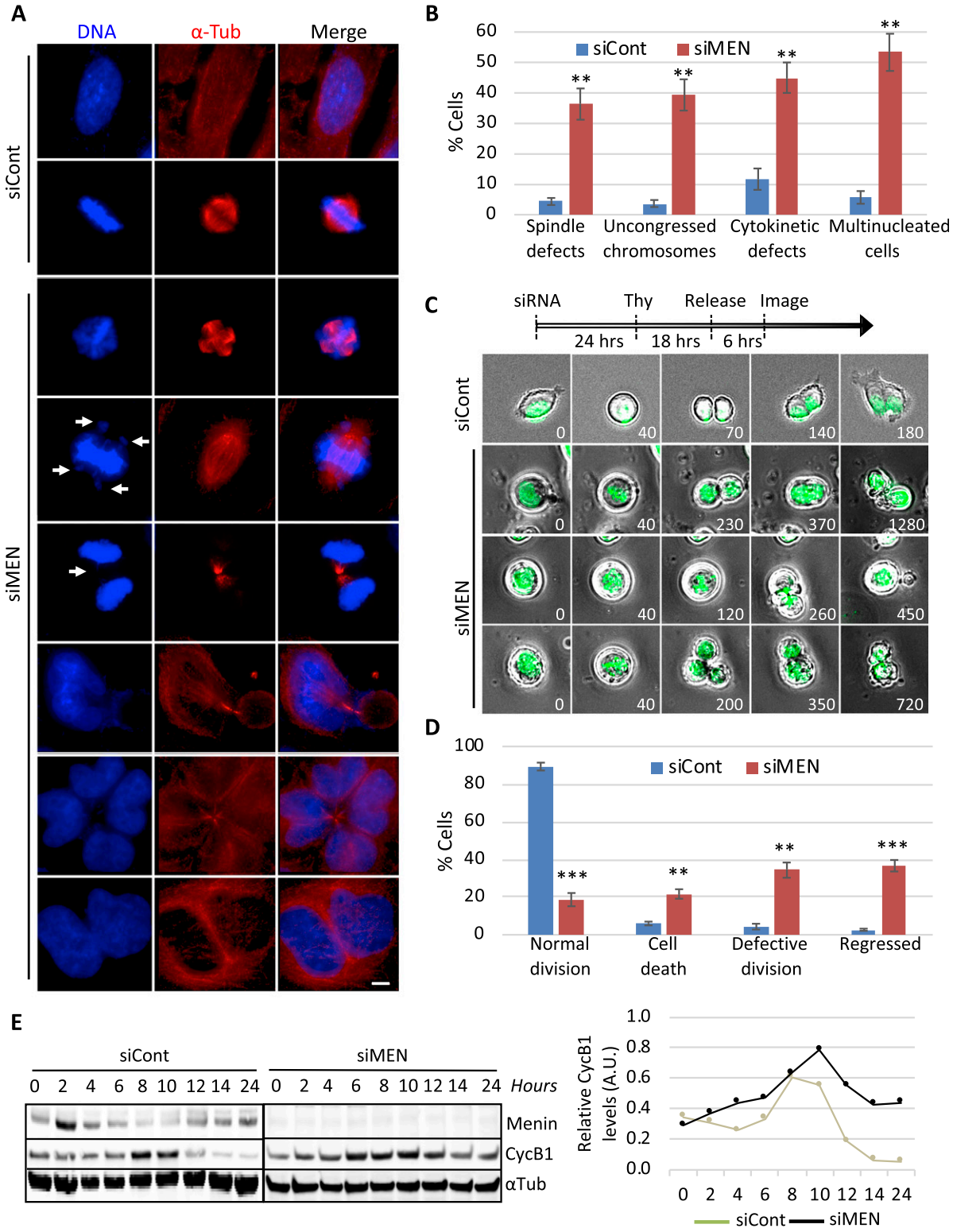

Figure 3. Depletion of menin leads to cell division defects. (A) Immunofluorescence microscopy of HeLa cells treated with siCont or siMEN for 48 h and stained for DNA and α-tubulin (α-Tub). Note that siMEN cells show multiple aberrancies, including multipolar spindles and unaligned chromosomes in metaphase, lagging chromosomes in anaphase, multipolar cytokinesis, and multinucleated interphase cells. Arrows point to uncongressed chromosomes in a metaphase cell (panel with four arrows) and lagging chromosomes in a telophase cell (panel with one arrow). Scale bar, 5 μm. (B) Quantification of the percentage of mitotic cells with defective spindles, uncongressed chromosomes, and cytokinetic defects and interphase cells with more than one nucleus (multinucleated). Data represent the mean ± SD of three independent experiments, 100 cells counted for each. $^{**}P < 0.001$. (C) Live-cell time-lapse microscopy snapshots of HCT116-GFP-H2B cells treated with siCont or siMEN (42). Representative cell division defects are shown, including cytokinetic arrest, multipolar cell division with cell death, and regression of a dividing cell into a binucleated cell. Time is in minutes. (D) The percentage of cells undergoing normal cell division, dying during cell division, undergoing aberrant cytokinesis and failing cytokinesis, and regressing to a binucleated state were quantified for

addition, the defects observed in siMEN cells were partially mitigated by *MEN1* cDNA overexpression (42). *MEN1* siRNA SR302867A (AGUACAGUCUGUAUCAAACCCACGA) used for these experiments maps to the 3′ untranslated region of menin and is therefore suitable to study menin overexpression rescue. These results indicated that depletion of menin led to defects in early mitosis and cytokinesis.

To characterize the cell division defects in live dividing cells, HCT116 cells (CCL-247 colon cancer cells) expressing GFP-tagged histone H2B were imaged after siRNA depletion of menin expression. Consistently, live-cell time-lapse fluorescent microscopy with HCT116-GFP-H2B cells showed that siMEN cells exhibited cell division defects, including cytokinetic arrest with the two cells linked by a cytokinetic bridge, a failure to divide with regression into one binucleated cell, and cell division followed by death of one or both cells [Fig. 3C and 3D; (42)]. Immunoblot analysis of HeLa cell extracts from cells synchronized in G_1/S with thymidine treatment and released into the cell cycle showed that siMEN cells sustained cyclin B1 levels for a longer time compared with siCont cells during cell division, indicative of slowed and/or aborted cell division (Fig. 3E). Together, these data indicated that depletion of menin led to defects in spindle assembly, chromosome congression, chromosome segregation, and cytokinesis, which resulted in multinucleated interphase cells and an increase in cell death.

Next, we asked if the menin-MLL1 interaction was important for cell division by analyzing the consequences of inhibiting the menin-MLL1 interaction pharmacologically with the small molecule inhibitor MI-2 (44). Menin binds the *N*-terminal subunit of MLL1 and this interaction is disrupted by MI-2. HeLa cells were synchronized in G_1/S, released, and treated with vehicle control DMSO or MI-2 (10 μM) 2 hours before mitotic entry. Nine hours after release, cells were fixed and stained for DNA and α-tubulin (Fig. 4A). Although MI-2–treated cells showed no apparent perturbation in the localization of MLL1-N and MLL1-C to the mitotic spindle and intercellular bridge microtubules, they did show an increase in the percentage of mitotic cells with defective spindles (multipolar and unfocused; MI-2: 21.3% ± 5.8%; $P < 0.01$ compared with DMSO: 4.3% ± 1.2%) and metaphase cells with uncongressed chromosomes, where all but a few (n = 1 to 3) chromosomes were aligned at the metaphase plate [MI-2: 19.3% ± 3.5%; $P < 0.01$ compared with DMSO: 3.7% ± 1.2%; Fig. 4A and 4B; (42)]. MI-2–treated cells also had a pronounced increase in the percentage of cytokinetic cells undergoing a defective cytokinesis (MI-2: 72.7% ± 5.0%; $P < 0.0001$ compared with DMSO: 12.3% ± 3.2%) and interphase cells with more than one nucleus (MI-2: 27.7% ± 5.5%; $P < 0.01$ compared with DMSO: 6.7% ± 2.9%; Fig. 4A and 4B). These results indicated that pharmacological inhibition of the menin-MLL1 interaction led to defects in early mitosis and cytokinesis.

Consistent with these results, live-cell time-lapse microscopy showed that MI-2–treated HCT116-GFP-H2B cells exhibited cell division defects, including cytokinetic arrest with the two cells linked by a cytokinetic bridge, a failure to divide with regression into one binucleated cell, and cell division followed by death of one or both cells [Fig. 4C and 4D; (42)]. Immunoblot analysis of HeLa cell extracts from cells synchronized in G_1/S with thymidine treatment and released into the cell cycle showed that MI-2–treated cells sustained cyclin B1 levels for a longer time compared with control DMSO-treated cells during cell division, indicative of slowed and/or aborted cell division (Fig. 4E). Together, these data indicated that disruption of the menin-MLL1 interaction led to defects in spindle assembly, chromosome congression, chromosome segregation, and cytokinesis, which resulted in multinucleated interphase cells and an increase in cell death.

Because MLL1 (specifically the C-terminal subunit) regulates Kif2A localization to the mitotic spindle to ensure proper chromosome alignment during mitosis (20), we analyzed whether menin similarly affected Kif2A localization during mitosis. HeLa cells were transfected with siCont or siMEN siRNA for 48 hours, fixed, and stained for DNA, α-tubulin, and Kif2A. Kif2A localized to the spindle microtubules in siCont- and siMEN-treated cells (Fig. 5A). Likewise, Kif2A localization was not affected by the treatment of the MLL1-menin inhibitor, MI-2, compared with the DMSO control (Fig. 5B). However, the ability of menin to localize to the mitotic spindle during mitosis was reduced in the presence of MI-2 treatment (Fig. 5C). Together, these results indicated that in contrast to depletion of MLL1, depletion of menin or inhibition of the MLL1-menin interaction did not affect the localization of Kif2A during mitosis. In addition, these results indicated that the MLL1-menin

Figure 3. (Continued). siCont- or siMEN-treated cells. Data represent the mean ± SD of three independent experiments, 50 cells counted for each. $^{**}P < 0.001$; $^{***}P < 0.0001$. (E) HeLa cells were treated with siCont or siMEN for 24 h, synchronized in G_1/S with thymidine (Thy), and released into the cell cycle. Cells were harvested at the indicated time after release and extracts were immunoblotted for menin, cyclin B1 (CycB1), and α-Tub. Line graph shows relative cyclin B1 levels over time (in hours) normalized to α-Tub.

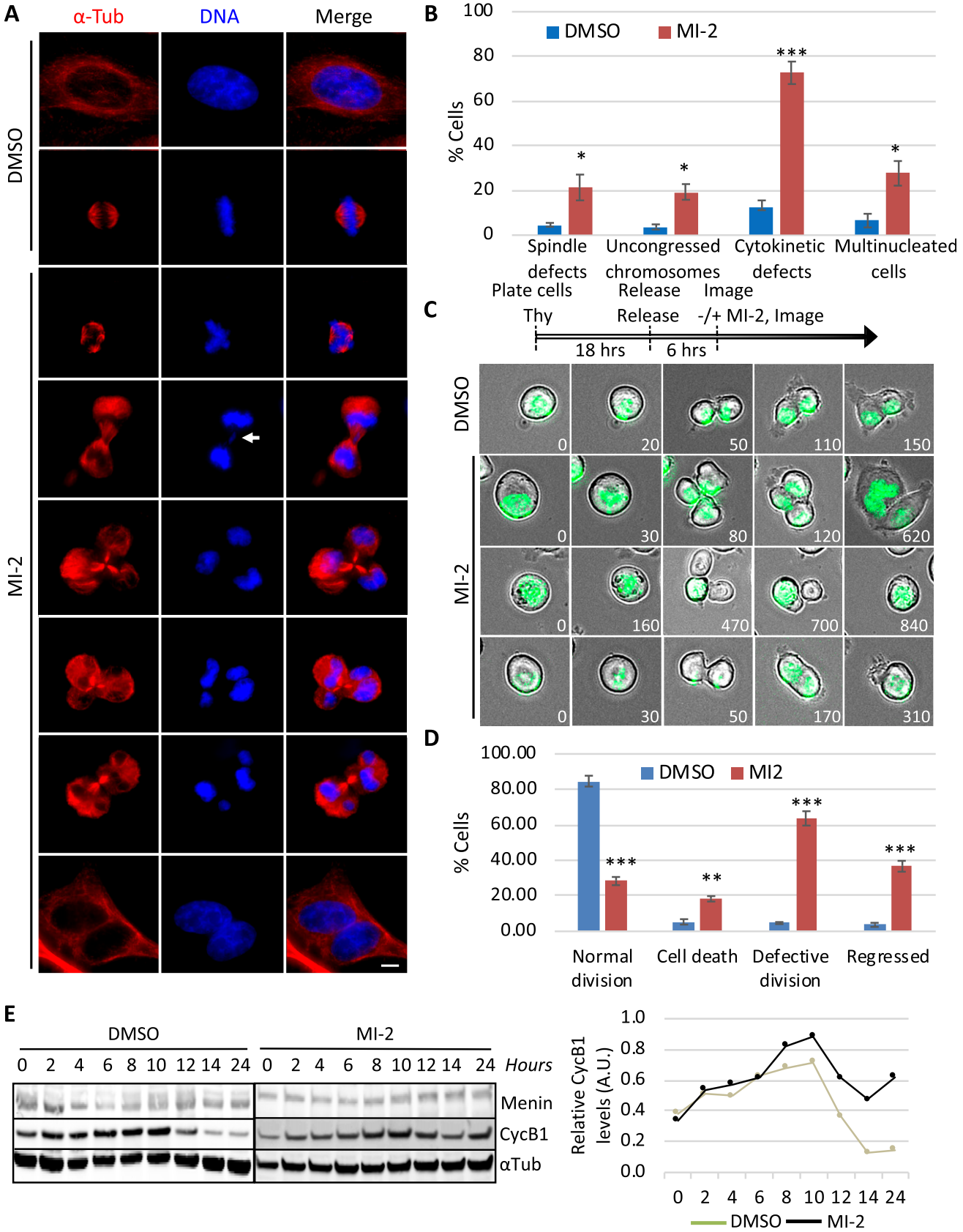

Figure 4. Pharmacological inhibition of the menin-MLL1 interaction with MI-2 leads to cell division defects. (A) Immunofluorescence microscopy of HeLa cells treated with DMSO or MI-2 (10 μM) for 2 h before mitotic entry and stained for DNA and α-tubulin (α-Tub). Note that MI-2–treated cells show multiple aberrancies, including multipolar spindles and unaligned chromosomes in metaphase, lagging chromosomes in anaphase, multipolar cytokinesis, and multinucleated interphase cells. The arrow points to lagging chromosomes in a telophase cell. Scale bar, 5 μm. (B) Quantification of the percentage of mitotic cells with defective spindles, uncongressed chromosomes, and cytokinetic defects and

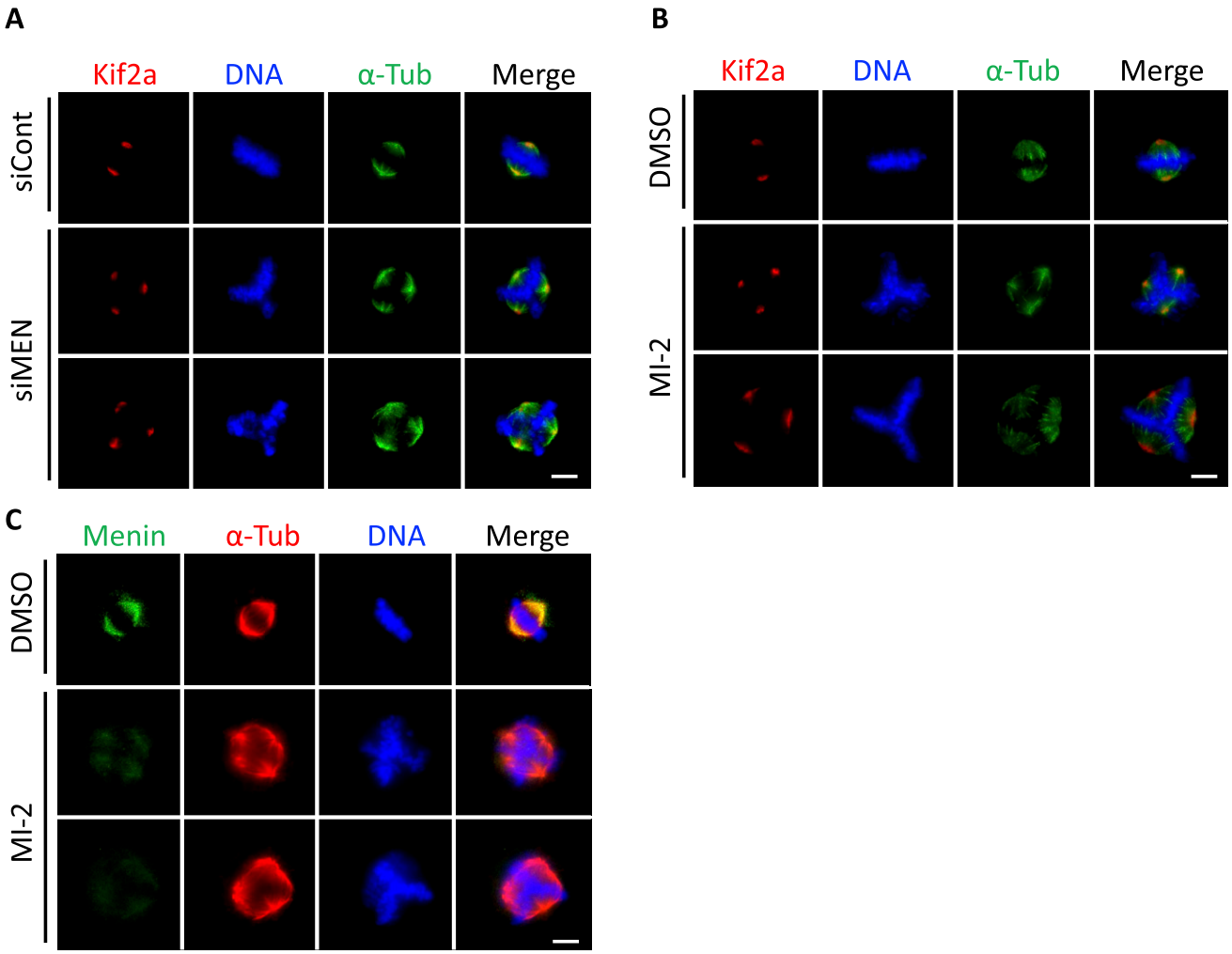

Figure 5. Depletion of menin or inhibition of menin-MLL1 interaction does not perturb Kif2A localization. (A) Immunofluorescence microscopy of HeLa cells treated with siCont or siMEN for 48 h and stained for DNA and α-tubulin (α-Tub). Note that Kif2A remains localized to the mitotic spindle in siMEN-treated cells. Scale bar, 5 μm. (B, C) Immunofluorescence microscopy of HeLa cells treated with DMSO or MI-2 (10 μM) for 2 h before mitotic entry and stained for (B) DNA, α-Tub, and Kif2A, or (C) menin. Note that Kif2A remains localized to the mitotic spindle in MI-2–treated cells, whereas menin localization to the mitotic spindle decreases. Scale bar, 5 μm.

interaction is important for the localization of menin to the mitotic spindle.

Discussion

Menin is mutated in patients with MEN1 syndrome and the related sporadic endocrine tumors (11). Most studies of menin function have focused on menin's role in MLL1- and MLL2-mediated epigenetic regulation of gene transcription (8, 9). It was previously reported that menin remains bound to chromatin with MLL1 during mitosis albeit at reduced levels compared with interphase, suggesting it shares a mitotic bookmarking role with MLL1 (19).We demonstrate that menin also has a nontranscriptional role during mitosis. Menin localized to the mitotic apparatus specifically to the spindle poles in early mitosis and intercellular bridge microtubules during cytokinesis, similar to the subcellular localization of MLL1. Diminished menin expression in HeLa and HCT116 cells by *MEN1* siRNA treatment led to defects in early mitosis and cytokinesis. In

Figure 4. (Continued). interphase cells with more than one nucleus (multinucleated). Data represent the mean ± SD of three independent experiments, 100 cells counted for each. $*P < 0.01$; $***P < 0.0001$. (C) Live-cell time-lapse microscopy snapshots of HCT116-GFP-H2B cells treated with DMSO or MI-2 (42). Representative cell division defects are shown, including multipolar cytokinesis and regression of dividing cells into binucleated cells. Time is in minutes. (D) The percentage of cells undergoing normal cell division, dying during cell division, undergoing defective divisions and failing cytokinesis, and regressing to a binucleated state were quantified for DMSO- or MI-2–treated cells. Data represent the mean ± SD of three independent experiments, 50 cells counted for each. $**P < 0.001$; $***P < 0.0001$. (E) HeLa cells were synchronized in G_1/S with thymidine (Thy) and released into the cell cycle in the presence of either DMSO or MI-2. Cells were harvested at the indicated time after release and extracts were immunoblotted for menin, cyclin B1 (CycB1), and α-Tub. Line graph shows relative cyclin B1 levels over time (in hours) normalized to α-Tub.

addition, live-cell time-lapse video microscopy showed that cell division in menin-depleted HCT116 cells frequently resulted in either cell death or multinucleated cells. On the basis of these observations, we propose that, like MLL1, menin plays a dual role during mitosis.

A nontranscriptional and methylation-independent role in mitosis was reported for MLL1 (20) and members of the associated WRAD (*i.e.*, WDR5, RbBP5, Ash2L, Dpy30) complex but not menin (21). The MLL1 mitotic-related function depends on the C-terminal proteolytic subunit of MLL1 but not the *N*-terminal MLL1 subunit, although both MLL1 subunits localize to the mitotic apparatus (20, 45). Interestingly, the results of our studies suggest there is a direct role for the MLL1 *N*-terminal subunit with menin during mitosis. Cells treated with a small molecule menin-MLL1 inhibitor (MI-2) demonstrated a similar mitotic defect as seen with *MEN1* siRNA, but neither treatment resulted in abnormal Kif2A localization, which is dependent on MLL1-C. On the basis of these data, we hypothesize that the mitotic role of menin depends on interaction with the *N*-terminal subunit of MLL1, although a transcriptional effect on mitosis cannot be entirely excluded.

One possible explanation for these observations may be that menin/MLL1-N and MLL1-C/Kif2A have distinct and functionally separable roles during mitosis. This hypothesis is supported by our observations that depletion of menin or inhibition of its interaction with MLL-N results in a different spectrum of mitotic defects compared with depletion of MLL1 alone, and inhibition of menin binding to MLL1-N does not affect Kif2A localization. Cells with diminished menin expression display defects in cytokinesis, spindle defects, and uncongressed chromosomes. Menin depletion did not cause spindle-pole lengthening or the pronounced chromosome misalignment reported with MLL1 depletion (20). Rather, in the case of menin depletion, only a few chromosomes were uncongressed in any given affected cell. The MLL-depleted chromosome misalignment phenotype that is characterized by most chromosomes missing from the metaphase plate was rescued with MLL-C, but the other MLL depletion–associated defects such as multipolar spindles were not quantified in the MLL-C rescue experiments, raising the possibility that these later defects could be related to menin/MLL-N function (20). Based upon these phenotype differences, menin's role with MLL1-N in the mitotic apparatus appears distinct from the MLL1-C regulation of Kif2A localization. An alternative mechanistic explanation for menin's role is to indirectly (through MLL-N) fine tune the function of MLL-C in mitosis; hence menin's absence might have a less dramatic phenotype. Because neither depletion of menin expression nor inhibition of menin-MLL1 interaction affect MLL1 localization (42), the function of menin is not to recruit MLL1 to the mitotic apparatus. Rather, inhibition of the interaction of MLL with menin reduces menin localization to the mitotic apparatus, suggesting MLL1 recruits menin during mitosis (Fig. 5C).

The *MEN1* gene is frequently mutated (30% to 44%) and inactive in sporadic pancreatic neuroendocrine tumors (46, 47). These tumors frequently display chromosome segregation abnormalities including recurrent whole-chromosome loss, aneuploidy, and polyploidy (48, 49). Based on our findings, inactivation of menin in these tumors could contribute to the observed chromosome copy number changes. Interestingly, a small percentage (6%) of these tumors have inactivating mutations of the histone methyltransferase SETD2/KMT3A, which also has both transcriptional and nontranscriptional actions during mitosis that could cause mitotic defects similar to menin/MLL1 in these tumors (48, 50, 51). Because inactivation of menin in these tumors is frequently the result of a point mutation of one allele with complete chromosome loss inactivating the remaining allele, it is possible that menin mutation (by reduced gene dose expression) leads to inactivation of its remaining normal allele through mitotic error (52–54). This could clarify the frequent propensity for MEN1 syndrome tumors and their sporadic counterparts to lose the remaining normal *MEN1* allele in susceptible cells that develop into tumors (55). In addition, cell types that do not gain the growth advantage with menin inactivation would not proliferate despite inactivation of both *MEN1* alleles. Such nonsusceptible cells may undergo apoptosis as a result of the mitotic errors or simply be unresponsive to the transcriptional effects of menin loss on specific downstream cell signaling in cell proliferation pathways (11, 49, 51). These effects combined could drive the tissue specificity of MEN1 syndrome.

Acknowledgments

Financial Support: This research was supported by National Institutes of Health, National Center for Advancing Translational Science, University of California, Los Angeles, Clinical and Translational Science Institute Grant UL1TR001881 to M.P.S.

Author Contributions: M.P.S., A.A.G., and J.Z.T. designed and performed experiments, discussed results, and wrote the paper.

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Disclosure Summary: The authors have nothing to disclose.

Data Availability: All data generated or analyzed during this study are included in this published article or in the data repositories listed in References.

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