CHK2–BRCA1 tumor-suppressor axis restrains oncogenic Aurora-A kinase to ensure proper mitotic microtubule assembly

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BRCA1 (breast cancer type 1 susceptibility protein) is a multifunctional tumor suppressor involved in DNA damage response, DNA repair, chromatin regulation, and mitotic chromosome segregation. Although the nuclear functions of BRCA1 have been investigated in detail, its role during mitosis is little understood. It is clear, however, that loss of *BRCA1* in human cancer cells leads to chromosomal instability (CIN), which is defined as a perpetual gain or loss of whole chromosomes during mitosis. Moreover, our recent work has revealed that the mitotic function of BRCA1 depends on its phosphorylation by the tumor-suppressor kinase Chk2 (checkpoint kinase 2) and that this regulation is required to ensure normal microtubule plus end assembly rates within mitotic spindles. Intriguingly, loss of the positive regulation of BRCA1 leads to increased oncogenic Aurora-A activity, which acts as a mediator for abnormal mitotic microtubule assembly resulting in chromosome missegregation and CIN. However, how the *CHK2–BRCA1* tumor suppressor axis restrains oncogenic Aurora-A during mitosis to ensure karyotype stability remained an open question. Here we uncover a dual molecular mechanism by which the *CHK2–BRCA1* axis restrains oncogenic Aurora-A activity during mitosis and identify BRCA1 itself as a target for Aurora-A relevant for CIN. In fact, Chk2-mediated phosphorylation of BRCA1 is required to recruit the PP6C–SAPS3 phosphatase, which acts as a T-loop phosphatase inhibiting Aurora-A bound to BRCA1. Consequently, loss of *CHK2* or *PP6C-SAPS3* promotes Aurora-A activity associated with BRCA1 in mitosis. Aurora-A, in turn, then phosphorylates BRCA1 itself, thereby inhibiting the mitotic function of BRCA1 and promoting mitotic microtubule assembly, chromosome missegregation, and CIN.

mitosis | chromosome segregation | aneuploidy | protein phosphatase | tumor suppressor

Breast cancer type 1 susceptibility protein (BRCA1) is a major and multifunctional tumor-suppressor protein involved in the regulation of chromatin organization, gene expression, DNA damage response, and DNA repair (1–3). In addition to its functions in interphase, BRCA1 also is required for faithful execution of mitosis. Consequently, loss of *BRCA1* results in abnormal mitotic progression, chromosome missegregation, and chromosomal instability (CIN), hallmark phenotypes of human cancer (4–6). However, the mitotic function of BRCA1 and its regulation during mitosis is little understood. BRCA1 localizes to centrosomes throughout the cell cycle and is phosphorylated during mitosis on S988 by the checkpoint kinase 2 (Chk2). This positive regulation is essential to ensure proper mitotic spindle assembly and chromosomal stability (5, 7, 8). Importantly, we recently have found that the loss of *CHK2* or of Chk2-mediated phosphorylation on BRCA1 causes an increase in microtubule plus-end assembly rates, which results in transient spindle geometry defects facilitating the generation of erroneous microtubule–kinetochore attachments. These defects finally lead to chromosome missegregation and the induction of aneuploidy, thus constituting CIN (6).

Interestingly, BRCA1 associates with the Aurora-A kinase during mitosis (6). Aurora-A is a key mitotic kinase encoded by the *AURKA* oncogene and is involved in various functions during mitosis, including centrosome separation and spindle assembly (9, 10). Aurora-A activation occurs at mitotic centrosomes and requires its autophosphorylation within the T-loop at threonine-288. Subsequent inactivation of Aurora-A can be mediated by the serine/threonine protein phosphatase 6 (PP6), which acts as the T-loop phosphatase for Aurora-A, and involves the catalytic subunit (PP6C) as well as regulatory subunits referred to as "Sit4-associated proteins" (SAPS1-3) and ankyrin repeat proteins (11–13). Significantly, in human cancer, *AURKA* frequently is overexpressed, and increased activity of *AURKA* is sufficient to induce enhanced microtubule plus end assembly rates, chromosome missegregation, and CIN (6). Thus, overexpression of *AURKA* and loss of the *CHK2–BRCA1* axis result in the same mitotic abnormalities triggering CIN. Moreover, loss of the Chk2-mediated phosphorylation of BRCA1 causes an increase in BRCA1-bound Aurora-A kinase activity at mitotic centrosomes, which mediates the induction of abnormal microtubule plus end dynamics and CIN (6). However, the molecular mechanism by which the loss of the *CHK2–BRCA1* tumor-suppressor axis unleashes oncogenic Aurora-A activity during mitosis and the

Significance

Inactivation of the tumor-suppressor gene breast cancer susceptibility type 1 (*BRCA1*) triggers abnormal microtubule assembly during mitosis, causing chromosome missegregation and aneuploidy. Unraveling how BRCA1 is regulated during mitosis and how it contributes to microtubule assembly is pivotal to understand its tumor-suppressor function. We showed that BRCA1 is positively regulated during mitosis by the tumor-suppressor kinase checkpoint kinase 2 (Chk2). This regulation is required for the recruitment of a protein phosphatase, which restrains oncogenic Aurora-A kinase activity bound to BRCA1. Unleashed Aurora-A, in turn, can negatively regulate the mitotic function of BRCA1. Thus we define a network in which the Chk2–*BRCA1* tumor-suppressor axis restrains oncogenic Aurora-A to ensure proper function of BRCA1 in mitosis essential for normal regulation of microtubule dynamics and chromosome segregation.

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mitotic target for Aurora-A relevant for the induction of increased microtubule dynamics and CIN remain unknown.

Results

The PP6C–SAPS3 Protein Phosphatase Associates with BRCA1 During Mitosis in a Chk2-Dependent Manner. Loss of Chk2-mediated phosphorylation of BRCA1 during mitosis causes increased binding of active Aurora-A kinase to BRCA1, thereby triggering an increase in mitotic microtubule assembly rates and leading to chromosome missegregation and CIN (6). To gain insights into the mechanism of how the *CHK2–BRCA1* tumor-suppressor axis negatively regulates the activity levels of oncogenic Aurora-A, we performed BRCA1 immunoprecipitation experiments combined with SILAC (stable isotope labeling with amino acids in cell culture)-based mass spectrometry analyses. This approach allowed the comparative and quantitative analyses of proteins bound to BRCA1 during mitosis in the presence (in HCT116 cells) or absence (in isogenic HCT116-*CHK2*$^{-/-}$ cells) of Chk2 as outlined in Fig. S1*A*. Mass spectrometry analyses identified a total of 515 proteins coimmunoprecipitating with BRCA1 (Dataset S1). The interaction of most of these proteins was not or was little regulated by Chk2, and among those were several known BRCA1-interacting proteins including BARD1, BRIP1/BACH1, and HSP90 (Fig. S1*B* and Dataset S1), thereby validating the approach. Importantly, among the proteins that showed reduced interaction with BRCA1 upon loss of *CHK2* [heavy/light (H/L) ratio >2], we found SAPS3, also known as PPP6R3 (H/L ratio = 2.7; Dataset S1: GI: 88941982). SAPS3 (GI: 194382030) also was identified in our previous mass spectrometry analyses (14) as a BRCA1-interacting protein. This protein immediately caught our attention, because SAPS3 represents a regulatory subunit of PP6 (12), which recently has been identified as a T-loop phosphatase for Aurora-A (11). Therefore, we reasoned that SAPS3-PP6 might represent the missing link for the Chk2–BRCA1–dependent regulation of Aurora-A activity during mitosis, and hence we focused on SAPS3 as a previously unidentified BRCA1-interacting protein.

Immunoprecipitation experiments using mitotic cell extracts verified the reduced interaction of SAPS3 and PP6C with BRCA1 upon loss of Chk2 (Fig. 1*A*). Loss of the PP6–BRCA1 interaction was accompanied by an increased binding of active, autophosphorylated Aurora-A (phospho-Thr-288) to BRCA1 (Fig. 1*A*). To test whether the Chk2-mediated phosphorylation of BRCA1 on S988 is required for the recruitment of PP6C-SAPS3 to BRCA1, we performed *BRCA1* reconstitution experiments. We expressed WT or nonphosphorylatable (S988A) or phospho-mimetic (S988E) mutants of *BRCA1* in HCT116 cells harboring a stable shRNA-mediated knockdown of *BRCA1* and analyzed the BRCA1–PP6 interaction. Indeed, the nonphosphorylatable BRCA1 mutant protein could no longer bind to PP6C-SAPS3, and, again, the loss of this interaction was associated with increased binding of active Aurora-A to BRCA1 (Fig. 1*B*). In contrast, the interaction of Aurora-A with its well-known binding partner TPX2 was not affected by the loss of *CHK2* or by the loss of the S988-phosphorylation site in BRCA1 (Fig. S1 *C* and *D*). These results suggest that loss of the recruitment of the phosphatase complex might be responsible for increased activity of Aurora-A bound to BRCA1. In line with this reasoning, treatment of cells with the serine/threonine protein phosphatase inhibitor okadaic acid also increased the amount of active Aurora-A kinase associated with BRCA1 (Fig. S1*E*).

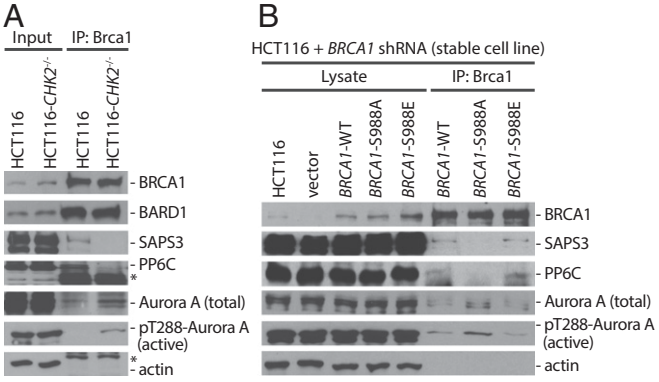

Fig. 1. The PP6C-SAPS3 protein phosphatase is recruited to BRCA1 in a Chk2-dependent manner. (*A*) Detection of PP6C and SAPS3 as BRCA1-interacting proteins. BRCA1 was immunoprecipitated from whole-cell lysates derived from HCT116 cells and from HCT116-*CHK2*$^{-/-}$ cells synchronized in mitosis, and BARD1, SAPS3, PP6C, and total and active Aurora-A (pT288) were detected on Western blots. Representative Western blots are shown. (*B*) The interaction of BRCA1 with PP6C-SAPS3 requires Chk2-mediated phosphorylation of BRCA1. HCT116 cells stably expressing shRNAs targeting *BRCA1* were reconstituted with BRCA1-WT or with the S988A or S988E mutants. BRCA1 proteins were immunoprecipitated, and SAPS3, PP6C, and total and active Aurora-A were detected on Western blots. Representative Western blots are shown.

PP6C-SAPS3 Restrains BRCA1-Bound Aurora-A Activity to Ensure Proper Microtubule Assembly Rates During Mitosis. To address whether PP6C-SAPS3 restrains the activity of Aurora-A bound to BRCA1, we depleted *SAPS3* and/or *PP6C* (Fig. S2*A*) and assessed Aurora-A bound to BRCA1. Indeed, loss of PP6C or SAPS3 was sufficient to increase a subpool of active Aurora-A in complex with BRCA1 but did not significantly alter the overall Aurora-A levels and activity in whole-cell lysates (Fig. 2*A*). Because our previous work has shown that the loss of the Chk2–BRCA1 axis results in an increase in Aurora-A activity localized at mitotic centrosomes (6), we performed quantitative immunofluorescence microscopy to detect Aurora-A spatially. We found that, very similar to the increase in Aurora-A activity seen after the loss of *CHK2* (6) and after the loss of the Chk2-mediated phosphorylation site of BRCA1 (Fig. S2*B* and ref. 6), loss of the PP6C or SAPS3 caused an ∼40% increase in active Aurora-A localized at mitotic centrosomes, but the overall levels of Aurora-A at centrosomes remained unchanged (Fig. 2*B*). As a consequence of the increase in centrosomal Aurora-A kinase activity upon loss of *PP6C-SAPS3* or after loss of the Chk2-mediated phosphorylation site of BRCA1, the phosphorylation of the bona fide centrosomal Aurora-A target TACC3, which represents a robust marker for centrosomal Aurora-A activity, was increased (Fig. S2 *C* and *D*) (6, 15, 16).

Because increased Aurora-A activity can trigger an increase in mitotic microtubule assembly rates as an underlying mechanism for subsequent chromosome missegregation and CIN (6), we determined microtubule plus-end assembly rates by live cell microscopy, tracking EB3-GFP comets in mitotic cells (Fig. S3 and Movies S1–S4) (6, 17). Very similar to the loss of *CHK2* (Fig. 3*C*) (6), the loss of *PP6C-SAPS3* was sufficient to trigger an increase in microtubule plus-end assembly rates in both bipolar metaphase spindles and monopolar spindles after synchronization of cells using a Eg5/KSP inhibitor (Fig. 2*C*). Importantly, this increase in microtubule growth was suppressed upon treatment with the Aurora-A–specific inhibitor MLN8054 (Fig. 2*C*), indicating that the induction of abnormal microtubule assembly rates in response to loss of the PP6C-SAPS3 phosphatase is mediated by increased Aurora-A activity. Moreover, we found that the ability of a phospho-mimetic mutant of *BRCA1* (S988E) to restore proper microtubule plus-end assembly rates in the absence of Chk2 requires SAPS3 and PP6C (Fig. S4 *A* and *B*), indicating that SAPS3 and PP6C act through BRCA1 to regulate microtubule plus-end assembly. Consistently, the suppression of active Aurora-A bound to BRCA1 upon mimicking the Chk2-mediated phosphorylation also was dependent on SAPS3 and PP6C (Fig. S4*C*). Together, our results strongly suggest that the S988-phosphorylation–dependent recruitment of PP6C-SAPS3

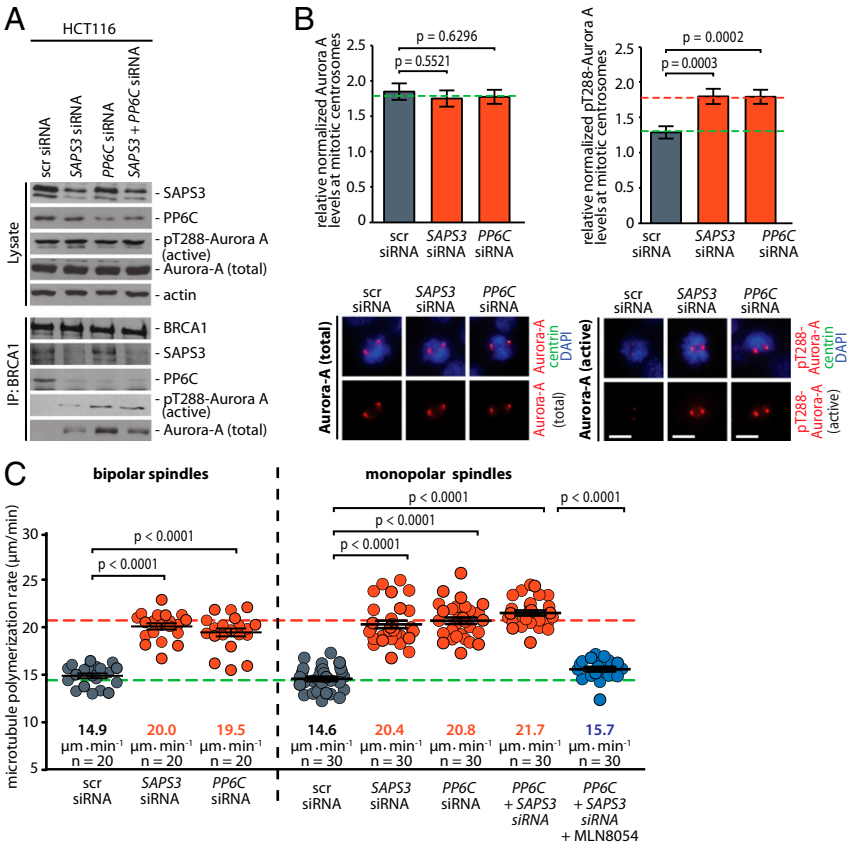

Fig. 2. Repression of *PP6C-SAPS3* is sufficient to increase the activity of centrosomal Aurora-A and accelerates microtubule assembly rates. (*A*) Repression of *PP6C* or *SAPS3* increases the binding of active Aurora-A to BRCA1. *PP6C* and/or *SAPS3* were repressed in HCT116 cells by siRNAs, and BRCA1 was immunoprecipitated from mitotic cell lysates. The indicated proteins were detected on Western blots. Representative Western blots are shown. (*B*) Repression of *PP6C* or *SAPS3* increases centrosomal Aurora-A activity. Detection of total or active Aurora-A (pT288) and centrin-2 at mitotic centrosomes in HCT116 cells upon siRNA-mediated repression of *PP6C* or *SAPS3*. (*Lower*) Representative immunofluorescence images of Aurora-A or active pT288-Aurora-A (red), centrin-2 (green), and chromosomes (blue). (Scale bar, 10 µm.) (*Upper*) The signal intensities were normalized to signals form centrosomal centrin-2 and quantified. Data are shown as mean ± SEM; *t* test; $n = 100$ cells. (*C*) Repression of *PP6C* or *SAPS3* increases mitotic microtubule assembly rates. Microtubule plus-end assembly rates were determined in bipolar metaphase or monopolar spindles after siRNA-mediated repression of *PP6C* or *SAPS3*. To inhibit Aurora-A activity partially, mitotic cells were treated with the Aurora-A inhibitor MLN8054 (0.06 µM) for 1 h. Scatter dot plots show average assembly rates (20 microtubules per cell). Green dashed line, normal values; red dashed line, abnormally increased values. Data are shown as mean ± SEM; *t* test; $n = 20$–30 cells from three independent experiments.

represents a key requirement to prevent an Aurora-A–mediated increase in microtubule plus-end growth.

Overexpression of *PP6C-SAPS3* Suppresses Increased Microtubule Assembly Rates in Colorectal Cancer Cells. Because loss of the Chk2-mediated phosphorylation of BRCA1 results in the removal of PP6C-SAPS3 from BRCA1, leading to the induction of Aurora-A activity and increased microtubule assembly rates (Figs. 1 and 2), we reasoned that overexpressing *PP6C-SAPS3* would suppress Aurora-A activity and might restore proper microtubule dynamics. Indeed, the overexpression of *SAPS3* or *PP6C* in *CHK2*-deficient HCT116 cells significantly suppressed the levels of active Aurora-A bound to BRCA1 (Fig. 3*A*), caused a significant reduction of TACC3 phosphorylation (Fig. 3*B*), and was sufficient to restore normal mitotic microtubule assembly rates (Fig. 3*C*). Similarly, centrosomal Aurora-A activity was suppressed and proper microtubule assembly rates were restored in response to *PP6C* or *SAPS3* overexpression in SW620 and SW480 colorectal cancer cells, which also are characterized by Aurora-A–mediated increased microtubule assembly rates (Fig. 3 *B* and *C*) (6).

Increased Aurora-A Activity Targets BRCA1 During Mitosis. Our data demonstrate that the loss of *CHK2* or *PP6C-SAPS3* results in increased Aurora-A activity that triggers an increase in microtubule assembly rates. What, however, is the crucial target for Aurora-A that mediates abnormal microtubule dynamics during mitosis? Because Aurora-A is bound to BRCA1, we wondered whether this interaction might reflect a kinase–substrate interaction. To address this possibility, we raised antibodies against phosphorylated S308 of BRCA1, which represents a putative Aurora-A phosphorylation site. Using these phospho-specific antibodies, we detected only very weak phosphorylation of BRCA1 in mitotic HCT116 cells. However, in isogenic *CHK2*-deficient cells, in which active Aurora-A bound to BRCA1 is increased, the S308 phosphorylation of BRCA1 was clearly increased also. Moreover, treatment of cells with MLN8054 abolished BRCA1-S308 phosphorylation, indicating that Aurora-A indeed mediates this phosphorylation of BRCA1 (Fig. 4*A* and Fig. S5*A*). Similarly, loss of the Chk2-target site of BRCA1 upon reconstitution with a S988A mutant not only led to an increase in active Aurora-A bound to BRCA1 but also induced BRCA1-308 phosphorylation (Fig. S5*B*). These results indicate that phosphorylation of BRCA1 on S308 is mediated by Aurora-A and that Aurora-A activity is predetermined by the Chk2-mediated phosphorylation of BRCA1 on S988. Because S988 phosphorylation is required for the recruitment of the PP6C-SAPS3 phosphatase complex to BRCA1, we consequently found that loss of the phosphatase also results in an increase in Aurora-A–mediated phosphorylation of BRCA1 on S308 (Fig. 4*B*). Vice versa, overexpression of *PP6C* or *SAPS3* in *CHK2*-deficient HCT116 cells not only caused a decrease in active Aurora-A bound to BRCA1 but also decreased BRCA1 phosphorylation on S308 (Fig. S5*C*). Thus, increased Aurora-A activity bound to BRCA1 causes increased BRCA1 phosphorylation on S308, and this increased phosphorylation might represent the relevant trigger for increased mitotic microtubule dynamics and chromosome missegregation.

Aurora-A–Mediated Phosphorylation of BRCA1 Determines Mitotic Microtubule Assembly Rates and Faithful Chromosome Segregation. To test whether Aurora-A–mediated phosphorylation of BRCA1 on S308 is indeed functionally involved in the regulation of mitotic microtubule plus-end assembly, we reconstituted *BRCA1*-knockdown cells with various *BRCA1* phospho-site mutants (Fig. S6*A*) and determined the microtubule assembly rates in mitosis. In fact, the loss of the Chk2-mediated phosphorylation site of BRCA1 (S988A mutant), which is associated with increased S308 phosphorylation of BRCA1 (Fig. 4*B*), results in an increase in microtubule assembly rates (Fig. 5*A*). Very

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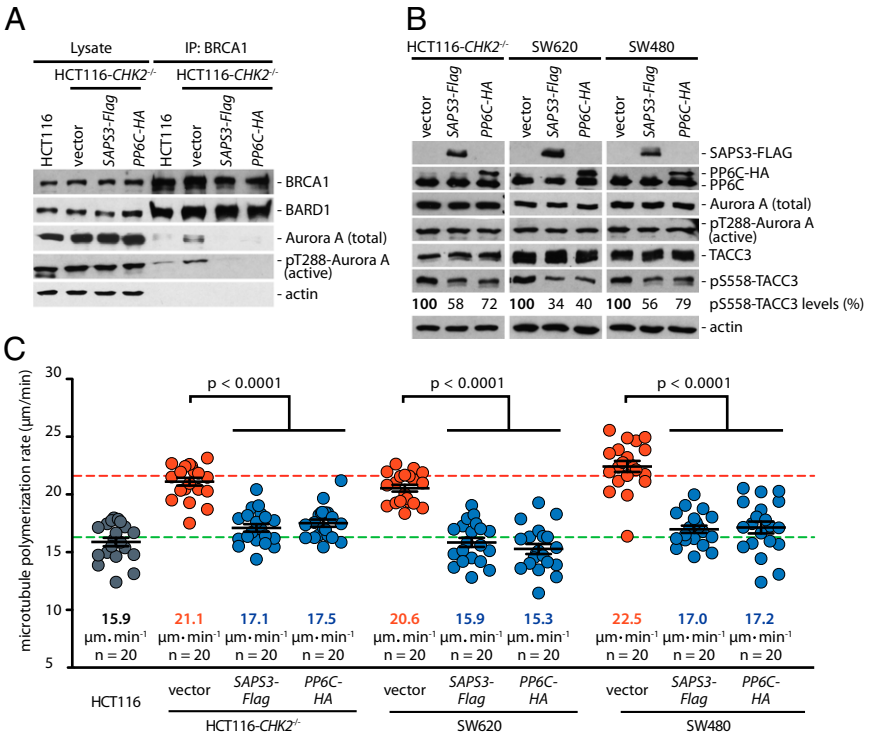

Fig. 3. Overexpression of *PP6C-SAPS3* inhibits centrosomal and BRCA1-bound Aurora A activity and suppresses increased microtubule assembly rates in colorectal cancer cells. (*A*) Overexpression of *PP6C* or *SAPS3* in *CHK2*-deficient HCT116 cells decreases the interaction of active Aurora-A with BRCA1. HCT116 cells overexpressing *PP6C-HA* or *SAPS3-FLAG* were synchronized in mitosis, and BRCA1 was immunoprecipitated. Total and active Aurora-A (pT288) bound to BRCA1 was detected on Western blots. Representative Western blots are shown. (*B*) Overexpression of *PP6C* or *SAPS3* decreases the centrosomal activity of Aurora A. *PP6C-HA* or *SAPS3-FLAG* was expressed in HCT116-*CHK2*$^{-/-}$, SW620, or SW480 cells and synchronized in mitosis. Total and phosphorylated TACC3 was detected as a marker for centrosomal Aurora-A activity on Western blots, and phosphorylated TACC3 was quantified. Representative Western blots are shown. (*C*) The overexpression of *PP6C* or *SAPS3* restores normal microtubule assembly rates in HCT116-*CHK2*$^{-/-}$, SW620, and SW480 cells. Microtubule plus-end assembly rates were determined in the different cell lines in the absence or presence of *PP6C* or *SAPS3* overexpression. Scatter dot plots show average assembly rates (20 microtubules per cell). Green dashed line, normal values; red dashed line, abnormally increased values. Data are shown as mean ± SEM; *t* test; *n* = 20 cells from three independent experiments.

similarly, mimicking a constitutive phosphorylation on S308 (S308E mutant), but not the loss of S308 phosphorylation (S308A mutant), was sufficient to cause an increase in microtubule plus-end assembly rates (Fig. 5*A* and Fig. S6*B*). Importantly, although the increased rates of microtubule growth in cells expressing the S988A mutant can be suppressed by inhibiting Aurora-A, this suppression is not possible in cells expressing the S308E mutant (Fig. 5*A*). This result is in line with our findings showing that the S988A mutant increases centrosomal Aurora-A activity (Fig. S2*B*), whereas the S308 phosphorylation status does not affect Aurora-A activity (Fig. S6*C*). Thus, these findings suggest that the Aurora-A–mediated (S308) rather than the Chk2-mediated (S988) phosphorylation of BRCA1 represents the crucial trigger for abnormal microtubule dynamics in mitosis. To support the hierarchy of these two phosphorylations on BRCA1 further, we combined S988 and S308 mutations. In fact, increased microtubule assembly rates triggered by the loss of the Chk2-mediated phosphorylation of BRCA1 (S988A mutant) can be fully suppressed not only by Aurora-A inhibition (Fig. 5*A*) but also by concomitant loss of the Aurora-A–mediated phosphorylation site (S308A mutant) (Fig. 5*B*). On the other hand, mimicking the phosphorylation of S308 (S308E mutant) increases microtubule growth rates regardless of the phosphorylation status of S988 (S988A or S988E) (Fig. 5*B*). Moreover, expression of the *BRCA1* mutant, which no longer can be phosphorylated by Aurora-A (S308A mutant), is sufficient to suppress the increase in microtubule growth in *CHK2*-deficient HCT116 cells as well as in SW620 and SW480 cells, where abnormal microtubule growth was shown to be dependent on increased Aurora-A activity (Fig. 5*C*) (6). Significantly, this suppression of abnormally increased microtubule growth also led to the suppression of the generation of lagging chromosomes during anaphase in those cells (Fig. 5*D*) indicating that the Aurora-A–mediated phosphorylation of BRCA1 on S308 indeed represents the key event that contributes to the increase in microtubule assembly rates leading to chromosome missegregation and CIN.

Discussion

The tumor suppressor BRCA1 is an important regulator of mitosis, and its loss is tightly linked to whole-chromosome CIN and the generation of aneuploidy (4–6, 18). Importantly, BRCA1 can ensure chromosomal stability by dampening microtubule plus-end assembly rates during mitosis (6). Our work presented here uncovered a dual regulatory circuit that controls the mitotic

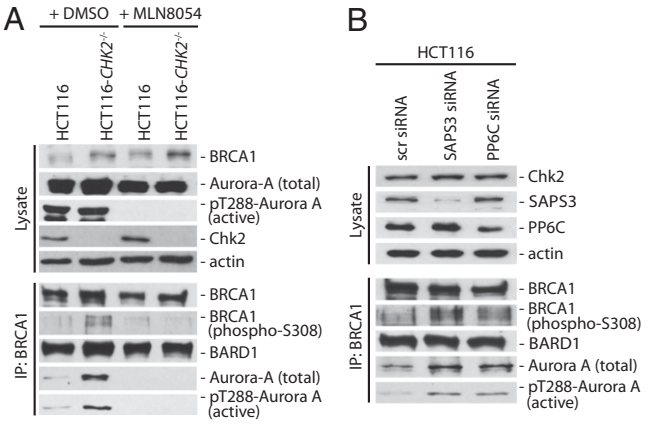

Fig. 4. Increased Aurora-A–mediated BRCA1 phosphorylation on S308 upon the loss of *CHK2* or after repression of *PP6C-SAPS3*. (*A*) Phosphorylation of BRCA1 on S308 is induced by the loss of *CHK2* and is dependent on Aurora A kinase activity. Parental HCT116 and HCT116-*CHK2*$^{-/-}$ cells were synchronized in mitosis, and BRCA1 was immunoprecipitated. Total and S308 phosphorylated BRCA1 and total and active (pT288) Aurora-A proteins were detected on Western blots. In addition, cells were treated with MLN8054 for 1 h to inhibit Aurora-A activity. Representative Western blots are shown. (*B*) Repression of *PP6C* or *SAPS3* induces BRCA1 phosphorylation on S308. HCT116 cells were transfected with siRNAs targeting *PP6C* or *SAPS3*, and BRCA1 was immunoprecipitated from mitotic cell lysates. The phosphorylation on S308 of BRCA1 and its binding to BARD1 and Aurora-A were analyzed. Representative Western blots are shown.

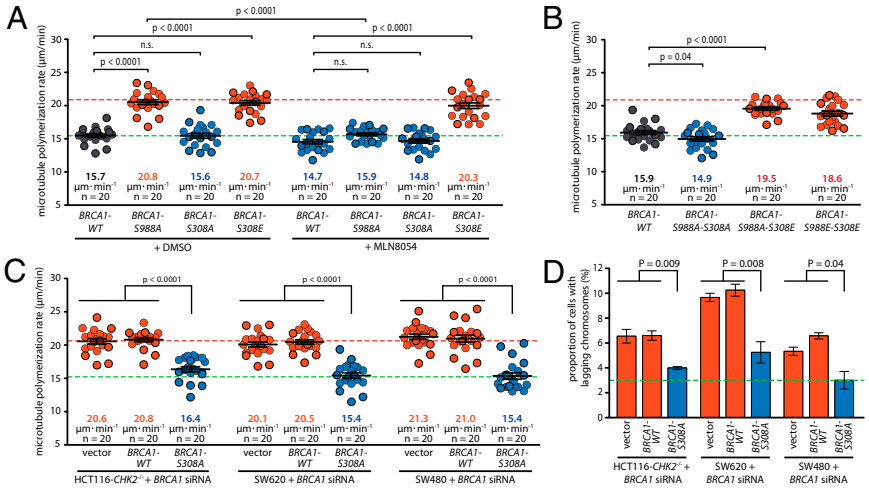

Fig. 5. Aurora-A–mediated phosphorylation of BRCA1 increases mitotic microtubule assembly rates. (*A*) Phosphorylation of BRCA1 on S308 is sufficient to increase microtubule plus-end assembly rates during mitosis. Stable *BRCA1*-knockdown cells were reconstituted with *BRCA1-WT* or with *BRCA1*-S988A, -S308A, or -S308E mutants and were treated with DMSO (control) or 0.06 μM MLN8054 to inhibit Aurora-A activity partially. Average mitotic microtubule assembly rates are based on the analyses of 20 microtubules per cell. Data are shown as mean ± SEM; *t* test; $n = 20$ cells from three independent experiments. (*B*) Phosphorylation of BRCA1 on S308 is a key event in regulating microtubule assembly rates. Mitotic microtubule plus-end assembly rates were measured in stable *BRCA1*-knockdown cells after reconstitution with the indicated double mutants of *BRCA1*. Scatter dot plots show average assembly rates (20 microtubules per cell). Data are shown as mean ± SEM; *t* test; $n = 20$ cells from three independent experiments. (*C*) Loss of S308 phosphorylation of BRCA1 restores normal microtubule assembly rates in colorectal cancer cells. Microtubule plus-end growth rates were determined in HCT116-*CHK2*$^{-/-}$, SW620, and SW480 cells with or without the replacement of endogenous BRCA1 with a *BRCA1-S308A* mutant. Scatter dot plots show average assembly rates (20 microtubules per cell). Data are shown as mean ± SEM; *t* test; $n = 20$ cells from three independent experiments. (*D*) Loss of the S308 phosphorylation of BRCA1 suppresses the generation of lagging chromosomes in colorectal cancer cells. HCT116-*CHK2*$^{-/-}$, SW620, and SW480 cells with or without replacement of endogenous BRCA1 with a *BRCA1-S308A* mutant were synchronized in anaphase, and the proportion of cells exhibiting lagging chromosomes was determined. Green dashed line, normal values; red dashed lines, abnormally increased values. Data are shown as mean ± SEM; *t* test; $n = 2$–4 with a total of 200–400 anaphase cells evaluated.

function of BRCA1 to ensure proper microtubule plus-end assembly and chromosomal stability (see the model in Fig. 6). We found that mitotic BRCA1 is inactivated through phosphorylation on S308 by the oncogenic Aurora-A kinase, leading to an increase in microtubule assembly rates, the induction of chromosome missegregation, and CIN (6). In normal cells, however, this unscheduled inactivation of BRCA1 is prevented by the tumor suppressor *CHK2*, which phosphorylates BRCA1 on S988, leading to the recruitment of the SAPS3-PP6C protein phosphatase complex. The PP6 phosphatase, in turn, keeps BRCA1-bound Aurora-A activity low by dephosphorylating its T-loop autophosphorylation (11). Thus, PP6 protects BRCA1 from being inactivated by Aurora-A during mitosis.

Currently, how BRCA1 dampens mitotic microtubule plus-end assembly is not known. However, possible targets of BRCA1 include proteins known as "+TIPs" that are localized to growing microtubule plus ends. +TIPs represent a protein network that shapes the microtubule plus ends and contributes to their dynamic behavior (19, 20). There is a growing list of +TIPs, but currently it is unclear how they act together to regulate microtubule dynamics (21, 22). Interestingly, we obtained the first evidence, to our knowledge, that the loss of the Chk2–BRCA1 axis might influence +TIPs. Increased microtubule plus-end growth upon loss of *CHK2* can be suppressed by partial repression of the processive plus tip-associated microtubule polymerase ch-TOG (6), indicating that the polymerase might be either hyperactive or hyper-recruited to plus tips in the absence of Chk2–BRCA1. However, Aurora-A, Chk2, and BRCA1 are localized to mitotic centrosomes (10, 23, 24), raising the question of how centrosomal proteins can regulate events at microtubule plus tips. Interestingly, in addition to their localization at the plus tips, several +TIPs, including ch-TOG, also are localized at mitotic centrosomes (25). Although the function of +TIPs at centrosomes is unclear, it is interesting to speculate that the Chk2–BRCA1 pathway might modulate a centrosomal subpool of +TIPs that subsequently are recruited to the microtubule plus end where they regulate microtubule dynamics.

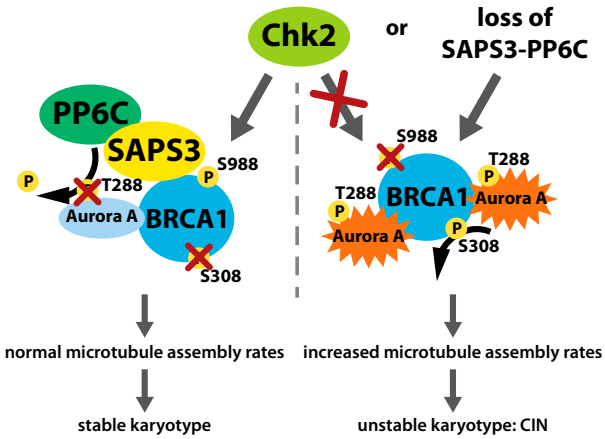

Fig. 6. Model summarizing the mitotic regulation of BRCA1 required for the maintenance of proper microtubule assembly and chromosomal stability. Aurora-A can inactivate the mitotic function of BRCA1 by direct phosphorylation on S308, resulting in increased microtubule assembly, chromosome missegregation, and CIN. During normal mitosis, however, BRCA1 is phosphorylated by Chk2 on S988, triggering the recruitment of the SAPS3-PP6 phosphatase complex to BRCA1 and thereby leading to dephosphorylation of Aurora-A restraining Brca1-bound Aurora-A activity. The limited Aurora-A activity, in turn, limits BRCA1 phosphorylation on S308 and thus, its inactivation. In cancer cells, the overexpression of oncogenic *AURKA* or the loss of the tumor suppressors *CHK2* or *PP6* leads to failure of this fail-safe mechanism and promotes the Aurora-A–dependent inactivation of BRCA1, resulting in CIN and aneuploidy as seen upon loss of *BRCA1*.

An important outcome from our work presented here is the identification of a regulatory network centered on the mitotic function of BRCA1 that is essential for the maintenance of chromosomal stability and therefore is important for our understanding of BRCA1-driven tumorigenesis and tumor progression (5, 26). Our results suggest that not only BRCA1, but also the positive BRCA1 regulators Chk2 and SAPS3-PP6, might represent important tumor-suppressor genes. Indeed, *CHK2* is a tumor suppressor that frequently is down-regulated in lung and colorectal cancer, and its loss mimics the loss of *BRCA1* and causes whole-chromosome missegregation and CIN (5, 6, 27, 28). Similarly, *PP6C* mutations that affect the formation of holoenzymes are detected in melanoma and have been shown to affect mitosis (29,30). On the other hand, Aurora-A can act as a negative regulator of BRCA1 in mitosis, and, as such, *AURKA* represents a well-established oncogene that is overexpressed in various human malignancies and is clearly associated with chromosome missegregation and CIN (6, 9, 10). In addition, recent work from our laboratories has identified the centrosomal protein Cep72 as a putative oncogene in colorectal cancer that can bind to BRCA directly and inhibits its mitotic function to regulate microtubule growth (14). In sum, our work not only reveals a key regulatory circuit on BRCA1 but also provides a network of cancer-associated genetic lesions that might contribute to the functional inactivation of BRCA1 during mitosis. These alterations of essential coregulators of BRCA1 might contribute to genome instability in tumor entities other than breast and ovarian cancer in which *BRCA1* mutations are prevalent.

Materials and Methods

Human Cell Lines, Cell Treatments, Antibodies, cDNAs, and si/shRNAs. Details on human cell lines, cell treatments, antibodies, cDNAs, and si/shRNAs are provided in *SI Materials and Methods*.

Mass Spectrometry Analyses. BRCA1 was immunoprecipitated from SILAC-labeled cells, and associated proteins were identified by mass spectrometry analyses as detailed in *SI Materials and Methods*.

Measurement of Microtubule Plus-End Assembly Rates. Determination of microtubule plus-end assembly rates was described previously (6).

Statistical Analyses. All data are shown as mean ± SEM. Where indicated, Student's *t* tests were calculated by using the Prism software package, version 4 (GraphPad).

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1. Silver DP, Livingston DM (2012) Mechanisms of BRCA1 tumor suppression. *Cancer Discov* 2(8):679–684.
2. Savage KI, Harkin DP (2015) BRCA1, a 'complex' protein involved in the maintenance of genomic stability. *FEBS J* 282(4):630–646.
3. Zhu Q, et al. (2011) BRCA1 tumour suppression occurs via heterochromatin-mediated silencing. *Nature* 477(7363):179–184.
4. Joukov V, et al. (2006) The BRCA1/BARD1 heterodimer modulates ran-dependent mitotic spindle assembly. *Cell* 127(3):539–552.
5. Stolz A, et al. (2010) The CHK2-BRCA1 tumour suppressor pathway ensures chromosomal stability in human somatic cells. *Nat Cell Biol* 12(5):492–499.
6. Ertych N, et al. (2014) Increased microtubule assembly rates influence chromosomal instability in colorectal cancer cells. *Nat Cell Biol* 16(8):779–791.
7. Kais Z, Parvin JD (2008) Regulation of centrosomes by the BRCA1-dependent ubiquitin ligase. *Cancer Biol Ther* 7(10):1540–1543.
8. Stolz A, Ertych N, Bastians H (2010) Loss of the tumour-suppressor genes CHK2 and BRCA1 results in chromosomal instability. *Biochem Soc Trans* 38(6):1704–1708.
9. Bischoff JR, et al. (1998) A homologue of Drosophila aurora kinase is oncogenic and amplified in human colorectal cancers. *EMBO J* 17(11):3052–3065.
10. Marumoto T, Zhang D, Saya H (2005) Aurora-A - a guardian of poles. *Nat Rev Cancer* 5(1):42–50.
11. Zeng K, Bastos RN, Barr FA, Gruneberg U (2010) Protein phosphatase 6 regulates mitotic spindle formation by controlling the T-loop phosphorylation state of Aurora A bound to its activator TPX2. *J Cell Biol* 191(7):1315–1332.
12. Stefansson B, Brautigan DL (2006) Protein phosphatase 6 subunit with conserved Sit4-associated protein domain targets IkappaBepsilon. *J Biol Chem* 281(32):22624–22634.
13. Bastians H, Ponstingl H (1996) The novel human protein serine/threonine phosphatase 6 is a functional homologue of budding yeast Sit4p and fission yeast ppe1, which are involved in cell cycle regulation. *J Cell Sci* 109(Pt 12):2865–2874.
14. Lüddecke S, et al. (2015) The putative oncogene CEP72 inhibits the mitotic function of BRCA1 and induces chromosomal instability. *Oncogene*, 10.1038/onc.2015.290.
15. Kinoshita K, et al. (2005) Aurora A phosphorylation of TACC3/maskin is required for centrosome-dependent microtubule assembly in mitosis. *J Cell Biol* 170(7):1047–1055.
16. LeRoy PJ, et al. (2007) Localization of human TACC3 to mitotic spindles is mediated by phosphorylation on Ser558 by Aurora A: A novel pharmacodynamic method for measuring Aurora A activity. *Cancer Res* 67(11):5362–5370.
17. Stepanova T, et al. (2003) Visualization of microtubule growth in cultured neurons via the use of EB3-GFP (end-binding protein 3-green fluorescent protein). *J Neurosci* 23(7):2655–2664.
18. Xu X, et al. (1999) Centrosome amplification and a defective G2-M cell cycle checkpoint induce genetic instability in BRCA1 exon 11 isoform-deficient cells. *Mol Cell* 3(3):389–395.
19. Galjart N (2010) Plus-end-tracking proteins and their interactions at microtubule ends. *Curr Biol* 20(12):R528–R537.
20. Akhmanova A, Steinmetz MO (2008) Tracking the ends: A dynamic protein network controls the fate of microtubule tips. *Nat Rev Mol Cell Biol* 9(4):309–322.
21. Gupta KK, Alberico EO, Näthke IS, Goodson HV (2014) Promoting microtubule assembly: A hypothesis for the functional significance of the +TIP network. *BioEssays* 36(9):818–826.
22. Tamura N, Draviam VM (2012) Microtubule plus-ends within a mitotic cell are 'moving platforms' with anchoring, signalling and force-coupling roles. *Open Biol* 2(11):120132.
23. Tsvetkov L, Xu X, Li J, Stern DF (2003) Polo-like kinase 1 and Chk2 interact and colocalize to centrosomes and the midbody. *J Biol Chem* 278(10):8468–8475.
24. Hsu LC, White RL (1998) BRCA1 is associated with the centrosome during mitosis. *Proc Natl Acad Sci USA* 95(22):12983–12988.
25. Jiang K, Akhmanova A (2011) Microtubule tip-interacting proteins: A view from both ends. *Curr Opin Cell Biol* 23(1):94–101.
26. Ricke RM, van Ree JH, van Deursen JM (2008) Whole chromosome instability and cancer: A complex relationship. *Trends Genet* 24(9):457–466.
27. Cybulski C, et al. (2004) CHEK2 is a multiorgan cancer susceptibility gene. *Am J Hum Genet* 75(6):1131–1135.
28. Zhang P, et al. (2004) CHK2 kinase expression is down-regulated due to promoter methylation in non-small cell lung cancer. *Mol Cancer* 3:14.
29. Hammond D, et al. (2013) Melanoma-associated mutations in protein phosphatase 6 cause chromosome instability and DNA damage owing to dysregulated Aurora-A. *J Cell Sci* 126(Pt 15):3429–3440.
30. Gold HL, et al. (2014) PP6C hotspot mutations in melanoma display sensitivity to Aurora kinase inhibition. *Mol Cancer Res* 12(3):433–439.
31. Jallepalli PV, Lengauer C, Vogelstein B, Bunz F (2003) The Chk2 tumor suppressor is not required for p53 responses in human cancer cells. *J Biol Chem* 278(23):20475–20479.
32. Lee JS, Collins KM, Brown AL, Lee CH, Chung JH (2000) hCds1-mediated phosphorylation of BRCA1 regulates the DNA damage response. *Nature* 404(6774):201–204.
33. Cox J, et al. (2011) Andromeda: A peptide search engine integrated into the MaxQuant environment. *J Proteome Res* 10(4):1794–1805.
34. Cox J, Mann M (2012) 1D and 2D annotation enrichment: A statistical method integrating quantitative proteomics with complementary high-throughput data. *BMC Bioinformatics* 13(Suppl 16):S12.
35. Wu et al. (1996) Identification of a RING protein that can interact in vivo with the BRCA1 gene product. *Nat Genet* 14(4):430–440.
36. Cantor et al. (2001) BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. *Cell* 105(1):149–160.
37. Stecklein SR, et al. (2012) BRCA1 and HSP90 cooperate in homologous and non-homologous DNA double-strand-break repair and G2/M checkpoint activation. *Proc Nat Acad Sci USA* 109(34):13650–13655.