Cell Cycle 12:12, 1821–1827; June 15, 2013; © 2013 Landes Bioscience

Cell Cycle News & Views

MISP: The missing link between extracellular matrix and astral microtubules

Comment on: Maier B, et al. Cell Cycle 2013; 12:1457–71;
PMID:23574715; http://dx.doi.org/10.4161/cc.24602

Amrinder S. Nain[1] and Daniela Cimini[2,*]; [1]Department of Mechanical Engineering; Virginia Tech; Blacksburg, VA USA; [2]Department of Biological Sciences; Virginia Tech; Blacksburg, VA USA; *Email: cimini@vt.edu; http://dx.doi.org/10.4161/cc.25171

Positioning of the cell division plane is critically important for tissue morphogenesis and architecture.[1] It is therefore not surprising that mitotic spindle orientation must be tightly regulated in living tissues, a phenomenon that is also observed in cells cultured in vitro. Because of the amenability of cultured cells to molecular and physical manipulation, many investigators have used such approaches to identify the unifying rules that control spindle positioning.[2,3] One long-standing idea is that in tissue culture cells, the long axis of the mitotic spindle aligns with the long axis of the cell.[1] However, many cell types round up during mitosis, which possibly erases pre-mitotic geometric cues. What then, if anything, controls spindle orientation in rounded mitotic cells? Some very elegant studies have recently shown that the extracellular matrix can control spindle positioning via forces that are transmitted through the plasma membrane and which are linked to the retraction fibers formed as the cell rounds up.[1-3] On the other (intracellular) side of the plasma membrane, spindle positioning is believed to be controlled by the astral microtubules interacting with the cell cortex via the dynein/dynactin complex.[4] What has been unclear so far is the force transmission mechanism linking extracellular space and astral microtubules. A new study by Maier et al. identifies MISP (mitotic interactor and substrate of Plk1) as the missing link in this network of force-transmitting elements[5] (**Fig. 1**). The authors had previously identified this protein in a genome-wide siRNA screen for proteins required for centrosome clustering in cancer cells with supernumerary centrosomes.[6] In the present study, the authors used MISP-siRNA and immunohistochemistry to better understand the role of this protein in spindle assembly and function. Key findings of this study include: (1) MISP depletion causes defects in spindle orientation and positioning, and (2) MISP colocalizes with the actin cytoskeleton and focal adhesions (specifically, the focal adhesion kinase, FAK).[5] The authors also find that MISP interacts with the plus-end-tracking protein EB1 and the p150^{glued} subunit of the dynein/dynactin complex, and that cells depleted of MISP display a mitotic arrest/delay.[5] Further insight on the role of MISP in spindle positioning was provided by another recent study also showing that MISP is an actin-associated protein important for spindle positioning.[7] In this study, using live-cell imaging, Zhu et al. showed that MISP depletion resulted in "unstable" spindle position, in which initially the spindle assembled correctly and the chromosomes aligned properly at the metaphase plate. However, spindle position and chromosome alignment could not be maintained, as the spindles frequently rotated and rocked inside the cell.[7] The authors attributed this behavior to the role of MISP in stabilizing astral microtubules and regulating the cortical distribution of p150^{glued}.[7] This behavior could also explain the presence of BubR1 (indicative of an active mitotic checkpoint) at the kinetochores of MISP-depleted cells.[5,7] This could happen because the observed instability may alter the balance of forces within the mitotic spindle, and thus reduce the stability of kinetochore-bound microtubules. This would then lead to the generation of unattached or partially unattached kinetochores causing checkpoint

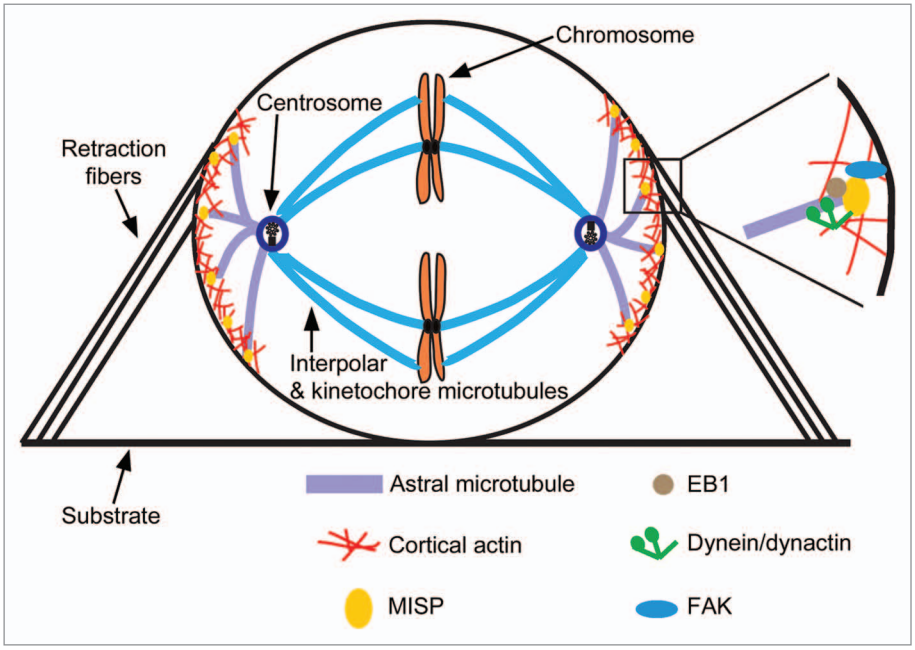

Figure 1. Schematic representation of MISP and its interactors in a rounded mitotic cell. Many different cell types round up during mitosis and form retraction fibers. During this process, focal adhesions are lost except at sites where retraction fibers persist, which also correspond to sites of enrichment of cortical actin. MISP (shown in yellow) may promote the orientation of the mitotic spindle, so that the two spindle poles are aligned with these regions of residual focal adhesions and high cortical actin density. A portion of the cell cortex (box) is shown as an enlarged view to the right, and shows that MISP promotes spindle positioning by simultaneously interacting with microtubule-associated proteins, such as dynein and EB1, cortical actin and focal adhesion-specific proteins, such as the focal adhesion kinase (FAK).

re-activation and mitotic arrest. The fact that MISP was initially identified because of its role in centrosome clustering[8] indicates that forces that are important for spindle positioning[5,7] are also important for maintenance of spindle structure,[5,7,8] and that MISP's function must be finely regulated in mitosis to ensure tight control of both spindle structure and spindle position/orientation. Evidence from these recent studies indicates that such tight regulation occurs via phosphorylation, and that MISP is a CDK1-primed[5,7] substrate of Plk1.[7] It is easy to envision how spindle positioning may be important in the context of a living organism/tissue, as abnormal orientation of the cell division plane would lead to disruption of tissue architecture. Equally important is the maintenance of spindle structure, as defects in spindle geometry, even if only transient, are a major cause of chromosome segregation errors,[8] which can, in turn, lead to cell death or transformation. The evidence provided so far convincingly shows that MISP plays a key role in both spindle structure and positioning in tissue culture cells. What remains to be explored is whether this is also true in living organisms/tissues, and this should be the focus of future studies.

References

1. Théry M, et al. Curr Opin Cell Biol 2006; 18:648-57; PMID:17046223; http://dx.doi.org/10.1016/j.ceb.2006.10.001
2. Fink J, et al. Nat Cell Biol 2011; 13:771-8; PMID:21666685; http://dx.doi.org/10.1038/ncb2269
3. Théry M, et al. Nat Cell Biol 2005; 7:947-53; PMID:16179950; http://dx.doi.org/10.1038/ncb1307
4. Sharp DJ, et al. Nature 2000; 407:41-7; PMID:10993066; http://dx.doi.org/10.1038/35024000
5. Maier B, et al. Cell Cycle 2013; 12:1457-71; PMID:23574715; http://dx.doi.org/10.4161/cc.24602
6. Leber B, et al. Sci Transl Med 2010; 2:33ra38; PMID:20505215; http://dx.doi.org/10.1126/scitranslmed.3000915
7. Zhu M, et al. J Cell Biol 2013; 200:773-87; PMID:23509069; http://dx.doi.org/10.1083/jcb.201207050
8. Silkworth WT, et al. Cell Div 2012; 7:19; PMID:22883214; http://dx.doi.org/10.1186/1747-1028-7-19

Mitotic checkpoints, MUGs and kinetochores

Comment on: Beeharry N, et al. Cell Cycle 2013; 12:1588–97; PMID:23624842; http://dx.doi.org/10.4161/cc.24740

Bill R. Brinkley; Department of Molecular and Cellular Biology; Baylor College of Medicine; Houston, TX USA; Email: Brinkley@bcm.edu;
http://dx.doi.org/10.4161/cc.25172

The kinetochore is a specialized locus located at the centromere (primary constriction) of mammalian mitotic chromosomes and serves as an attachment site for spindle microtubules. Thus, kinetochores are essential for the correct movement, alignment and partitioning of chromosomes during mitosis. With exception of the minute centromeres of yeasts, i.e., *S. cerevisiae*, much remains to be learned about the molecular organization of DNA and protein components of larger, more complex centromeres/kinetochores of chromosomes of higher eukaryotic organisms. This has been due, for the most part, to the lack of reliable procedures for isolating and purifying functional kinetochores of the higher eukaryotes. Hope came unexpectedly approximately 30 y ago, when we utilized a method developed by Schlegel and Pardee[1] for driving metaphase-arrested cells into mitosis prematurely, bypassing S-phase and DNA synthesis. We termed these "mitotic cells with unreplicated genomes" or MUGS, and to our surprise, when MUGs were examined by EM, we discovered numerous kinetochores that had become detached from the condensed chromatin.[2] These laminar-like elements were essentially identical to kinetochore lamina or plates normally seen at the centromere of mitotic chromosomes (**Fig. 1**) and were mostly attached or associated with mitotic spindle microtubules. This fortuitous and unexpected discovery enabled us to ascertain that the kinetochores from metaphase chromosomes were more structurally complex than anticipated, consisting of repeated protein subunits interspersed by DNA linkers.[3] Moreover, we determined that

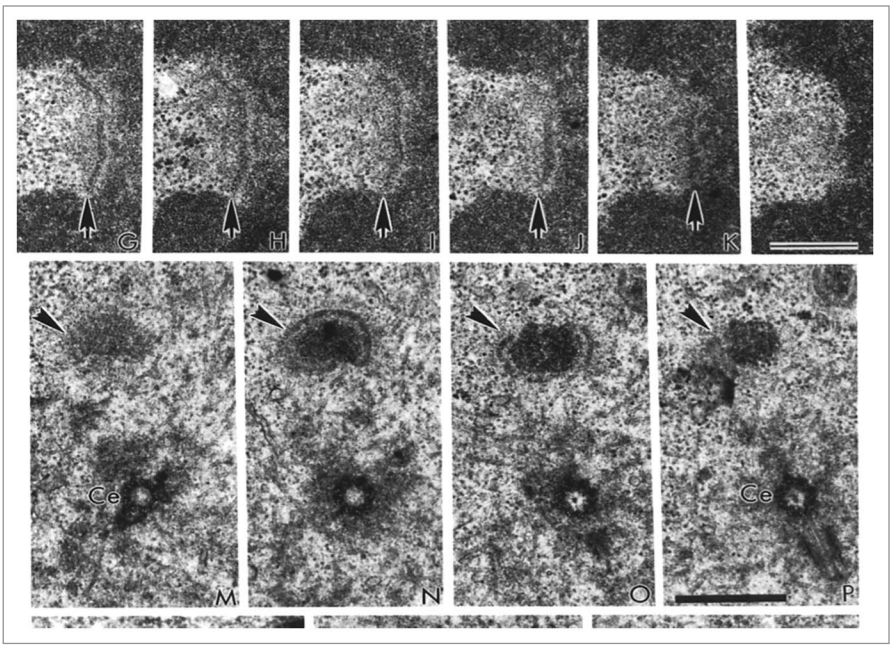

Figure 1. Electron micrographs of contiguous serial sections of normal attached kinetochore (**G–K**) and detached kinetochores from MUGs (**M–P**) are shown below. Reproduced from reference 3 with permission.

the number of detached kinetochores in each MUGs was 2–5 times greater than the actual diploid chromosome number, consistent with the notion that kinetochores were structurally repetitive.

Initially we were optimistic that MUGS offered a potential strategy for the purification and isolation of kinetochores from human chromosomes. However, this notion was threatened initially when MUGS were thought to be produced in only a limited number of mammalian cell lines, i.e, hamsters, rats and deer. Subsequently, however, Balczon[4] found that by overexpressing cyclin A, MUGS could be readily induced in HeLa cells. In a later study, Wise and Brinkley[5] reported that kinetochore fragments of MUGS, although fully detached from chromosomes, could undergo both normal prometaphase movements and equatorial alignment via spindle microtubules, even in the absence of paired sister kinetochores, as seen in normal mitosis. Therefore, it was concluded that "information" needed for proper chromosome alignment at metaphase, resides largely within the mitotic spindle per se and is not as a function of kinetochores. It was confirmed, however, that detached kinetochores of MUGS, although properly aligned on the metaphase spindle, were incapable of undergoing anaphase movement and segregation to spindle poles without attachment to chromosomes.

In view of the plethora of new knowledge on the regulation of cell cycle and spindle checkpoints, it should be possible to establish a more efficient molecular rationale for MUG induction and perhaps decipher more clearly the molecular mechanisms associated with centromere fragmentation and kinetochore detachment. Although the methodology offers a logical approach for fractionation of centromere/kinetochores in human cells, could the induction of such catastrophic events in mitotic cells have potential application to cancer chemotherapy? A recent report by Beeharry et al.[6] offers a reasonable rationale for such an approach. In their search for chemosensitization agents that could be useful tools for overriding cell cycle checkpoints and inducing cell death (mitotic catastrophe), these investigators re-discovered MUGs after almost 30 y of quiescence. When S-phase cells were treated with gemcitabine in combination with Chk1 inhibitors, S-phase checkpoints were overridden, and the cells displayed detached kinetochores essentially identical to those previously in our original reports. Even greater efficiency and more relevant results were obtained when topoisomerase II-mediated S-phase-arrested cell were used. Perhaps of more significance was their success in inducing MUGs in cells derived directly from primary human pancreatic tumors (EGF-1 cells). Previous studies of MUGS have all been limited to establish cells in vitro. MUGS represent manifestations of severe mitotic catastrophe, and that end-point itself, may have relevance for novel strategies in the realm of cancer chemotherapy. However, MUG technology as a strategy for isolating pure fractions of functional kinetochores needed in the construction of artificial chromosomes remains a worthwhile goal.

References

1. Schlegel R, et al. Science 1986; 232:1264-6; PMID:2422760; http://dx.doi.org/10.1126/science.2422760
2. Brinkley BR, et al. Nature 1988; 336:251-4; PMID:3057382; http://dx.doi.org/10.1038/336251a0
3. Zinkowski RP, et al. J Cell Biol 1991; 113:1091-110; PMID:1828250; http://dx.doi.org/10.1083/jcb.113.5.1091
4. Balczon RC. Chromosoma 2001; 110:381-92; PMID:11734996; http://dx.doi.org/10.1007/s004120100157
5. Wise DA, et al. Cell Motil Cytoskeleton 1997; 36:291-302; PMID:9067624; http://dx.doi.org/10.1002/(SICI)1097-0169(1997)36:3<291::AID-CM9>3.0.CO;2-A
6. Beeharry N, et al. Cell Cycle 2013; 12:1588-97; PMID:23624842; http://dx.doi.org/10.4161/cc.24740v

MicroRNA-181a/b: Novel biomarkers to stratify breast cancer patients for PARPi treatment

Comment on: Bisso A, et al. Cell Cycle 2013; 12:1679–87; PMID:23656790; http://dx.doi.org/10.4161/cc.24757

Federica Mori,[1] Sabrina Strano[1] and Giovanni Blandino[2,*]; [1]Molecular Chemoprevention Unit; Regina Elena National Cancer Institute; Rome, Italy; [2]Translational Oncogenomics Unit; Regina Elena National Cancer Institute; Rome, Italy; *Email: blandino@ifo.it; http://dx.doi.org/10.4161/cc.25173

Deregulation of the DNA damage response (DDR) is a main feature of cancer progression and a common finding in breast cancer. While a clear correlation between mutations of BRCA1/2 and ATM in cancer development has been found in hereditary breast cancers, the molecular basis of DDR deregulation of sporadic breast cancers is still unclear. Triple-negative (TNBC) and basal-like (BLBC) breast cancers are very aggressive sporadic tumors that relapse very frequently. Only 2% of sporadic tumors bear detectable BRCA1/2 and ATM mutations. Interestingly, TNBCs and BLBCs present a histoclinical phenotype comparable to that observed in patients with hereditary germline BRCA1/2 mutations ("BRCAness" phenotype). However, while BRCA1 mutated breast cancers are sensitive to PARP inhibitors, both TNBCs and BLBCs are poorly responsive to anticancer treatments. Recent evidence has shown that the altered expression of microRNA genes can be associated with diverse types of cancers. microRNAs are small non-coding RNA molecules that negatively regulate gene expression at the post-transcriptional level. They bind through partial sequence homology to the 3′-untranslated region of target mRNAs and cause translational inhibition and/or mRNA degradation. In the June 1, 2013 issue of *Cell Cycle*, Del Sal's group surveyed public breast cancer data sets for miR-181a/b expression.[1] They found that increased expression of miR-181a/b correlated with shorter disease-free survival and early onset of metastatic disease. The expression of miR-181a/b was also assessed on more than 100 primary breast cancer samples and was found upregulated in tumor specimens when compared with normal tissues. This also correlated with tumor aggressiveness. miR-181a/b have been shown to promote migration and invasion of breast cancer cells as well as the expansion of breast cancer stem-like cells.[2] miR181a/b expression is regulated by transforming growth factor-b,[3-5] whose pathway is aberrantly activated in breast cancer transformation.[6,7] At the molecular level, Del Sal group showed that miR-181a/b targeted ATM mRNA, thereby impinging severely on DDR response of breast cancer cells. BRAC1 phosphorylation was reduced in miR181a/b-overexpressing

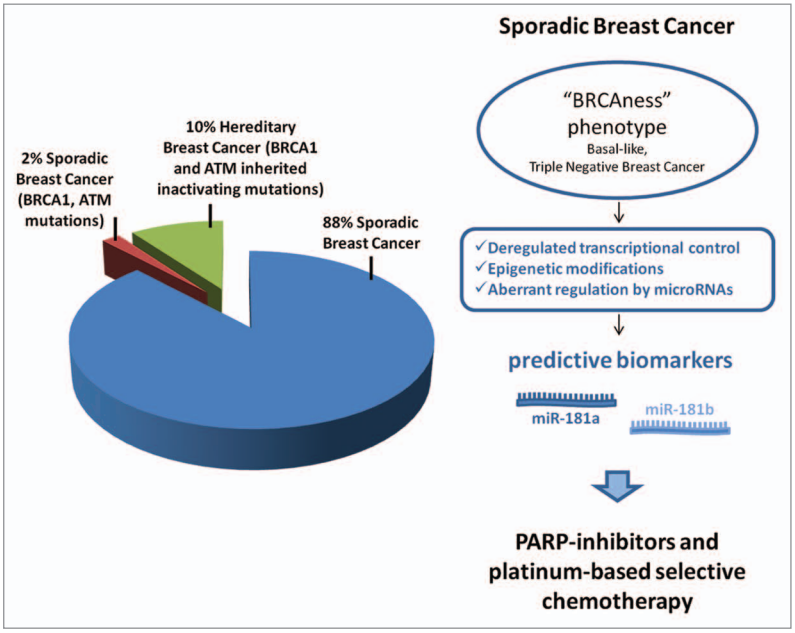

Figure 1. Hereditary breast cancers and sporadic breast cancers presenting BRCA1 mutations account for the 12% of the total breast tumors. Basal-like and triple-negative breast cancers among the sporadic breast tumors (88%) that present "BRCAness" phenotype and may be screened for miR-181a/b expression to treat selectively with PARP inhibitor.

cells, suggesting that miR-181a/b increased expression may dampen DDR and DNA double-strand breaks repair, leading to the accumulation of unfixed DNA lesions in breast cancer cells. This might unveil the oncogenic value of miR-181a/b overexpression and lead to the development of potential therapeutic strategies aimed at overcoming its pro-tumorigenic effects in breast cancers. It is becoming increasingly clear that a detailed molecular cancer portrait of a single patient is required to design the most effective cancer treatment. This is ineludible, as the development of resistance to target therapy for diverse types of tumors, including breast cancer, significantly increases. Indeed, chemoresistance remains a major clinical obstacle to successful treatment of breast cancer and a source of poor prognosis. The power of cancer patient response prediction to given treatments needs to be strongly improved. It is reasonable to think that the more molecular variables we can include in the prediction model the better prediction we may expect. Notably, in this issue of *Cell Cycle*, Bisso and colleagues reported that miR-181a/b overexpressing cells are sensitive to pharmacological inhibition of PARP. miR-181a/b overexpression sensitized breast cancer cells to PARP inhibition, as for Olaparib (AstraZeneca). This raises the intriguing possibility that overexpression of miR-181a/b could be regarded as a potential biomarker that allows the identification of a subgroup of TNBC and BLBC patients who may benefit from the treatment with PARPi. The miR-181a/b overexpression Del Sal's group observed in diverse tumors types could also have major implications for other types of human cancers. In light of these findings, additional work attempting the functional validation of novel miR-181a/b mRNA targets might provide novel insight into the molecular characterization of aggressive cancer subtypes and hold important therapeutic potential. (**Fig. 1**)

References

1. Bisso A, et al. Cell Cycle 2013; 12; PMID:23656790
2. Lopez-Camarillo C, et al. Int J Mol Sci 2012; 13:1347-79; PMID:22408395; http://dx.doi.org/10.3390/ijms13021347
3. Neel JC, et al. Cell Signal 2013; 7:1556-66; http://dx.doi.org/10.1016/j.cellsig.2013.03.013
4. Wang Y, et al. Oncogene 2011; 30:1470-80; PMID:21102523; http://dx.doi.org/10.1038/onc.2010.531
5. Taylor MA, et al. J Clin Invest 2013; 123:150-63; PMID:23241956; http://dx.doi.org/10.1172/JCI64946
6. Seton-Rogers SE, et al. Cell Cycle 2004; 3:597-600; PMID:15107620; http://dx.doi.org/10.4161/cc.3.5.886
7. Cesi V, et al. Cell Cycle 2011; 23:4149-61; http://dx.doi.org/10.4161/cc.10.23.18346

Gender, mutant p53 and PML: A growing "affaire" in tumor suppression and oncogenesis

Comment on: Haupt S, et al. Cell Cycle 2013; 12:1722–31; PMID:23656786; http://dx.doi.org/10.4161/cc.24805

Silvia Di Agostino,[1] Sabrina Strano[2] and Giovanni Blandino[1,*]; [1]Translational Oncogenomics Laboratory; Regina Elena National Cancer Institute; Rome, Italy; [2]Molecular Chemoprevention Laboratory; Regina Elena National Cancer Institute; Rome, Italy; *Email: blandino@ifo.it; http://dx.doi.org/10.4161/cc.25174

P53 mutations occur in more than half of all human cancers. Mutant p53 proteins are mostly full-length with a single residue change, remarkably stable and abundantly present in tumor cells. Mutant p53 proteins do not represent only the mere loss of wt-p53 activity, but also the gain of oncogenic functions that strongly contribute to the establishment, the maintenance and the spreading of a given tumor. The last decade has witnessed remarkable experimental efforts to elucidate the molecular mechanisms underlying the oncogenic role of mutant p53 proteins. To date, two main molecular scenarios are intensively investigated: (1) mutant p53 protein can function as an oncogenic transcription factor as a component of large transcriptional competent protein complexes that also include transcription factors, acetylases, deacetylases and scaffold proteins.[1,2] This leads to the aberrant expression of distinct sets of genes; (2) mutant p53 protein physically interacts with and inactivates tumor suppressor gene products, which are exemplified by the p53 family members p73 and p63.[3] Previous work from the Haupt's group showed that PML interacts with and enhances mutant p53 transcriptional activity (**Fig. 1A**).[4] PML was required for aberrant proliferation and colony-forming ability of mutant p53 cancer cells.[4] This,

intriguingly, highlighted that, unlike the case of p73 and p63 proteins, which are bound and inactivated by mutant p53, binding to PML allows mutant p53 to parasitize PML activities and consequently enhance its pro-tumorigenic effects (**Fig. 1A**). Thus, PML nuclear bodies might endow mutant p53 proteins with gain of function activity. In a previous issue of *Cell Cycle,* Sue Haupt and colleagues provide novel in vivo evidence, which further strengthens the concept of a close relationship existing between gain of function mutant p53 protein and the tumor suppressor protein PML.[5] They elegantly investigated the contribution of PML to mutant p53-driven tumorigenesis in a mouse model harboring a p53 mutation ($p53^{wild\text{-}type/R172H}$) that recapitulates a frequent p53 mutation ($p53^{R175H}$) in human sporadic and Li-Fraumeni cancers. First, loss of PML expression unleashed a pro-tumorigenic response, as manifested by increased protein levels of $p19^{ARF}$, c-MYC and mutant p53 (**Fig. 1B**). Second, loss of PML expression dictated tumor manifestation of $p53^{wild\text{-}type/R172H}$ mice reducing the incidence of lymphomas and increasing the frequency of sarcomas (**Fig. 1B**). Third, PML loss in male mutant p53 mice led to reduced survival when compared with that of females (**Fig. 1B**). These findings have several implications. Previous work has shown that mutant p53 protein is unstable in untransformed tissues.[6] The transformed cellular context plays a pivotal role in the accumulation of mutant p53 proteins. Haupt's findings indicate that loss of PML expression might represent a major oncogenic event, leading to accumulation of mutant p53 protein and thereby enhancing its oncogenic effects.[5] The fine deciphering of mutant p53 accumulation upon loss of PML might unveil molecular targets whose aberrant activity could be pharmacologically tackled either to attenuate its oncogenic effects, through the reduction of its half-life, or to promote anti-tumoral effects, by switching its conformation to that of wt-p53 protein. Mutant p53 has been shown to interact with diverse transcription factors, ranging from NF-Y and VDR to ETS1, SP1, ZEB-1 and E2F-1.[1,2,7,8] It's reasonable to speculate that each of these protein complexes might drive the aberrant expression of selected genes whose protein products drive oncogenesis of specific subsets of human tumors. This may explain why loss of PML through the re-wiring of the network of mutant p53 protein interactions determined a switch in the tumor manifestation of p53 mutant mice. Gender appears to be an additional layer of complexity that can have a profound impact in the development of specific subsets of human tumors. Although further work is required to corroborate Sue Haupt's findings, loss of PML expression emerges to be a candidate factor for determining gender-related cancer disease. Mutant p53 mice with PML exhibited perturbation of the hematopoietic compartment manifested as lymphomas or extramedullary hematopoiesis (EMH). EMH was associated with leucocytosis and macrocytic anemia indicative of myeloprolferative/myelodisplastic overlap. Thus, EMH underlines a molecular lesion that may provide an early prediction for the development of hematopoietic tumors in the context of mutant p53 mice.

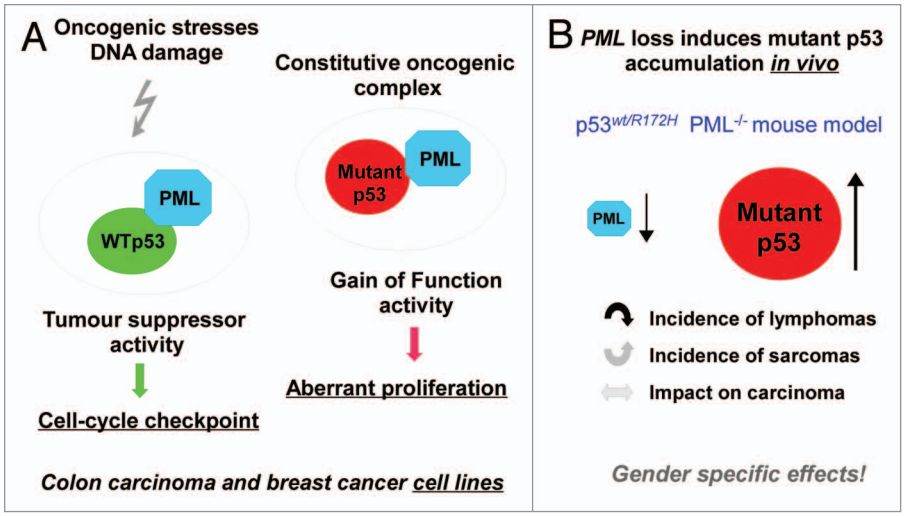

Figure 1. Cross-talk between PML and mutant p53 proteins. (**A**) Gain-of-function mutant p53 proteins parasite PML tumor suppressor activity and enhance their pro-tumorigenic effects. (**B**) Loss of PML expression accumulates mutant p53 protein and dictates gender-associated tumor spectrum.

Collectively, a functional cross-talk between gain-of-function mutant p53 proteins and PML exists; the fine molecular dissection might hold great therapeutic potential for the treatment of mutant p53-driven tumors.

References

1. Di Agostino S, et al. Cancer Cell 2006; 10:191-202; PMID:16959611; http://dx.doi.org/10.1016/j.ccr.2006.08.013
2. Fontemaggi G, et al. Nat Struct Mol Biol 2009; 16:1086-93; PMID:19783986; http://dx.doi.org/10.1038/nsmb.1669
3. Di Agostino S, et al. Cell Cycle 2008; 7:3440-7; PMID:18948736; http://dx.doi.org/10.4161/cc.7.21.6995
4. Haupt S, et al. Cancer Res 2009; 69:4818-26; PMID:19487292; http://dx.doi.org/10.1158/0008-5472.CAN-08-4010
5. Haupt S, et al. Cell Cycle 2013; 12; PMID:23656786
6. Lozano G. Curr Opin Genet Dev 2007; 17:66-70; PMID:17208429; http://dx.doi.org/10.1016/j.gde.2006.12.003
7. Stambolsky P, et al. Cancer Cell 2010; 17:273-85; PMID:20227041; http://dx.doi.org/10.1016/j.ccr.2009.11.025
8. Masciarelli S, et al. Oncogene 2013; PMID:23584479; http://dx.doi.org/10.1038/onc.2013.106

Therapeutic S and G_2 checkpoint override causes centromere fragmentation in mitosis

Comment on: Beeharry N, et al. Cell Cycle 2013; 12:1588–97;
PMID:23624842; http://dx.doi.org/10.4161/cc.24740

Ailine Stolz and Holger Bastians*; Institute for Molecular Oncology; University Medical Center (UMG) and Goettingen Center for Molecular Biosciences (GZMB); Georg-August University; Goettingen, Germany; *Email: holger.bastians@uni-goettingen.de; http://dx.doi.org/10.4161/cc.25175

Abrogation of DNA damage-induced cell cycle checkpoints represents a promising concept for chemosensitization during anticancer treatment. A prominent example is the Chk1 inhibitor UCN-01 that is currently used in clinical trials in combination with various DNA damaging or DNA replication inhibiting drugs. It is well-established that inhibition of Chk1 in the presence of DNA damage leads to an override of the G_2 cell cycle checkpoint, resulting in a premature mitotic entry.[1] Damaged cells that are forced to enter mitosis show a transient mitotic arrest mediated by the spindle assembly checkpoint (SAC). At the same time, massive chromosome fragmentation is commonly observed, and this seems to be a prerequisite for the subsequent mitotic catastrophe, leading to the induction of apoptosis during mitosis, which involves a Mad2-dependent proapoptotic pathway[2] (**Fig. 1**). However, the exact nature of mitotic defects triggering mitotic catastrophe remains unclear, but it is investigated in the work presented by Beeharry et al.[3]

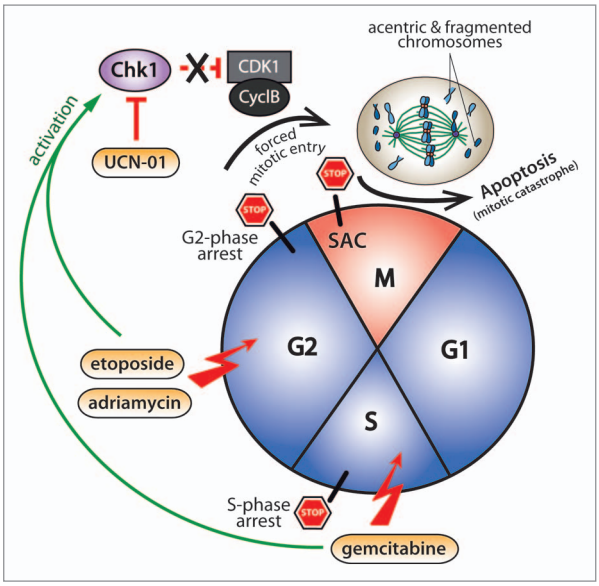

Figure. 1. Model illustrating mitotic centromere fragmentation after UCN-01-mediated S and G_2 checkpoint override. Replication-stress (gemcitabine treatment) or inhibition of topoisomerase II (etoposide or Adriamycin treatment) leads to cell cycle arrest in S- and G_2-phase, respectively. Abrogation of the damage-induced cell cycle checkpoints by treatment with the Chk1 inhibitor UCN-01, forces cells to enter mitosis with incompletely replicated DNA leading to the formation of acentric and fragmented chromosomes. As a consequence of MUGing (mitosis with unreplicated genomes), the cells transiently arrest in mitosis in a spindle assembly checkpoint (SAC)-dependent manner before undergoing mitotic catastrophe and subsequent apoptosis.

In the May 15, 2013 issue of *Cell Cycle*, Beeharry and colleagues provide interesting new insights into the mitotic defects that result from abrogation of a DNA damage-induced S- or G_2-phase checkpoint arrest. Immunofluorescence and electron microscopy analysis of a panel of different pancreatic cancer cell lines revealed that sequential treatment with the DNA replication inhibitor gemcitabine or with topoisomerase II inhibitors such as etoposide and adriamycin in combination with UCN-01 leads to the generation of MUGs (mitosis with unreplicated genomes). Here, MUGs are characterized by severe fragmented mitotic chromosomes with unreplicated centromeres and kinetochores detached from the bulk chromosomes. MUGs were first described by Brinkley et al. some 30 years ago,[4] but Beeharry and colleagues now provide the first evidence that MUGs might be the primary outcome of G_2 checkpoint override after treatment with chemotherapeutic DNA replication inhibitors. In contrast, G_2 checkpoint override after treatment of cells with alkylating agents such as methyl methanesulfonate (MMS), albeit inducing DNA damage and arresting cells in G_2 phase of the cell cycle, do not induce MUGs, and accordingly, cells progress though mitosis without further delay. Interestingly, inhibition of topoisomerase II by etoposide and adriamycin, although not grossly inhibiting DNA replication, still causes MUGing. By using FISH analysis with a centromere-specific probe hybridizing to the centromere region of chromosome 7, the authors found that inhibition of topoisomerase II, like inhibition of DNA replication by gemcitabine, results in unreplicated centromeres.[3] It was previously shown that replication of centromeric DNA proceeds until metaphase,[5] supporting the notion that the replication of centromeres might be sensitive toward topoisomerase II inhibition. However, it remains an open question how topoisomerase II inhibition blocks centromere replication.

The work presented by Beeharry et al. suggests that the underlying cause for mitotic catastrophe observed after G_2 checkpoint override might be a lack of replication and subsequent fragmentation of the centromere, resulting in acentric genomes. These interesting results might also explain why cells cannot satisfy the SAC after forced entry into mitosis and arrest in a metaphase-like state.[2] Furthermore, it would be interesting to address the question of whether this mitotic arrest or the centromere fragmentation is directly related to the massive chromosome fragmentation that is commonly seen in mitotic cells after checkpoint override. In fact, it might be possible that chromosome condensation that

occurs on chromosomes after forced entry into mitosis can generate forces that tear chromosomes apart.

From the clinical point of view, this study provides insights on the choice of chemotherapeutic drugs that are to be combined with G_2 checkpoint inhibitors. At least in pancreatic cancer cells, as shown here, the combination of inhibitors of centromere replication and G_2 checkpoint inhibitors might be the most powerful combination, in particular for cancer cells lacking functional p53. Appropriate clinical trials will show whether this holds true in a clinical setting. Also, additional studies investigating other cancer entities are needed to provide a more general view on the efficacy of chemotherapeutic drugs that affect centromere replication and S or G_2 checkpoint inhibitors.

References

1. Tse AN, et al. Cancer Res 2004; 64:6635-44; PMID:15374978; http://dx.doi.org/10.1158/0008-5472.CAN-04-0841
2. Vogel C, et al. Cancer Res 2007; 67:339-45; PMID:17210716; http://dx.doi.org/10.1158/0008-5472.CAN-06-2548
3. Beeharry N, et al. Cell Cycle 2013; 12:1588-97; PMID:23624842; http://dx.doi.org/10.4161/cc.24740
4. Brinkley BR, et al. Nature 1988; 336:251-4; PMID:3057382; http://dx.doi.org/10.1038/336251a0
5. Madan K, et al. Exp Cell Res 1976; 99:438-44; PMID:57878; http://dx.doi.org/10.1016/0014-4827(76)90604-2