

Cell Cycle News & Views

BMPs morph into new roles in ovarian cancer

Comment on: Ma W, et al. *Cell Cycle* 2013; 12:88–97;
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Our understanding of cancer pathogenesis is being revolutionized by the acceptance and continued molecular dissection of two central hypotheses of cancer biology. First, the “cancer stem cell” hypothesis is advancing our understanding of cancer progression and recurrence. Second, the “seed and soil” hypothesis defines a critical role for the tumor microenvironment in cancer growth and metastasis. In a recent issue of *Cell Cycle*, Ma et al. further define the interplay between these two hypotheses in ovarian cancer by demonstrating a regulatory link between the stem cell factor Lin28 and the signaling molecule bone morphogenic protein 4 (BMP4).¹

Lin28 is a stem cell factor that binds to and blocks downstream effects of the microRNA let-7, thus maintaining stem cell pluripotency. Several recent reports indicate that Lin28 is also an important factor in promoting tumorigenesis (Fig. 1). Lin28 was found to promote breast cancer growth via increased translation of the HER2 protein² and to promote breast and ovarian cancer growth via increased expression of the cell cycle proteins CDK2, cyclin D1 and cell division cycle 25 homolog A (CDC25A).³ Ma et al. now demonstrate an additional tumorigenic role for Lin28. Lin28 binds to BMP4 at a newly identified Lin28-responsive element (LRE), leading to upregulation of BMP4 at the post-transcriptional level in epithelial ovarian cancer cells.¹

BMPs are members of the transforming growth factor- β (TGF- β) family of secreted peptides that function in both normal developmental tissue homeostasis and tumorigenesis.⁴ This current study adds to a growing literature supporting a critical role for BMPs in ovarian cancer by defining a potential autocrine function for BMPs expressed by cancer cells. We have previously reported a critical role for increased BMP2, BMP4 and BMP6 expression by mesenchymal stem cells in the tumor stroma. Increased BMP expression in the stroma increases the pool of cancer

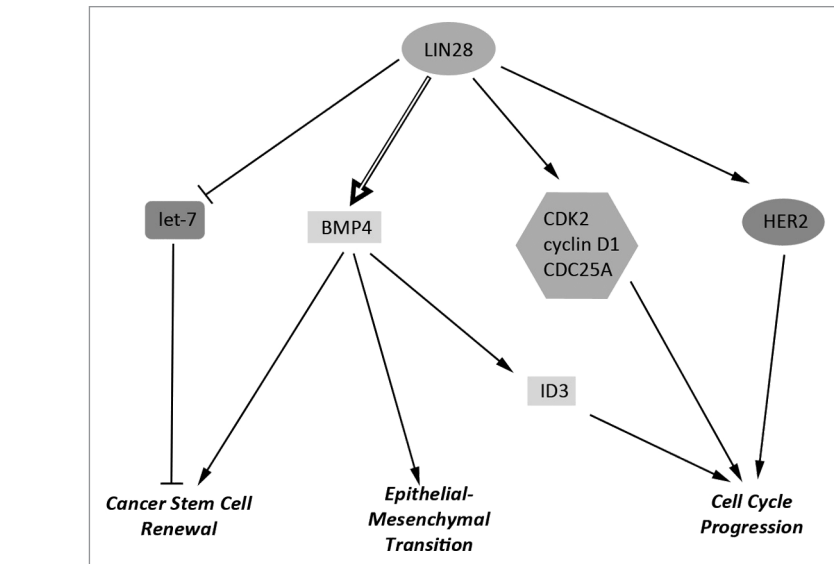


Figure 1. Lin28/BMP4 signaling pathways promote tumorigenesis. The newly defined regulatory role for Lin28 upregulating BMP4 (outlined arrow) adds to our understanding of Lin28 downstream signaling that promotes multiple aspects of the tumor phenotype (italics).

stem cells, resulting in enhanced tumorigenesis.⁵ BMP4 has also been shown to directly upregulate ID3 proto-oncogene expression in human ovarian cancer cells.⁶ Additionally, exogenous BMP4 treatment of ovarian cancer cells results in epithelial-mesenchymal transition (EMT), with increased cellular adhesion, motility and invasion.⁷ All of these studies support a pro-tumorigenic role of BMP overexpression in ovarian cancer (Fig. 1). These downstream effects of BMP signaling are consistent with the finding that overexpression of BMP2, a closely related family member of BMP4, is associated with poorer prognosis in ovarian cancer patients.⁸

Ma et al. also extend the clinical implications of Lin28-mediated upregulation of BMP4 by looking at the prognostic implications of Lin28 overexpression. Immunohistochemical analysis of more than 300 primary ovarian cancers, looking at the expression of Lin28 and the stem cell factor Oct4, demonstrated that

overexpression of Lin28 and Oct4 together is correlated with decreased patient survival.¹ Interestingly, Lin28 and Oct4 define a subset of cells in ovarian cancer with stem-like properties.⁹ These findings, together with the role of BMPs in promoting ovarian cancer stemness, suggest an important interplay between Lin28/Oct4/BMP that impacts cancer stem cells, tumorigenesis and, ultimately, patient outcomes.

Ovarian cancer is a disease plagued by recurrences with progressive chemoresistance, ultimately leading to uncontrolled cancer growth resulting in patient death. The challenge that lies ahead is integrating our improved molecular understanding of ovarian cancer pathogenesis with novel therapeutic options to improve patient outcomes. Considering the BMP pathway, further studies are necessary to continue to tease out the relative roles of epithelial and stromal production of BMPs in ovarian cancer and the subsequent

autocrine and paracrine effects of the aberrant BMP expression. Hopefully continued characterization of the newly identified Lin28/Oct4/BMP pathway will provide potential targeted therapy options for patients.

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Wip1 regulation: Who controls a reset button?

Comment on: Macurek L, et al. *Cell Cycle* 2012; 12:251–62;

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Wip1/PPM1D is a type 2C family serine/threonine phosphatase able to dephosphorylate central players in the DNA damage response (DDR). Wip1 removes DNA damage-induced phosphorylation in p53, H2AX, ATM, Chk2 and p38MAPK among others.¹⁻³ Therefore, Wip1 works like a reset button to inactivate the DNA damage response when the DNA is repaired. Since many of the known targets of Wip1 are tumor suppressors, amplification of the PPM1D gene occurs in some primary cancers, and its deletion in mice causes a tumor resistance phenotype.^{3,4}

To accomplish these important functions in the cell, Wip1 should be tightly controlled at several levels. In spite the remarkable interest in studying how Wip1 activity/levels are controlled, not much is yet known. For example, Wip1 expression is negatively controlled by miR-16, a microRNA-induced at early time points after DNA damage, to avoid Wip1 activity at the initiation of the DDR.⁵ Also, Wip1 is a transcriptional target for p53, working in a negative feedback loop to inactivate p53.⁶

The elegant work of Macurek and colleagues sheds additional light on the mechanism of regulation of Wip1.⁷ Using state-of-the-art techniques and different rigorous approaches, they demonstrated that the levels of Wip1 are regulated during the cell cycle: high during the S and G₂ phases of the cell cycle and low during mitosis. Wip1 regulation during mitosis is mediated by ubiquitin-dependent proteasome degradation controlled by the anaphase-promoting

complex/cyclosome (APC/C) and its activator Cdc20.

Moreover, Macurek and coworkers showed regulation of Wip1 enzymatic activity by phosphorylation of multiple sites in the N-terminal catalytic domain. By mass spectrometry they identified several residues, which, when mutated, did not produce a gel-mobility shift during mitosis that was observed with the wild-type version of the protein. One of these residues (among seven) was shown to be phosphorylated by Cdk1 in vitro. Interestingly, the phosphorylation of these serines and/or threonine inactivates Wip1, as mutations to alanine do not affect the in vitro activity, but a phospho-mimicking mutant is phosphatase inactive.

Consequently, Macurek and colleagues unequivocally showed that Wip1 is degraded and inactivated during mitosis. Why does this occur? The importance of these findings does not seem to be the regulation of mitosis, as the lack of Wip1 did affect mitotic progression, but Wip1 regulation is related to the DDR modulation. Wip1 maintains cells competent for re-entry into the cell cycle after DNA damage in G₂ phase. Therefore, Wip1 high levels and/or activity during G₂ compared with mitosis are likely to be required for this purpose. Does the degradation of Wip1 have a functional role then? Macurek and colleagues suggest that it is, indeed, important for the DDR. During mitosis, DNA damage largely remains unrepaired, but repair can start during the next G₁ phase.^{8,9} Therefore, the low levels and/or activity of Wip1 during mitosis might lead to

a decrease in the threshold for the DDR (mainly H2AX phosphorylation), which may help in the subsequent repair during the following G₁ phase. Hence, cells may be able to sense low levels of endogenous DNA damage that occurs at underreplicated chromatin regions during normal mitotic progression.⁹ Indeed, Macurek et al. showed that the overexpression of wild type Wip1 during mitosis led to a decrease of γ H2AX during mitosis and less 53BP1 focus formation during G₁. In contrast, the expression of a phosphomimicking mutant had a low impact on 53BP1 focus formation during G₁ phase.

The article by Macurek et al. also leaves some open questions that will be interesting to address in the future. From the mechanistic point of view, the work suggests the existence of other post-translational modifications in Wip1 (possible phosphorylations) that might be dependent on cell cycle and/or DNA damage. The molecular details of how Cdc20 recognizes Wip1 to target it for degradation also remain to be elucidated. Finally, the kinase(s) responsible for the mitotic phosphorylation of Wip1 and investigating if these kinases are themselves targets of the phosphatase will be subjects to study. From a pathological point of view, this work opens the possibility to examine the impact of a non-degradable Wip1 or mutants in phosphorylated residues during mitosis on cancer predisposition in mice. Especially interesting to study is the phosphomimicking mutant, as it possibly may be more resistant to tumor formation but might not show some of the secondary effects that the Wip1-knockout mouse has.

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Wip1 downregulation conserves truncated DNA damage response (DDR) in mitosis

Comment on: Macurek L, et al. *Cell Cycle* 2012; 12:251–62; PMID:23255129; <http://dx.doi.org/10.4161/cc.23057>

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Our genomes are attacked constantly by reactive oxygen species generated as by-products of metabolic processes or induced by exogenous sources, such as UV light and cigarette smoke. Tens of thousands of DNA lesions are estimated to occur daily in each human cell. To cope with these genomic insults and preserve their genetic information, cells have evolved a set of conserved mechanisms collectively called the DNA damage response (DDR). These highly orchestrated signaling and DNA repair networks are tightly controlled by various post-translational modifications (PTMs), including, predominantly, phosphorylation and ubiquitination.¹

Double-strand breaks (DSBs) are the most deleterious type of DNA lesion. Under physiological conditions, the majority of DNA breakages that enter or arise in mitosis originate during S phase, when DNA replication machinery approaches unstable or challenging DNA structures, such as fragile sites.² Once a DSB occurs, the protein kinases ataxia-telangiectasia mutated protein (ATM) and DNA-dependent protein kinase (DNA-PK) activate the DDR by phosphorylating H2AX to generate γ -H2AX. This factor recruits mediator of DNA damage checkpoint 1 (MDC1), which mediates the DDR in the vicinity of a DSB. MDC1 promotes the phosphorylation-dependent recruitment of DDR-sensor proteins, such as the Mre11-Rad50-Nbs1 complex (MRN).¹ This formation is deemed the “proximal” DDR, and its components are early markers of DSBs. MDC1 also functions as a platform for the integration of ubiquitination events. As a second PTM, ubiquitination orchestrates the “distal”

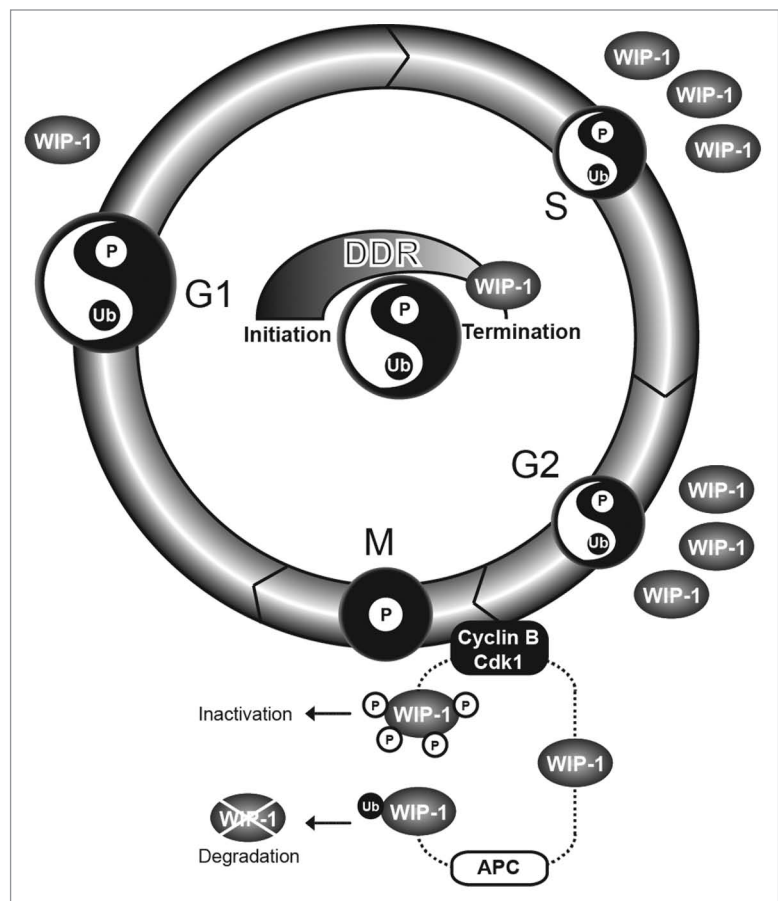


Figure 1. Wip1 inactivation and degradation lead to truncated DDR in mitosis. Wip1 phosphatase terminates the DDR. Here, the concept of yin-yang is used to represent the harmony of phosphorylation (p = Yin-dark) and ubiquitination (Ub = Yang-white) events in the coordination of the fully activated DDR during G₁, S, and G₂. Only the fully activated DDR can execute DNA repair. The domination of Yin (P) in the DDR results from the truncated and inactive DDR during mitosis (M), which can identify, but not repair, DNA damage. The inactivation and degradation of Wip1 enables the existence of truncated DDR (Yin) in mitosis. Once the cell exits mitosis, Wip1 expression gradually increases.

DDR. Phosphorylated MDC1 recruits the E3 ubiquitin ligase, RNF8, which mobilizes two parallel ubiquitin chain cascades: Lys48-linked ubiquitin and Lys63-linked ubiquitin.³ These chains are essential for the recruitment of "caretaker" gene products and DNA repair proteins, such as 53BP1, BRCA1 and Rad51, which constitute the fully activated DDR.^{4,5} Precise spatiotemporal synchronization of proximal and distal DDR events is essential for efficient DNA repair.

The fully activated DDR, coordinated by phosphorylation and ubiquitination, can occur during G₁, S or G₂ phases of the cell cycle. In contrast, only the proximal DDR exists in mitosis.⁶ This truncated DDR can sense and mark DNA damage but cannot execute DNA repair, owing to its inability to activate ubiquitin signaling cascades. Cells in mitosis preserve their damaged DNA until mitotic exit, when the DDR resumes full activation, and the lesion can be repaired.⁷ Inactivation of DNA repair in mitosis likely occurs because of compacted and condensed mitotic chromosomes that obstruct RNF8 and the other distal DDR components to the proximal DDR assembly. In addition, the complexity of mitotic processes and the limited duration of mitosis could explain why chromosomal segregation and cytokinesis preclude DNA repair.

In a recent issue of *Cell Cycle*, Macurek et al.⁸ described the maintenance of the proximal DDR during mitosis and the mechanisms by which damaged mitotic DNA is marked and preserved but not repaired. These authors identified the strict, cell cycle-dependent regulation of Wip1/PPM1D, a phosphatase that dephosphorylates γ -H2AX and other elements of the DDR.⁹ Wip1 protein expression and enzymatic activity are low during G₁, increase gradually as cells enter S phase and peak during G₂. When cells enter mitosis, Wip1 phosphatase activity is downregulated rapidly by ubiquitin-dependent Wip1 proteasomal degradation coordinated by APC/C^{Cdc20} (Fig. 1). Macurek et al.⁸ demonstrated that the Wip1 catalytic domain is phosphorylated at multiple sites during mitosis by cyclin B/Cdk1 and other unidentified kinases. Phosphorylation of Wip1 inactivates its phosphatase activity. Endogenous Wip1 phosphorylation may regulate its phosphatase activity or may only be a prerequisite for ubiquitination and consequent proteasomal degradation. Nevertheless, the work of Macurek et al.⁸ provides evidence that phosphorylation and proteasomal degradation occur cooperatively to inactivate and remove Wip1 from the cellular milieu during mitosis. Wip1 is essential for the timely inactivation of the DDR. After damaged DNA is

repaired, the removal of Wip1 as the cell proceeds to mitosis allows only the proximal DDR to assemble on existing DNA lesions. Macurek et al.⁸ elucidate the function of Wip1 phosphatase during the cell cycle and underscore the importance of Wip1 downregulation in the preservation of a truncated DDR during mitosis. Additional research is warranted to address the molecular details of distal DDR inactivation during mitosis.

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P-TEFb as a target to reactivate latent HIV: Two Brds are now in hand

Comment on: Boehm D, et al. *Cell Cycle* 2012; 12:452-62; PMID:23255218; <http://dx.doi.org/10.4161/cc.23309>

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P-TEFb is a protein kinase required for RNA polymerase II transcriptional elongation of most, if not all, mammalian protein-coding genes. P-TEFb is believed to be a therapeutic target for cancer, and inhibitors of P-TEFb are currently being evaluated in clinical trials. Additionally, P-TEFb has potential as a therapeutic target for HIV infection, as transcriptional elongation of the integrated virus is dependent upon the viral Tat protein's recruitment of P-TEFb to the TAR RNA element at the 5' end of nascent viral transcripts.

Resting CD4⁺ T lymphocytes that contain integrated but transcriptionally silent HIV are clinically significant, as when patients stop antiviral drugs that effectively suppress viral replication, some viruses in this latent reservoir

reactivate and rekindle infection. Substantial effort in the HIV/AIDS field is currently directed toward identifying cell-permeable small molecules that can reactivate latent viruses and thereby reduce or even purge the latent reservoir, perhaps curing infection. This research activity is illustrated by five recent publications, including one from Boehm and colleagues in a recent issue of *Cell Cycle*, which shows that a molecule termed JQ1 targets P-TEFb and reactivates latent HIV under some conditions.¹⁻⁵

Although P-TEFb exists in multiple complexes in cells, its core is composed of CDK9 and either Cyclin T1 or Cyclin T2, with Cyclin T1 being the predominant subunit in most human tissues examined. Three P-TEFb complexes have been biochemically characterized:

core P-TEFb + the bromodomain protein Brd4, the 7SK snRNP and the super elongation complex.⁶ JQ1 was identified as a molecule that recognizes protein bromodomains, or acetylysine recognition motifs, and it has highest specificity for Brd4, although it also has specificity for Brd2 and Brd3.⁷

All five of the recent publications found that JQ1 can reactivate HIV in cell line models of latency. P-TEFb is clearly involved in this reactivation, as shRNA depletions of Cyclin T1 largely abolished JQ1's activity in cell lines.⁵ Reactivation of latent virus by JQ1 is, however, not strictly dependent upon the viral Tat protein, as reactivation was observed in a cell line harboring a latent virus that lacked the *Tat* gene.⁵ JQ1 appears to function as an

antagonist of Brd4 and Brd2, and shRNA depletions of either protein also reactivated latent HIV in cell lines.^{2,4,5} The mechanisms whereby Brd4 and Brd2 mediate JQ1's reactivation activity remain to be clarified, but both proteins were found in complexes with P-TEFb in a large-scale co-immunoprecipitation study.⁸

Although its HIV reactivation activity in cell lines suggests that JQ1 and related molecules have therapeutic potential, a critical difference between cell lines and primary resting CD4⁺ T lymphocytes is that P-TEFb is expressed at high levels in cell lines but is repressed in resting lymphocytes. In primary resting CD4⁺ T cells, very low levels of Cyclin T1 are expressed, and phosphorylation of the CDK9 T-loop is absent, a modification required for P-TEFb catalytic activity.⁹ JQ1's abilities to reactivate latent HIV in CD4⁺ cells from patients or primary cell models of latency were mixed in the recent studies. JQ1 reactivated the virus in one of three patients' samples in one study,¹ while its effects were variable in another study, either enhancing or suppressing reactivation

of virus in patients' samples when used in combination with prostratin or SAHA, other molecules that have some ability to reactivate latent HIV.⁴ JQ1 activity in primary CD4⁺ T cell models of latency was also variable—it was able to reactivate latent virus in a model developed by the Siliciano lab but was inactive in an alternative model developed by the Planelles lab.⁵ Elucidating JQ1's differential activity in these two primary cell models may give important clues into mechanisms of HIV latency, as may the study of the roles of Brd2 and Brd4 in latency and reactivation.

Similar to effective suppression of HIV replication by a combination of antiviral drugs that target different steps in the viral life cycle, it is thought that reactivation of latent viruses will require a combination of small molecules that reactivate latent virus through different mechanisms. The identification of JQ1 and both Brd2 and Brd4 as targets for reactivation are hopefully steps forward in the development of effective strategies to reactivate latent viruses in infected individuals.

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