Wiping DNA Methylation: Wip1 Regulates Genomic Fluidity on Cancer

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Wip1 phosphatase plays an important role in cancer by inactivating p53 and INK4a/ARF pathways. In this issue of *Cancer Cell*, Filipponi and colleagues further connect the oncogenic role of Wip1 with heterochromatin dynamics, transposable element expression, and a mutation-prone environment that may enhance heterogeneity and ultimately contribute to tumor evolution.

The protein phosphatase Mg/Mn dependent 1D (PPM1D), also known as wild-type p53-induced phosphatase 1 (Wip1), plays an important role in cancer by promoting the termination of the DNA damage response pathway and cell cycle progression. In concordance with this oncogenic activity, *Wip1/PPM1D* is often overexpressed in breast tumors and other types of human tumors. In this issue of *Cancer Cell*, Filipponi et al. (2013) reveal a new role for Wip1: connecting heterochromatin dynamics, transposable element (TE) expression, and, ultimately, genomic fluidity in cancer.

TE-derived sequences comprise more than half of the human genome, and the activity of currently active TEs continues to generate genomic fluidity in our genome (Beck et al., 2011). In the human genome, *l*ong *in*terspersed *e*lement class-1 (LINE-1 or L1) is the only active autonomous TE. LINE-1s comprise 20% of the human genome, although only 80–100 L1s remain retrotranspositionally active (Beck et al., 2011). Additionally, two nonautonomous retrotransposons, Alu and SVA (comprising 10% and <1% of our genome, respectively), can mediate their mobilization using the LINE-1-encoded enzymatic machinery (reviewed in Beck et al., 2011). These three non-LTR retrotransposons move in our genome by a "copy and paste" mechanism using an intermediate RNA. Active LINE-1 s are 6-kb-long elements containing a 5′ untranslated region (UTR) with internal promoter activity, two ORFs encoding protein products required for retrotransposition, and end in a short 3′UTR with a poly(A) tail. As "mobile" DNA, new LINE-1 insertions can be mutagenic by a myriad of mechanisms, leading to gene inactivation and/or gene deregulation processes. Thus, the host has evolved multiple mechanisms aimed to reduce the mutagenic load generated by LINE-1 s. Controlling LINE-1 transcription is the most effective manner to control TE activity, because it will abolish mobilization of not only LINE-1, but also Alu and SVA. Notably, the 5′UTR of mammalian LINE-1 s contains a canonical CpG island that serves to regulate its expression by DNA-methylation (Yoder et al., 1997). Somatic tissues and germ cells efficiently silence LINE-1 expression by DNA methylation, avoiding accumulation of LINE-1 insertions. However, the LINE-1 promoter is hypomethylated during embryogenesis (leading to the accumulation of new LINE-1 s that will be transmitted to newborns) and in several types of tumors. Indeed, recent studies have demonstrated that selected TEs are active in lung, colorectal, prostate, ovarian, myeloma, glioblastoma, and hepatic tumors (Iskow et al., 2010; Lee et al., 2012; Shukla et al., 2013; Solyom et al., 2012). These data indicate that, on top of other forms of cellular stress, cancer cells can be impacted by potentially mutagenic new TE insertions.

DNA damage response (DDR) is often deregulated in cancer, leading to genomic instability. Wip1 is a DDR regulator in the germline. Additionally, the *Wip1/PPM1D* gene is often amplified in human cancers, whereas genomic depletion in mouse models suggests that Wip1 may act as a tumor regulator. Interestingly, Filipponi et al. (2013) now demonstrate that Wip1 maintains the epigenetic homeostasis of TEs associated to heterochromatin (including active LINE-1s), providing a mechanistic link between DNA-methylation and LINE-1 expression in the germline and in cancer.

In a mouse knockout (KO) model for *Wip1*, Filipponi et al. (2013) confirmed that *Wip1* deletion results in attenuated spermatogenesis. Surprisingly, *Wip1* deletion also results in increases of heterochromatin centers in germ cells and a concomitant reduction in the expression levels of LINE-1 and intracisternal A-type particle endogenous retrovirus mRNAs. Whether fewer TE insertions accumulate in these mice remains to be determined. Importantly, Wip1 might be the first positive regulator of LINE-1 expression and retrotransposition. Further experiments revealed that depletion of *Wip1* in human cancer cell lines results in increased levels of repressive histone marks on LINE-1 promoters and reduced levels of expression. Furthermore, depletion of *Wip1* in human cancer and mouse germ cells led to reduced DNA-methylation levels in LINE-1 (and endogenous retroviruses) promoters. Consistently, increased LINE-1 mRNA expression levels (and reciprocal changes in histone marks and DNA methylation) were detected upon Wip1 overexpression in cancer cells. Altogether, these data indicate that Wip1 participates in controlling the homeostasis of TE epigenetic regulation.

Consistent with previous reports, Filipponi et al. (2013) confirmed that the

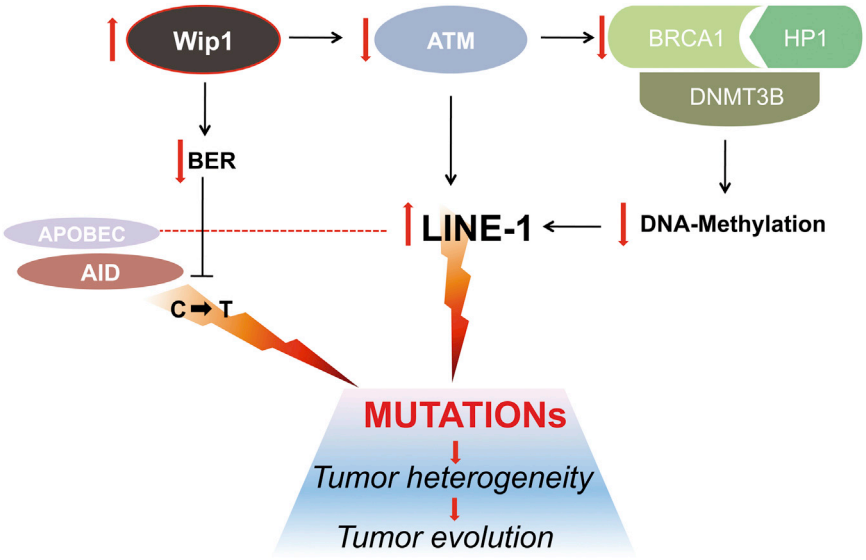

Figure 1. Wip1 Overexpression Is often Observed in Breast Cancer, Suppressing ATM Signaling

Suppression of ATM signaling avoids formation of a BRCA1-HP1-DNMT3B complex and subsequent DNA methylation. DNA hypomethylation on LINE-1s upon overexpression of Wip1 results in increased L1-mRNA expression and likely subsequent retrotransposition. Increased LINE-1 retrotransposition is a source of genomic fluidity and can potentially create mutagenic insertions. Also depicted in the diagram is the direct regulation of LINE-1 retrotransposition by ATM. Additionally, Wip1/AID/APOBEC overexpression can act as a source of somatic mutations in cancer (C-to-T), mostly on non-methylated cytosines. AID performs the conversion of cytosine to uracil, which can be repaired by base excision repair (BER). However, BER is negatively regulated by Wip1, leading to mutation accumulation. Thus, there are two sources of genomic fluidity—AID-unrepaired substitutions and potential LINE-1 insertions—that can impact tumor evolution.

ATM-dependent signaling pathway is constitutively activated upon *Wip1/PPM1D* deletion, resulting in the activation of the downstream effector BRCA1. Additional data demonstrates that BRCA1 interacts with the DNA methyltransferase DNMT3B through *HP1* binding, forming a BRCA1-HP1-DNMT3B complex that modulates the LINE-1 promoter DNA-methylation (Figure 1). These data strongly suggest that BRCA-1 is involved in DNA-methylation in an ATM-dependent manner. In the germline, depletion of the *DNMT3L* results in global reduction of retrotransposon DNA methylation and significant increases in retrotransposon mRNA levels (Bourc'his and Bestor, 2004). It has been suggested that meiotic failure of *DNMT3L*-KO mice is a result of retrotransposons overexpression and deregulated retrotransposition. Thus, it is likely that a balance between BRCA1-HP1-DNMT3B and DNMT3L complexes on TE DNA methylation allows normal meiosis to succeed in germ cells, regulating a physiological level of TE expression and retrotransposition.

Furthermore, elegant genetic experiments demonstrate that defects in spermatogenesis in *Wip1-KO* mice can be rescued by the deletion of a single ATM allele, suggesting that ATM is required to establish DNA methylation upon *Wip1/PPM1D* depletion. Interestingly, previous studies have demonstrated that ATM depletion results in increased levels of engineered LINE-1 retrotransposition in a mouse model and human cell lines (primary and transformed) (Coufal et al., 2011). These data reveal that there is complex regulation of LINE-1 expression and retrotransposition involving ATM-signaling and Wip1 pathways (Figure 1).

In human cancer cell lines, Wip1 overexpression correlate with increased levels of LINE-1 mRNAs, including mRNA derived from potentially active elements. Filipponi et al. (2013) further examined whether alterations in *Wip1/PPM1D* expression, heterochromatin dynamics, and DNA methylation correlate with genomic fluidity observed in breast cancer samples. Indeed, cytidine deaminases are involved in generating point mutations in an epigenetically dependent manner. Activation-induced cytidine deaminase (AID) removes the amino group from a cytosine base and turns it into uracil (which is recognized as a thymine). This deamination may lead to mutation if not corrected by DNA repair. Notably, AID is enriched in TE sequences in a Wip1-dependent manner. Consistent with this, breast cancer samples with an increased copy number of *Wip1/PPM1D* contain more C-to-T mutations, likely mediated by differential AID binding in a *Wip1*-dependent manner (Figure 1). Additionally, the accumulation of the AID mutations is also favored, because Wip1 overexpression inhibits base excision repair. Interestingly, a strong correlation was observed between the expression level of the APOBEC3B cytidine deaminase, AID, Wip1, and overall C-to-T substitution load. APOBEC3B has been recently shown to generate genomic editing in cancer genomes (Burns et al., 2013), consistent with the findings reported by Filipponi et al. (2013). Intriguingly, several APOBEC cytidine deaminases are known to efficiently inhibit LINE-1-engineered and Alu-engineered retrotransposition by an elusive mechanism (Beck et al., 2011). Thus, it is tempting to speculate that a balance between AID, APOBECs, and Wip1 might regulate the load of somatic retrotransposition observed in different human cancers. Future experiments are required to determine to what extent Wip1 modulate the somatic load of LINE-1 retrotransposition in cancer.

In conclusion, DNA hypomethylation in *Wip1/PPM1D*-overexpressing tumors may promote genomic fluidity by the accumulation of mutations induced by TE transposition and AID deamination (Figure 1). These data also reveal how the regulation and the impact of retrotransposition in cancer development might be more complex than previously anticipated.

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Cancer-Associated Osteoclast Differentiation Takes a Good Look in the miR(NA)ror

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Tumor-bone cell interactions are critical for the development of metastasis-related osteolytic bone destruction. In this issue of *Cancer Cell*, Ell and colleagues show how a discrete miRNA network regulates osteoclastogenesis during breast cancer bone metastasis. A signature of upregulated miRNAs may have diagnostic and therapeutic implications for bone metastases.

Advanced breast cancer commonly metastasizes to bone, where it causes osteolytic bone destruction and associated bone pain and fracture, hypercalcemia, and paralysis due to spinal cord compression. In the bone microenvironment, tumor cells hijack the bone remodeling process, normally orchestrated by osteoclasts, osteoblasts, and osteocytes, to wreak havoc and weaken the bone. Osteoclast differentiation and bone resorption is dependent on macrophage colony-stimulating factor and receptor activator of NF-κB ligand (RANKL) (Boyle et al., 2003). Once in the bone, breast cancer cells release factors that send osteoclasts into overdrive by recruiting preosteoclasts and inducing their differentiation. Osteoclastic bone resorption releases growth factors stored in the bone, such as transforming growth factor β (TGF-β), which in turn drives tumor cell production of factors that further increase osteoclast activity (Weilbaecher et al., 2011). This feed-forward vicious cycle creates a fertile microenvironment for tumor growth in bone to drive the devastating effects of bone destruction and render the tumor incurable.

Therapy for patients with bone metastases attacks the tumor cells as well as the bone microenvironment. Antiresorptive therapy, bisphosphonates (zoledronic acid), and the RANKL antibody (denosumab) are standard-of-care to target osteoclast hyperactivity. These drugs effectively reduce skeletal-related events due to bone metastases but do not cure disease. Further, it is difficult to predict who will develop bone metastases due to lack of broadly applicable biomarkers to better guide long term preventive therapy.

In this issue of *Cancer Cell*, Ell et al. (2013) propose a single approach to treat and predict bone metastases based on microRNA (miRNA). Specifically, they identify a miRNA signature induced by highly metastatic tumor cells that stimulates differentiation of osteoclasts and recruits preosteoclasts to the site of the tumor-bone interface (Figure 1). miRNAs repress gene expression through complementary binding to the "seed sequence" of mRNAs (Bartel, 2009) and are important for osteoclastogenesis (Mizoguchi et al., 2010; Sugatani and Hruska, 2007; Zhang et al., 2012). Here, the authors demonstrate how breast cancer cell invasion in the bone co-opts this normal process to hyperactivate osteoclasts and prime the bone for osteolytic destruction. This represents significant insight into our understanding of the organ-specific function and pathological activity of miRNAs, which could lead to improvements in diagnosis, treatment, and prevention of bone metastases and elucidates a unique aspect of the bone microenvironment to support tumor growth in bone.

To identify miRNAs modulated during osteoclastogenesis, the authors used