Cancer Cell
Previews

Epigenetic Deficiencies and Replicative Stress: Driving Cancer Cells to an Early Grave

Muhammad Shoaib[1] and Claus Storgaard Sørensen[1,*]
[1]Biotech Research and Innovation Centre (BRIC), University of Copenhagen, Ole Maaløes Vej 5, 2200 Copenhagen N, Denmark
*Correspondence: claus.storgaard@bric.ku.dk
http://dx.doi.org/10.1016/j.ccell.2015.10.009

Cancer cell-specific synthetic lethal interactions entail promising therapeutic possibilities. In this issue of *Cancer Cell*, Pfister et al. describe a synthetic lethal interaction where cancer cells deficient in H3K36me3 owing to SETD2 loss-of-function mutation are strongly sensitized to inhibition of WEE1, a cell cycle controlling kinase.

Epigenetic alterations are important emerging players in cancer causation and progression (Morgan and Shilatifard, 2015). In the last decade, high throughput sequencing and proteomic studies have highlighted epigenetic abnormalities as an important hallmark of cancer cells (Shen and Laird, 2013). The field of cancer epigenetics was initially largely centered upon studying abnormalities in patterns of DNA methylation and developing targeted therapies directed toward DNA methyltransferases. However, in the past decade, this focus has merged with a burst of data linking covalent histone modifications to gene expression patterns and cancer (Morgan and Shilatifard, 2015).

The epigenetic information in the form of histone modifications is dynamically regulated by chromatin modifying enzymes and helps create global and local chromatin states, regulating DNA-templated processes. Consequently, any disturbance in transcriptional pattern or mutations in chromatin modifiers critically affects these processes and can lead to initiation and progression of various cancers. In this regards, recent years have seen great progress in targeting specific chromatin modifying enzymes, and, in particular, those that regulate histone acetylation and methylation (Campbell and Tummino, 2014). Inhibitors targeting histone methyltransferases EZH2 and DOT1L and histone deacetylase inhibitors are currently in clinical trials (Campbell and Tummino, 2014). Despite these discoveries and advances, scientific challenges remain, in particular, due to the fact that there are few mutations, translocations, or synthetic lethal relationships known in cancers that directly implicate chromatin modifications and the enzymes that create them.

Synthetic lethality (SL) describes the relationship between two factors whereby loss or inhibition of either is compatible with cell viability, but loss or inhibition of both results in cell death. SL-based screening has shown great promise in identifying targets with high therapeutic efficacy, low toxicity, and high selectivity against tumor cells (Fece de la Cruz et al., 2015). Because, chromatin modifiers are critically involved in DNA-based cellular processes, their inactivating mutations in a variety of cancer types could potentially be exploited in combination with drugs targeting other DNA-templated processes. In this issue of *Cancer Cell*, Pfister et al. (2015) describe a synthetic lethal interaction where SETD2 loss-of-function mutation renders cancer cells extremely sensitive to inhibition of WEE1, a cell cycle controlling kinase.

SETD2 is the sole histone H3K36me3 methyltransferase and has recently been highlighted as a tumor suppressor in a variety of cancer types. Furthermore, its role in transcription, DNA repair, and chromatin structure modulation has also been established, highlighting SETD2 inactivating mutations as potential targets for SL-based cancer therapy. Interestingly, by identifying a critical SL interaction between H3K36me3 deficiency and WEE1 inhibition, Pfister et al. (2015) targeted replicative stress, a common hallmark of all cancer cells. Although replicative stress is one of the drivers of malignant transformation, further heightening replicative stress in a catastrophic manner may make it the Achilles' heel of tumor cells and could be therapeutically targeted to drive them to cell death (Dobbelstein and Sørensen, 2015). Replicative stress is the direct consequence of both endogenous and exogenous obstacles to DNA replication. Furthermore, an insufficient supply of key DNA building blocks such as deoxyribonucleoside triphosphates (dNTPs) or dNTP precursors decrease the overall activity of DNA polymerases, leading to replicative stress (Dobbelstein and Sørensen, 2015).

The SL described by Pfister et al. (2015) involves a critical within-pathway interaction between SETD2 loss and WEE1 inhibition, resulting in extremely low levels of RRM2 protein and enhanced replicative stress. In addition to this within-pathway interaction, WEE1 inhibition allows firing of inactive DNA replication origins, which further heightens the replicative stress to a critical level that eventually leads to cell cycle arrest and cell death (Figure 1). Through their research in the genetic model organism *Schizosaccharomyces pombe*, the authors first observed SL between combined loss of Set2 (*SETD2* ortholog) and Wee1 (a *WEE1* ortholog). Later on, they extended this finding to human cancer cell lines selectively deficient in H3K36me3, which were found to be extremely sensitive to AZD1775, an inhibitor of WEE1.

WEE1 has a very well-established role in guarding timely entry into mitosis through control of Cyclin B1-CDK1 activity; however, more recent findings have identified a role for WEE1 in suppression of replication stress (Beck et al., 2010). This is mediated by WEE1 suppression of CDK activity in S phase, which secures an orderly replication program without premature initiation of DNA replication at multiple sites. Notably, the WEE1 inhibitor AZD1775 blocked DNA replication,

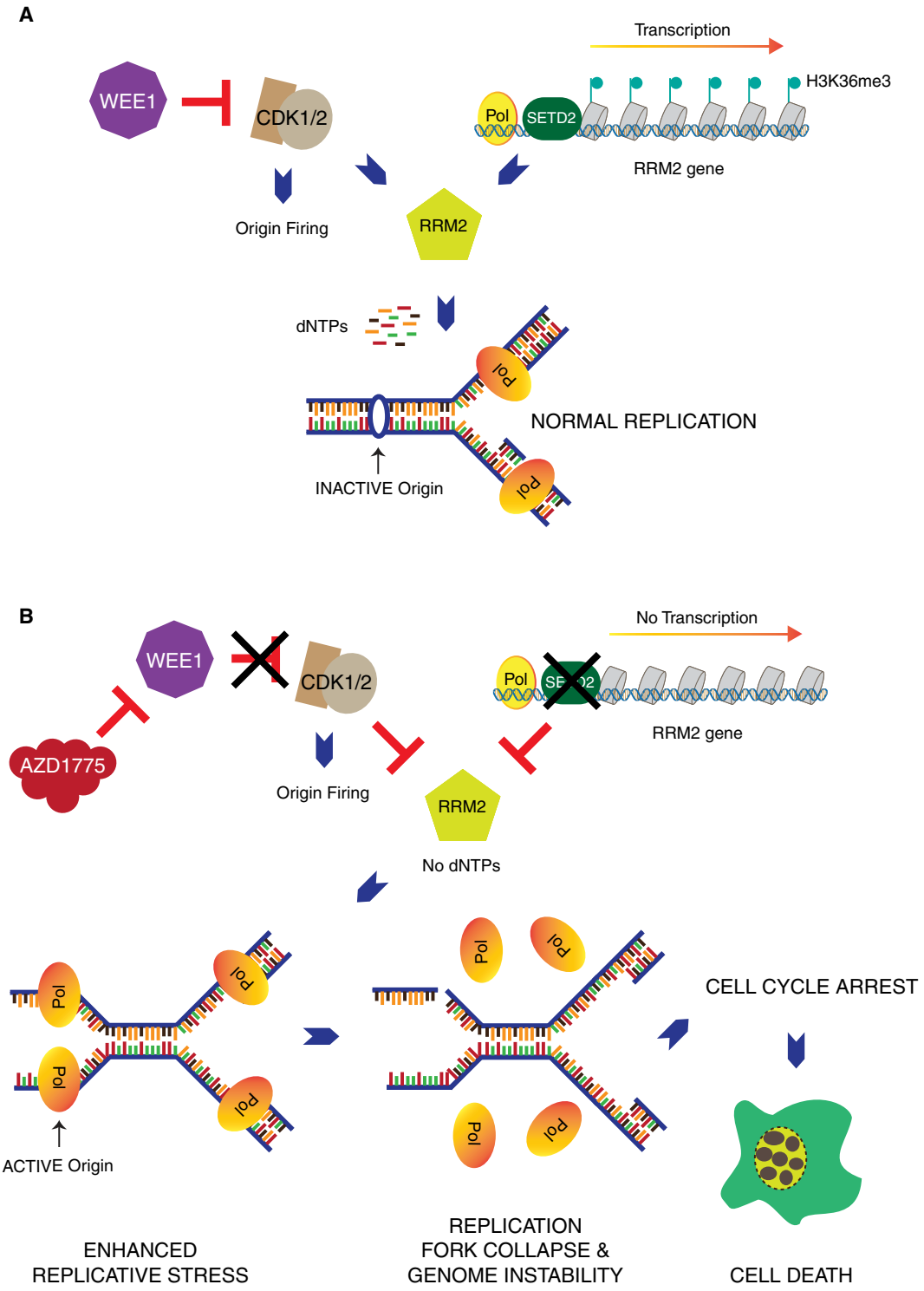

Figure 1. Synthetic Lethal Interaction between Epigenetic Deficiency and Replicative Stress

(A) In normally proliferating cells, RRM2 levels are regulated at transcriptional level by SETD2-dependent H3K36me3 and at protein level by WEE1 dependent suppression of CDK1/2 activity. Furthermore, WEE1, by inhibiting CDK1/2, also controls firing of inactive replication origins. Both SETD2 and WEE1, therefore, control the rate of DNA replication by a regular supply of dNTPs and keeping replicative stress to a minimal level.

(B) In cancer cells carrying a loss-of-function mutation in SETD2 leading to loss of H3K36me3, the transcription of *RRM2* is severely affected, leading to low levels of dNTPs. In this background, treatment of cells with AZD1775, a selective WEE1 inhibitor, caused RRM2 to be degraded via a CDK1/2-dependent pathway, causing critically low levels of dNTPs. Lack of DNA building blocks severely hampers DNA replication and causes replicative stress. In addition, inhibition of WEE1 leads to activation of previously inactive replication origins, which further heightens the replicative stress due to dNTP starvation. Subsequently, the replication fork collapses, leading to genome instability and cell cycle arrest and drives cancer cells to an early grave.

particularly in SETD2-deficient cells. This was not linked with obvious DNA repair deficiencies or the p53 response, but rather, RRM2 levels were severely reduced by SETD2 and WEE1 ablation. RRM2 is a subunit of ribonucleotide reductase (RNR) that catalyzes the formation of dNTPs, the key building blocks for DNA duplication. In the absence of SETD2-mediated H3K36me3, RRM2 mRNA levels were more than 2-fold reduced. Reduced *RRM2* transcription was not simply a generalized transcriptional effect resulting from H3K36me3 loss, because previous RNA-seq studies found limited expression changes in SETD2-deficient cells. The underlying mechanism for the RRM2 specificity remains to be determined.

Next, the authors investigated how WEE1 inhibition contributes to lethality. Here, two modes of action were identified. First, WEE1 contributes via previously discovered control of replication initiation (Beck et al., 2012). Second, WEE1 promotes RRM2 stability. This is a so-called within-pathway interaction whereby WEE1 secures RNR activity (Figure 1A). In the absence of WEE1, elevated CDK1/2 activity phosphorylates RRM2, promoting untimely degradation via the Cyclin F ubiquitin ligase. This results in RRM2 degradation at times when dNTPs are still needed for DNA replication. Notably, the RRM2 inhibitors hydroxyurea (HU) and gemcitabine (GM) may not replace AZD1775 in targeting H3K36me3-deficient cells. This is likely because the within-pathway effect is insufficient to promote cell death and the second effect of WEE1 inhibition, aberrant origin firing (Beck et al., 2012), is required to enhance replicative stress levels, leading to SL (Figure 1B). Finally, the authors confirmed their findings in vivo, because AZD1775 regressed SETD2-deficient xenograft tumors.

SL provides a conceptual framework for discovering drugs that selectively kill cancer cells while sparing normal tissues. The close interplay between chromatin modifiers and pathways regulating DNA replication could be further exploited in the context of discovering new SL links between chromatin modifiers and inducers of replicative stress. WEE1 inhibition in the context of H3K36me3 deficiency is one such approach that resulted in increased replicative stress and has been demonstrated by Pfister et al. (2015) to have the potential to treat cancers deficient in H3K36me3. This study paves the way for further research into discovering links between cancers deficient in chromatin modifications and agents enhancing replication stress. Furthermore, it significantly underscores the idea of exploiting heightened replicative stress in cancer cells as a source of intoxication, which would turn their selective growth advantage into a lethal disadvantage. It will be highly relevant in this regard to direct future screening efforts toward developing SL-based therapies by inducing replicative stress using inhibitors for WEE1, ATR, or CHK1 for treating cancers with aberrations in mitogenic pathways, DNA damage response factors, anti-apoptotic pathways, and immunomodulators. We hope that the concept of mechanism-based target identification will uncover new vulnerabilities in cancer cells that can be leveraged to develop new treatment modalities. The therapeutic targeting of "Hallmarks of Cancer" (Hanahan and Weinberg, 2011) in combination with enhancing replicative stress holds exciting potential to develop precision cancer therapy and improve clinical outcome in cancer patients.

REFERENCES

Beck, H., Nähse, V., Larsen, M.S., Groth, P., Clancy, T., Lees, M., Jørgensen, M., Helleday, T., Syljuåsen, R.G., and Sørensen, C.S. (2010). J. Cell Biol. *188*, 629–638.

Beck, H., Nähse-Kumpf, V., Larsen, M.S., O'Hanlon, K.A., Patzke, S., Holmberg, C., Mejlvang, J., Groth, A., Nielsen, O., Syljuåsen, R.G., and Sørensen, C.S. (2012). Mol. Cell. Biol. *32*, 4226–4236.

Campbell, R.M., and Tummino, P.J. (2014). J. Clin. Invest. *124*, 64–69.

Dobbelstein, M., and Sørensen, C.S. (2015). Nat. Rev. Drug Discov. *14*, 405–423.

Fece de la Cruz, F., Gapp, B.V., and Nijman, S.M. (2015). Annu. Rev. Pharmacol. Toxicol. *55*, 513–531.

Hanahan, D., and Weinberg, R.A. (2011). Cell *144*, 646–674.

Morgan, M.A., and Shilatifard, A. (2015). Genes Dev. *29*, 238–249.

Pfister, S.X., Markkanen, E., Jiang, Y., Sarkar, S., Woodcock, M., Orlando, G., Mavrommati, I., Pai, C.C., Zalmas, L.P., Drobnitzky, N., et al. (2015). Cancer Cell *28*, this issue, 557–568.

Shen, H., and Laird, P.W. (2013). Cell *153*, 38–55.