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WIP1 and senescence: Oxygen matters

Comment on: Sakai H, et al. Cell Cycle 2014; 13:1015–29; PMID:24552809; http://dx.doi.org/10.4161/cc.27920

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Initiation of DNA damage response (DDR) signaling receives significant interest, in particular the biological consequences of p53 activation. Perhaps equally important and often overlooked are the mechanisms employed to return to a normal state. The targets of WIP1 (encoded by *Ppm1d*) include ATM-S1981, p53-S15, and γH2AX-S139 and clearly highlight the key roles this phosphatase has in reversing the DDR. *Ppm1d* is a well-characterized p53-target gene that promotes proliferation and blocks entry into senescence. Previous work demonstrated that loss of *Ppm1d* leads to functional activation of the $p19^{ARF}$ and p53 pathways and increased senescence.[1] Loss of p53 or overexpression of WIP1 leads to accelerated tumorigenesis due to decreased senescence.[2]

In the March 15, 2014 issue of *Cell Cycle*, Sakai et al. continued the study of the effect of loss of *Ppm1d* in mouse embryonic fibroblasts (MEFs), but have considered that because MEFS are particularly sensitive to oxidative stress, physiologically relevant levels of oxygen are important.[3] It is important to note that this study has not been conducted in hypoxia, which by definition refers to conditions of insufficient oxygen, but instead at physiologically relevant levels of oxygen (3%) in contrast to standard tissue culture (20%). The need to consider oxygen concentration is often overlooked, despite a pivotal study which demonstrated that whereas primary MEFs quickly senesce in standard tissue culture, this does not occur at physiologically relevant levels of oxygen.[4]

As expected in this study, when MEFs (+/– *Ppm1d*) were cultured at 20% oxygen they underwent premature senescence, and this was increased in the $Ppm1d^{-/-}$ cells. This was alleviated by incubation in conditions, which more closely resemble physiological conditions (3% O_2). Surprisingly however, the loss of *Ppm1d* still increased the rate of premature senescence even at 3% oxygen, indicating that even when the levels of oxidative stress are reduced, loss of *Ppm1d* still increases senescence rates.

Increased senescence in response to Ppm1d loss was attributed to increased p53 activity and subsequent transactivation of p21.[5] This study also revealed a remarkable *Ppm1d*-dependent difference in the levels of p16 and p19, which could also contribute to the induction of senescence.[6]

Sakai and colleagues then went on to measure reactive oxygen species (ROS) levels, and found that at 20% oxygen the $Ppm1d^{-/-}$ cells accumulated more ROS than the wild type. To investigate the consequences of increased ROS, they interrogated the levels of 8-oxodG, which is a marker of ROS-induced DNA damage.[7] Their data was supportive of the hypothesis that the $Ppm1d^{-/-}$ accumulate more ROS-induced damage compared with the wild-type cells and, therefore, enter senescence prematurely. However, when this analysis was repeated at the physiological levels of oxygen, they found, as expected, that the levels of ROS-induced damage were reduced, but that there was no *Ppm1d*-dependent difference. This is in contrast to the original hypothesis, and suggests that the premature senescence observed in $Ppm1d^{-/-}$ cells does not result from an increased accumulation of ROS-induced DNA damage. Again, when they investigated 8-oxodG accumulation, they found the KO had higher levels of damage at 20%, but there was no apparent difference at 3%.

γH2AX was investigated as both a marker of double-strand breaks and DDR signaling and was found to be increased in $Ppm1d^{-/-}$ cells. This raises the interesting question of whether some other form of DNA damage accumulates in these cells or whether there is a persistent DDR (as WIP1 dephosphorylates γH2AX).[8] The increase in γH2AX occurred specifically in S-phase $Ppm1d^{-/-}$ cells and was ATM-dependent.

Finally, when DNA damage was induced using an agent that is known to induce S phase-specific DSBs, the $Ppm1d^{-/-}$ underwent higher rates of senescence. From this, the authors suggest that WIP1 plays a role in reducing senescence through attenuating DDR signaling initiated by replication-induced DSBs.

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