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Guarding the Guardian:

Mdmx plays important roles in setting p53 basal activity and determining biological responses in vivo

Yunyuan V. Wang, **Mark Wade**, and **Geoffrey M. Wahl**
The Salk Institute for Biological Studies, La Jolla, CA USA

Substantial research over the past two decades has been focused on how activation of the p53 tumor suppressor in response to diverse stresses preserves genomic stability.[1] However, very little attention has been paid to how its activity is kept at bay during homeostatic growth. This is clearly essential as errant activation can result in permanent arrest or cell death. Here, we focus on the importance of regulating p53 basal activity in determining the magnitude of p53-dependent biological responses. We also discuss the critical role of the lesser known of the p53 negative regulators, Mdmx (Mdm4), in basal level control.

Genetic studies show that Mdm2 and Mdmx are the two main negative regulators of p53, as the embryonic lethality caused by their deletion is rescued by concomitant deletion of p53.[2] Mdm2 and Mdmx are close structural homologs, but their failure to compensate for one another in vivo suggests they perform non-overlapping functions.[2] Mdm2 function has been extensively studied and reviewed,[3] yet despite the discovery of Mdmx only four years later, we know much less about its role in p53 regulation.

Studies on Mdmx function using overexpression in cell culture have generated some conflicting results. While some data show that Mdmx promotes Mdm2-mediated ubiquitination and degradation of p53,[4] others suggest that overexpression of Mdmx protects p53 from degradation.[5, 6] Indeed, the work of Yuan and colleagues suggested that differences in the relative abundance of Mdm2 and Mdmx could affect p53 levels and transcriptional activity.[7] Our recent quantitative analyses demonstrated that Mdmx is present at 1/5-1/10 the level of Mdm2 in several types of normal human cells and is consequently stoichiometrically limiting.[8] This provides an explanation of why altering Mdmx abundance could lead to discrepancies among the in vitro studies, and provides a molecular basis for understanding how changing p53, Mdm2, or Mdmx gene dosage in mice can profoundly affect p53 function.

Mdmx degradation following DNA damage correlates with the onset of p53 transcriptional activity, indicating that controlling Mdmx abundance and stability is critical for p53 activation. There is a consensus of opinion that phosphorylation of three conserved serine residues (S342, S367, and S403) in Mdmx by DNA damage-activated kinases plays an important role in Mdmx stability, which in turn regulates p53 activity in response to DNA damage.[2] However, since the mechanism was deduced from transfection approaches that are unable to reveal tissue-specific effects in a physiologically relevant setting, it was important to generate a mouse model to investigate these issues. We, therefore, made a mouse mutant ($Mdmx^{3SA}$) in which these residues were mutated to prevent their posttranslational modification.[9] Consistent with in vitro studies, we showed that phosphorylation of Mdmx is important for Mdmx degradation control in vivo. These analyses also proved that one or more of these phosphorylations is critical for regulating the interaction with 14-3-3 proteins, which in turn regulates association of the deubiquitinating protein HAUSP.[2] We infer that the inability of $Mdmx^{3SA}$ to bind 14-3-3 enables more persistent HAUSP association, which engenders increased Mdmx stability. However, in contrast to in vitro studies, blocking Mdmx phosphorylation did not prevent its

nuclear entry.[9, 10, 11] While the reasons for this difference remain obscure, it is conceivable that in vitro Mdmx overexpression might sequester cellular factors such as 14-3-3 to perturb Mdmx subcellular localization.

One of the most surprising results of our analysis was the effect of the $Mdmx^{3SA}$ mutation on p53 basal level and activity. We consistently observed lower absolute transcript levels of the p53 target genes p21 and puma in $Mdmx^{3SA}$ cells prior to genotoxic stress. We also observed that the fold induction of these genes is similar between WT and $Mdmx^{3SA}$ cells after DNA damage. Due to the lower basal level expression, with a similar fold-induction, the *absolute* transcript levels in $Mdmx^{3SA}$ cells never reach the same levels observed in wild type cells. This had several profound biological consequences. First, $Mdmx^{3SA}$ mice were remarkably resistant to ionizing radiation, being able to survive many months after exposure to 10Gy of whole body gamma-irradiation. This level of exposure introduces 400-800 double strand breaks per cell, and is sufficient to kill 100% of wild type mice within two weeks. This result clearly indicates that it is not the DNA damage that kills the animals, but the p53 responses that it elicits. On the other hand, insufficient p53 activation caused by $Mdmx^{3SA}$ renders the mutant mice more susceptible to some oncogenic stimuli, such as c-Myc over-expression, leading to precocious tumor development. Notably, the $Mdmx^{3SA}$ allele also increases the frequency at which differentiated cells can be reprogrammed to a pluripotent state.[12] This demonstrates that controlling p53 activity through Mdmx is critical during cell reprogramming. Together, these data emphasize the importance of Mdmx regulation for setting basal p53 activity, and for tuning the p53 response to damage and oncogenic stresses. Since p53 regulation has been reported to be crucial in stem cell renewal, embryogenesis, and reproduction, we suggest that an important role of Mdmx is to buffer p53 activity to the diverse stresses that normally impinge on cells in vivo and that may result from exposure to oxygen rich environments, inflammation, etc.

Mdmx is viewed as an oncogene because its amplification and overexpression is often found in human tumors and in most pre-B ALL, in which p53 mutations rarely occur. Small molecules such as Nutlin were identified by their ability to bind Mdm2 and activate p53 by preventing Mdm2-p53 binding.[13] However, none of the currently available Mdm2-binding drugs binds Mdmx sufficiently well to disrupt p53-Mdmx interaction. Our data indicate that small changes in Mdmx stability can also promote tumorigenesis [9], raising the possibility that factors involved in its degradation may also contribute to modifying tumor risk. Therefore, targeting Mdmx degradation or disrupting Mdmx-p53 interactions to reactivate p53 provides a potential therapeutic strategy for cancer treatment. Conversely, *preventing* Mdmx modification/degradation may be beneficial in order to prevent cytotoxicity in normal tissues exposed to environmental or chemotherapeutic genotoxins. Our results also indicate that antagonism of p53 activation should be explored as a strategy to increase the efficiency of cell reprogramming.

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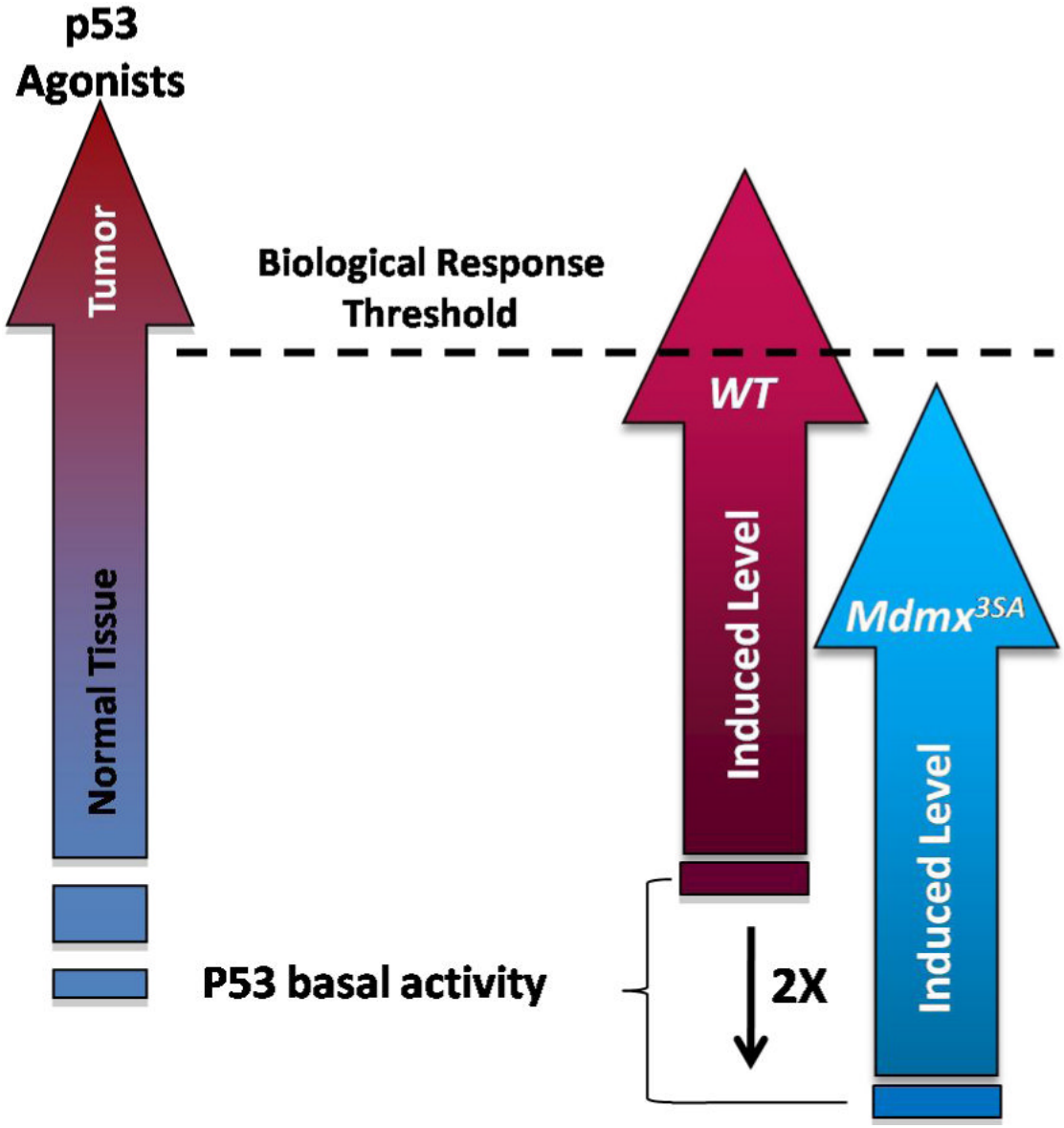

Figure 1.

Mdmx contributes to p53 baseline activity and is a determinant of the biological consequences of p53 activation. Mdmx3SA causes a 2-fold decrease of p53 basal activity in vivo. Although the fold-induction of p53 activity in response to DNA damage is similar between WT and $Mdmx^{3SA}$ cells, the absolute transcript abundance of p53 target genes does not reach the threshold required for biological responses. This renders $Mdmx^{3SA}$ mice extraordinarily resistant to 10Gy of whole body gamma-irradiation, but susceptible to oncogenic stimuli for tumor development. Down-regulating p53 pathway by $Mdmx^{3SA}$ also contributes to increased somatic cell reprogramming.