



The MDM2/MDMX/p53 axis in the adaptive stress response

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Abstract: Via regulation of cellular stress responses, p53 contributes to the maintenance of homeostasis. Contrary to its well-established pro-death function, p53 is also implicated in promoting cell survival by mediating the adaptive stress response. Emerging data reveal that the adaptive stress response is coupled with p53 decline that is a prerequisite for the induction of pro-survival pathways augmenting cell fitness. However, if the adaptive stress responses persist or become chronic, the sustained p53 downregulation would result in a permanent loss of p53 function and p53-dependent homeostasis. The available information suggests a model in which cells respond to different levels of stress by governing the activity and abundance of p53 that, in turn, determines the cell fate dependent on not only the intensity but also the duration of stress.

Keywords: The MDM2/MDMX/p53 axis; mild stress; adaptive stress response; cell fate

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Introduction

A proper stress response is critical for the maintenance of homeostasis. To preserve their fitness in an energetically sensible way, cells must constantly make precise decisions that range between survival and death according to the level of stress. To cope with harsh stresses that can potentially threaten their genome stability and the integrity of other essential structures, cells must sense cellular damage and promptly activate an appropriate response, such as cell cycle arrest, DNA repair, or, if the damage is unrepairable, apoptosis. However, living cells or organisms are also exposed to temporary and modest levels of stress that are more often encountered in our living environment. In response to such transient and mild stresses, arresting cell cycle progression or inducing cell death would be insensible. In this context, cells have to calibrate their stress response to the perturbation based on the level of stress.

Relative to severe stress, the cellular response to mild stress is less well characterized.

As a protein extremely responsive to stress, p53 plays a central role in the maintenance of homeostasis by regulating a number of cellular pathways in response to both endogenous and exogenous stress cues (1). Under the condition of severe stress, p53 is robustly activated, inducing the expression of a host of genes whose products mediate either apoptosis or senescence to eliminate irreparably damaged cells, which is critical for protecting organismal fidelity. However, growing evidence also implicates p53 in promoting adaptation and survival responses to mild stress in support of organismal fitness. It appears that p53 is a multifaceted stress-responsive protein that can sometimes mediate seemingly opposing effects on biological pathways, such as the pro-survival activity, which is in clear conflict with p53's canonical pro-death functions. While the

intricate stress responses mediated by p53 are likely context dependent, the role of p53 in cellular response to mild stress is much understudied relative to severe stress.

To understand the p53 response to stress, it is of importance to appreciate how p53 is regulated. Among several other players, MDM2 and MDMX are the two essential negative regulators of p53, as convincingly demonstrated by genetic studies where knock out either MDM2 or MDMX resulted in p53-dependent embryonic lethality (2-5). However, it was initially unclear why these two p53 inhibitors could not compensate for the loss of each other since in MDM2 knockout mice the MDMX expression was intact and vice versa. Later studies performed independently by Huang *et al.* and Pant *et al.* demonstrated that disassociation of the MDM2/MDMX complex in mice is associated with embryonic lethality. Of importance is that this lethality is completely rescued by concomitant deletion of p53, establishing that an integral MDM2/MDMX complex is essential for effective p53 control, at least during the embryonic stage (6,7). Via their respective RING domain, MDM2 and MDMX bind to each other to form a complex (8). Structural studies with crystal and NMR analysis have revealed that the heterocomplex of MDM2/MDMX is more energetically favorable than the MDM2 homocomplex (9). In support of the heterocomplex being more stable, the MDM2/MDMX complex is the dominant form found in cells. Upon binding to MDMX, MDM2 becomes more stable and functions as a better E3 ligase for p53. In the absence of MDM2 expression, the MDMX protein is localized in the cytoplasm due to a lack of the nuclear localization sequence. As a result, MDMX alone is unable to bind to and inhibit p53 as the latter exists primarily in the nucleus. Binding to MDM2 however brings MDMX into the nucleus where the complex can effectively inhibit p53 activity. The mutual dependency between MDM2 and MDMX underpins the requirement of the intact MDM2/MDMX complex in p53 control (10). In line with this model, various stress signals converge on the MDM2/MDMX complex to modulate p53 activity. For instance, DNA damage induced MDMX phosphorylation by ATM and Chk2 stimulates MDM2-dependent MDMX ubiquitination/degradation resulting in reduced MDM2/MDMX complex levels and subsequent p53 activation (11-13). Oncogenic stresses also target MDMX for phosphorylation, which however is associated with enhanced stability of the MDM2/MDMX complex and subsequently increased p53 degradation (14-16). Ample information indicates that the MDM2/MDMX

complex integrates diverse intra- and extra-cellular stress signals regulating p53 response to perturbations of cellular homeostasis (17). Given that the severe stress-induced p53 response is fairly well understood, we sought to focus our discussion on the p53-mediated cellular response to mild stress.

Evidence supporting p53-dependent response to mild stress

Exposure of cells to high doses of ionizing radiation (IR) induces considerable DNA damage resulting in injury that is associated with robust p53 activation, whereas low doses of radiation (LDR) induces an adaptive response, as reflected by enhanced survival. Such a dose-dependent stress response is also widely observed with many other different types of stress (18). We thus choose LDR as a representative to discuss the p53 response to mild stress. Other types of stress will be included when data are available. We define LDR as the dose of radiation below the threshold able to induce detectable DNA damage. Because the direct biological effects of LDR are relatively subtle and difficult to measure, the effects of LDR are often investigated in the context of the radioadaptive response, which is characterized as an enhanced resistance to high dose radiation-induced harmful effects by pretreatment with LDR. This adaptive response is in fact part of a general cellular response to stress that is evolutionally conserved and has been observed from single cell organism such as yeast to mammalian cells (18). Various readouts including DNA damage, chromosomal aberration, cell death, mutagenesis and among others, have been used to determine the radioadaptive response. In the context of DNA damage, it was reported that pretreatment of C57BL/6N mice with 500 mGy during a span of 23 days considerably reduced a challenge dose of radiation-induced DNA damage in the spleen, a very radiosensitive tissue (19). The radioadaptive response was also reported with an end point of cancer development. Treatment of Swiss mice with a priming dose of 10 mGy daily for 5 or 10 days was associated with a significant decrease in the lymphoma incidence induced by 2Gy of irradiation (20). A critical contribution of p53 to the radioadaptive response was first reported by Horie and his colleagues. Using p53^{-/-}, p53^{+/-} and p53^{+/+} mice, the authors showed that pretreatment of mice with a priming dose of irradiation (0.45 Gy) induced marked radioadaptive response in wildtype mice but not p53^{-/-} mice. Of note is that p53^{+/-} mice exhibited an intermediate radioadaptive response, consistent with a p53

gene-dosage dependent radioadaptive response (21). A critical role of p53 in mediating the radioadaptive response was further corroborated by the study from Jiang *et al.* who reported that the radioadaptive response was specific to normal but not tumor cells (22).

Although animal studies provided solid evidence to support the concept of radioadaptive response, it remained a topic of intensive debate because many observations derived from animal experiments could not be recapitulated in cell-based *in vitro* studies. While there might be a number of factors contributing to this disparity, it was found that the difference in oxygen concentration between *in vivo* and *in vitro* conditions played a major role (23). In the standard tissue culture conditions, the oxygen pressure is around 20–21% whereas the O₂ concentration *in vivo* is approximately 5%. As a result, cells cultured *in vitro* experience an excess amount of oxygen often causing oxidative stress. Such stressful *in vitro* culture conditions would render cells insensitive to LDR treatment because LDR-induced effects are largely mediated by a modest increase in ROS. The small amount of ROS is readily masked under the *in vitro* tissue culture condition due to the high O₂ concentration. Indeed, in contrast with the standard tissue culture condition, cells cultured under the physiological O₂ concentration (3–5%) exhibited comparable radioadaptive responses to those observed *in vivo*. Pretreatment of cells with LDR induced significant resistance to subsequent challenging dose IR-induced DNA damage. Additional evidence supporting a crucial role of ROS came from the observation that treatment of cells with N-acetyl cysteine, an antioxidant, abrogated the radioadaptive response. The importance of physiological oxygen concentration may have very broad implication in the study of cellular response to stress in general and to the moderate level of stress in particular.

Mechanisms behind p53-mediated radioadaptive response

Although p53 was reported to play a role in the radioadaptive response, the underlying mechanism remained unclear, which to a large extent is due to the fact that LDR typically induces little DNA damage and does not overtly activate p53. Indeed, it was found that p53 was not downregulated in LDR-treated cells (23). Remarkably, this p53 decline was essential for inducing the radioadaptive response. LDR-induced p53 downregulation was mediated by ROS, which stimulates p38, a stress-responsive kinase. Upon activation,

p38 phosphorylates MDMX resulting in an increase in the stability and activity of the MDM2/MDMX complex. As a consequence, MDM2/MDMX-mediated p53 turnover is increased resulting in a reduction in p53 levels (16).

The association of p53 decline with LDR-induced survival is not in conflict with the canonical pro-death function of p53. Interestingly, LDR-induced p53 downregulation was accompanied with an induction of HIF1, a master transcription factor critical for metabolic regulation (24), providing an interesting link to metabolism. In accordance, the radioadaptive response was associated with a metabolic switch from catabolic oxidative phosphorylation to anabolic glycolysis (23). This metabolic response is analogous to the Warburg effect, a phenomenon initially thought as a unique metabolic feature of transformed cells. However, growing evidence indicates that the Warburg metabolism is the anabolic program necessary for supporting cell growth and proliferation shared by most growing cells (25). In the context of the radioadaptive response, HIF1-mediated anabolic metabolism is essential for LDR-induced survival, representing a metabolic mechanism behind the radioadaptive response. Such anabolic metabolism-mediated adaptive response was also observed when cells were treated with low levels of arsenic, H₂O₂ and among others (26), implicating the metabolic response as a common mechanism underlying the adaptive stress response. The available information implicates that p53 under physiological conditions keeps anabolic metabolism in check. Either an increase or decrease in the p53 level would impact cellular metabolic programs. In this context, a low level of stress induces p53 decline enabling the induction of anabolic metabolism to augment the adaptive response. In the event of severe stress or very high of an increase in ROS, p53 is activated, resulting in the induction of either cell cycle arrest, senescence or apoptosis where the anabolic metabolism is suppressed (27). In addition to the stress response, p53 downregulation was reported to be required for the induction of anabolic metabolism to support T cell activation and proliferation (28). It was also shown that p53 reduction is critical to allow mTOR-mediated anabolic metabolic pathway augmenting cell fitness (29). Further studies will be necessary to understand how p53 restrains anabolic metabolism to preserve homeostasis.

In summary, the adaptive stress response, if transient or temporary, is largely protective or beneficial because it induces the cellular pathways that can not only minimize potential damage but also enhance robustness. However,

if stress conditions persist or become chronic, the adaptive response could become detrimental resulting in pathological consequences. With the finding that the adaptive response is associated a decline in p53 levels, it becomes clear why prolonged or chronic adaptive stress response is largely harmful because the sustained p53 downregulation would cause a permanent loss of p53-dependent homeostasis leading to pathological outcomes. The available information suggests a model in which cells respond to different levels of stress by governing the activity and abundance of p53 that, in turn, determines the cell fate dependent on not only the intensity but also the duration of stress.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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