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Signalling networks in focus

Regulation of the p53 pathway by ubiquitin and related proteins

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ABSTRACT

The p53 tumour suppressor protein is subject to many levels of control, including modification with ubiquitin and related proteins such as SUMO and NEDD8. These modifications regulate p53 at a number of levels, including control of protein turnover, alterations in sub-cellular localization and changes in the ability to regulate gene expression. Numerous E3 ligases that can mediate these modifications of p53 have been described, some of which promote conjugation with more than one ubiquitin-like protein. Understanding the complexity of this mechanism of p53 regulation will help in the development of therapeutic drugs that function to modulate these events.

1. Introduction

The importance of p53 in tumour suppression is well established in a wide variety of species, including mouse and human. p53 participates in a complex pattern of responses that can lead to many different outcomes, including cell survival, damage repair, metabolic adaptation, cell death and senescence (Vousden and Prives, 2009). Integration of these disparate responses is influenced by signal- and context-dependent factors, and how the ultimate response to p53 is determined is still not clear. However, identification of the breadth of cellular responses that can be driven by p53 has been accompanied by the realisation that p53 can have numerous activities beyond the regulation of cancer development, with contributions to various aspects of health and disease (Vousden and Prives, 2009).

Notwithstanding the potential survival benefits of p53 activation, it is very clear that even minimal alterations in p53 expression can be highly deleterious to the organism. While loss or mutation of p53 strongly predisposes to cancer, very subtle changes in p53 levels promote enhanced aging while a more profound deregulation of p53 is lethal. Robust control p53 of function is therefore essential, and numerous such mechanisms exist. Virtually every aspect of p53 expression, localization, stability and activity is tightly regulated by a plethora of systems. In this short review we have focused on the regulation and consequences of post-translational modification of p53 by ubiquitin-like proteins (UBLs) (Fig. 1), and how these may be harnessed for the development of therapeutic drugs.

2. Regulation of p53 by ubiquitination

One of the key mechanisms by which p53 activity is regulated is through control of protein stability. Under normal growth conditions, p53 protein levels are kept low as a consequence of rapid degradation through the proteasome. It is well established that MDM2 is a key negative regulator of p53 and that control of MDM2 activity is critical to both restraining p53 function under normal conditions, and allowing a rapid accumulation of p53 in response to stress. MDM2 is the major ubiquitin ligase (E3) for p53, regulating its stability by directly assembling polyubiquitin chains on p53 and so targeting it for proteasomal degradation (Lee and Gu, 2010). MDMX, a related protein, also binds p53 but has no intrinsic E3 activity. Many cell culture and *in vivo* models have shown that depletion of either MDM2 or MDMX results in the activation of p53. Both MDM2 and MDMX can directly block the transcriptional activity of p53, and can both be recruited to p53 target promoters (Tang et al., 2008). However, an elegant knock in study in which a catalytic inactive point mutant of MDM2 replaced the wild-type locus has shown that E3 ligase activity is indispensable for the control of p53 *in vivo*, and that binding alone may be insufficient for keeping p53 activity in check (Itahana et al., 2007).

It is clear however, that the regulation of p53 by MDM2 is more complex than simply promoting polyubiquitination and degradation. Mutational analysis has shown that ubiquitination of p53 by MDM2 can be separated from degradation, and a role for MDM2 in targeting p53 to the proteasome has also been described (Kulikov

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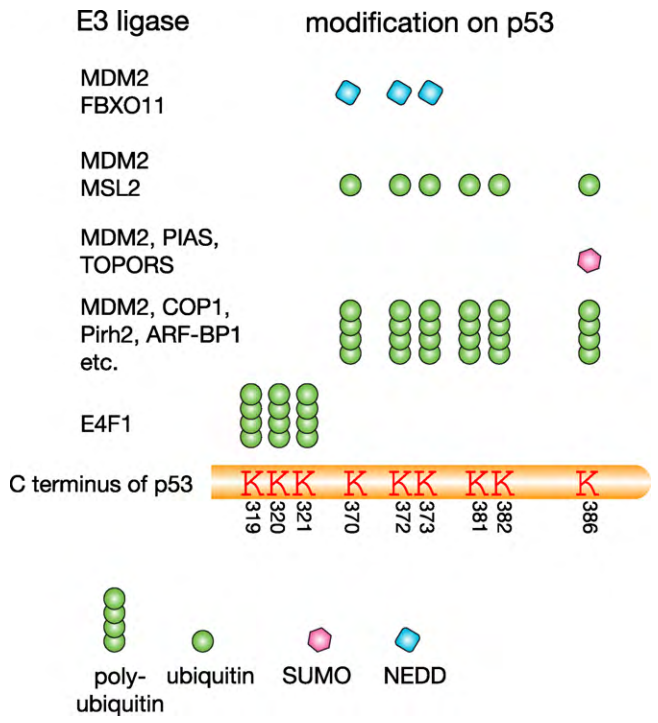

Fig. 1. Ubiquitin-like post-translational modifications of p53. Schematic diagram of the p53 C-terminus that details the location of lysines (K) and how they are modified by various E3 ligases.

et al., 2010). Furthermore, monoubiquitination of p53 by MDM2 controls the sub-cellular localization of p53 by promoting nuclear export (Lee and Gu, 2010). While this would lead to an inhibition of p53's transcriptional activity, export to the cytoplasm may be necessary for some recently described transcriptionally independent activities of p53, including an ability to promote apoptosis and inhibit autophagy (Green and Kroemer, 2009).

Besides MDM2, a number of other ubiquitin ligases have been shown to regulate the stability of p53, including COP1, Pirh2, ARF-BP1, CHIP, Synoliolin, CARP1, CARP2 and TRIM24 (Lee and Gu, 2010). However, none of these proteins can compensate for the loss of MDM2 function *in vivo*, and further studies are needed to clarify the context under which these E3 ligases control p53. Additionally, E3s that ubiquitinate p53 without targeting it for degradation have also been described. These include MSL2 and WWP1, which drive cytoplasmic localization of p53 (Laine and Ronai, 2007; Kruse and Gu, 2009) and E4F1, which ubiquitinates a lysine distinct from those targeted by MDM2 and promotes the ability of p53 to drive cell cycle arrest (Le Cam et al., 2006). Finally, the effects of these E3s can be counteracted by the activity of deubiquitinating enzymes (DUBs). For example, USP10 had recently been shown to regulate the sub-cellular localization and stability of p53 by opposing the effects of MDM2 (Yuan et al., 2010).

3. Regulation of p53 by SUMOylation and NEDDylation

In addition to ubiquitination, a number of other UBL modifications of p53 have been described. p53 can be NEDDylated on several lysines within the C-terminus (residues that coincide with some of the ubiquitinated lysines) and, interestingly, this modification can also be promoted by MDM2. Lysine 320 and 321 of p53 can also be NEDDylated by the F-box protein FBXO11 (Abida et al., 2007). NEDDylation of p53 reduces transcriptional activity, at least on some targets, while opposing monoubiquitination and nuclear export of p53 (Liu and Xirodimas, 2010).

A number of SUMO E3 ligases that can modify p53 on a single lysine in the C-terminus (K386) have also been described. Principal amongst these are the PIAS family (Stehmeier and Muller, 2009), although MDM2 can also participate in the SUMOylation of p53 in cooperation with p14ARF, a protein that binds MDM2 and inhibits ubiquitination. The effects of SUMO modification of p53 are not completely clear, but have been associated with a modest increase in p53's transcriptional activity that is accompanied by an enhanced p53-dependent senescence (Bischof et al., 2006). However, SUMOylated p53 has also been reported to have reduced transcriptional activity because SUMOylation of p53 leads to reduced acetylation and decreased affinity to chromatin (Wu and Chiang, 2009). SUMOylation is also likely to regulate the subcellular localization of p53, possibly contributing to the movement of p53 to nuclear bodies (Stehmeier and Muller, 2009). A role for Sumoylation in allowing the release of MDM2 following monoubiquitination, to allowing nuclear export, has also been proposed (Carter et al., 2007).

Taken together, there is clearly a complex interplay between various UBL modifications of p53, which is further complicated by competition with other modifications of the same lysines, such as acetylation and methylation. Attempts to determine the importance of these modifications by mutation of the acceptor lysines have been frustrated by weak effects that many of these changes have on p53 activity *in vivo* – although in some cases this reflects a failure to consider all the modification sites. However, it is also clear that subtle changes in p53 activity can have extremely profound effects on biological outcomes, and modest changes in p53 activity following UBL modification may be extremely important in the physiology of the p53 response (Fig. 2).

4. Regulating the regulators

The importance of proper regulation of p53 by MDM2 is reflected by an intricate network of systems to regulate MDM2. Various spliced forms of MDM2 are expressed, each of which may modify the control of p53. Fine-tuning of MDM2 expression through a polymorphism in the MDM2 promoter leads to a significant effect on MDM2 function and, by extension, p53 activity and tumour suppression (Bond et al., 2004). The stability of the MDM2 protein is also under tight control and although MDM2 is capable of targeting itself for degradation via autoubiquitination, inactivating the E3 ligase activity of MDM2 in mice leads neither to its accumulation nor to a prolonged half-life of the protein (Itahana et al., 2007). Under these circumstances, MDM2 is still ubiquitinated and turned over via the proteasome, suggesting that other E3s can target MDM2 for degradation. Regulation of MDM2 stability is an important component of the response to stress signals such as DNA damage, which induces the rapid degradation of MDM2 allowing for the stabilization of p53.

MDM2 interacts with numerous binding partners, many of which play an important role in the regulation of the MDM2/p53 pathway. MDM2 functions as a dimer, either in association with itself or by binding to the related protein MDMX (Linke et al., 2008). Like MDM2, MDMX is a binding partner for p53 and plays an essential non-redundant role in negatively regulating p53. While MDMX possesses no intrinsic E3 activity, there is accumulating evidence that the heterodimer between MDM2 and MDMX is the more active E3 for p53 (Okamoto et al., 2009).

Besides targeting p53 for degradation, the stability of MDM2 can also be regulated by DUBs that control the rate of deubiquitination. HAUSP – or USP7 – functions primarily to regulate MDM2 stability, such that knock down of USP7 expression results in the destabilization of MDM2 and consequent stabilization of p53 (Lee and Gu, 2010). *In vivo* studies have shown that deletion of USP7 causes