Regulation of the p53 Tumor Suppressor Protein

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Mutations in the p53 tumor suppressor gene occur in about 50% of all human tumors, making it the most frequent target for genetic alterations in cancer (for recent reviews on p53 see Refs. 1–5). Such mutations probably facilitate carcinogenesis primarily through abrogating the tumor suppressor activities of the wild type p53 protein, although at least some forms of tumor-associated mutant p53 proteins may also contribute overt oncogenic activities (gain of function). Excessive wild type p53 activity gives rise to a variety of cellular outcomes, most notably cell cycle arrest and apoptosis. These cellular effects of wild type p53 can reduce cancer incidence through elimination of cancer-prone cells from the replicative pool. However, such effects might become very undesirable if occurring in a normal, unperturbed cell. p53 activity must therefore be kept under tight control, being unleashed only when a cell accumulates lesions that may otherwise drive it into a cancerous state. The signals and mechanisms that regulate p53 activity, maintaining it at low levels under normal conditions and turning it on in cancer-prone cells, are the subject of this review.

p53-activating Signals

Under normal conditions, p53 is most probably latent. Consequently, it does not interfere with cell cycle progression and cell survival. Moreover, p53 knock-out mice appear in most cases to undergo proper development and maturation (6), suggesting that p53 is not essential for the normal performance of cells within the body. However, a variety of conditions can lead to rapid induction of p53 activity (Fig. 1). The common denominator of these conditions is that they represent various types of stress, which are likely to favor the emergence of cancer-bound cells. Such conditions include direct DNA damage (7–9) as well as damage to components involved in the proper handling and segregation of the cellular genetic material (e.g. the mitotic spindle (10)), ribonucleotide depletion (11), hypoxia (12), heat shock (13), and exposure to nitric oxide (NO) (14). Accumulation of genomic aberrations is a key carcinogenic mechanism; the rapid induction of p53 activity in response to DNA damage within cells serves to ensure that cells carrying such damage are effectively taken care of. Furthermore, p53 may also contribute, directly or indirectly, to particular DNA repair processes (15, 16). The pivotal role of p53 in maintaining genomic integrity has earned it the nickname “guardian of the genome” (17). As described later, induction of the p53 response upon stress occurs largely through alterations in the p53 protein. Changes in the rate of transcription of the p53 gene play a minor role, if any, in such induction. Consequently, the transcriptional regulation of the p53 gene has received very little attention during recent years. This need not imply that the regulation of p53 gene expression is totally irrelevant. In fact, it was observed long ago that p53 mRNA levels rise substantially upon serum stimulation (22). This rise may be because of the presence of binding sites for serum-induced factors in the p53 promoter (23) as well as to the ability of the p53 gene to bind the c-Myc protein and to be transcriptionally stimulated by excess c-Myc (24). The induction of an anti-proliferative gene, p53, by serum and growth factors may at first glance seem paradoxical. However, it does make good sense. Cells undergoing DNA replication and extensive proliferation are at higher risk of acquiring DNA damage and giving rise to multiple cancer-prone progeny than quiescent cells. Induction of higher p53 mRNA levels under such conditions places the cells in a state of anticipation; as long as there is no DNA damage or other stress, p53 remains latent and does not interfere with normal cellular transactions. However, if conditions emerge that call for a p53 response, the presence of higher levels of p53 mRNA ensures that such a response will be rapid and effective.

Activation of p53 by Post-transcriptional Mechanisms

Exposure of cells to p53-activating signals can lead within a relatively short time to a marked elevation in p53 protein. To some extent, this can be achieved by increased translation of the p53 mRNA, probably involving relief of a translational repression mechanism operating through the 3′-untranslated region of this mRNA (25). There also exists evidence that p53 itself can inhibit p53 synthesis through binding to its own mRNA (26, 27). Yet, it is generally accepted that the accumulation of active p53 in response to stress occurs mainly through post-translational mechanisms. Pivotal is the increase in the protein half-life of p53. p53 is usually a very labile protein, turning over with a half-life sometimes as short as a few minutes (28). In response to DNA damage and other types of stress, p53 is markedly stabilized (7, 8). A rapid increase in p53 concentration without a need for de novo transcription is particularly advantageous in cells with severely damaged genomes. In addition, there is most probably a qualitative conversion of p53 from latent to active form. The best documented change concerns the sequence-specific DNA binding activity of p53. p53 operates as a gene-specific transcriptional activator, which relies on its ability to bind defined sequence elements within target genes (1–5). The sequence-specific DNA binding activity of p53 is subject to constitutive negative regulation, primarily through its inhibitory C-terminal domain (29–31). Relief of this inhibition upon exposure to stress results in increased DNA binding (32–34) and consequently increased biochemical and biological activity. The transcriptional activity of p53 may also be induced by changes in other regions, e.g. modifications in its N-terminal transactivation domain, enabling a more efficient recruitment of components of the transcription machinery (35). Finally, p53 activation may also involve a change in subcellular localization; whereas latent p53 may often be cytoplasmic, at least during part of the cell cycle (36), exposure to stress results in its accumulation in the nucleus, where it is expected to exert its biochemical activities.

The p53-Mdm2 Loop

A key player in the regulation of p53 is the Mdm2 protein. Mdm2 is the product of an oncogene, whose excess activity facilitates several types of human cancer (for reviews see Refs. 37–39). Mdm2 exhibits a unique relationship with p53. On the one hand, the Mdm2 protein binds to p53 and inactivates it (40–42). The binding occurs right within the p53 transactivation domain, interfering with recruitment of basal transcription machinery components (43, 44). Moreover, Mdm2 is required for the transformation of p53. Importantly, Mdm2 binding can also lead to complete elimination of p53 through proteolytic degradation. On the other hand, p53 binds specifically to the mdm2 gene and stimulates its transcription (46, 47). This duality defines a negative feedback loop (Fig. 2), which probably serves to keep p53 in tight check and to terminate the p53 signal once the triggering stress has been effectively dealt with. In some situations, mdm2 transcription

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is induced later than that of other p53 target genes (48, 49); this may set a time window within which p53 is allowed to exert freely its biochemical and biological effects. The critical importance of the p53-Mdm2 loop is best illustrated by the analysis of mdm2 knockout mice. Inactivation of the mdm2 gene results in early embryonal lethality, but this is completely prevented by simultaneous inactivation of p53 (50, 51). Conceivably, in the absence of functional Mdm2 protein, p53 becomes strongly deregulated to the extent that its excess activity leads to embryonic death. The other side of the coin is revealed in certain human cancers; excessive Mdm2 expression, achieved through mdm2 gene amplification (52) or other mechanisms (53), can lead to constitutive inhibition of p53 and thereby promote cancer without a need to alter the mechanisms (53), can lead to constitutive inhibition of p53 and thereby promote cancer independently of p53 (54, 55).

Regulation of p53 Protein Stabilization

Much of the activation of p53 is achieved through p53 protein stabilization. This realization has markedly accelerated research on p53 degradation. It is now well established that the rapid demise of p53 is achieved largely through the ubiquitin-proteasome pathway (56), although a role for other proteolytic enzymes such as calpain has also been implied (57). Mdm2 plays a pivotal role here as well. Elevated Mdm2 levels result in rapid p53 degradation, which is dependent on the ability of the two proteins to engage in direct binding (58, 59). Furthermore, interference with p53-Mdm2 binding by monoclonal antibodies or competitor peptides results in a dramatic stabilization and accumulation of p53 in non-stressed cells (60). This strongly argues that the low basal levels of p53 in such cells are due primarily to continuous Mdm2-promoted degradation.

How does Mdm2 promote p53 degradation? When the proteolytic activity of the proteasome is blocked by specific inhibitors, excess Mdm2 augments the accumulation of ubiquitinated forms of p53 (58, 59), suggesting that Mdm2 facilitates p53 ubiquitination. Strong support for this conclusion was provided by showing that Mdm2 can directly function in vitro as a p53-specific E3 ubiquitin-protein ligase, which covalently attaches ubiquitin groups to p53 (61, 62) (Fig. 3A). It remains to be determined whether Mdm2 operates alone in vivo or is part of a larger E3 complex.

Although Mdm2 emerges as the key regulator of p53 stability, other mechanisms for p53 ubiquitination and degradation also exist. Of particular interest is the possible role of the c-Jun N-terminal kinase (JNK); in vitro and in vivo studies suggest that the binding of JNK to p53 results in ubiquitination and proteolytic removal of p53 (63). The "division of labor" between Mdm2 and JNK is presently unclear; however, there are strong indications that it changes during the cell cycle (63).

Covalent Modifications of p53

Rapid post-translational activation of signaling proteins is often achieved through covalent modifications, particularly protein phosphorylation. It was thus conceivable that the rapid stabilization and activation of the p53 protein upon stress also involves stress-induced covalent modifications of p53. Indeed, there is mounting evidence in support of this conjecture. p53 becomes phosphorylated on multiple sites in vivo in response to various types of stress, and many stress-activated kinases can phosphorylate p53 in vitro (reviewed in Refs. 5 and 64–67). A potential outcome of such phosphorylation might be the stabilization of p53 through inhibition of p53 ubiquitination and degradation. The pivotal role of Mdm2 in these processes suggests several likely scenarios. For instance, because degradation requires the binding of Mdm2 to p53 (58, 59), phosphorylation of residues positioned within the binding interface of either protein may interfere with binding and lead to p53 stabilization (Fig. 3B). In the case of p53, several candidate sites within its Mdm2-binding domain have been identified which are modified in response to DNA damage and whose phosphorylation reduces the affinity of p53 for Mdm2 (68–70). Of particular interest are serines 15 and 20 and threonine 18 of human p53, all located within or very close to the Mdm2-binding domain of p53. Serine 15 has been studied particularly closely, as it is the site of p53 phosphorylation by the ATM kinase (71, 72), whose activity is required for p53 stabilization in response to ionizing radiation and some other types of DNA damage (73, 74). It should be noted that although the idea that such phosphorylation events are responsible for p53 stabilization is very attractive, the in vivo relevance of this idea has been challenged recently (75, 76). Hence, the effect of p53 phosphorylation on stability may depend on the intracellular context and particularly on the availability of alternative mechanisms for p53 degradation.

Stabilization of p53 might be achieved by modifying not only p53 but also Mdm2. In a simple scenario, Mdm2 may become phosphorylated in a manner that disrupts its interaction with p53 (Fig. 3B). In fact, a candidate phosphorylation site within Mdm2 has been

1 The abbreviation used is: JNK, c-Jun N-terminal kinase.
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The ability of Mdm2 to promote p53 ubiquitination can be modulated not only by covalent modifications but also by the binding of other regulatory proteins. The most vivid and perhaps most important example is provided by the ARF protein. This small protein arises through translation of an alternative reading frame derived from the INK4A tumor suppressor gene (82). Of note, ARF binds to Mdm2 and to a lesser extent also to p53, and this binding prevents Mdm2-mediated p53 proteolysis (82–85), apparently by blocking the ligase activity of Mdm2 (62). The interaction between Mdm2 and ARF is therefore another attractive candidate for modulation by stress signals.

p53 and Oncogenic Stress

Of particular interest is the activation of the p53 response by oncogenic stress, such as the deregulated expression of oncoproteins like adenovirus E1A, Ras, Myc, and β-catenin (Fig. 1). Although the importance of this response to tumor suppression is very obvious (Fig. 4A), its biochemical basis has remained unknown until the discovery of ARF and its role in p53 stabilization. Recent work has revealed that excess activity of several oncoproteins leads to massive induction of ARF (86–88). This induction is primarily because of enhanced transcription, at least some of which is mediated through the E2F transcription factor (89). The induced ARF protein then binds to Mdm2, thus preventing p53 ubiquitination and degradation (Fig. 3D). Obviously, the inhibitory effects of p53 are not triggered when Myc or Ras proteins are recruited as part of a properly orchestrated growth response, initiated by the binding of a growth factor to its receptor, or else such cells would not be able to execute a mitogenic response. The question that comes to mind is: how can p53 tell between such “healthy” activation of Ras, Myc, or E2F and one that occurs independently of a proper growth signal and may lead to cancer? One possible difference may lie in the more transient nature of the activation in the first case. However, it is also conceivable that when a cell is exposed to a growth factor, one arm of the response drives the neutralization of p53 concurrent with the activation of Myc, Ras, and E2F by the other arm. Support for this notion comes from the observation that factors such as basic fibroblast growth factor and insulin-like growth factor-1 up-regulate Mdm2 and quench p53 activity (90, 91). This mechanism could also contribute to the anti-apoptotic potency of these factors. In other cases, receptor-generated signals may act downstream of p53 to abolish its cellular effects without impairing on p53 itself.

Obviously, p53 can respond to a plethora of stress conditions. This versatility is provided through its intricate regulation, which allows it to collect inputs from very diverse signaling pathways.

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...described (77). Alternatively, phosphorylated Mdm2 may retain p53 binding but become impaired with regard to its E3 ubiquitin ligase activity (Fig. 3C). This may particularly apply to the C-terminal part of Mdm2, known to be required for p53 ubiquitination (61, 78).

Finally, both p53 and Mdm2 may also be subject to other types of modifications. Acetylation of p53, leading to increased DNA binding, has been well documented (33, 79). p53 glycosylation has also been reported, and it too may increase DNA binding (80). The role of covalent modifications in p53 activation by stress remains a very challenging area of research.

In addition to covalent modifications, protein-protein interac-
