PERSPECTIVE

Building p53

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The intricacies of p53 regulation just got more complex. While much is known about the transcriptional regulation of p53 target genes, Chen and Kastan (pp. 2146–2156) uncovered a new mechanism regarding the making of the p53 protein itself. In the October 1, 2010, issue of *Genes & Development*, they introduced us to a novel mechanism of p53 translational control, by which a 5′–3′ cap-independent, poly(A)-independent, RNA–RNA interaction enhances p53 translation by binding the ribosomal protein RPL26 following DNA damage. Oligonucleotides designed against this 5′–3′ untranslated region (UTR) duplex disrupted the binding of RPL26 to p53 mRNA and reduced p53 synthesis and, therefore, function. This study reveals an alternate mechanism of translational control to regulate p53 levels.

The p53 tumor suppressor is tightly regulated and maintained at low physiological levels in unstressed cells to avoid its deleterious effects on living organisms. Thus, p53 expression is regulated by a variety of means, including transcriptional and translational control, alternative splicing, changes in protein degradation rate or stability, and regulation of subcellular localization (Hollstein and Hainaut 2010). All of this is to ensure that p53 is maintained at low levels, since it is a master regulator of genes involved in important processes such as apoptosis, cell cycle progression, senescence, differentiation, and DNA repair (Levine and Oren 2009; Vousden and Prives 2009). Therefore, deregulation of p53 levels has serious consequences on cell growth and survival (when overexpressed) or confers an increased risk for cancer (haploinsufficiency, for example).

In response to multiple stimuli, including DNA damage, the p53 protein is rapidly stabilized and activated (Horn and Vousden 2007). p53 stabilization is ensured primarily by uncoupling its main negative regulator, the E3 ubiquitin ligase MDM2, which regulates ubiquitination, degradation (Haupt et al. 1997; Honda et al. 1997; Kubbutat et al. 1997), and nuclear export (Toledo and Wahl 2006). Under stress, post-translational modifications of both proteins are induced to disrupt the p53/MDM2 complex, resulting in an increase in p53 half-life and a rise

in cellular protein levels (Meek and Anderson 2009). On the other hand, MDM2 and p53 form an autoregulatory feedback loop where *MDM2* is the transcriptional target of p53 (Barak et al. 1993; Wu et al. 1993; Lahav et al. 2004), which also contributes to regulation of p53 levels.

A number of other mechanisms contribute to p53 stabilization in response to different stimuli; translational control of p53 mRNA is one such example. This type of regulation has considerable impact on p53 levels and may be critical for its tumor-suppressive function (Halaby and Yang 2007). Translation occurs in the ribosome, and several proteins govern assembly and processing of mRNAs. The ribosomal protein RPL26 in particular binds to p53 mRNA, increasing its translation. RPL26 also binds to MDM2, and is polyubiquitinated and targeted for proteasomal degradation (Ofir-Rosenfeld et al. 2008). Since the binding of RPL26 to MDM2 diminishes its availability for interaction with p53 mRNA, p53 translation and activity are reduced under normal physiological conditions (Ofir-Rosenfeld et al. 2008). When stressed, MDM2 is subjected to post-translational modification that is proposed to render it less efficient at disrupting RPL26–p53 mRNA binding, leading to up-regulation of p53 translation (Takagi et al. 2005; Ofir-Rosenfeld et al. 2008). Takagi et al. (2005) demonstrated that optimal p53 induction after DNA damage calls for a robust increase in p53 mRNA translation and efficient binding of RPL26 to the 5′ untranslated region (UTR) of p53 mRNA. Thus, RPL26 is a bona fide positive regulator of p53. While RPL26 overexpression directly increased p53 translation, cellular levels, and subsequent functions (G1 arrest and apoptosis), RPL26 knockdown with siRNAs attenuated p53 translation and weakened its apoptotic response to DNA damage (Takagi et al. 2005). Another protein, nucleolin, competes with RPL26 for binding to the 5′ UTR of p53 mRNA to influence translation. Its overexpression has the opposite effect of diminishing p53 protein levels and inhibiting the ionizing radiation (IR)-induced increase in p53 translation and induction. Moreover, inhibition of nucleolin enhanced p53 translation (Takagi et al. 2005). These proteins and others that bind to the 5′ and 3′ UTR of p53, controlling its translation in response to stress, effectively alter wild-type and, likely, mutant p53 cellular levels and functions. Higher levels of p53 will eliminate damaged cells and ensure exclusive survival of intact cells in the face of a general translational decrease. Thus, translational control of p53 in stressed cells is critical.

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The Kastan group (Takagi et al. 2005) demonstrated previously the pivotal role the 5' UTR of *p53* mRNA plays in DNA damage-induced translational control.

Yet another layer of complexity of *p53* regulation is presented by Chen and Kastan (2010), and shows a novel model of DNA damage-induced translational control that modulates *p53* synthesis and activity. Chen and Kastan (2010) show an RPL26-dependent, cap-independent, and poly(A)-independent RNA base-pairing interaction between the 5' and 3' UTR sequences induced by DNA damage that is required for optimal *p53* mRNA translation (Fig. 1). Disrupting mutations (as few as 3 nucleotides) in this predicted dsRNA region interrupted the binding of RPL26 to human *p53* mRNA and decreased its induction. These mutations occur in the last 3 base pairs of the 5'–3' UTR-interacting region. Functional reporter expression assays as well as elegantly designed experiments based on creating compensatory mutations that restored complementarity of this UTR interaction demonstrated the importance of the 3' UTR *p53* region and the dependence of RPL26 on the dsRNA sequence for optimal binding to *p53* mRNA, and its positive translational regulation induced by DNA damage.

While alterations of many ribosomal proteins are capable of inducing a change in *p53* protein levels, RPL26 is uniquely involved in *p53* translation. Since the increase in binding to *p53* mRNA after DNA damage occurs in the nucleus, the findings of Chen and Kastan (2010) shed light on an extraribosomal function of RPL26 that selectively targets *p53* activation. Exactly how RPL26 binds to *p53* remains to be elucidated.

Figure 1. Schematic representation of DNA damage-induced translational regulation of *p53* by RPL26. DNA damage promotes the binding of ribosomal protein RPL26 to a double-stranded UTR of *p53* mRNA. This region is made from the base-pairing of 5' and 3' UTR of *p53* mRNA, resulting in its circularization. RPL26 binding to *p53* is required for optimal *p53* translation. MDM2 keeps *p53* protein levels in check and physiologically low by inhibiting its activity, monoubiquitinating and targeting it for degradation by the proteasome. MDM2 also binds to RPL26, polyubiquitinates it, and promotes its degradation. Furthermore, MDM2 disrupts the binding of RPL26 to *p53* mRNA. Thus, RPL26 exhibits positive effects on *p53* activity.

Commonly, mRNA-specific translational regulation is determined by protein complexes that recognize particular regulatory sequences or structural features that are present mainly in the 5' and/or 3' UTRs (Standart and Jackson 1994; Gebauer and Hentze 2004). These regions are generally relatively conserved, which highlights their functional importance with a more recognized role of the 5' UTR in translational modulation and the 3' UTR in RNA stability. Their importance in *p53* mRNA translation has also been established (Mosner et al. 1995; Fu and Benchimol 1997; Mazan-Mamczarz et al. 2003; Schumacher et al. 2005). However, the discovery by Chen and Kastan (2010) of this 5'–3' UTR RNA–RNA base-pairing phenomenon—rather than through protein mediated, cap-poly(A) bridging and circularization of the mRNA—represents an original and intriguing regulatory mechanism for *p53* translation (Fig. 1). While this type of cap-independent 5'–3' UTR communication is uncommon in eukaryotic cells, it is a known mechanism in viruses (such as viruses in the *Luteoviridae* and *Tombusviridae* families) (Fabian and White 2004; Miller and White 2006) and bacteria (Franch et al. 1997). In viruses, the RNA forms a closed loop through direct long-range RNA–RNA communication between the 5' and 3' UTR ends of mRNA. This mRNA circle brings the 3' UTR end in close proximity to the 5' UTR, where the 3' UTR regulatory factors that control the initiation of translation reside (Gebauer and Hentze 2004; Miller and White 2006). In some cases, this bridging seems to play a role in viral RNA replication, with no obvious impact on translation (Villordo and Gamarnik 2009). In other viral examples, such as the Barley yellow dwarf (Guo et al. 2001; Miller and White 2006) and Tomato bushy stunt viruses [both lack the 5' cap and 3' poly(A)] (Fabian and White 2004; Miller and White 2006), this mechanism regulates translation. Similar 5'–3' RNA bridging was observed in multiple viral RNAs, and could represent a widespread strategy for viral RNA replication but not translation (Filomatori et al. 2006). Over and above the significance of the findings of Chen and Kastan (2010) regarding the regulation of *p53* protein levels and the consequent therapeutic implications, the mechanism of 5'–3' UTR base-pairing that increases the efficiency of mRNA translation will be viewed with much interest by those studying rapid environmental response genes, not only in viruses and bacteria, but especially in eukaryotic cellular systems.

Chen and Kastan (2010) also highlighted the need for therapeutic agents that limit the physiological effects of *p53* activation after DNA damage by transiently limiting the transactivation capacity of *p53*. In cases of accidental exposure to radiation, therapeutic irradiation, or other stresses (such as hypoxia induced by heart attack or stroke) that may result in *p53*-mediated cytotoxicity, a means of modulating the *p53* response will be a valuable clinical tool in enhancing survival and limiting tissue damage. To this end, Chen and Kastan (2010) designed and synthesized small single-stranded oligonucleotides that cover the crucial sequences of the 5'–3' RNA base-pairing area. Cell culture experiments were used to

successfully demonstrate that these synthetic oligonucleotides were able to limit p53 translation by blocking RPL26 binding to p53 mRNA (without completely inhibiting p53 induction), thereby restraining the p53 response to diverse types of DNA damage and stresses without affecting p53 mRNA levels. It is important to keep in mind, however, that the same 5' and 3' sequences are present in mutant p53 mRNA, and that missense mutations in p53 commonly observed in human tumors manifest gain-of-function phenotypes (Brosh and Rotter 2009). This is in agreement with the fact that mouse models of a p53^{R172H} knock-in allele with increased mutant p53 protein levels may present with accelerated tumor progression and increased metastasis (Lang et al. 2004; Olive et al. 2004; Terzian et al. 2008). We hypothesize that therapeutic strategies that reduce protein levels of mutant p53 may limit tumor progression and/or metastasis. On the other hand, while the possibilities offered by therapeutic interruption of 5'–3' UTR base pair-enhanced p53 translation are intriguing, it is unclear whether this may be a more or less effective strategy than a conventional siRNA-mediated approach to p53 silencing. Moreover, the most likely roadblock to the future clinical use of oligonucleotide-based therapeutic strategies remains the success of the delivery systems currently under development.

Finally, this original finding will set the stage for a plethora of clinical and therapeutic strategies in disease and cancer. This novel p53 translational regulation is definitely one area that we “guessed or thought of” that will need to be studied further and “will become clear and obvious over the next decade” (Levine and Oren 2009). Since p53 is important in maintaining tissue homeostasis and cancer prevention, mastering the fine tuning of its production will be a challenge and will serve as an example in studies of other crucial proteins. The ability to modulate p53 protein levels in specific settings or under certain forms of stress using these types of oligonucleotides should have minimal risk for secondary cancers. Furthermore, testing this approach in vivo on mouse or other models will permit the validation of this tool as a valuable addition to our arsenal of therapies against deleterious effects of high p53 levels.

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