PERSPECTIVE

Insights from a novel p53 isoform in zebrafish want to reel us in, but will we take the bait?

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The p53 tumor suppressor has been reported to be expressed as multiple isoforms. In this issue of *Genes & Development*, Chen and colleagues (pp. 278–290) use a zebrafish model to show that one such isoform, $\Delta 113\text{p53}$, is a target for transcriptional activation by p53 and can, in turn, inhibit the activity of the full-length p53 protein, establishing a novel negative feedback loop centered on the p53 locus itself. The challenge will be to determine whether these intriguing results in zebrafish will pave the way for new insights into several perplexing issues in p53 biology that will impact human disease.

The p53 tumor suppressor has been extensively studied, in no small part due to its clear relevance in the genesis of human cancers. p53 acts primarily as a transcription factor to regulate expression of specific target genes (Vousden and Lane 2007; Riley et al. 2008). More recent studies have implicated a cytoplasmic role for p53 in influencing cell death mediated by the intrinsic pathway and mitochondria (Vousden 2006). In spite of the efforts of a large number of laboratories, several key questions involving p53 have remained unanswered. Among others, these include what determines the cellular outcome to p53 activation, whether p53 itself plays a role in embryonic development, and how exactly does the p53 pathway exert a tumor suppressor activity that is altered in human cancers.

p53 plays a well-characterized role in the cellular response to DNA damage as well as a variety of other stresses including oncogenic activation (Vousden and Lane 2007; Riley et al. 2008). There are two main outcomes to activation of p53 under such circumstances, namely, cell cycle arrest (that, if persistent, becomes senescence), or apoptosis. The cellular determinants that dictate which of these two actually occur have been elusive. A variety of hypotheses have been generated, including regulation at the level of target gene selectivity, the ability to interact with a subset of other cellular proteins, as well as the

extant molecular milieu in a particular cellular setting (Vousden 2006).

p53 is evolutionarily conserved from flies to humans (Lu and Abrams 2006). Tumorigenesis is associated with aging, and typically does not occur in animals until well after breeding age. Thus, it is unlikely that the tumor suppressor function of p53 is the driving selective pressure for its conservation from invertebrates through humans. Yet, a germline deletion of p53 in the mouse does not affect normal embryonic development (Donehower et al. 1992). Dysregulation of p53 in mouse embryos through genetic alteration of its negative regulators Mdm2 or MdmX/Mdm4 clearly produces developmental phenotypes (Marine et al. 2006). Some developmental defects have been reported occasionally in p53-null animals (Vousden and Lane 2007). Nevertheless, a clear role for p53 in development remains to be elucidated.

Missense mutations of p53 are commonly found in particular types of human tumors (Petitjean et al. 2007). Other alterations in p53 regulators have been implicated as contributing to the loss of p53 tumor suppressor activity (Vazquez et al. 2008). Mdm2 has been shown to act as a ubiquitin ligase for p53 as well as to bind and inhibit its transcriptional activity. MdmX cooperates with Mdm2 in its regulation of p53 as well as also binding and inhibiting the function of p53 as a transcription factor. Both of these proteins have been shown to be overexpressed in human cancer and to have oncogenic activity in model systems (Marine et al. 2006). The ARF protein inhibits the function of Mdm2 and has been shown to be down-regulated or lost in a subset of tumors (Sherr 2001). In cervical cancer, the expression of the human papilloma virus E6 protein leads to the targeting of p53 for ubiquitin-mediated degradation, thereby serving as an additional means to inactivate the pathway (Hietanen et al. 2000). Nevertheless, there remain a large number of cancer types in which alterations in either p53 itself or one of these regulators only accounts for a minority of tumors. This group includes common cancers, including those of the breast (Lacroix et al. 2006) and prostate (Downing et al. 2001), as well as tumors that are especially aggressive and difficult to treat such as melanoma (Olivier et al. 2002). One can speculate that p53 may play no role in these settings. It is much more

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likely, however, that there are mechanisms for inactivation that have yet to be fully elucidated.

Certainly, a great deal of knowledge about p53 has been derived from studies in human cell culture as well as the use of mouse models. With the identification of conserved p53 pathways in lower model organisms including *Drosophila*, *Caenorhabditis elegans*, and Zebrafish, hopes were raised that the ability to exploit the powerful genetics of these models would lead to major advances in our understanding (Lu and Abrams 2006). In this issue of *Genes & Development*, Chen et al. (2009) exploit the use of zebrafish to provide new and exciting leads to address some of these critical issues in p53 biology.

Several years ago, Bourdon et al. (2005) made the remarkable discovery that p53 actually can be expressed as multiple isoforms in human cells. This occurs through a combination of alternative splicing as well as the use of an additional promoter contained within intron 4. In retrospect, these findings were to be expected. The related family members p63 and p73 are expressed in a similarly complex manner (Murray-Zmijewski et al. 2006). As studies in lower organisms suggested that, in fact, p53 came along much later in evolution than its siblings, it followed that p53 would have such a genomic expression pattern. Sequences with promoter activity were noted within intron 4 in studies with the mouse previously as well (Beenken et al. 1991; Lozano and Levine 1991). Importantly, p53 isoform expression was shown to be conserved from zebrafish to humans (Fig. 1; Bourdon 2007). The digestive organ expansion factor (*def*) gene in zebrafish regulates digestive organ development. Notably, a loss of function mutant in the *def* gene showed selective up-regulation of one such isoform $\Delta 113p53$ (Fig. 1), and this was shown to contribute to arrest in the cell cycle leading to defects in digestive organ development (Chen et al. 2005).

In the current study (Chen et al. 2009), the promoter contained within intron 4 is shown to be, in fact, p53-responsive. p53 can be shown to occupy intron 4 in chromatin immunoprecipitation assays with reporter assays

confirming that intron 4 has promoter activity. Full-length p53 is well known to be up-regulated in response to DNA damage in a manner independent of its mRNA levels, but rather due to post-translational protein stabilization. Consistent with this, Chen et al. (2009) showed that $\Delta 113p53$ is up-regulated transcriptionally under conditions in which full-length p53 mRNA levels are unchanged. Given that the $\Delta 113p53$ retains the oligomerization domain of p53 but lacks the N-terminal activation region (Fig. 1), it was not surprising that $\Delta 113p53$ could inhibit the ability of full-length p53 to trigger cell death. It was to be expected that it would act in a dominant-negative manner. The surprise came when gene expression patterns were examined. It was shown that the $\Delta 113p53$ had no effect on the ability of p53 to up-regulate targets such as p21 and Mdm2. Rather, it affected the expression of the gene encoding the anti-apoptotic *bcl2L*. *Bcl2L* is a zebrafish protein that is most similarly to mammalian *Bcl-xL*. In zebrafish, p53 normally down-regulates *bcl2L*, but in the presence of the truncated isoform, *bcl2L* levels actually increase. Manipulating *bcl2L* expression itself confirmed that this was an important contributor to p53-dependent apoptosis. These findings indicate (1) $\Delta 113p53$ does not act in a dominant-negative manner as expected, and (2) the effects of the truncated isoform lead to selective alterations in p53-dependent gene expression. Of course, the key question remains: How does it do this?

Chen et al. (2009) make a reasonable argument that the mechanism of action of $\Delta 113p53$ is not via a conventional dominant-negative effect, as there is no inhibition of transcriptional activation of Mdm2 or p21. If anything, the latter shows enhanced up-regulation in the presence of the truncated isoform. It is possible that $\Delta 113p53$ does not interact with full-length p53, but rather serves to sequester particular cellular factors, thereby influencing the ability of p53 to transcriptionally regulate target genes. For example, the ASPP family of proteins has been shown to interact with the DNA-binding domain of p53 and selectively influence transcription of apoptotic targets

Figure 1. Structure of full-length and a truncated isoform of human (Hu) and zebrafish (Zf) p53. The isoforms are designated as $\Delta 133p53$ (human) and $\Delta 113p53$ (zebrafish). (A) Amino acid sequences for each protein are aligned with orange shading indicates identical amino acids. Adapted from Chen et al. (2005). (B) Schematic diagrams of each protein are shown with relevant domains of the p53 protein, indicated by colored boxes: transcription activation (yellow), sequence-specific DNA binding (green), and tetramerization (red). Two regulatory regions are also shown: one that is proline-rich (PXXP, blue) and another at the C terminus (basic, gray). Although human $\Delta 133p53$ initiates at amino acid 133 and the zebrafish $\Delta 113p53$ at amino acid 113, the resulting proteins are identical in size (261 amino acids).

(Samuels-Lev et al. 2001). Little is known about the activity of p53 to down-regulate Bcl-xL in mammalian cells. There have been a few reports to argue that the human Bcl-2 gene is a target for repression by p53 (Miyashita et al. 1994a,b). Mapping of the relevant response element has suggested that the mechanism of p53 action is not via direct binding of the Bcl-2 promoter, but may be mediated via protein–protein interactions (Miyashita et al. 1994a). Further studies are needed to address the basis for down-regulation of the zebrafish bcl2L levels to determine the relevance of the studies performed on human Bcl-2. Alternatively, it is possible that $\Delta 113p53$ does, indeed, interact with full-length p53 and forms a mixed oligomer. In so doing, it may influence its ability to interact with specific binding sites or influence its interaction with cellular proteins that contribute to target gene selectivity. The fact that down-regulation of Bcl2L may be the relevant means by which p53 triggers cell death, at least in the settings used here, reinforces the notion that transcriptional repression represents an important means by which p53 elicits particular cellular outcomes.

Importantly, these findings in zebrafish have implications for the role of p53 in human tumorigenesis. Although mutation of p53 is a common event in some tumors, direct genetic alteration of p53 is often not found in other cancer types (Downing et al. 2001; Olivier et al. 2002; Lacroix et al. 2006). A noteworthy example is human breast cancer where it was noted that the mutation rate as determined by sequencing of genomic p53 was often lower than the incidence of immunopositive staining for p53. As mutant p53 often has a much longer half-life than its wild-type counterpart, immunohistochemical detection of p53 in primary tumor samples has been used as an indication of genetic alteration in the p53 gene itself. This appears not to be the case in studies of human breast cancer samples (Lacroix et al. 2006). This has led to speculation that there are alternate ways to inactivate p53 besides mutation. Initial studies suggested that this may be due to cytoplasmic sequestration of p53 in breast cancer cells (Moll et al. 1992). Although this possibility has been elegantly explored in neuroblastoma (Moll et al. 1995, 1996), a clear molecular understanding of this in breast cancer has not been achieved. An intriguing possibility is that the over-expressed p53 that is detected in such tumors may be the human ortholog of zebrafish $\Delta 113p53$. Thus, the apoptotic activity of p53 may be inhibited by high levels of a truncated isoform. Although p53 levels appear high, it is not the full-length species that is overexpressed. The generation of isoform-specific reagents that have utility in histopathological analysis of primary tumor samples will be critical to address this exciting possibility. Should this be the case, new avenues for efficacious therapeutic approaches can be explored. Indeed, a recent study suggests altered expression of specific isoforms in human tumors (Boldrup et al. 2007).

Since Bourdon et al. (2005) reported the existence of multiple p53 isoforms, there have been limited studies by other laboratories to explore the significance of those initial exciting findings. Use of zebrafish as a model

system has confirmed that a subset of these isoforms play important roles both in development as well as in the DNA damage response (Chen et al. 2005, 2009). The work of Chen et al. (2009) provides an important framework for future studies in mammalian systems. Given the high relevance of p53 in human disease, especially cancer, it is imperative that these findings be pursued with rigor. It will be important to confirm that the promoter activity contained within intron 4 of the human p53 gene is similarly p53-responsive, and that the human ortholog $\Delta 133p53$ affects full-length p53 in a manner akin to that of zebrafish $\Delta 113p53$. The studies in zebrafish have revealed exciting possibilities. The existence of p53 isoforms has been the so-called “elephant in the room” for many p53 researchers. Certainly, it is now time for p53 isoforms to be fully acknowledged and integrated into studies relevant to p53 biology.

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