Mutant p53 gain of function: The NF-Y connection

The molecular mechanisms underlying mutant p53 gain of function are becoming increasingly complex. In this issue of *Cancer Cell*, Di Agostino et al. identify the heterotrimeric transcription factor NF-Y as an interacting partner of mutant p53. They show that mutant p53/NF-Y complexes bind to NF-Y target promoters and recruit p300 in response to DNA damage, resulting in aberrant transactivation of NF-Y target genes and cell cycle deregulation. These data thereby implicate transcriptional activation by mutant p53 as a key mechanism responsible for its oncogenic activity.

p53 is fairly unique among the family of tumor suppressors in that about 50% of all human tumors express, at relatively high levels, p53 protein with missense mutations in the DNA binding domain. In fact, innumerable clinical reports have documented mutation of virtually every amino acid in this ~200 amino acid region, with some "hot spot" mutations occurring at a markedly higher frequency than the others. Such p53 mutations can arise sporadically or are inherited as a germline mutation in Li-Fraumeni syndrome. While they usually weaken or abrogate sequence-specific interactions with DNA, the fact that these mutant proteins are stable and present at high levels in the nucleus has led to speculation that they are not neutral in cells, but rather confer advantages for tumor growth. Indeed, many patients with p53 missense mutations have an increased resistance to chemotherapy and poorer prognosis than those who have wild-type or no p53 protein (Soussi and Beroud, 2001). The challenge has been to obtain experimental proof of, and mechanistic insight into, mutant p53 oncogenic gain of function.

There is strong experimental evidence to support dominant-negative and gain-of-function activities of mutant p53 (Lang et al., 2004; Olive et al., 2004). Mutant p53 knockin mice on two genetic backgrounds that either were heterozygous for mutant p53 or expressed only mutant p53 (R172H or R270H missense mutations) developed tumor spectra distinct from that of $p53^{+/-}$ and $p53^{-/-}$ mice.

Two different models to explain mutant p53 gain of function have been put forward. The first proposes that mutant p53 regulates a specific set of genes that mediate its oncogenic activities. Indeed, several lines of evidence exist that p53 mutants not only possess altered target gene specificity compared to wild-type proteins, but also differentially regulate p53 target genes (Kim and Deppert, 2004; Sigal and Rotter, 2000). Furthermore, chromatin immunoprecipitation analyses have revealed that mutant forms of p53 physically associate with several promoters, for example MSP/MST-1 (Zalcenstein et al., 2006), although whether mutant p53 proteins bind directly to DNA or indirectly through other sequence-specific transcription factors remains less clear.

The second model proposes that some mutant forms of p53 acquire gain of function through their interactions with the p53 family members p63 and p73. The ability of mutant p53 to bind to and inhibit the activities of p63 and p73 has been shown by several groups (Moll et al., 2001), and taken together with data obtained from human tumor cells (Irwin et al., 2003) and the above-mentioned mouse models (Lang et al., 2004; Olive et al., 2004), strongly implicates the inhibition of p63 or p73 as a potential mechanism for mutant p53 gain of function.

In this issue of *Cancer Cell*, Di Agostino et al. provide evidence for the first hypothesis as being responsible for p53 mutant gain of function following DNA damage (Di Agostino et al., 2006). The authors show that three different mutant p53 proteins interact with the heterotrimeric transcription factor NF-Y in vivo, and that these mutant p53/NF-Y complexes modulate the expression of key NF-Y-regulated cell cycle genes after adriamycin treatment. In fact, wild-type p53 has previously been shown to form a complex with NF-Y on CCAAT box-containing promoters, and upon DNA damage this complex recruits histone deacetylases (HDACs) and releases histone acetyltransferases (HATs), coinciding with the repression of key cell cycle

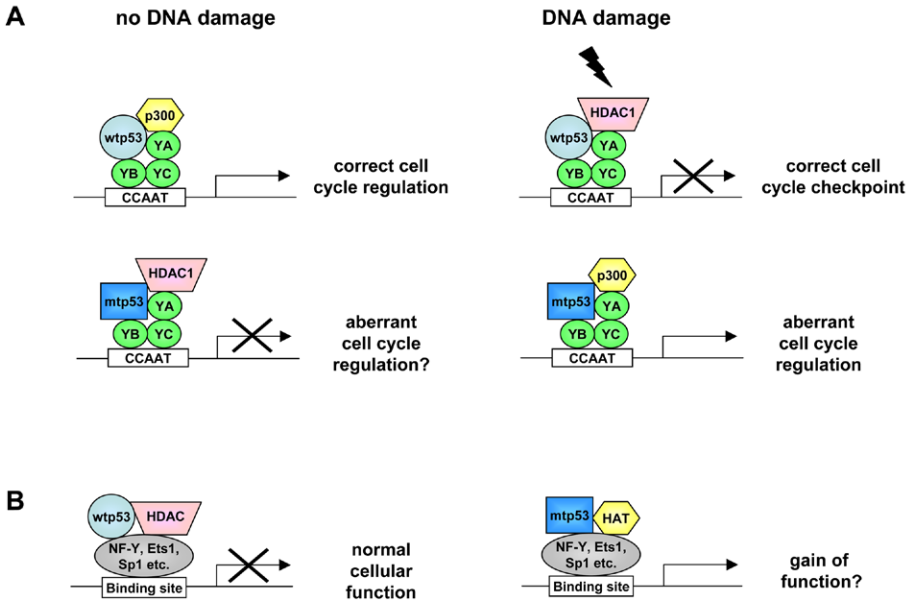

Figure 1. Transcriptional activities underlying mutant p53 gain of function

A: Model proposed by Di Agostino et al. (2006) in which the transcriptional regulation of NF-Y target genes by wild-type and mutant p53 are opposite following DNA damage due to the recruitment of opposing chromatin-modifying enzymes, conferring oncogenic "gain-of-function" properties to mutant p53 proteins.

B: Speculative model of mutant p53 gain of function, whereby the activities of a variety of transcription factors, such as NF-Y, Ets1, Sp1, or other factors are differentially regulated by wild-type or mutant p53 due to specific interactions with HDACs or HATs.

control genes (Imbriano et al., 2005). Strikingly, Di Agostino et al. show that mutant p53/NF-Y complexes have the opposite effect on transcription to wild-type p53/NF-Y complexes and transactivate proliferative genes such as cyclin A, cyclin B2, cdk1, and cdc25C following DNA damage, resulting in activation of cyclin/cdk1 kinase complexes and aberrant cell cycle regulation.

Further, Di Agostino et al. show that NF-Y and mutant p53 are present at the promoters of NF-Y-regulated genes along with HDAC1 independent of DNA damage. Association of mutant p53 with these promoters, dependent on the presence of NF-Y and CCAAT box integrity, is increased after adriamycin treatment, and the p300 HAT is then recruited in a manner that requires mutant p53. The switch between HDAC1 and p300 is accompanied by increased acetylation and reduced methylation of neighboring histones on the cyclin B2 and cdk1 promoters. In contrast, wild-type p53 interacts with HDAC1 upon DNA damage to repress NF-Y target genes.

To gain in vivo relevance for their observations, Di Agostino et al. reduce the expression of NF-YA, a subunit of NF-Y, or mutant p53 using siRNA or shRNA, respectively. Downregulation of either protein impairs the induction of NF-Y target genes and reduces S phase accumulation following adriamycin treatment. Moreover, mutant p53 contributes to chemoresistance of cells to DNA damage. Therefore, the ability of mutant p53 to interact with NF-Y and control important cell cycle regulatory genes defines new oncogenic gain-of-function properties for these proteins.

It should be noted that mutant p53 has previously been shown to switch a repressive transcriptional response to an active one. For example, wild-type p53 has been reported to inhibit Sp1- and Ets1-dependent activity, whereas the mutant p53 interaction enhances transcription mediated by these factors (Kim and Deppert, 2004). However, the mechanism behind these differential effects has remained speculative. The work of Di Agostino et al. suggests that specific recruitment of chromatin-modifying enzymes is responsible (Figure 1A). Given that mutant p53 retains the ability to bind a similar repertoire of transcription factors to wild-type p53, aberrant transactivation of p53 target genes by mutant p53 may be a widespread mechanism underlying mutant p53 gain of function (Figure 1B).

The observations made by Di Agostino et al. raise some interesting questions. First, since both wild-type and mutant p53 can interact with HDAC1 or p300 in complexes containing NF-Y, how are HDAC1 or p300 recruited in an opposite fashion by wild-type or mutant p53 following DNA damage? Second, what is the function of mutant p53/NF-Y/HDAC1 complexes under unstressed conditions? Further, which of the myriad of different tumor-derived p53 mutants interact with and coregulate genes with NF-Y, and what are the structural features of mutant p53 and NF-Y that are necessary for their physical interaction? In this paper the authors use both a DNA contact point mutant and a conformational mutant to show that the gain-of-function activity mediated by mutant p53/NF-Y complexes does not depend on a specific type of p53 mutation. Nevertheless, it would be interesting to confirm the universality of their observations by testing a wider spectrum of p53 mutations. Recent studies have shown that various p53 missense mutants differentially regulate gene expression, impacting their oncogenicity, and in turn, tumor phenotype (Menendez et al., 2006). Moreover, different p53 mutant alleles display distinct tumor spectra, providing clear evidence that various mutants possess their own gain-of-function characteristics (Olive et al., 2004). Taken together, these data suggest that transcriptional complexes containing mutant p53 may differ depending upon the type of p53 mutation.

Finally, given the results of Di Agostino et al., is the second mechanism whereby mutant p53 downregulates the proapoptotic activities of p63 and p73 still valid? Since mutant forms of p53 are frequently present at significantly higher levels in cells than either of its siblings, p63 and p73 (C.P., unpublished data), there may well be enough mutant p53 protein in tumor cells to perform multiple functions. Thus, in the absence of contrary data, there is presently no need to exclude either model.

In summary, the data presented by Di Agostino et al. provide support for mutant p53 facilitating NF-Y transactivation of proliferative genes as being a critical component of their gain of function in response to DNA damage. Additionally, they provide even more impetus to identify means to reactivate the wild-type activity and conformation of p53 mutant proteins, goals that will undoubtedly be eagerly sought in the future.

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Selected reading

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