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p53 Function and Dysfunction

Minireview

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The word "cancer" is used to describe a group of heterogeneous pathologic states in which cells multiply abnormally and invade surrounding tissues. There are hundreds of different kinds of cancers, at least one originating from nearly every cell type in the mammalian organism. One long-standing hope has been that the same biochemical pathway for controlling growth is disrupted in many different kinds of cancers, despite their biologic heterogeneity; this would provide a common denominator for understanding, treating, and preventing these diseases. The pathway involving p53 fulfills this hope, as alterations of this tumor suppressor gene appear to be involved, directly or indirectly, in the majority of human malignancies. This has in turn stimulated an intense search for the biochemical functions of p53 and the effects of mutation on these properties.

Biochemical Activities of the Wild-Type p53 Protein

Two lines of p53 investigation have converged in the past year. First, it was noted that p53 contained an acidic domain near its N-terminus that was similar to those previously noted in well-characterized transcription factors (Fields and Jang, 1990; Raycroft et al., 1990). When this acidic domain was fused to the DNA-binding region of GAL4, the resulting chimeric protein could activate transcription from a *GAL4* operon. The activation domain has been recently mapped to the region lying between codons 20 and 42 (Unger et al., 1992; Miller et al., 1992) (Figure 1). Although many proteins contain such acidic regions, the strength of the activation and the nuclear localization of p53 suggest that p53 is involved in transcriptional control, either directly or through a complex with other proteins that bind to specific genes.

The second line of investigation illuminates this latter point. Through the testing of a large number of human genomic DNA clones, several have been identified that can bind to p53 in vitro (Kern et al., 1991; El-Deiry et al., 1992). Mapping of the p53-binding sites within these clones reveals that each contains two copies of the 10 bp motif 5′-PuPuPuC(A/T)(A/T)GPyPyPy-3′. One copy of the 10 bp motif is insufficient for binding, but binding is preserved when the two copies are separated by up to 13 bp of random sequence. The p53-binding sites have an obvious symmetry—four copies of the half-site 5′-(A/T)GPyPyPy-3′ are oriented in opposing directions. This suggests that p53 may bind to these sites as a tetrameric protein, which is consistent with biophysical studies indicating that p53 exists as a tetramer in solution (Stenger et al., 1992).

Two additional studies have confirmed that p53 can specifically bind to such sequences. From a large pool of random oligonucleotides, a small subset is selectable by virtue of binding to p53 (Funk et al., 1992). These synthetic oligomers share a 20 bp sequence very similar to the p53-binding sites described above. Anti-p53 antibodies and unidentified proteins from nuclear extracts appear to stabilize the complex of p53 with its binding sites (Funk et al., 1992; El-Deiry et al., 1992). The SV40 genome also contains a weak binding site for p53, which matches the 20 bp p53-binding site at 16 positions (Bargonetti et al., 1991).

If p53 binds DNA specifically and contains an acidic activation domain at its N-terminus, one would expect that p53 could activate the expression of genes adjacent to a p53-binding site. This expectation has been confirmed: cotransfection of a p53 expression vector with a plasmid containing a p53-binding site upstream of a reporter gene results in a high level of reporter activation in mammalian cells (Kern et al., 1992; Funk et al., 1992). This activation could have been an indirect one, perhaps in response to the numerous changes in gene expression and growth parameters induced by artificially high levels of wild-type p53. Indeed, p53 has been shown to affect the expression of several genes, few of which are likely to contain p53-binding sites (Ginsberg et al., 1991; Weintraub et al., 1991). Several additional experiments, however, strongly argue that p53 can directly activate transcription from p53-binding sites. First, the level of transcriptional activation precisely correlates with the strength of binding to p53-binding sites in vitro (Kern et al., 1992). Second, p53-binding site–mediated transactivation of reporter genes by p53 can be observed in yeast as well as in mammalian cells (Scharer and Iggo, 1992; Kern et al., 1992). If such effects are indirectly mediated through the induction of another gene product that actually binds to p53-binding sites in vivo, then the induction and function of this second gene product would have to be remarkably conserved in evolution, even though there is no known p53 homolog in

Figure 1. p53 Domain Map

HSP, a domain (amino acids 13–29) implicated in binding of heat shock proteins to mutant p53 (Lam and Calderwood, 1992); ACT, domain (amino acids 20–42) that, when fused to the DNA-binding domain of GAL4, activates transcription of genes downstream from GAL4-binding sites (Fields and Jang, 1990; Raycroft et al., 1990; Unger et al., 1992); HOT, "hotspots" (amino acids 129–146, 171–179, 234–260, 270–287) corresponding to evolutionarily conserved domains containing the most frequent sites of missense mutation (Hollstein et al., 1991); AB, region (amino acids 213–217) binding to mAb240 in some p53 mutants (Stephen and Lane, 1992); NUC, major nuclear localization signal (amino acids 316–325; Shaulsky et al., 1990); CDC2, serine-315 phosphorylated by $p34^{cdc2}$ kinase (Bischoff et al., 1990); CK2, serine-392 phosphorylated by casein kinase 2 (Fakharzadeh et al., 1991); OLIGO, domain (amino acids 344–393) required for p53 oligomerization (Milner and Medcalf, 1991). Human p53 contains 393 amino acids.