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Commentary

The Two Faces of Tumor Suppressor p53

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Mammalian cells have evolved complex responses to genotoxic and other stresses. A historical account of DNA-damage inducible (DDI) responses reveals a unifying theme in that, generally speaking, they serve a protective function.[1–3] DDI genes associated with enhanced cell survival would obviously include genes whose products function in DNA repair or repair-related functions, or cell cycle checkpoint activation. It is widely held that checkpoints function by allowing additional time for DNA repair at critical junctures in cell cycle progression.[4] An important cell cycle checkpoint in mammalian cells, which acts in the G1 phase of the cell cycle, is mediated by the p53 tumor suppressor gene;[5] and G2/M phase checkpoints have recently been shown to be influenced by p53 as well.[6] Cancer cells generally exhibit loss or deregulation of cell cycle checkpoints, a property associated with genomic instability, and tumor progression. It is widely held that cell cycle checkpoint loss results from strong selection during the clonal evolution of cancer cells;[7] the most commonly reported genetic alteration associated with human carcinogenesis is loss of p53 function, usually by mutation.[8]

If cell cycle checkpoints allow additional time for DNA repair, then checkpoint loss by p53 mutation[5] or loss of p53 function by other causes[9] should result in decreased DNA repair and, consequently, increased mutagenesis after entry into S phase or mitosis. In addition, if p53 affects DNA repair rates directly, then decreased repair would occur independently of any checkpoint abnormalities. Loss of cell viability might also be predicted under conditions of increased mutagenesis, presumably because vital cellular "housekeeping" genes are targeted at the same time that oncogenic growth-promoting alterations are acquired. These predictions, though highly logical, have only recently been borne out by experimental data. Loss of p53 function results in decreased DNA repair, specifically of UV-type DNA damage,[10,11] and decreased DNA repair capacity results in increased mutagenesis, at least at certain gene loci.[12,13] Havre et al[12] reported a 73-fold increase in mutation frequency in human RKO colon carcinoma cells transfected with the human papillomavirus E6 gene, which abrogates p53 function. Increased mutagenesis owing to loss of p53 function results in decreased cell viability,[10,12, 14,15] but only in cell types that are not prone to undergo apoptosis as a prominent and early response to DNA damage. Apoptosis is an active process that can also be triggered by p53, but because dead cells tell no tales, the otherwise intuitively obvious protective nature of p53 activation has remained elusive until recently.[10–13,16,17] Thus, p53 activation may elicit protective responses such as cell cycle arrest and DNA repair, on the one hand, or may trigger the cell's demise, on the other (Figure 1). Some cell types, such as lymphoid or myeloid, undergo apoptosis quite readily after DNA damage, whereas other cell types are resistant to apoptosis[2,18] and this may in large part determine the final outcome of the p53-mediated stress response.

Conventional wisdom had suggested that p53-mediated G1 arrest simply provided more time for DNA repair to take place. Recent evidence suggests that p53 may play a more active role in DNA repair.[10,11,19] Cells lacking p53 exhibit a condition of genomic instability,[20,21] and mice lacking functional

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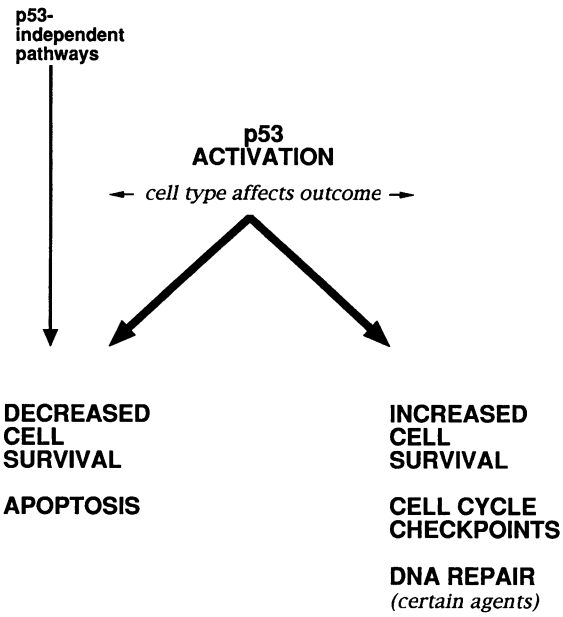

Figure 1. *The two faces of p53. In cells such as myeloid and lymphoid lineages, activation of p53 by DNA damage usually triggers apoptosis. P53 may directly trigger apoptosis*[35] *and p53-regulated effector genes such as BAX also induce apoptosis*[36,37]. *In cancer cells, genetic alterations such as c-*myc *or bcl-2 overexpression also play a role in cellular sensitivity to apoptosis, thereby altering the outcome of DNA-damaging treatments, such as cancer chemotherapy or radiotherapy. Apoptosis can also be triggered by p53-independent mechanisms* (**left**). *In cells that are not particularly prone to undergo apoptosis, the protective function of p53 is unveiled* (**right**). *P53-activated cell cycle checkpoints provide additional time for DNA repair, and p53 probably plays a more direct role in DNA repair. Collectively, these activities act to promote cell survival. In the first example* (**left**), *human cancers carrying mutant p53 genes tend to be resistant to chemotherapy or radiotherapy*[16,38]. *In the second example* (**right**) *human cancers carrying mutant p53 genes are more sensitive to killing by chemotherapy drugs, such as cisplatin, that produce DNA damage of a type that is repaired by the NER pathway*[14,39].

p53 show increased tumorigenesis.[22,23] Genomic instability and increased spontaneous tumorigenesis occur in the absence of exogenous sources of DNA damage, even though probably the bulk of cancer-promoting gene mutations in the real world arise from endogenous sources, such as oxidative stress.[24] These observations suggested that when p53 mutation occurs early in carcinogenesis, as might be the case in some chemical carcinogen models, that additional cancer-promoting genetic alterations will follow. The cancer-prone Li-Fraumeni syndrome in humans exemplifies this concept. In essence, germline p53 mutations in Li-Fraumeni patients provide essentially a first "hit" in multistage carcinogenesis. Proneness to cancer stems not only from cell cycle checkpoint loss, but a recent study shows that Li-Fraumeni fibroblasts have reduced nucleotide excision repair (NER) function as well,[11] which appears to be independent of the checkpoint. Host-cell reactivation experiments, using UV-damaged reporter genes, showed that p53-deficient cells were less proficient in reactivation of the damaged reporter than isogenic cells in which p53 function was retained.[10] Because only the reporter plasmids, and not the cells, were treated with UV, this finding suggests that p53 functions affected repair independently of G1 arrest.[10] Along these same lines, even though p21 is a major mediator of p53-induced G1 arrest[25] and $p21^{Waf1/Cip1}$-deficient mice showed loss of the G1 checkpoint, the mice did not display increased tumorigenesis,[26] again suggesting that genomic instability associated with p53 loss is due to additional activities linked to the p53 pathway, such as in DNA repair.

The p53 protein itself has been implicated in the direct recognition of DNA damage,[27,28] perhaps as a component of the multiprotein complex TFIIH.[13] In addition to its role in DNA damage recognition, TFIIH is also a necessary component of the NER complex.[29] If p53 affects TFIIH activity *in vivo*, as appears to be the case *in vitro,*[13] then p53 may directly augment or facilitate DNA repair. As p53 is a transcription factor that transactivates a fair number of downstream "effector" genes, its is very likely that downstream effector genes of the p53 pathway may also encode proteins that either directly or indirectly affect DNA repair.[17]

Finally, these findings linking p53 function to DNA repair, specifically of UV-type DNA damage,[10–12,30] may prove useful clinically in that some types of cancer cells carrying mutant p53 genes may be more sensitive to certain chemotherapy drugs than cells that retain wild-type p53 function. Because p53 mutation is associated with increased malignancy and metastasis in many types of human cancers, including brain,[7] breast,[31] and colon cancers,[32] the preferential killing of cells having increased metastatic potential would be obviously desirable. Fan et al[14] showed that MCF-7 breast carcinoma cells carrying a mutant p53 transgene were preferentially killed by the chemotherapy drug cisplatin, which produces intrastrand crosslinks and diadducts that are repaired by NER.

The paper by Li et al[30] in this issue underscores the significance of these findings linking p53 function to DNA repair, in the context of a mouse skin model of carcinogenesis. Using an antibody that detects pyrimidine dimers in radioimmunoassay experiments, the authors show that UV-radiation-induced lesions are removed slowly in transgenic mice carrying mutant p53 genes. Because some mutant p53 alleles are able to block or override wild-type p53 when present in heterozygous condition, the suggestion is made that DNA repair is decreased after the occurrence of a single "hit" targeting the p53

gene during carcinogenesis, despite the presence of the remaining wild-type allele. These findings are again reminiscent of Li-Fraumeni syndrome in humans,[11] even though the particular mutant p53 allele present in the transgenic mice may be oncogenically more potent and may be expressed at higher levels than endogenous Li-Fraumeni alleles.

The finding that apoptosis was not altered in UV-irradiated skin of p53 transgenic mice might suggest that p53-independent mechanisms of apoptosis may predominate in this model. On the other hand, other authors have shown decreased apoptosis in UV-irradiated skin of mice carrying homozygous deletions of p53, suggesting that p53 does mediate apoptosis in this system.[33] These authors suggested that cells that had sustained UV-radiation damage were eliminated by apoptosis in normal (p53 wild-type) mice, but not in the p53-null mice, with escape from apoptosis thereby providing a mechanism for promotion and progression of initiated cells.[33] It remains possible that mutant p53 genes are not merely the functional equivalent of p53 loss by deletion. Indeed, transgenic mice carrying the mutant Val-135 p53 allele exhibited accelerated tumor development and an altered spectrum of tumors compared with the p53-null mice,[34] suggesting a gain-of-function for some p53 mutant alleles. In human cancers, mutation is by far the most frequent mechanism for p53 inactivation.[8] Because p53 mutations of the type induced by UV-radiation (specifically C>T or CC>TT mutations) are found in over 90% of human squamous cell carcinomas p53 mutations are presumably initiating events in these cancers.[33] The concept put forth by Ziegler et al, that "sunlight can act twice: as tumor initiator and tumor promoter," would seem to apply equally to the paper by Li et al in this issue.

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