Minireview

p53 in Life and Death

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Introduction

One of the supreme challenges faced in cancer therapy is the small therapeutic window afforded by chemotherapeutic agents and radiation therapy. Many of these agents act by damaging DNA. This DNA damage is believed to be directly lethal to rapidly growing cancer cells but also produces toxicity through effects on normal cells. A major therapeutic research goal in molecular oncology is to identify factors and pathways through effects on normal cells. A major therapeutic research goal in molecular oncology is to identify factors and pathways through effects on normal cells. A major therapeutic research goal in molecular oncology is to identify factors and pathways through effects on normal cells. A major therapeutic research goal in molecular oncology is to identify factors and pathways through effects on normal cells. A major therapeutic research goal in molecular oncology is to identify factors and pathways through effects on normal cells. A major therapeutic research goal in molecular oncology is to identify factors and pathways through effects on normal cells. A major therapeutic research goal in molecular oncology is to identify factors and pathways through effects on normal cells. A major therapeutic research goal in molecular oncology is to identify factors and pathways through effects on normal cells.
p53 disrupt the degradation pathway, resulting in particularly high levels of the mutant protein. Thus, p53 overexpression (recognized immunohistochemically, for example) indicates the presence of a p53 mutation commonly. If this mutant protein forms oligomers with wild-type p53 in the cell, such high protein levels likely would exacerbate the dominant-negative effects.

p53 in Tumorigenesis

Multiple lines of evidence point to a key role of p53 in tumor suppression. Loss of functional p53 by either gene mutation or deletion is associated with oncogenesis. Genetically altered mice lacking p53 (homozygous p53 ‘knockout’) are developmentally normal but exhibit a nearly 100% incidence of neoplasia in the first year of life (17). Functional inactivation of p53 through binding by specific viral or cellular oncoproteins is also associated with oncogenesis. This is seen, for example, in cervical carcinoma, in which the human papilloma virus E6 protein promotes ubiquitin-mediated p53 degradation (18), and in soft tissue sarcomas, which have an amplified mdm-2 oncogene that leads to disruption of the transcriptional activity of p53. Point mutations or allelic loss of p53 are found commonly in diverse human cancers. Furthermore, the Li-Fraumeni syndrome, characterized by increased familial risk for a variety of neoplasms at an early age, is associated with germ line mutations in one p53 allele (19). Members of these families are heterozygous for p53 mutations in nonneoplastic tissues but carry mutations in both p53 alleles in the neoplastic cells. Animal studies also have used transgenic mice to demonstrate the role of p53 in modulating tumorigenesis (20). Mice were engineered to develop T antigen-induced tumors in genetic backgrounds of either wild-type p53, p53 knockout, or p53 heterozygote (+/-). Although abnormal cell proliferation (hyperplasia) occurred in wild-type p53 and heterozygotes, frankly malignant tumors formed when p53 was homozygously deficient. Interestingly, when p53 was present, a significant number of spontaneously apoptotic cells were observed, suggesting that p53 might suppress tumorigenesis in part by triggering apoptosis in cells with a dysregulated proliferative apparatus. This p53-dependent apoptosis seemed to be a barrier to a discrete (late) stage in malignant transformation. In human colon carcinoma, in which multiple steps in the molecular transformation have been elaborated elegantly, p53 aberrations also occur commonly as a late step (21).

p53 Function: Cell Cycle Regulation

Cells containing wild-type p53 undergo G1 and G2-M arrest following DNA damage by ionizing radiation. These cycle-specific pauses, known as checkpoints, are thought to ensure that critical events (such as genome replication or chromosome segregation) occur only when chronologically appropriate. Fibroblasts lacking functional p53 fail to arrest in G1 following treatment with ionizing radiation (22–24). Thus, p53 seems to regulate a G1 checkpoint, a pivotal function because it occurs just prior to replicating a potentially damaged genome. With insufficient DNA repair, damaged DNA might be replicated, with resultant genetic instability. Such increased genomic plasticity has been observed in cells lacking functional p53 (25, 26), although it remains to be seen exactly which forms of DNA repair are affected by the protective activity of p53. These effects could be due to p53-dependent stimulation of the DNA repair machinery (see below). Alternatively, p53 may provide a purely temporal barrier (G1 arrest), during which repair occurs with unchanged kinetics.

A number of important clues have come to light regarding the mechanism by which p53 regulates cell cycle progression. DNA damage induced by ionizing radiation leads to rapid increases in p53 protein by posttranslational changes in protein stability. In turn, the abundant p53 protein leads to transcriptional activation of certain target genes, including p21/WAF1/CIP1 (27–29), a cell cycle regulator, the expression of which is regulated profoundly (although not exclusively) by p53. p21/Waf1 inhibits cyclin-dependent kinase activity, which in turn is required for progression into S-phase (DNA replication). p21/Cip1/Waf1 also binds and inhibits a subunit of DNA polymerase called PCNA2 (30) directly, thereby blocking DNA replication itself. Several additional, p53-independent regulators of p21/Cip1/Waf1 expression include MyoD (31–33), a transcription factor that promotes a program of muscle differentiation, and a serum-responsive element still to be characterized (34, 35).

p53 also may function to stimulate the DNA repair machinery. GADD45, a p53-regulated protein, is induced by stresses that arrest cell growth and agents that cause DNA damage. GADD45 has been shown recently to bind PCNA to stimulate excision repair of damaged DNA (36). The mechanism by which the interaction between GADD45 and PCNA stimulates DNA repair is under active investigation. p53 also binds ERCC3, an excision repair molecule that recognizes and removes damaged DNA segments (37). These data suggest that p53 may inhibit DNA replication via p21 while stimulating DNA repair via GADD45 or ERCC3 simultaneously.

The addition of methylxanthines such as pentoxifylline or caffeine, both known to disrupt cell cycle arrest particularly at the G2 checkpoint, leads to increased sensitivity of cultured cells to radiation or chemotherapy. Cells lacking functional p53 are more sensitive to the addition of caffeine or pentoxifylline than are cells containing wild-type p53 (38, 39). Cells lacking functional p53 have lost the ability to arrest in G1 already; therefore, the further loss of the G2 checkpoint seems to have an additive effect. This observation carries intriguing implications for future therapeutic strategies, because p53-negative tumor cells may be more responsive to a combination of DNA-damaging agents and agents that abrogate the G2 checkpoint.

p53 also has been suggested to play a potential role in regulating cell cycle events distinct from those incurred by DNA damage. For example, p53 was found to regulate an apparent spindle checkpoint, which ensures the maintenance of chromosomal diploidy. p53-deficient cells treated with spindle inhibitors failed to complete chromosome segregation and accumulated tetraploid and octoploid chromosomal complements (40).

2 The abbreviation used is: PCNA, proliferating cell nuclear antigen.
p53 and Apoptosis

Because p53 loss is apparently associated with a diminished capacity for at least certain forms of DNA repair, one might predict that tumor cells deficient in p53 would be relatively radiosensitive. Paradoxically, the opposite seems to be the case; thymocytes (41, 42) and tumor cells containing wild-type p53 (43-45) are significantly more radiosensitive than genetically matched cells lacking p53. p53 has been found to modulate the susceptibility to apoptosis, a function that may explain this apparent paradox.

Apoptosis is a cellular program inherent to multicellular organisms, which, when triggered, sets off a biochemically and morphologically recognizable cascade resulting in cellular suicide. Apoptosis is distinct from necrosis, wherein cells swell, trigger significant inflammation, and die of externally inflicted insults. In contrast, apoptosis plays an essential role in normal development, organogenesis, and probably senescence. Apoptosis also may be initiated by nonphysiological external stimuli, such as toxins and ionizing radiation, or aberrant internal genetic stimuli, such as oncogenic lesions, which dysregulate growth. The morphological signature of apoptosis is cell shrinkage, nuclear condensation, and fragmentation. A biochemical hallmark of apoptosis is endonucleolytic DNA cleavage, sometimes resulting in genomic fragmentation down to the size of individual nucleosome units (150–200 bp in length).

Early evidence that p53 may modulate apoptosis came from studies in which p53 function was restored to a p53-deficient myeloid leukemia cell line (44). The result was rapid apoptosis. When p53 knockout mice were obtained, their thymocytes were shown to be substantially resistant to the induction of apoptosis by radiation, compared with p53 wild-type littermates of otherwise identical genetic background (41, 42). Subsequently, it was shown that oncogenically transformed fibroblasts derived from either p53 wild-type or p53 knockout mice differed dramatically in their susceptibility to the induction of apoptosis by radiation and chemotherapy (43). In all cases, wild-type p53 conferred drug sensitivity in vitro, whereas p53 loss was associated with resistance. Importantly, because p53 loss is associated with genomic instability, it remains possible that at least some of this effect could be due to secondary mutations, although early passage cells have been examined in these studies for this reason. In the context of solid tumors in nude mice, the same effect was observed; the presence of p53 correlated with responsiveness to radiation and multiple chemotherapies, whereas the absence of p53 produced resistance to all radiation and multiple DNA-damaging chemotherapies (43). Furthermore, in the p53 wild-type solid tumors, acquired radioresistance was produced using repeated low doses of radiation. In about 50% of cases, the newly resistant tumors were found to contain acquired mutations in p53. The emergence of p53-deficient tumor cells may reflect their selection and growth advantage over the more sensitive wild-type p53 cells, although a direct mutagenic effect of radiation also may play some role. When responding to therapies, these solid tumors were also found to die by apoptosis, whereas essentially no apoptosis was seen in histological sections of resistant, p53-null tumors after treatment in vivo (46).

Studies using human cancer cell lines illustrate the complexity of the p53 story further. Studies with Burkitt’s lymphoma and lymphoblastoid cell lines (47, 48), ovarian cancer cell lines (49), and breast cancer cell lines (38) all demonstrate an increased sensitivity to radiation or specific chemotherapeutic agents in the presence of wild-type p53. Transfection of dominant-negative mutant p53 genes into these cell systems led to abrogation of the G1 arrest and a diminished response to radiation or chemotherapy. In contrast, studies with colorectal carcinoma cells (50) and head and neck squamous cell cancer cell lines (51) containing or lacking functional p53 failed to demonstrate any significant differences in sensitivity to radiation. This was observed despite the disruption of G1 arrest following introduction of a dominant-negative mutant p53 gene into the colorectal cancer cells. Thus, the effect of functional disruption of p53 may vary between cell lines. One potential reason for such variation may lie in differences in the regulatory signals for apoptosis lying upstream or downstream from p53.

p53 and Apoptosis: The Therapeutic Index

For an anticancer therapy to be effective in vivo, it must produce substantially greater toxicity for the tumor cells than for normal cells. The role of p53 in modulating apoptosis has suggested that it may affect exactly such a therapeutic index significantly (Fig. 1). Analysis of primary, untransformed fibroblasts treated with radiation revealed that, whereas p53-null cells arrest in G2, having lost the G1 checkpoint (23), untransformed fibroblasts undergo cell cycle arrest at low doses of radiation, which otherwise produce apoptosis in E1A/Ras-transformed cells of the same genetic origin (38). This difference potentially constitutes a therapeutic index for antineoplastic agents. p53 null-transformed cells, however, display profound radioresistance both in vitro and as solid tumors in vivo (41), potentially contributing to the worsened prognosis of many p53-deficient human tumors.
tumor cells specifically and thereby may underlie a therapeutic index. Importantly, however, p53-deficient tumor cells seem to have lost the apoptotic triggering pathway and are strikingly radiotherapy and chemotherapy resistant in vitro (43, 46). Importantly, the apoptosis mediated by p53 does not, a priori, prevent oncogenic transformation, at least with the E1A/Ras oncogene combination. However, it may be an important determinant of response to many standard cancer therapies, explaining why diseases such as testicular carcinoma and pediatric acute lymphoblastic leukemia (mostly wild type for p53) are highly curable.

Triggers of p53-mediated Apoptosis

An important, although still poorly understood, question is the identification of upstream events that activate p53-dependent programs. In response to DNA damage, a number of proteins that recognize or respond to DNA double-strand breaks and could play a role in signaling the accumulation of p53 have been identified. Interestingly, p53 has been shown recently to modulate apoptosis from triggers distinct from DNA damage, such as growth factor deprivation (52, 53). In these cases, Myc-transformed fibroblasts were shown to enter apoptosis on growth factor (serum) deprivation in a fashion dependent on the presence of wild-type p53. Among antineoplasics, drugs known to be DNA damaging trigger apoptosis with much greater efficiency when p53 is intact (43). It is formally possible that the resistance to apoptosis seen with the loss of p53 could be the indirect result of genomic instability and secondary genetic events. The reversibility of the loss of p53-deficient apoptosis using inducible p53 genes and the ability of cells from p53-deficient transgenic mice to undergo apoptosis induced by alternate stimuli argue against this possibility at least in these systems. Another important possibility, however, is that p53 modulates apoptosis by these triggers directly. If so, the diversity of these stimuli suggests that p53 might be a fairly general "stress response" protein. An understanding of the molecular mechanisms underlying such a stress response by p53 may shed important light on its role in apoptosis.

Downstream events in p53-mediated apoptosis remain equally unclear (Fig. 2). For example, it remains uncertain whether p53 mediates apoptosis by a distinct mechanism from that which regulates the cell cycle. It is tempting to speculate, for example, that the activity of p53 inhibits cell cycle progression, but that in certain contexts (such as oncogene overexpression), these cell cycle inhibitory and cell cycle stimulatory activities "clash," thereby resulting in apoptosis. Alternatively, p53 may trigger apoptosis by a distinct or "dual" mechanism, which is independent of its cell cycle regulation (54). Along these lines, it is intriguing that p53-mediated apoptosis from radiation (55) or growth factor deprivation (53) seemed to proceed despite treatment with actinomycin D, a nonspecific transcriptional inhibitor. Additional studies have demonstrated the ability of p53 to activate the Bax gene, a bcl-2 dimerization partner with preapoptotic activity, transcriptionally (56). Although this activity may contribute to the induction of apoptosis by p53, it remains to be seen how surges in p53 protein level result sometimes in cell cycle arrest and in other instances in apoptosis. The determinants of arrest versus apoptosis may prove to be critical, both for an understanding of the biology of p53 and also for rational therapies aimed at manipulating this choice in tumor cells.

Prospects

The roles of p53 in cancer biology have placed it in a position of potential importance with regard to cancer therapy. For example, families carrying a germ line mutation in p53 are at a higher risk of developing numerous neoplasms. This argues for cancer screening to be initiated at a younger age in these patients. Recommendations regarding lifestyle choices to minimize exposure to carcinogens might be reinforced strongly. Importantly, bioethical questions are also raised with genetic screening proposals, particularly because the information obtained may have enormous health ramifications but would not necessarily lead to a simple, effective treatment option. The recognition that p53 may be prognostically important in human cancer also carries several implications. As with any statistically sound risk factor, if verified, tumors harboring p53 mutations might be managed best with particularly aggressive or experimental treatment protocols. If p53 deficiency modulates treatment resistance purely through the indirect mechanism of increased genomic instability, then therapeutic options may be limited, perhaps including attempts at restoring p53 function (no simple task). If, alternatively, p53 activates an apoptotic pathway directly, as a significant body of data suggests, then therapeutic strategies might focus on triggering this pathway despite p53 deficiency. In fact, p53-independent apoptosis occurs clearly (e.g., in the normal embryological development of p53 knockout mice (17) and by glucocorticoid treatment of p53 knockout thymocytes (41, 42)). If more agents can be identified that trigger p53-independent apoptosis with tumor cell selectivity, they may offer new hope in the quest for rational cancer therapy in the many human tumors deficient in p53.

References


