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The p53/IGF-1 Receptor Axis in the Regulation of Programmed Cell Death

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The loss or functional inactivation of tumor suppressor genes appears to be one of the most fundamental genetic mechanisms of tumorigenesis, and rational insights into the signaling pathways of tumor suppressor genes have emerged as a successful strategy of identifying novel drug discovery targets downstream of the tumor suppressor protein itself. Elucidation of novel pathways downstream of p53 have established a link between this important tumor suppressor gene and the insulin-like growth factor-1 receptor (IGF-1r), either via direct regulation of IGF-1 receptor levels, or modulation of IGFs via transactivation of the insulin-like growth factor-binding protein 3 (IGF-BP3) gene. Binding of IGF-BP3 to IGFs inhibits both their mitogenic and cell survival functions, highlighting a novel pathway whereby p53 may regulate apoptosis in tumor cells.

Key Words: p53; tumor supressors; apoptosos; insulin-like growth factor-1 receptor (IGF-1r); insulin-like growth factor binding protein 3 (IGF-BP3); c-myc.

Introduction

The induction and activation of p53 is a critical response to various forms of cellular stress, most notably genotoxic stress, and is essential in safeguarding the replication and propagation of intact chromosomal DNA. Loss or inactivation of p53 lead to genetic instability and promote cellular transformation and tumorigenesis.

The best characterized cellular functions of p53 that are associated with its properties as a tumor suppressor, are those implicating it as a regulator of cell cycle progression and apoptosis (for recent review *see* ref.*1*). In response to DNA damage, p53 may induce cell cycle arrest, which may concur with ongoing DNA repair, or, alternatively, it may trigger signals that lead cells down the path of programmed cell death/apoptosis. Whether a cell is eliminated by a process of p53-induced cell death may depend on a number of factors, including the level of induction of p53 protein, cell-specific genetic determinants (e.g., normal or tumor cell) and the trophic environment *(1)*. Integration of these various signals appears to be particularly important in the regulation of cell fate of oncogene-transformed cells or tumor cells, which in many cases are inherently more susceptible to the induction of cell death by p53 than normal cells *(2)*, and consequently, more dependent on the presence of trophic factors for survival following damage.

It is now well established that the sequence-specific transcription activation function of p53 is an important activity associated with its role in cell cycle control and apoptosis *(1,2)*. Nearly all tumor-derived mutations of p53 reside in the sequence-specific DNA binding domain of p53 and completely abolish or severely reduce the ability of p53 to bind to various genomic p53 binding sites. Mutations may cause structural changes associated with loss of wild type p53 protein conformation or, more subtly, affect residues directly involved in interactions at the protein/DNA interphase *(1,3)*. These genetic and structural analyses of p53 highlight the importance of the DNA binding function of p53 in tumor suppression. Consistent with a role of transcription in p53 function, mutations in the transactivation domain that affect interaction of p53 with TAFII31 and abolish p53-mediated transactivation of gene expression, may also interfere with the ability of p53 to regulate cell cycle progression and apoptosis *(4)*. Transcription-independent activities of p53 have also been suggested to play a role in growth regulation and apoptosis by p53, although these are not yet as well-characterized *(5–7)*. Transcription repression through interference with the function of components of the basal transcription machinery

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(TFIID) has been forwarded as a possible mechanism of action of p53 *(1)*. However, direct transcriptional repression of endogenous genes by p53 has not yet been demonstrated, although it should be noted that downregulation of at least two genes in response to p53 induction have recently been reported and include the microtubule-associated-protein MAP4 *(8)* and the insulin-like-growth factor-1 receptor (IGF-1r) *(9,10)* genes. In contrast, a number of p53-induced genes have been identified that link p53 to regulators of the cell cycle and cell death machineries (for review, *see* ref. *1* and references therein). Mdm-2, the first p53-interacting cellular protein to be identified, is encoded by a p53-response gene, and its presumed role is to exert negative feedback control on p53. The p21/WAF1 target gene encodes a general inhibitor of cyclin-dependent kinases and is required for p53 to induce efficient cell cycle arrest. GADD45 may represent yet another cell cycle regulator. Bax, a member of the bcl-2 family of proteins is a positive effector of cell death, and has been implicated as a mediator of p53 function. Most recently, the authors identified several additional novel p53 target genes, among which the gene encoding IGF-BP3 provides a link between p53 and the IGF-1 receptor axis which plays an important role in cellular growth, transformation, and survival.

The p53/IGF-BP3/IGF-1 Receptor Axis

Various approaches have been taken to identify p53 target genes encoding mediators of p53 function. These include differential cloning approaches using cDNA libraries derived from cells carrying inducible wild type p53 transgenes or cells expressing temperature-sensitive mutants of p53. Alternatively, p53-dependent differential induction of genes has been identified in cells subjected to genotoxic stress.

Using such differential cloning strategies, the authors recently identified the gene encoding insulin-like growth factor-binding protein 3 (IGF-BP3) as a p53 target gene *(11,12)*. IGF-BP3 transcripts are rapidly induced in response to p53 activation, concomitant with a significant accumulation of IGF-BP3 in the extracellular medium. Furthermore, a p53-dependent induction of IGF-BP3 transcripts was observed in cells in response to genotoxic stress, indicating that the IGF-BP3 gene is a target for endogenous p53. Consistent with a direct activation of the IGF-BP3 gene by p53, two distinct p53-responsive elements were identified in the first and second intron of the IGF-BP3 gene. These elements were shown to bind highly purified wild type, but not mutant p53 protein in vitro, and confer p53-inducibility to a heterologous promoter when introduced into cultured cells. As observed for p53-responsive elements identified in other p53 target genes such as the p21, mdm-2, gadd45, and bax genes, the sequence of the IGF-BP3 gene elements match closely the p53 consensus binding site $(RRRC(A/T\ A/T)GYYY)_2$.

The slight deviations of known genomic p53 binding sites from the consensus p53 binding site may provide an important basis for a differential induction of these genes in response to different levels or activated forms of p53 in different cell types. Clearly, not all p53 binding sites have the same affinity for p53 *(1,13)*. Recent studies have shown that p53 may induce cell cycle arrest or apoptosis in a manner that is dependent on its level of expression *(14)*. It will be interesting to determine whether different sets of p53 target genes are induced under these conditions. A classification of p53 target genes with respect to p53-inducibility has recently been approached by a study using a particular tumor-derived mutant of p53 that retains the ability to induce cell cycle arrest, but is deficient in the induction of apoptosis. The same mutant p53 protein was determined to still effectively activate the p21 promoter, but to be defective in the activation of p53-responsive elements from the bax and IGF-BP3 genes, which display a weaker affinity for p53 *(7,15)*. Thus, both bax and IGF-BP3 genes may represent important targets of p53 that link it to the regulation of programmed cell death.

The authors have recently shown that p53-induced IGF-BP3 accumulating in the extracellular medium of stimulated cells effectively inhibits the mitogenic function of IGF-1 *(12)*, consistent with the known function of IGF-BP3 to form complexes with IGFs, and thereby preventing these growth factors from interacting with the IGF-1 receptor. However, the ability of IGFs to function as cell survival factors appears to be of even greater importance in regulating cell fate of oncogene-transformed or tumor cells (for review, *see* ref. *16*). A number of studies have shown that IGFs can prevent apoptosis, for instance, cell death induced by cytotoxic agents such as etoposide *(17)*, or in response to overexpression of the c-myc oncogene in conjunction with serum deprivation *(18)*. Efficient induction of apoptosis by c-myc requires a functional p53 protein and has been suggested to be mediated, at least in part, via induction of p53 *(19)*. Further consistent with a role of IGF-1 receptor signaling in inhibition of p53-induced cell death, IGF-1 effectively inhibit apoptosis induced upon activation of a temperature-sensitive mutant of p53 in murine fibroblasts overexpressing c-myc and maintained in low-serum conditions (Fig.1). IGF-BP3 counteracts the protective effects of IGF-1 (Fig.1). Based on these findings, one may propose a model in which p53 further sensitizes cells to apoptosis by interfering with the survival function of IGFs via induction of IGF-BP3. Furthermore, recent studies indicate that p53 may down-regulate expression of the IGF-1 receptor in hematopoietic cells *(10)* or Saos-2 osteosarcoma *(20)*, highlighting a second mechanism whereby p53 may regulate cell survival.

Future challenges will reside in elucidating the signaling pathways associated with IGF-1 receptor-mediated rescue from cell death. Preliminary studies indicate that this may occur downstream of p53 and activation of related target

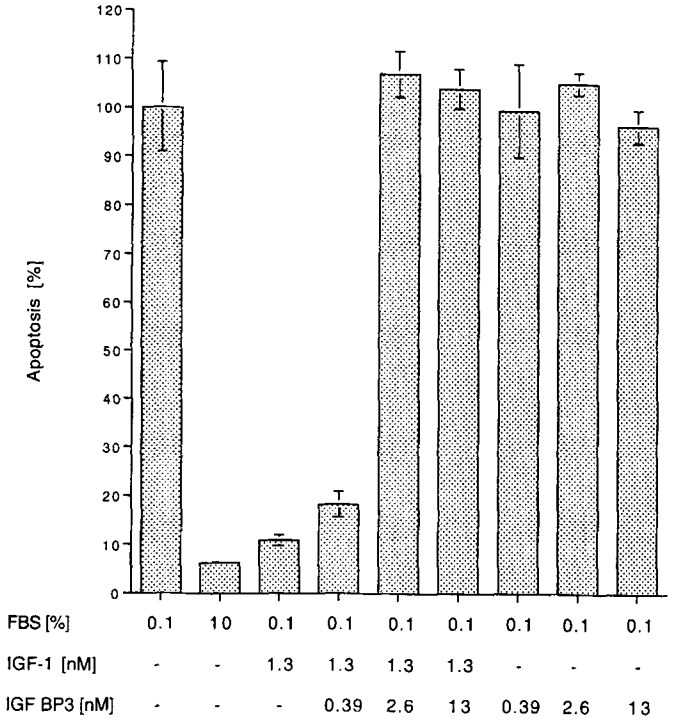

Fig. 1. IGF-BP3 inhibits IGF-1 mediated suppression of p53-induced apoptosis. VM10 murine fibroblasts (p53 -/-), constitutively expressing a temperature-sensitive mutant p53 protein (p53V135A) and the c-myc oncoprotein *(8)*, were incubated for 4 1/2 h at 32.5°C (permissive temperature) vs 39°C in DMEM supplemented with 0.1% fetal bovine serum (FBS). Effects of 10% FBS, recombinant IGF-1 and IGF-BP3 on p53-induced apoptosis at 32.5°C in 0.1% FBS, are shown. The extent of apoptosis [% + S.D.] as shown, represents the average of three independent experiments. Apoptosis was quantitated with a Boehringer Mannheim Cell Death Detection ELISA^{PLUS} Kit determining the amount of fragmented DNA present in the cytosolic fraction of cells, as recommended.

genes. Consistent with IGF-1r signaling interfering with the cell death process further downstream in the cell death signaling pathway, the IGF-1 receptor has been implicated in tumorigenic growth of cells deficient in endogenous p53 *(16)*. Furthermore, IGF-1 may inhibit apoptosis associated with overexpression of ICE protease *(21)*. Recent studies further show that IGF-1 inhibits the proteolytic activation of endogenous ICE/CPP32-like protease in response to p53 activation *(22)*. It therefore appears that IGF-1 interferes with signals that promote protease activation, rather than the active protease itself or signals downstream thereof. Thus, elucidation of transducing signals implicated in IGF-mediated protection from cell death may provide new insights into mechanisms associated with activation of apoptotic proteases and are expected to yield important new clues in the search for therapeutic targets in the treatment of cancer.

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