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Editorial

E2F and p53 make a nice couple: converging pathways in apoptosis

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It has been known for some time that p53 activation elicits growth arrest in normal cells, whereas activation of this tumor suppressor in transformed cells results in apoptosis (reviewed in Ko and Prives[1]). However, the mechanisms underlying this differential response are not well understood. Furthermore, inactivation of the RB pathway (through loss of the *RB* or *p16* gene or amplification of *cyclin D*) occurs in a large fraction of human tumors, and one result of such deregulation is the enhancement of E2F activity. E2F has a well-established role in cell cycle progression, and numerous studies suggest additional roles in p53-dependent and p53-independent apoptosis.[2–4] A very interesting question thus emerges: What are the mechanistic underpinnings that explain the observation that tumor cells show increased sensitivity to p53-induced apoptosis as compared to their normal counterparts? Therefore, a precise articulation of the molecular determinants that sensitize tumor cells to p53-induced death would present a valuable direction for attacking cancer cells. Many p53 targets that play a role in growth arrest or apoptosis have been identified, but given the frequent occurrence of mutations in the RB pathway and established connections between RB/E2F and p53, a more detailed understanding of how both pathways converge may present an even more effective way to approach the issue of driving tumor cells toward apoptosis. Four new papers in CDD have begun to tackle precisely this question.[5–8]

In addition to the well-studied role of p53 in apoptosis, activation of the E2F transcription factor also promotes cell death. E2F plays the paradoxical role of both oncogene and tumor suppressor.[9] A prevailing notion is that E2F could serve as a guardian of the genome by eliminating cells in which an oncogene has been activated, thus providing its tumor suppressor role. In support of this role, E1A can in some settings push cells toward p53-dependent apoptosis, presumably as a result of deregulated E2F activity.[10] Several groups have implicated the activator class of E2Fs (E2F1, E2F2, and E2F3) in apoptosis, although other laboratories have emphasized the importance of E2F1 over the other members of the family.[11] E2F propagates both p53-dependent and p53-independent apoptotic signaling cascades via several distinct mechanisms. First, E2F binds and regulates p14ARF, which in turn stabilizes p53. It is clear that p14ARF is not the sole E2F target that mediates the effects of p53, as evidenced by mouse knockout studies.[12,13] Second, E2F has now been shown to activate directly proapototic genes including Apaf-1 and BH3-only proteins (PUMA, Noxa, Bim, and Hrk/DP5) as well as multiple caspases.[14–17] Several of these genes appear to be regulated by both p53 and E2F (*Apaf-1*, *PUMA*, and *NOXA*). Third, E2F can induce expression of the p53-related protein p73.[18] Thus, E2F-mediated apoptosis occurs through channels that either involve mitochondria (regulation of Apaf-1 and BH3-only proteins) or bypass (direct induction of caspases) this organelle. Although clear links have been forged between E2F and downstream events in the apoptotic pathway in general and between p53 and E2F specifically, the molecular mechanisms governing E2F-induced apoptosis and the signals that generate sufficient E2F activity to begin the cascade of events in the first place are not well defined, and it is clear that other E2F targets beyond p14ARF involved in apoptosis induction have yet to be discovered.

Four laboratories have now made significant in-roads into understanding E2F targets that play a role in p53-mediated apoptosis, and in so doing, have shed valuable new light on the problem of killing tumor cells.[5–8] These groups have found that two members of the *ASPP* (*a*poptosis-*s*timulating *p*roteins of *p*53) family, *ASPP1* and *ASPP2* (also known as 53BPL2), are transcriptional targets of E2F.[5–7] This is an interesting finding because these proteins are thought to function as proapoptotic cofactors of p53 by helping tip the balance of p53 activity toward upregulation of apoptotic rather than growth-inhibitory targets subsequent to death-inducing insults. This could be achieved through the ability of ASPP1 and ASPP2 to enhance p53 (as well as p63 and p73) binding and transactivation of proapoptotic genes such as BAX *in vivo*.[19,20] *ASPP* genes are induced by ectopic E2F expression or as a result of E1A expression, and induction in the presence of cycloheximide suggests that transcription may be directly enhanced by E2F.[5] Importantly, each group has shown that E2F binding is direct and occurs under physiological conditions using the chromatin immunoprecipitation (ChIP) assay. Motif-finding algorithms identify at least one E2F binding site in each proximal promoter or just downstream from predicted transcription start sites. In addition, Ginsberg and colleagues identified two other E2F targets, *JMY* and *TP53INP1* (or *p53DINP1* or *SIP*), each of which is thought to play a role as a cofactor in mediating p53-dependent apoptosis.

A second study by Lopez and colleagues suggests that regulation of the *ASPP2* promoter may be complex and could involve coregulation by additional mitogen-stimulated factors beyond E2F.[6] *ASPP2* expression is induced upon serum stimulation of quiescent cells, and like many E2F-responsive genes, it is maximally expressed in the S phase. Their studies suggest that this promoter may be regulated by both repressor and activator E2Fs. Consistent with the possible regulation by a repressor E2F, Hershko *et al.*[5] found significant recruitment of E2F4 to this promoter. In a separate study, the Lu laboratory identified *ASPP1* and *ASPP2* as E2F targets and showed that E2F1 activated both promoters in a dose-dependent manner.[7] Mutagenesis of the *ASPP1* promoter suggested that the E2F binding site lies just downstream of the transcription start site. Ectopic expression of any of the three activator E2Fs stimulated both promoters, perhaps explaining why certain labs observe apoptosis upon expression of any of these E2Fs. Endogenous E2F is capable of activating the *ASPP1* promoter, since inactivation of the RB pathway via E1A results in the upregulation of this promoter. Interestingly, in contrast with *ASPP1*,

the authors showed that E1A did not significantly alter expression from the *ASPP2* promoter.

A fourth group, utilizing expression profiling data, showed that an uncharacterized gene (KIAA0767) induced by ectopic E2F had interesting proapototic properties.[8] Adenovirus-mediated expression of this gene led to apoptosis, and unlike p53, killing was observed in both normal and tumor cells. Hence, this gene has been named death-inducing protein (*DIP*). Immunofluorescence and subcellular fractionation suggested that DIP accumulates in mitochondria upon expression of E2F1. Importantly, in experiments that address whether *DIP* is an essential target of E2F1 involved in E2F-mediated killing, the authors suppressed DIP levels using RNAi and demonstrated that they could partially reverse the apoptotic effects of enhanced E2F activity. At least some of the apoptosis-inducing effects of DIP may be caspase independent, as a pancaspase inhibitor led to an incomplete block of DIP-mediated apoptosis. It remains to be determined (through ChIP analysis for example) whether *DIP* is a *bona fide* physiological target of E2F and whether endogenous DIP accumulates in the mitochondria and under what conditions. In addition, it will be interesting to uncover the ways in which this protein interconnects with other proapoptotic proteins to provoke cell death, and whether this protein is modified post-translationally, as suggested by the presence of several such motifs.

These studies suggest many new ways to understand the role of E2F and p53 in mediating an apoptotic response. Of course, a large number of questions remain, and our tool kit for attacking tumors is probably much less than half full. Beyond loss of the RB pathway in tumors, what is the nature of other potential signals that activate E2F in the first place? Are there ways in which we can coax the induction of genes such as *ASPP1* and *ASPP2* that would push tumors toward p53-mediated death? The first piece of the puzzle – the regulation of these genes by the E2F transcription factor – is now in hand. Understanding in greater detail how these genes are regulated in response to apoptotic stimuli in normal and transformed cells could very well provide the remaining pieces and provide novel chemotherapeutic strategies. It is very likely that we have just scratched the surface in terms of defining the extent of interplay between these key growth regulatory proteins.

Keypoints

E2F is a key player in mediating apoptosis in response to oncogenic activation that results in loss of RB.

The ASPP family of proteins (which includes ASPP1 and ASPP2) plays a role in enhancing p53 activity and/or sensitivity to p53-mediated apoptosis and promotes p53-mediated apoptosis rather than growth arrest.

ASPP1, ASPP2, JMY, TP53INP1, and DIP have been identified as E2F target genes.

DIP is a novel E2F target whose ectopic expression results in the accumulation of DIP in mitochondria and p53-independent apoptosis. DIP depletion through RNAi partially rescues the apoptotic effects of enhanced E2F activity.

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