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Editorial

Apoptosis and the cell cycle: the p53 connection

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The p53 gene in its wild-type (wt) form is defined as a tumor suppressor gene. Indeed, mutations in p53 were found to occur in high frequency in most of the common types of human cancer (Hollstein *et al*, 1991). Stabilization and activation of p53 following a variety of signals, such as genotoxic insults, result in the execution of its biological activities. The best characterized activities of p53 are the induction of a cell growth arrest or of apoptosis.

Induction of a growth arrest by p53 was shown to depend on its ability to act as a sequence-specific transcriptional activator (Crook *et al*, 1994, Pietenpol *et al*, 1994). An important target gene in the growth arrest pathway is WAF1/CIP1 (El-Deiry *et al*, 1993; Harper *et al*, 1993). The protein product of this gene, p21, binds to cyclin-dependent kinases and inhibit their action, thereby blocking cell proliferation (Xiong *et al*, 1993). Mice lacking p21 were shown to be defective in the G1 checkpoint control (Brugarolas *et al*, 1995; Deng *et al*, 1995). However, the G1 checkpoint was only partially impaired, indicating that p21 does not play an exclusive role in this pathway.

The mechanism of p53-induced apoptosis is still not well characterized, and it appears that p53 may mediate apoptosis both through transcriptional activation-dependent and independent pathways.

Whether a cell would undergo growth arrest or apoptosis following p53 activation appears to depend on a variety of factors, such as environmental conditions and the cell type. As reviewed by Kasten and Giordano 1998 (see this issue), the loss of the tumor suppressor pRb function may contribute to p53-induced apoptosis: the activity of pRb and/or other pRb-related proteins was shown to be necessary for the induction of a G1 arrest by p53 following DNA damage (Demers *et al*, 1994; Hickman *et al*, 1994; Slebos *et al*, 1994; White *et al*, 1994), and pRb may have a protective effect on p53-induced apoptosis in some cells (Qin *et al*, 1994; Haupt *et al*, 1995) but not in all systems (Hsieh *et al*, 1997). In cells having a functional pRb, induction of p21 would lead to inactivation of cyclin-dependent kinases and therefore to inhibition of pRb phosphorylation. The hypophosphorylated pRb retains transcription factors of the E2F family, which are necessary for the G1/S transition, thus imposing a p53-induced G1 arrest. In the absence of functional pRb, p21 will still be induced by p53 activation but cells will be unable to growth-arrest and may therefore be 'forced' to die through inappropriate cell proliferation signals by entering into S phase (Kasten and Giordano, 1998, see this issue). In this context, deregulated expression of E2F was shown to induce p53-mediated apoptosis (Qin *et al*, 1994; Shan and Lee 1994; Wu and Levine 1994; Almasan *et al*, 1995; Logan *et al*, 1995). E2F1-DP1 complex was reported to bind to and to induce p53, thereby overriding survival factors to induce apoptosis (Hiebert *et al*, 1995; O'Connor *et al*, 1995). Without both Rb and p53, E2F activation would stimulate cell proliferation and permit tumor formation, as was demonstrated by the development of retinal tumors in HPV E7 transgenic mice (Howes *et al*, 1994; Pan and Griep 1994). However, recent publications have demonstrated that E2F-1-induced apoptosis does not require transactivation and DNA synthesis and can occur in the absence of p53 (Hsieh *et al*, 1997; Nip *et al*, 1997; Phillips *et al*, 1997). Thus, the reported repression function of the Rb-E2F-1 complex (Zacksenhaus *et al*, 1996) may play an important role in regulation of apoptosis by these cell cycle proteins. Interestingly, pRb cleavage following caspases activation in several apoptotic pathways was observed (Kasten and Giordano 1998, see this issue, and references therein), suggesting that apoptosis may be incompatible with functional Rb, and linking apoptosis to cell cycle.

Another link between cell death and regulation of cell proliferation is the c-Myc proto-oncogene. c-Myc plays a role as a positive regulator of cellular proliferation, but its activation can also result in apoptosis under certain environmental conditions such as serum deprivation or hypoxia (Evan *et al*, 1992; Graeber *et al*, 1996). Several studies have suggested a role for p53 in c-Myc-induced apoptosis upon serum withdrawal in fibroblasts (Hermeking and Eick, 1994; Wagner *et al*, 1994; Yu *et al*, 1997; Han *et al*, 1997). Using Rat-1 fibroblasts expressing a conditional c-Myc, Rupnow *et al*, 1998 (see this issue) demonstrate that cells expressing antisense p53 are more resistant to c-Myc-induced apoptosis under hypoxic or low serum conditions. In this system, c-Myc activation also sensitized Rat-1 cells to radiation-induced apoptosis, and there again cells expressing antisense p53 were more resistant to apoptosis induced by the combined effect of c-Myc activation and γ-irradiation. Thus, p53 appears to be an important mediator of c-Myc-induced cell death under a variety of environmental stress signals.

The cellular response to p53 activation may depend not only on the cell type and factors such as Rb, as described above, but also on the level of p53 expression. Thus, high levels of p53 expression induced apoptosis while low levels induced cell cycle arrest in Saos-2 and H1299 cells (Chen *et al*, 1996). Using a similar approach of introducing a tetracycline-regulated inducible p53 expression into a p53-null cell line of small cell lung carcinoma (SCLC), Adachi *et*

al, 1998 (see this issue) assessed the effect of the level of p53 expression on these cells. Apoptosis was induced in SCLC cells by high levels of p53 expression, while low expression levels induced a G1 arrest. However, G1-arrested cells underwent apoptosis after further cultivation. In agreement with previous reports in other cell types, p21 was induced by low and high levels of expression of p53 but it does not appear to play a role in p53-induced apoptosis (reviewed by Yonish-Rouach, 1996). Indeed, expression of exogenous p21 induced G1 arrest but not apoptosis in the SCLC cells. On the other hand, high levels of p53 down-regulated Bcl-2 expression in SCLC cells while Bax expression was not modified irrespective of p53 expression level. The authors suggest that p53-mediated apoptosis and G1 arrest depend on the level of p53 expression in SCLC cells, and that the relative high expression of Bax over low expression of Bcl-2 at high level of p53 expression is involved in the induction of apoptosis. It is interesting to note that there is no G1 arrest at high level of p53 expression prior to the induction of apoptosis and concomitantly with down-regulation of Bcl-2. Recent data provided evidence that Bcl-2 is yet another link between cell death and cell cycle regulation, since in addition to its anti-apoptotic function it can also restrain cell cycle entry (O'Reilly *et al*, 1996). Thus, the low expression of Bcl-2 imposed by high level of p53 expression may play a role in escaping a G1 arrest.

Finally, the importance of p53-mediated apoptosis is demonstrated in an *in vivo* study by Reichel *et al*, 1998 (see this issue). Previous experiments have shown that a significant fraction of p53 null mice have developmental abnormalities, including profound neural-tube defects associated with overgrowth of neural tissue, and the affected embryos frequently undergo resorption (Armstrong *et al*, 1995; Sah *et al*, 1995). In the present study, Reichel *et al*, have identified a role for p53-dependent apoptosis in the regression of the hyaloid vasculature and tunica vasculosa lentis, thus providing further evidence for the importance of p53 in normal development. This study also demonstrates for the first time a role for p53 in postnatal development in remodelling of the developing eye.

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