Activation of p53 by oncogenes

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Abstract
p53 is activated by a variety of cellular stresses, including DNA damage, hypoxia, and mitogenic oncogenes, but the extent to which each signal engages p53 as a tumour suppressor remains unknown. In non-immortal cells, the adenovirus E1A oncogene activates p53 to promote apoptosis, whereas oncogenic ras activates p53 to promote cellular senescence. Inactivation of p53 prevents E1A-induced apoptosis or Ras-induced senescence, allowing proliferation to continue unabated. In each instance, the ability of the oncogene to activate p53 involves the same functions as are required for their transforming potential, implying that p53 activation acts as a fail-safe mechanism to counter hyperproliferative signals. Furthermore, p19ARF is strictly required for oncogene signalling to p53. The fact that ARF - itself a tumour suppressor - acts as an intermediary in this response argues that the tumour suppressor activity of p53 can arise from its ability to eliminate oncogene-expressing cells.

Introduction


Oncogenes can also induce p53, leading to increased apoptosis or premature senescence (Lowe & Ruley 1993, Hermeking & Eick 1994, Serrano et al. 1997). For example, the adenovirus E1A oncogene induces p53 and promotes apoptosis in primary cells (Debas & White 1993, Lowe & Ruley 1993, Querido et al. 1997, Samuelson & Lowe 1997), which is reflected by the remarkable ability of E1A to enhance radio- and chemosensitivity (Lowe et al. 1993). Although E1A is a mitogenic oncogene, p53 acts to limit its oncogenic potential. Thus, p53-deficient primary fibroblasts expressing E1A are resistant to apoptosis and become oncogenically transformed (Lowe et al. 1994b). The ability of E1A to activate p53 is not unique, as c-Myc activates p53 to promote apoptosis (Hermeking & Eick 1994) and oncogenic ras induces p53, leading to premature senescence (Serrano et al. 1997). How oncogenic signals activate p53 is unknown, and it is conceivable that they induce p53 by inadvertently damaging DNA. Nevertheless, the general involvement of p53 in the cellular response to oncogenes raises the possibility that these stimuli are relevant triggers of the tumour suppressor activity of p53.

The INK4a/ARF locus is second only to p53 in the frequency of its disruption in human cancer (reviewed by Haber 1997). This locus encodes p16INK4a, a cyclin-dependent kinase inhibitor that acts upstream of retinoblastoma protein (RB) to promote cell-cycle arrest (Serrano et al. 1993). Whereas compelling evidence indicates that p16INK4a is an important tumour suppressor, the INK4a/ARF locus encodes a second protein translated in an alternate reading frame, designated p19ARF (Quelle et al. 1995), p19ARF and p16INK4a are often co-deleted in tumour cells, but mice lacking p19ARF alone are highly susceptible to cancer (Kamijo et al. 1997; reviewed by Haber 1997). p19ARF promotes cell-cycle arrest (Quelle et al. 1995), whereas ARF-null primary mouse embryo fibroblasts (MEFs) do not undergo replicative senescence...
and are transformed by oncogenic ras alone (Kamijo et al. 1997). Thus ARF is a bona fide tumour suppressor.

Several observations suggest that p19ARF may function in a genetic and biochemical pathway that involves p53. At the level of the organism, the consequences of deleting p53 and ARF are remarkably similar (Donehower et al. 1992, Kamijo et al. 1997). In either case, the mutant mouse develops normally, but is highly predisposed to malignant tumours of a similar overall pattern and latency. At the cellular level, enforced expression of p19ARF can induce cell-cycle arrest in cells harbouring wild-type, but not mutant, p53 (Kamijo et al. 1997). In turn, p19ARF can physically associate with p53 itself, with Mdm2, or with both, to alter p53 levels and activity (Kamijo et al. 1998, Zhang et al. 1998). Nevertheless, ARF is not required for the p53 response following DNA damage, because radiation induces G1 arrest in ARF-deficient fibroblasts and apoptosis in ARF-deficient thymocytes (Kamijo et al. 1997, 1998). Thus an understanding of the signals that activate p19ARF may help to explain its role as a tumour suppressor, in addition to that of p53.

**E1A functions that activate p53**

To determine how E1A induces p53 and promotes apoptosis, a series of E1A mutants were introduced into primary human and mouse fibroblasts using high-titre recombinant retroviruses, allowing analysis of E1A in genetically normal cells outside the context of adenovirus infection (Samuelson & Lowe 1997). In primary human and mouse fibroblasts, E1A mutations that prevented p53 accumulation and apoptosis separated into two complementation groups, which correlated precisely with the ability of E1A to associate with either the p300/CRE binding protein (CBP) or RB-related proteins. Furthermore, E1A mutants incapable of binding RB, p107, and p130 induce p53 and promote apoptosis in fibroblasts derived from RB-deficient mice, but not in fibroblasts from mice lacking p107 or p130. Hence, inactivation of RB, but not p107 or p130, is required for p53 accumulation and apoptosis induced by E1A. Interestingly, the regions of E1A that promote apoptosis are precisely those required for the oncogenic potential of E1A (Whyte et al. 1988), suggesting that apoptosis and p53 accumulation are a response to oncogenic ‘stress’ rather than a direct effect of E1A.

**Ras functions that activate p53**

We also examined the Ras signalling pathway(s) responsible for p53 activation and premature senescence of non-immortal human and mouse fibroblasts. Known Ras effectors include Raf-1 and components of the MAPK cascade, phosphoinositide-3-kinase, Akt (protein kinase B), c-Jun-N-terminal kinase, Rac, the Rho proteins, and NF-xB (Ridley et al. 1992, Finco & Baldwin 1993, Van Aelst et al. 1993, Derijard et al. 1994, Franke et al. 1997, Robinson & Cobb 1997, Rodriguez-Viciana et al. 1997). In normal cells, Ras induces premature senescence through activation of the MAPK cascade - the same pathway important for Ras-induced mitogenesis in immortal cells (Lin et al. 1998; see also Zhu et al. 1998). In human fibroblasts, Ras ‘effector loop’ mutants that retain their ability to bind Raf-1 promote premature senescence and, among a series of Ras downstream components examined, only activated Raf-1 and MEK induced p53, p16, and features of senescence. Moreover, a MEK inhibitor (PD98059) prevents Ras-induced cell-cycle arrest and senescence. In primary murine fibroblasts, activated MEK arrested wild-type MEFs, but forced uncontrolled mitogenesis and transformation when expressed at comparable levels in p53−/− or INK4aΔ16/Δ16 MEFs. The precisely opposite response of normal and functionally immortal cells to constitutive mitogen-activated protein kinase (MAPK) activation implies that premature senescence acts as a fail-safe mechanism to limit the transforming potential of excessive Ras mitogenic signalling. Thus constitutive MAPK signalling activates p53 and p16 as tumour suppressors.

**Oncogene signalling to p53 involves p19ARF**

Using MEFs derived from wild-type, ARF−/−, and p53−/− mice, we investigated whether oncogene signalling to p53 involves p19ARF. E1A induces p19ARF mRNA and protein, and the ability of E1A to induce p53 and its transcriptional targets is severely compromised in ARF-null cells (de Stanchina et al. 1998). ARF−/− cells expressing E1A are resistant to apoptosis, albeit to a lesser extent than their p53-null counterparts. Re-introduction of p19ARF restores p53 accumulation and resensitizes ARF-null cells to apoptotic signals. Like E1A, oncogenic ras induces p19ARF in wild-type cells. Moreover, Ras is unable to arrest ARF−/− cells, which continue to grow at rates similar to p53−/− and INK4aΔ16/Δ16 MEFs. Ras-induced p53 transcriptional targets are induced in wild-type, but not ARF−/− cells (unpublished observations). Thus ARF is required for p53 activation and cell-cycle arrest induced by oncogenic Ras. Our data, together with those of others (Bates et al. 1998, Palmero et al. 1998, de Stanchina et al. 1998, Zindy et al. 1998) argue that ARF mediates p53 activation by oncogenes. The ARF-p53 tumour suppressor pathway

Although p53 integrates signals from multiple stress-induced pathways, its action can, in turn, lead to several
possible anti-proliferative responses, depending upon cellular context. For example, enforced ARF expression in MEFs induces cell-cycle arrest, but cells overexpressing p19ARF together with E1A or Myc undergo apoptosis (de Stanchina et al. 1998, Zindy et al. 1998), which is potentiated by withdrawal of serum survival factors (Evan et al. 1992, Lowe & Ruley 1993, Lowe et al. 1994a). ARF-null MEFs are resistant to both E1A- and Myc-induced apoptosis and escape Ras-induced senescence, bypassing the p53-dependent fail-safe mechanism that normally protects them from these oncogenic signals, and thereby enabling mitogenic oncogenes to function as pure growth promoters.

Like p53, ARF has no overt role in normal cell cycle control or development; hence, the physiological circumstances in which it might become activated to inhibit proliferation or suppress tumour growth are not obvious. Our studies suggest that p19ARF can be activated to suppress proliferation by the E1A oncogene through mechanisms that correlate with its binding to both p300/CBP and RB (de Stanchina et al. 1998). These same functions are required for E1A to induce p53 and to promote apoptosis in primary fibroblasts (Samuelson & Lowe 1997) and, remarkably, are also required for the transforming potential of E1A (Whyte et al. 1988, 1989).

Loss of RB greatly increases ARF expression, consistent with the possibility that ARF is an E2F-responsive gene (DeGregori et al. 1997). Indeed, enforced expression of E2F-1 induces p19ARF and, conversely, ARF-null cells are resistant to E2F-1-induced apoptosis (Bates et al. 1998, Zindy et al. 1998). In contrast, Ras induces ARF through the MAPK cascade (unpublished observations), but whether this acts as a direct or indirect effect remains to be established. In any case, p19ARF function, like that of p53, depends upon the mutational status of RB, and upon both c-myc and ras proto-oncogene activities. Irrespective of the precise outcome, ARF mutations compromise p53 activation and reduce its ability to counter uncontrolled proliferation.

Our studies provide additional insights into the role of p53 in tumour suppression. The predominant view of p53 action centres around its ability to function in the cellular response to DNA damage. Although this stimulus is undoubtedly important for the tumour suppressor activity of p53 and may contribute to the outcome of cancer therapy (Lowe et al. 1993, 1994a), p53 activation in response to oncogenes provides an alternative pressure to mutate p53 during tumorigenesis (Lowe et al. 1994b, Symonds et al. 1994). In this view, p53 normally acts to limit the consequences of uncontrolled mitogenesis by promoting cell-cycle arrest or apoptosis, whereas its loss allows proliferation to continue unabated. The fact that disruption of the ARF-p53 pathway occurs in the vast majority of human cancers underscores its global importance in suppressing proliferation of oncogene-expressing cells.

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