PERSPECTIVE

Tumor surveillance via the ARF–p53 pathway

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The retinoblastoma (*Rb*) and *p53* genes are not essential for completion of the cell division cycle, but disruption of their functions is central to the life history of most, if not all, cancer cells (for review, see Weinberg 1995; Sherr 1996; Levine 1997). Surprisingly, Rb and p53 are themselves regulated by two proteins encoded by a single genetic locus, *INK4a/ARF*, the products of which, $p16^{INK4a}$ and $p19^{ARF}$, are also potent tumor suppressors. The role of $p16^{INK4a}$ as an inhibitor of cyclin D-dependent kinases has been appreciated since its discovery (Serrano et al. 1993). Now, emerging evidence is providing valuable insights into the molecular circuitry through which $p19^{ARF}$ modulates p53 activity as part of a checkpoint response to oncogenic, hyperproliferative signals.

Regulation of cell cycle progression by pRb and p53

During most of G_1 phase of the mammalian cell cycle, Rb in its hypophosphorylated form binds to several transcription factors of the E2F family, constraining their activity on some promoters and actively repressing transcription from others (see Dyson 1998). Phosphorylation of Rb by cyclin-dependent kinases (cdks) in the mid-to-late G_1 phase of the cycle untethers Rb from the E2Fs. In turn, this enables the E2Fs to activate a series of target genes, the expression of which is required for cells to enter S phase, thereby stimulating proliferation (Fig. 1). The cyclin D-dependent kinases cdk4 and cdk6 trigger Rb phosphorylation, which is likely completed by cyclin E–cdk2 as cells approach the G_1-to-S phase transition. Because induction and assembly of cyclin D-dependent kinases is dependent on mitogenic signaling, cancellation of Rb's growth-suppressive activity is coupled to extracellular stimuli. By inhibiting cdk4 and cdk6, a family of INK4 proteins can prevent cells with functional Rb from entering S phase. The prototypic member, $p16^{INK4a}$ (Serrano et al. 1993), is distinguished from its close relatives ($p15^{INK4b}$, $p18^{INK4c}$, and $p19^{INK4d}$) in its role as a potent tumor suppressor. Disruption of the $p16^{INK4a}$–cyclin D1/cdk4–Rb pathway is a common event in human cancer, either resulting from loss of function of one of the two negative regulators ($p16^{INK4a}$ or Rb) or from events leading to overexpression of one of the two proto-oncogenes (cyclin D1 or cdk4) (for review, see Weinberg 1995; Sherr 1996; Ruas and Peters 1998).

The p53 protein is a transcription factor that can inhibit cell cycle progression or induce apoptosis in response to stress or DNA damage, and inactivation of p53 attenuates both of these cellular responses (for review, see Ko and Prives 1996; Levine 1997; Giaccia and Kastan 1998). Elimination of functional p53 through various mechanisms is the single most common event in human cancer, occurring in over half of all tumors (Hollstein et al. 1994). The p53 protein is short-lived and expressed at very low levels in normal cells but it is stabilized and accumulates in cells that have sustained genotoxic damage (Fig. 1). Among the gene products induced by p53 is the cdk inhibitor $p21^{Cip1/Waf1}$, which can effect cell cycle arrest (El-Deiry et al. 1993; Harper et al. 1993; Xiong et al. 1993). Another key target is Mdm2, which acts in a feedback loop to limit the action of p53 (Barak et al. 1993; Wu et al. 1993), both by inhibiting its transactivating activity and by catalyzing its destruction (Haupt et al. 1997; Honda et al. 1997; Kubbutat et al. 1997). Mutation of p53 compromises cell cycle arrest, attenuates apoptosis induced by DNA damage, predisposes cells to drug-induced gene amplification, affects centrosome duplication, and rapidly leads to changes in chromosome number and ploidy (Kastan et al. 1991, 1992; Kuerbitz et al. 1992; Livingstone et al. 1992; Yin et al. 1992; Clarke et al. 1993; Lowe and Ruley 1993; Lowe et al. 1993; Fukusawa et al. 1996; Jacks and Weinberg 1996; Hermeking et al. 1997; Paulovich et al. 1997; Gualberto et al. 1998; Lanni and Jacks 1998). The resulting genomic instability greatly increases the probability that p53-null cells will evolve toward malignancy.

Cooperation between the Rb and p53 pathways has been amply demonstrated. Classic examples involve oncoproteins encoded by the DNA tumor viruses, which both cancel Rb function to drive cells into S phase and neutralize p53 to prevent host cell suicide (for review, see White 1996). Loss of function by Rb and related family members can bypass p53-mediated G_1 arrest (Demers et al. 1994; Slebos et al. 1994), but Rb loss induces E2F and p53-dependent apoptosis (Lowe and Ruley 1993; Howes et al. 1994; Morgenbesser et al. 1994; Pan and

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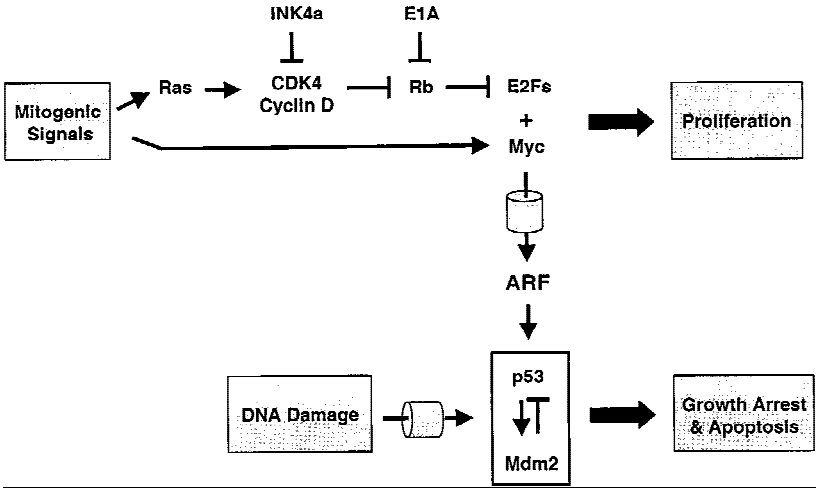

Figure 1. ARF checkpoint control. *ARF* responds to proliferative signals that are normally required for cell proliferation. When these signals exceed a critical threshold, the ARF-dependent checkpoint (gray vertical barrel) is activated, and ARF triggers a p53-dependent response that induces growth arrest and/or apoptosis. Signals now known to induce signaling via the ARF–p53 pathway include Myc, E1A, and E2F-1. In principle, 'upstream' oncoproteins, such as products of mutated Ras alleles, constitutively activated receptors, or cytoplasmic signal transducing oncoproteins, might also trigger ARF activity via the cyclin D–cdk4–Rb–E2F or Myc-dependent pathways, both of which are normally necessary for S-phase entry. In inhibiting cyclin D-dependent kinases, $p16^{INK4a}$ can dampen the activity of mitogenic signals. E1A is shown to work, at least in part, by canceling Rb function, although its ability to inhibit p300 contributes to the response by interfering with *mdm2* expression. Again for simplicity, Myc and E2F-1 are only shown to activate p53 via ARF. However, highly overexpressed levels of these proteins can activate p53 in *ARF*-negative cells, albeit with an attenuated efficiency. ARF activation of p53 likely depends on inactivation of some Mdm2-specific function (implied by the unfilled box bracketing the latter two proteins). DNA damage signals (ionizing and UV radiation, hypoxic stress, genotoxic drugs, etc.) access p53 through multiple signaling pathways shown, again for simplicity, as a single DNA damage checkpoint (gray horizontal barrel). Signals through the ARF and DNA damage pathways can synergize in activating p53.

Griep 1994; Qin et al. 1994; Shan et al. 1994; Symonds et al. 1994; Wu and Levine 1994). In short, mutational events that disable the $p16^{INK4a}$–cyclin D1/cdk4–Rb pathway and enforce cell proliferation are counterbalanced by a p53-dependent apoptotic response that can eliminate incipient cancer cells. The ability of E2F to trigger p53-dependent cell suicide implies that a biochemical connection links their functions. Other cellular oncogenes, such as *myc*, also induce p53-dependent apoptosis (Hermeking and Eick 1994; Wagner et al. 1994). Hence, p53 is not only activated by DNA damage, but it provides an 'oncogene checkpoint' function that guards cells against hyperproliferative signals (for review, see Van Dyke 1994; Jacks and Weinberg 1996; White 1996; Levine 1997). This is the setting in which $p19^{ARF}$ plays a key role.

The *INK4a/ARF* locus and tumor suppression

The manner by which a single genetic locus encodes both $p16^{INK4a}$ and $p19^{ARF}$ is unprecedented in mammals (Quelle et al. 1995) (Fig. 2). $p16^{INK4a}$ is encoded by three closely linked exons (designated 1α, 2, and 3). An RNA segment arising from an alternative first exon (1β), which maps 13–20 kb upstream in the human, mouse, and rat genomes, is spliced to exon 2, yielding a β transcript that is almost identical in size to the α transcript that encodes $p16^{INK4a}$ (Duro et al. 1995; Mao et al. 1995; Quelle et al. 1995; Stone et al. 1995; Swafford et al. 1997). The initiator codon in exon 1β is not in frame with sequences encoding $p16^{INK4a}$ in exon 2, so the β transcript specifies a novel polypeptide. In the mouse, this 19-kD protein consists of 65 amino acids encoded by exon 1β, and 105 amino acids arising from the alternative reading frame (ARF) of exon 2 (Quelle et al. 1995). The human protein terminates farther upstream in exon 2 and it has a predicted molecular mass of only 14 kD. Mouse $p19^{ARF}$ and human $p14^{ARF}$ are highly basic nuclear proteins that induce G_1- and G_2-phase arrest when introduced into a variety of different cell types (Quelle et al. 1995; Stott et al. 1998). *INK4a/ARF*-null cells are susceptible to ARF-induced arrest, so this activity of $p19^{ARF}$ does not depend upon $p16^{INK4a}$.

Mutations that inactivate the cdk inhibitory function of $p16^{INK4a}$ occur frequently in a wide spectrum of human cancers (for review, see Ruas and Peters 1998). For example, certain inactivating point mutations impinge on exon 1α, some of which are inherited in melanoma kindreds (Kamb et al. 1994a,b; Gruis et al. 1995). Although many point mutations in exon 2 of *INK4a* are also predicted to alter $p19^{ARF}$, those that have been tested experimentally have been found to inactivate $p16^{INK4a}$ without affecting the ability of $p19^{ARF}$ to induce cell cycle arrest. Moreover, the amino-terminal moiety of ARF (amino acids 1–64), encoded entirely by exon 1β, is sufficient to induce cell cycle arrest when overexpressed (Quelle et al. 1997; Zhang et al. 1998), although tumor-specific point mutations in this domain have not been described (Stone et al. 1995; Ruas and Peters 1998). Together, these data suggest that $p16^{INK4a}$ is disrupted frequently by point mutations in human cancer, but $p19^{ARF}$ is not. However, the common occurrence of homozygous deletions of *INK4a/ARF* in a wide range of human tumors leaves open the possibility that *ARF* plays an independent role as a tumor suppressor (see below).

Functional ablation of *INK4a/ARF* in mice by elimination of exons 2 and 3 (Fig. 2) revealed that derived

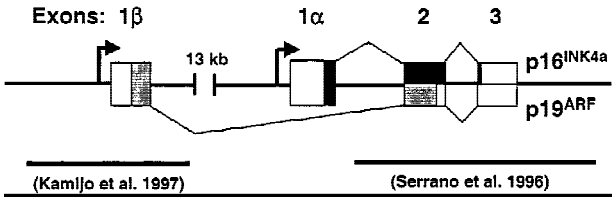

Figure 2. The *INK4a/ARF* locus. Genomic sequences encoding $p16^{INK4a}$ are defined by completely filled regions within the boxes designating exons 1α, 2, and 3, whereas the segments of exons 1β and 2 that encode ARF are defined by shaded areas. Unfilled portions of the exons correspond to noncoding 5′ and 3′ regions. Splicing between the exons is indicated by the connecting lines, and exons 1α and 1β are indicated to have separate promoters (→). In the mouse genome, the alternative first exons are separated by ~13 kb of intervening sequences. Segments of the genes that were disrupted by Serrano et al. (1996) and Kamijo et al. (1997) are designated by horizontal lines below the schematic.

nullizygous animals were highly prone to tumor development (Serrano et al. 1996). Tumors arose early in life, and their appearance was accelerated by irradiation of newborn mice or by their treatment with chemical carcinogens. Intriguingly, mouse embryo fibroblasts (MEFs) explanted from the *INK4a/ARF* knock-out mice did not undergo replicative senescence in culture. Like many established mouse cell lines, but unlike normal primary MEFs, they could be transformed by oncogenic *ras* alleles without a requirement for so-called immortalizing oncogenes such as *myc* or adenovirus *E1A*. MEFs from *p53*-null mice exhibit similar properties (Harvey et al. 1993), and p53-inactivating mutations are the most common single events in the spontaneous conversion of MEF strains into continuously growing cell lines (Harvey and Levine 1991). Results with both *INK4a/ARF*-null or *p53*-null MEFs directly contrast with those obtained with normal primary MEF strains, in which introduction of oncogenic *ras* instead provokes a state of growth arrest resembling senescence, associated with accumulation of both p53 and $p16^{INK4a}$ (Serrano et al. 1997). Initially, it was reasoned that the phenotype observed in *INK4a/ARF*-null mice depended on the loss of $p16^{INK4a}$ function (Serrano et al. 1996). It followed that both $p16^{INK4a}$ and p53 acted as determinants of cell senescence in MEFs, with the loss of either leading to establishment and immortalization. Release of the senescence block by disruption of $p16^{INK4a}$ or p53 would be necessary for transformation of mouse fibroblasts by oncogenic *ras* (Serrano et al. 1997; for review, see Weinberg 1997). A persistent ambiguity is whether these mice lack *ARF* function completely. This is likely, because the targeting cassette disrupted the mRNA polyadenylation signals as well as the *INK4a* and *ARF* carboxy-terminal coding equences (Fig. 2). However, the issue formally remains unresolved, because it is conceivable that a truncated ARF protein might somehow arise from undisrupted exon 1β.

Surprisingly, when pure *ARF*-null mice were created that lacked only the exon 1β sequences (Fig. 2), their phenotype was indistinguishable from that attributed previously to $p16^{INK4a}$ disruption (Kamijo et al. 1997). Importantly, functional $p16^{INK4a}$ was expressed in normal tissues of *ARF*-null mice, in cultured MEFs, and in cells from spontaneously arising tumors. Therefore, *ARF* functions as a bona fide tumor suppressor, and the phenotype initially ascribed to INK4a loss is instead likely due to ARF inactivation. In turn, the phenotypic consequences of $p16^{INK4a}$ loss in mice remain uncertain, and construction of a pure *INK4a* knockout strain is warranted.

The ARF–p53 pathway

A cardinal feature of *ARF*-null MEFs is their capacity to grow as established cell lines and to be transformed by oncogenic *ras* genes alone (Kamijo et al. 1997). Approximately 20% of spontaneously established fibroblast cell lines derived from MEFs of wild-type mice undergo biallelic *ARF* loss. MEF strains that are hemizygous for *ARF* lose their remaining functional *ARF* allele and spontaneously immortalize at a faster rate than wild-type strains. In each case, established MEF cell lines that lacked *ARF* preserved p53 function, whereas those that retained *ARF* had sustained p53 mutations. These results suggested that $p19^{ARF}$ and p53 might function in the same biochemical pathway. Consistent with this hypothesis, cells lacking a functional *p53* gene are resistant to $p19^{ARF}$-induced cell cycle arrest, implying that p53 acts downstream of *ARF* (Kamijo et al. 1997). However, *ARF*-null cells exhibit an intact p53 checkpoint following ionizing or UV irradiation, so $p19^{ARF}$ does not relay signals to p53 in response to DNA damage (Fig. 1). Loss of p53 can occur in cancer cells that arise in *ARF*-null mice, again indicating that ARF plays a more specialized role in tumor suppression than p53, and that selection against p53 can further contribute to malignancy (Kamijo et al. 1997).

Evidence supporting direct biochemical interactions between $p19^{ARF}$ and p53 is now in hand. Ectopic ARF expression stabilizes p53 and induces p53-responsive genes, Mdm2 among them. ARF can physically interact with Mdm2, and its binding blocks both Mdm2-induced p53 degradation and transactivational silencing (Kamijo et al. 1998; Pomerantz et al. 1998; Stott et al. 1998; Zhang et al. 1998). The interaction between Mdm2 and $p19^{ARF}$ depends on the carboxy-terminal half of Mdm2 and on the ARF amino-terminus (i.e., the active exon 1β-coded segment) (Zhang et al. 1998). Because Mdm2 binds to p53 through its amino-terminal domain, ARF can enter into ternary complexes with both Mdm2 and p53.

Although human $p14^{ARF}$ appears not to interact with p53 directly (Pomerantz et al. 1998; Stott et al. 1998; Zhang et al. 1998), there is some evidence that the mouse ARF protein can bind to p53 even in the absence of Mdm2 (Kamijo et al. 1998). For example, in electrophoretic mobility shift assays performed with purified, activated p53 and a labeled oligonucleotide containing tandem p53 consensus DNA-binding sites from the

p21^{Cip1} promoter, addition of recombinant p19^{ARF} retarded the mobility of the p53–oligonucleotide complexes. In these assays, the otherwise latent DNA-binding capability of p53 needed to be activated by antibodies directed to a carboxy-terminal p53 epitope, and p19^{ARF} was unable to substitute for the antibody in activating DNA binding. These observations raise the possibility that interactions between p19^{ARF} and p53 can occur on chromatin, although there is no direct evidence that ARF plays any physiologic role as a p53 coactivator.

ARF requires p53 to induce growth arrest, but the direct physical interactions among p19^{ARF}, p53, and Mdm2 in various binary and ternary complexes suggest that some p53 functions may reciprocally depend on ARF. Overexpression of p19^{ARF} in *ARF*-null NIH-3T3 cells induced expression of a *p21*^{Cip1} promoter-driven reporter gene in a manner that depended on endogenous p53. Paradoxically, ectopic overexpression of wild-type p53 itself in *ARF*-null cells did not activate the reporter, indicating that simple increases in the amount of p53 were insufficient to activate transcription in this setting. p53-Dependent reporter gene expression was restored when subliminal amounts of *ARF* expression vector were reintroduced together with increasing concentrations of *p53*, so p19^{ARF} can provide some type of activating signal that facilitates p53-dependent transcription (Kamijo et al. 1998). In this respect, the functions of p53 and p19^{ARF} are interdependent.

Zhang and colleagues (1998) reported that ARF accelerated Mdm2 turnover in HeLa cells cotransfected with vectors encoding ARF and Mdm2. They proposed that destabilization of Mdm2 by ARF was the mechanism underlying p53 accumulation. However, experiments by others have yielded conflicting results. The idea that ARF destabilizes Mdm2 seems to be at odds with observations that ARF activation in MEFs induces endogenous Mdm2 to accumulate in a p53-dependent manner (de Stanchina et al. 1998; Kamijo et al. 1998; Zindy et al. 1998). Stott and coworkers (1998) confirmed that in a variety of cell types cotransfected with Mdm2 and p53, introduction of ARF overcame the ability of Mdm2 to induce p53 degradation. However, in the presence or absence of exogenous p53, ARF caused Mdm2 to accumulate. Moreover, coexpression of the E6 protein of human papilloma virus 16, which independently targets p53 for degradation, did not interfere with the ability of ARF to stabilize cotransfected Mdm2. Minimally, it seems reasonable to conclude that ARF can antagonize Mdm2 function through a mechanism that does not depend on increased Mdm2 turnover.

How, then, does ARF stabilize p53? One possibility is that ARF interferes with Mdm2's ability to trigger p53 polyubiquitination. Supporting this idea, Mdm2 seems to induce the appearance of polyubiquitinated forms of p53, which are much less abundant in cells that overexpress p19^{ARF} (Pomerantz et al. 1998). Mdm2 and p19^{ARF} also colocalize in the nucleoli of cells transfected with both genes (Pomerantz et al. 1998). Because p53 degradation depends upon its Mdm2-mediated nuclear export (Roth et al. 1998), ARF could conceivably retain Mdm2–p53 complexes in the nucleolus, preventing their degradation in the cytoplasm.

Finally, in cells lacking p53, ARF levels are significantly elevated (Quelle et al. 1995; Stott et al. 1998), but reintroduction of wild-type p53 into *p53*-null MEFs can restore p19^{ARF} to normal levels (Kamijo et al. 1998). Similarly, in human Saos-2 osteosarcoma cells lacking endogenous p53 function, expression of p14^{ARF} was down-regulated when cells were induced to express either tetracycline-regulated or temperature-sensitive p53 (Stott et al. 1998). Therefore, not only can ARF stabilize p53, but ARF expression is in turn controlled by p53 through negative feedback. Again, the underlying mechanism needs to be clarified. Among the possibilities is that the *ARF* gene might be repressed by p53, or the ARF protein could itself be a target of Mdm2-induced turnover.

Oncogenic signals induce ARF

The fact that ARF-null MEFs grow as established cell lines and can be transformed by oncogenic *ras* mimics effects induced by so-called immortalizing oncogenes, like *myc* and *E1A* (Land et al. 1983; Ruley 1983). It therefore seems paradoxical that *myc* and E1A are also potent inducers of apoptosis (Askew et al. 1991; White et al. 1991; Evan et al. 1992; Rao et al. 1992), a process aggravated by depriving MEFs of serum survival factors (Evan et al. 1992; Lowe et al. 1993). These contrasting outcomes of Myc and E1A action—extended life versus accelerated death—can be reconciled by observations that their overexpression provides a strong selective pressure for events that dismantle apoptotic signaling pathways, with *ARF* being a key target.

Overexpression of Myc, E1A, or E2F-1 in primary MEFs rapidly induces *ARF* gene expression and leads to p53-dependent apoptosis. However, *ARF*-null and *p53*-null MEFs resist these effects (de Stanchina et al. 1998; Zindy et al. 1998). Similarly, wild-type or *ARF* hemizygous MEFs that survive Myc overexpression generally sustain either p53 mutation or *ARF* loss, but not both, rapidly yielding established cell lines that tolerate supraphysiologic Myc levels even in the absence of survival factors (Zindy et al. 1998). Myc and E1A can induce p53 through both *ARF*-dependent and *ARF*-independent pathways, but much higher levels of oncoprotein expression are required to activate p53 when *ARF* is absent. Under the latter conditions, the p53 response is attenuated and cells resistant to oncogene-induced killing rapidly emerge. Reintroduction of p19^{ARF} into surviving *ARF*-null cells expressing either Myc or E1A resensitizes them to apoptosis, indicating that the attenuation of death is a direct consequence of *ARF* loss and does not result from other cryptic mutations. Therefore, Myc, E1A, and E2F-1 trigger a p53-dependent oncogene checkpoint gated by ARF (Fig. 1). Although the ARF–p53 pathway is not essential for normal proliferation, the checkpoint could provide a fail-safe function during embryonic development. For example, in a model of the developing

murine lens, Rb deficiency triggers apoptosis in a p53-dependent manner (Morgenbesser et al. 1994), but the process is attenuated in lenses from animals lacking *INK4a/ARF* (Pomerantz et al. 1998).

One component of the E1A response involves its ability to activate p300, a coactivator required for p53-dependent *mdm2* transcription (Thomas and White 1998). But the ability of E1A to induce ARF in MEFs is likely mediated by the E2Fs, as E1A mutants that bind p300 but do not interact with Rb are highly defective in this regard (de Stanchina et al. 1998) (Fig. 1). Conditional expression of E2F-1 in Saos-2 cells was followed temporally by increased *ARF* mRNA and protein expression (Bates et al. 1998). Cotransfection experiments indicated that wild-type E2F-1 activated transcription from a minimal *ARF* promoter, whereas an E2F-1 mutant defective in transactivation was devoid of activity. Despite the fact that Myc also induces $p19^{ARF}$ to accumulate very rapidly (Zindy et al. 1998), it is presently unclear whether Myc activates the *ARF* promoter directly.

Cooperation between *myc* and oncogenic *ras* (Land et al. 1983; Ruley 1983) can be viewed to involve the ARF–p53 pathway indirectly. Cultured MEFs achieve replicative immortality by inactivating *ARF* or *p53*, and by promoting cell death, oncogenes such as *E1A* and *myc* provide a strong selective pressure for disabling ARF or p53 function. Because enforced expression of $p19^{ARF}$ arrests wild-type MEFs but does not kill them (Quelle et al. 1995), other functions of Myc and E1A in addition to *ARF* induction are required for this process. The growth promoting properties of Myc and E1A are important because without them, the selection for immortal cells would likely not occur. This is even more obvious in other cell types in which transformation and tumorigenesis strongly depend upon Myc's growth promoting functions even in the absence of *p53* (see, for example, Metz et al. 1995). In turn, Myc and E1A seem to inactivate cellular responses that are normally required for Ras-mediated inhibition of cell proliferation, thereby converting *ras* into a growth-promoting gene (Franza et al. 1986; Hicks et al. 1991; Hirakawa et al. 1991; Lloyd et al. 1997; Serrano et al. 1997). The fact that oncogenic *ras* alone can transform MEFs lacking *ARF* or *p53* argues that their inactivation is key.

Because $p19^{ARF}$ addresses p53 through a pathway that is distinct from those activated by DNA damage (Fig. 1), induction of *ARF* by oncogenes may sensitize cells to the effects of genotoxic drugs that are used to treat cancer. Indeed, MEFs expressing E1A are significantly more sensitive to killing by adriamycin than their normal counterparts, whereas E1A-expressing *ARF*-null MEFs no longer manifest this synergy (de Stanchina et al. 1998). The ability of ARF to sense hyperproliferative stimuli must be important in tumor surveillance, because *ARF* loss strongly predisposes to spontaneous cancer development and accelerates the frequency of tumor induction by irradiation or carcinogens (Serrano et al. 1996; Kamijo et al. 1997). Indeed, in the absence of *ARF* emergence of *p53*-negative tumor cells that are resistant to DNA damaging agents should still occur (Fig. 1).

ARF in human cancer

Much of the experimental work on *ARF* to date has involved murine systems. Senescence (and conversely, immortalization) of human cells is likely to be subject to additional and more stringent controls, particularly in light of our longer life span. Whereas p53 and Rb inactivation can endow human fibroblasts with increased proliferative potential, cells lacking these functions are not immortal, and chromosomal telomere shortening soon limits continued cell proliferation (Bodnar et al. 1998). In contrast, mouse chromosomes have much longer telomeres, and mice lacking telomerase activity must be bred through many generations before the deleterious effects of telomere shortening are manifest (Blasco et al. 1997; Lee et al. 1998).

Despite fundamental differences of this type, *ARF* is likely to function as a tumor suppressor in humans. Certain cancers such as melanomas, biliary tumors, non-small cell lung carcinomas, pancreatic, and esophageal carcinomas frequently sustain *INK4a* point mutations. Other tumor types, however, such as T- and B-cell acute lymphoblastic leukemias, bladder and nasopharyngeal carcinomas, mesotheliomas, anaplastic astrocytomas, and glioblastoma multiforme routinely exhibit *INK4a/ARF* deletions rather than point mutations (Ruas and Peters 1998). Whether or not these homozygous deletions target both *ARF* and *INK4a* or *ARF* alone, their high frequency of occurrence strongly argues that *ARF* loss contributes significantly to human cancer. This makes good sense. If p53 is directly targeted in >50% of human malignancies, then p53-positive tumors have likely sustained epistatic mutations such as *Mdm2* amplification or *ARF* loss. The concept that ARF monitors proliferative signals rather than DNA damage helps to expand our understanding of p53 action and provides a further rationale for *ARF* inactivation through chromosomal deletion in many forms of cancer.

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