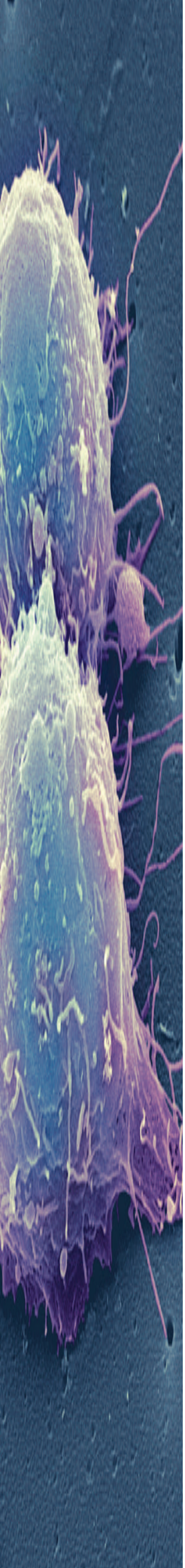

Intrinsic tumour suppression

Scott W. Lowe[1], Enrique Cepero[1] & Gerard Evan[2]

[1]*Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, New York 11724, USA*
[2]*Cancer Research Institute, University of California at San Francisco, San Francisco, California 94143, USA (e-mail: lowe@cshl.edu)*

Mutations that drive uncontrolled cell-cycle progression are requisite events in tumorigenesis. But evolution has installed in the proliferative programmes of mammalian cells a variety of innate tumour-suppressive mechanisms that trigger apoptosis or senescence, should proliferation become aberrant. These contingent processes rely on a series of sensors and transducers that act in a coordinated network to target the machinery responsible for apoptosis and cell-cycle arrest at different points. Although oncogenic mutations that disable such networks can have profound and varied effects on tumour evolution, they may leave intact latent tumour-suppressive potential that can be harnessed therapeutically.

Cancers arise by an evolutionary process as somatic cells mutate and escape the restraints that normally rein in their untoward expansion. Suppressing the emergence of such autonomous cells is an evolutionary imperative of metazoans, particularly in large, long-lived organisms where cells in regenerative tissues retain the potential for neoplastic havoc throughout life. Consequently, multiple mechanisms have arisen to forestall uncontrolled cell division. Some of these are devices within the cell, such as those that limit cell-cycle progression, whereas others are social signals that prompt a cell to remain within its supportive microenvironment. In combination, these tumour-suppressing mechanisms are remarkably effective; on average, cancers arise less than once in a human lifetime, despite trillions of potential target cells, each harbouring hundreds of susceptible cancer-causing genes, all subject to a significant mutation rate. Yet more remarkable is the fact that our tumour-defence systems can discriminate between neoplastic (abnormally growing) and normal cellular states and efficiently quell the former without suppressing the latter.

Insight into the mechanisms that constrain neoplastic progression has come from the realization that many, perhaps all, networks that drive cell proliferation harbour intrinsic growth-suppressive properties. Such innate inhibitory functions obscure any immediate selective advantage that mutations in such pathways might otherwise confer. Because no single pathway confers a net growth advantage, any proto-cancer cell acquiring any single oncogenic mutation is effectively trapped in an evolutionary cul-de-sac. By contrast in normal cells, coordinated extracellular cues activate multiple pathways in concert. In this way, the inherent growth-suppressive activity of each pathway is gated by another, thereby unlocking the cell's proliferative potential (Fig. 1). The nature of the coupling of growth-inhibitory programmes to proliferative networks, and its implications for understanding the evolution and treatment of cancers, are the focus of this review.

Oncogene-induced apoptosis

Cell proliferation and cell death are such diametrically opposed cellular fates that the discovery that the two are linked and interdependent processes was a great surprise[1,2]. There is little mechanistic overlap between the machineries driving proliferation and apoptosis. Rather, the two processes are coupled at various levels through the individual molecular players responsible for orchestrating cell expansion. Importantly, the same players are often targets for oncogenic mutations, and in many instances, mutations that drive proliferation cooperate with those that uncouple proliferation from apoptosis during transformation and tumorigenesis[2,3]. But, although the phenomenon of oncogene-induced apoptosis is now generally accepted as an innate tumour-suppressive mechanism, we have only recently begun to glimpse the diversity and complexity of mechanisms by which oncogenic lesions engage the cell suicide machinery.

At least two distinct general programmes trigger apoptosis, each regulated at many levels (Fig. 2). The 'intrinsic' pathway is the primary death programme responsive to the signals of survival factors, cell stress and injury[4–6]. The central conduit of this pathway is the mitochondrion, the intermembrane space of which sequesters a variety of pro-apoptotic effectors that, when released, trigger cellular demise. Mitochondrial permeability is, in turn, determined by the balance between the pro-apoptotic Bax/Bak proteins and their anti-apoptotic Bcl2/BclXL cousins. The activity of these proteins are positively or negatively regulated by the various BH3-only members (Bcl2 family members that contain a single Bcl2 homology-3 domain), each acting as the terminal effector of distinct signalling pathways. According to this simple model, apoptosis occurs when the protective Bcl2/BclXL buffer is breached by the sum of all the active BH3-only proteins, resulting in the net dominance of the pro-apoptotic Bax/Bak proteins, which then permeabilize the mitochondria to release pro-apoptotic factors. One such factor, cytochrome *c*, acts together with

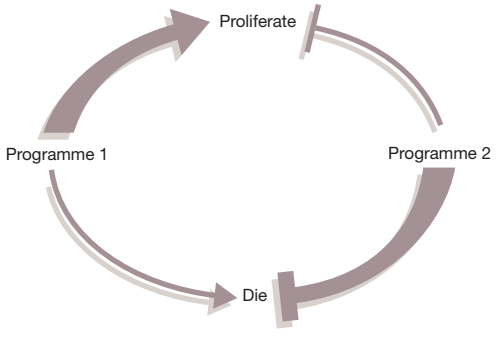

Figure 1 Example of an obligate combinatorial signalling network. Programme 1 drives proliferation and apoptosis, and Programme 2 blocks both. For each cell fate, dominant components are shown as thick lines. Concerted activation of both programmes together leads to cell expansion because Programme 1 overcomes the growth inhibition of Programme 2, and Programme 2 overcomes the lethality of Programme 1. However, activation of either programme on its own triggers cell-death (Programme 1) or senescence (Programme 2).

©2004 Nature Publishing Group

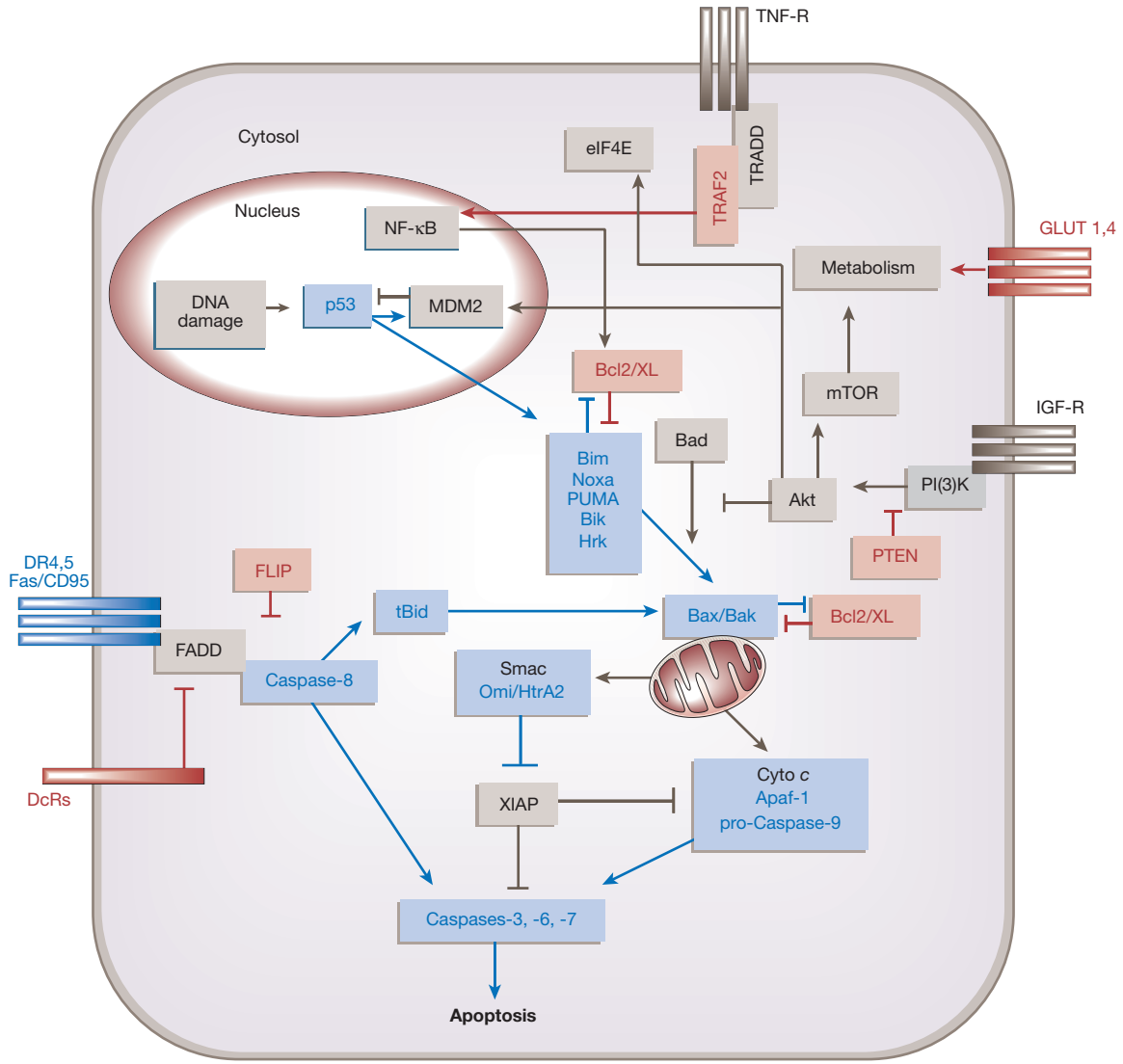

Figure 2 Oncogenic signalling targets many levels of the apoptotic machinery. Shown are key components of the extrinsic and intrinsic apoptotic programmes, as well as some key regulators. Such a network organization allows the cell to sense many aspects of the intracellular and extracellular milieu, yet ensures that cell death proceeds efficiently once activated. Excessive oncogenic signalling is coupled to apoptosis by a complex mechanism that targets key control points in the pathways. Components highlighted in red can be downregulated by pro-apoptotic oncogenes, whereas components highlighted in blue are often upregulated.

the cell-death adaptor Apaf-1 to trigger the activation of caspase-9, a cysteine protease that initiates a downstream proteolytic cascade that also involves caspase-3 and caspase-7. Once activated, caspases cleave proteins important for cell and genome integrity, orchestrating the orderly death and engulfment of the cell. Regulation of the intrinsic cell-death pathway occurs at many levels, including transcriptional and post-transcriptional regulation of the Bcl2/BH3-only family members, and expression of death-effector components and a class of caspase inhibitors known as 'inhibitors of apoptosis' (IAPs).

The 'extrinsic' cell-death pathway is activated through ligation of cell-surface 'death receptors', such as Fas/CD95, TNFR (tumour necrosis factor receptor) and DF-5, with their respective cognate ligands FasL, TNFα and TRAIL (ref. 7). Once ligated, these receptors form the 'death-inducing signalling complex' (DISC), which activates the apical caspase-8. In some cell types, this alone is sufficient to trigger the downstream caspase cascade and consequent apoptosis. In other cells, however, death-receptor-induced apoptosis also requires recruitment of the mitochondrial pathway through caspase-8-mediated activation of the BH3-only protein Bid (refs 7–9). The extrinsic pathway is subject to modulation by decoy receptors, which bind ligand but are defective in signalling, and by intracellular molecules such as FLIP that compete with caspase-8 for binding to the DISC (ref. 7). In addition, IAPs modulate the activity of both apical and effector caspases in the pathway and, in cells where the intrinsic mitochondrial pathway is co-opted, so do Bcl2 family proteins[4,8,9]. Remarkably, signals that initiate cell division (mitogenic signals) can interface with the intrinsic and extrinsic programmes at several points.

p53 is a master regulator

p53 is a transcription factor that establishes programmes for apoptosis, senescence, and repair in response to a variety of cellular stresses, including DNA damage, hypoxia, and nutrient deprivation[3,10]. Known transcriptional targets for p53 in promoting apoptosis include various pro-apoptotic Bcl2 members, including *puma*, *noxa*, *bid* and *bax* (ref. 3), as well as components of death-receptor signalling (for example, DR5, Fas/CD95), the apoptotic-effector machinery

©2004 Nature Publishing Group

(for example, caspase-6, Apaf-1, PIDD) and others with less well-defined roles (for example, PERP, PML, p53AIP)[3,10]. Additionally, p53 might directly facilitate cytochrome *c* release[11].

p53 is also induced by many oncogenes, including E1A, Myc and E2F (refs 3, 10). Moreover, p53 inactivation severely compromises oncogene-induced apoptosis in many instances. Consistent with this role in coupling proliferation to cell death, inactivation of p53 potently cooperates with diverse oncogenes to promote transformation *in vitro* and tumorigenesis *in vivo*. For example, p53 inactivation relieves the requirement for E1B in adenovirus transformation of rodent fibroblasts[12], and dramatically potentiates the abilities of Myc, E2F and forms of T antigen that do not bind p53, to promote tumorigenesis in transgenic mouse models[13–15]. Moreover, studies in mice indicate that selective disruption of the apoptotic machinery downstream of p53 can substitute for p53 loss in promoting tumorigenesis. For example, inactivation of the *bax* or *puma* genes promotes tumorigenesis, despite the presence of wild-type p53, and gene expression of *bcl2* or *bclXL* cooperates with Myc as effectively as p53 loss[16–19]. Such studies demonstrate that apoptosis is a significant component by which p53 suppresses tumorigenesis.

An especially important mediator of oncogene-dependent activation of p53 is the tumour suppressor ARF (refs 20, 21). Thus, the ability of Myc and E1A to activate p53 is severely compromised in ARF-null cells, which consequently show marked resistance to apoptosis following withdrawal of growth factors[22,23]. By contrast, ARF is not required for the p53-dependent response to DNA damage[24], although it might contribute to a more robust response to DNA damage in oncogene-expressing cells through its stabilization of p53 (refs 23, 25). *In vivo* studies confirm the importance of ARF for oncogene signalling to p53. Disruption of ARF in mice dramatically accelerates Myc-induced lymphomas and carcinomas in a manner broadly comparable to p53 inactivation[13,26,27]. Deregulated expression of Bmi-1, a polycomb group protein that acts as a negative regulator of the *INK4a/ARF* genetic locus, similarly accelerates Myc-induced tumours[26].

Despite its importance, ARF is not the sole conduit through which oncogenes signal to p53. Indeed, in some mouse models of tumorigenesis, ARF inactivation does not appreciably accelerate oncogene-initiated tumorigenesis, even though loss of p53 does[28,29]. Some evidence suggests that oncogenes can induce genotoxic stress directly and thereby activate p53. Consistent with this, studies also suggest that lesions in DNA-damage repair and response machinery can compromise oncogene-induced apoptosis[30,31]. However, the relevance of oncogene-induced DNA damage, and whether disruption of the DNA-damage response eliminates oncogene surveillance mechanisms *in vivo*, remains unclear[32]. Perhaps the machinery that senses DNA damage also mediates responses to non-genotoxic signals that might accompany increased proliferation or transformation, such as an increased nuclear/cytoplasmic ratio[33]. Alternatively, given the well-known synergy between DNA damage and pro-apoptotic oncogenes in promoting cell death[3], ablating the DNA-damage component might confer protection from apoptosis without it being involved directly in the relationship between the activated oncogene and p53.

p53-independent mechanisms of apoptosis

Although p53 has gained legendary status as our principal defender against malignancy, there are other parallel networks connecting proliferation and apoptosis. The *p53* gene itself is a member of a family that includes *p63* and *p73*, both of which encode proteins implicated in apoptosis and several other processes[4]. Disruption of p63 or p73, either alone or in combination, ameliorates apoptosis in cultured fibroblasts[34], and both can induce p53 transcriptional targets and apoptosis when overexpressed[35,36]. Moreover, p73 is a direct transcriptional target of E2F and Myc, and both p63 and p73 can act to redirect p53 to the promoters of pro-apoptotic genes[34], although such mechanisms are not universal[37]. In addition, p73 can promote apoptosis in p53-deficient cells, a property that can be blocked by a specific cadre of 'gain-of-function' p53 mutants that are able to associate physically with p73 (refs 38–40). Nonetheless, mice heterozygous for either p63 or p73 are not overtly tumour-prone[35,36], so the exact extent of the contribution of the p53 siblings to tumour suppression *in vivo* remains uncertain.

Oncogenes can also target various components of the cell-death machinery independently of p53. Thus, E1A suppression of FLIP sensitizes cells to death-receptor-induced apoptosis[41], and Myc sensitizes cells to death-receptor signalling by recruiting the mitochondrial pathway[42]. Additionally, Myc, E2F and E1A have pleiotropic effects on the expression of pro- and anti-apoptotic members of the Bcl2 family. For example, Myc represses expression of *bcl2* and *bclXL*, whereas E1A and E2F suppress another Bcl2-family gene *mcl-1* (refs 43, 44). Myc and E2F also induce expression of several BH3-only killer proteins, including Bim (refs 45, 46). Finally, E2F can induce several downstream effectors of the apoptotic machinery, including various caspases[47].

The relative importance of p53-independent versus p53-dependent apoptotic mechanisms in suppressing tumorigenesis remains unclear. Experimental overexpression of Bcl2 can relieve selection against loss of p53 in the Eμ-Myc mouse model of lymphoma[18] (where the myc oncogene is expressed from an immunoglobulin enhancer), whereas inactivation of even a single allele of *bim*, a Myc target, dramatically accelerates Myc-induced lymphomagenesis in the same model[45]. Nonetheless, it seems likely that the relative contributions of p53-dependent and p53-independent apoptotic pathways will vary depending on tumour type, and on the nature and sequence of oncogenic mutations within any specific cancer.

Overlapping mechanisms of oncogene-induced cell death

If oncogenic lesions engage a variety of effector molecules that modulate cell proliferation in diverse ways , then how can each be coupled to the same core death programme? The simplest possibility is that pro-apoptotic oncogenes act at different points in a single linear pathway that is coupled to apoptosis through some downstream node. The mechanisms by which the Myc and E2F oncogenes promote apoptosis illustrate this point. Myc activates E2F, and there are consensus Myc-binding sites in at least one E2F promoter[48]. Moreover, in cultured mouse embryonic fibroblasts (MEFs), Myc-induced apoptosis can be dependent on E2F1 (refs 49, 50). Accordingly, both deregulated Myc expression and inactivation of the tumour-suppressing retinoblastoma (Rb) protein exert some of their apoptotic action through common downstream E2F effectors.

Still, there are clear differences in the apoptotic modes of action of Myc and E2F. For example, both *in vitro* and *in vivo* studies indicate that ARF is more important for apoptosis induced by Myc than for that induced by ectopic E2F expression or Rb inactivation[29,51]. Consistent with this, Myc can induce ARF through E2F1-independent mechanisms[52]. Furthermore, E2F1, but not Myc, augments apoptosis following cytosolic injection of holocytochrome *c*, indicating that E2F directly influences components of the apoptotic effector machinery downstream of the mitochondrial switch[47]. Indeed, E2F1 directly controls the expression of certain caspases[47], an activity that Myc does not share (Z. Nahle and S. W. L., unpublished work).

In reality, dissecting the precise interrelationship between Myc and E2F1 in apoptosis signalling is complicated by the multiplicity of E2F proteins, each of which can induce and compensate partially for the others, at least when overexpressed[53,54]. Indeed, that Myc and E2F normally act in a highly integrated signalling network makes it difficult, even in principle, to assign individual contributions to each. Probably, Myc and E2F promote apoptosis by targeting multiple processes (some that converge on common targets and others that are distinct), that then act collectively to engage the apoptotic programme. It appears that the cell-proliferative and cell-death machineries are not coupled through a single conduit but that evolution has employed a variety of redundant mechanisms to link the two.

©2004 Nature Publishing Group

Coupling proliferation to senescence

Apoptosis is not the only anti-proliferative response coupled to oncogenic signalling. Activated oncogenes can also trigger cellular senescence[55–57], a state characterized by permanent cell-cycle arrest and specific changes in morphology and gene expression that distinguish the process from quiescence (reversible cell-cycle arrest)[58,59]. Whereas 'replicative' senescence is triggered by the erosion of telomeres during cell divisions, a similar phenotype can occur in 'young' cells in response to oncogenes, DNA damage or oxidative stress[58,59]. Consistent with their roles in mediating cell-cycle checkpoints and tumour suppression, both Rb and p53 tumour suppressors are key regulators of the senescence programme.

Oncogenic Ras promotes cellular senescence in non-immortal human and rodent cells in a manner that depends on one or both products of the *INK4a/ARF* locus, which encodes the tumour suppressor proteins p16 and ARF (Fig. 3)[20,21]. The mitogen-activated protein kinase (MAPK) signalling cascade appears to be the principal Ras-effector pathway responsible for cellular senescence by inducing p16 and/or ARF, and ultimately by activating Rb and p53, respectively[20,59]. p53 and Rb then promote senescence by controlling a number of effectors, including p21CIP1/WAF1, PML, and various chromatin-modifying factors that produce a repressive state that buffers proliferative genes from mitogenic signalling[60–64]. The respective contributions of Rb and p53 to senescence are apparently cell-type dependent: thus, MEFs depend primarily on the ARF–p53 axis, whereas human fibroblasts and some rodent haematopoietic cells also rely on p16–Rb functions[20].

Escape from oncogene-induced senescence is a prerequisite for the transformation of cells that probably explains the oncogenic cooperation between Ras and so-called 'immortalizing' oncogenes *in vitro*. Thus, in mouse embryonic fibroblasts or dermal keratinocytes, disruption of either ARF or p53 abrogates Ras-induced cytostasis and permits oncogenic transformation[20,21]. In human cells, the situation is more complex, often requiring additional oncogenic lesions to thwart senescence; for example, *INK4a* loss[20,21]. High Ras levels are frequently observed in tumour cells and are probably required for malignant conversion[65]. Cancers must therefore acquire cooperating lesions that uncouple mitogenic Ras signalling from senescence. Such secondary lesions that thwart senescence are likely to be required for tumour maintenance, as suggested by the observation that suppression of the p53-inactivating E6 oncoprotein rapidly triggers senescence in human cervical carcinoma cells[66].

In general terms, both senescence and apoptosis seem to serve the same ends in tumour suppression. Both represent an irrevocable growth-inhibitory cellular response to oncogenic stress that acts as a potent barrier to the further evolution of any pre-neoplastic cell. Indeed, many of the signals that promote apoptosis in one cell type induce senescence in others. For example, both E2F and Myc can be either pro-apoptotic or pro-senescent depending on the cell type, the levels to which they are expressed, and the extent of other pro-apoptotic and growth signals received by the cell[55,67]. It is plausible that both programmes are induced by the same generic processes and have been structured by evolution to serve as backups for each other.

Is senescence relevant?

Although it is generally accepted that oncogene-induced apoptosis is a bona fide tumour-suppressor mechanism, the role of oncogene-triggered senescence is more contentious because the programme has not been observed definitively *in vivo*. Even *in vitro*, oncogenic Ras does not always trigger senescence in primary cells[68]. This is most notable when it is expressed from its endogenous locus[69,70], raising the troubling possibility that the whole phenomenon of Ras-induced senescence is an artefact of overexpression *in vitro*. Such a possibility has devastating ramifications, because most of our current understanding of genetic interactions in cancer depends on studies involving Ras overexpression. Unfortunately, defining any role for senescence in tumour suppression *in vivo* is complicated by extreme difficulty in identifying senescence *in vivo*, and our relatively rudimentary understanding of the mechanisms that regulate it.

Studies in mouse models provide circumstantial evidence that senescence acts to counter tumorigenesis induced by mitogenic mutations. In chemically induced skin carcinogenesis in mice, the initiating carcinogen induces mutations in the endogenous *H-ras* gene in multiple target cells. However, progression of such incipient proto-tumour cells into malignant tumours requires obligate secondary mutations in the *p53*, *p16INK4a, ARF* or *p21CIP1/WAF1* genes—precisely those that mediate Ras-induced growth arrest in cultured dermal keratinocytes[71,72]. Likewise, enforced E2F expression in the mouse pituitary gland initially promotes proliferation and tissue expansion that then stalls because of a progressively increasing insensitivity of the affected cells to further mitogenic stimulation (K. Helin, personal communication). The non-dividing tissue displays significant upregulation of p16 and other markers of senescence—offering strong evidence that a senescence programme suppresses aberrant proliferation *in vivo*.

Both apoptosis and senescence involve integrating diverse extracellular and intracellular influences into a binary live/die or go/stop cellular decision. For example, we know that Myc-induced apoptosis is a contingent phenomenon that is potently inhibited by survival factors and greatly exacerbated by additional insults with pro-apoptotic signals. In effect, Myc activation contributes only one component to the net pro-apoptotic load of any individual cell: whether that is enough to breach the apoptotic firing threshold depends on a host of contributing factors including level of Myc expression, cell type, location and availability of trophic survival signals, and differentiation and stress status. Such contingency is clearly observed in studies of transgenic mice that show that, when activated *in vivo*, Myc is a powerful destroyer of certain cell types but not others[19,73]. By analogy to apoptosis, therefore, we might expect senescence to be dramatically influenced by the cellular microenvironment. Consequently, Ras might induce senescence only in certain cell types and, even then, perhaps only in combination with other simultaneous insults, such as DNA damage or growth-factor deprivation. Such 'contingent' senescence would not be readily apparent in conventional transgenic studies, but it could explain why oncogenic Ras, similar to Myc, is only capable of directly inducing tissue expansion in a subset of tissues[70].

Crossing thresholds

The decisions whether to live or die, to proliferate or arrest are choices a cell must make in the face of many disparate influences. Furthermore, once a threshold for firing such programmes has been breached, they necessarily run to completion. During oncogene-induced apoptosis, the threshold is probably crossed when the pro-apoptotic influences far outweigh the anti-apoptotic buffer (Fig. 3). The ability of BH3-only proteins to integrate apoptotic signals offers an explanation of why diverse stimuli, such as DNA damage, death-receptor signals and activated oncogenes show synergy[2,4]. One consequence of such signal integration is that it is neither possible nor meaningful to attribute the ultimate outcome to any one signal, because elimination of any single component might be sufficient to drop the system below the firing threshold. When operating close to its firing thresholds, the relative contributions of individual components to a particular biological process are not additive.

The lessons of thresholds in the control of apoptosis are important. Just because deletion of a specific gene causes a 90% reduction in apoptosis does not mean that all the other pro-apoptotic influences together account for the remaining 10% of cell death. Accordingly, a mutation in any one of the downstream pathways by which oncogenes promote apoptosis might be sufficient to suppress a significant degree of cell death and so confer a significant growth advantage. Such a scenario might explain how deletion of *bim* accelerates Myc-induced lymphomagenesis or compensates for spontaneous *p53*

©2004 Nature Publishing Group

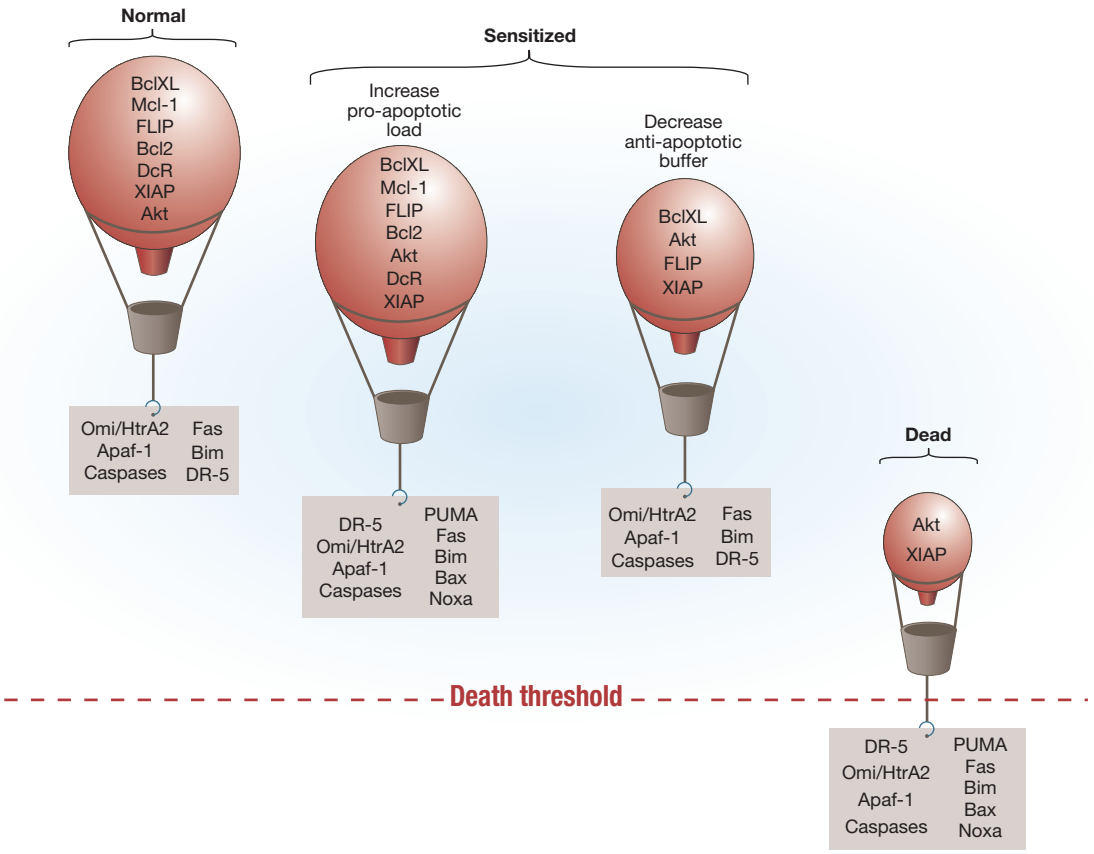

Figure 3 Crossing the apoptotic threshold. Apoptosis is tightly controlled by the ability of the cell to integrate many pro- and anti-apoptotic signals into a binary life/death decision. In one model, apoptosis occurs when the pro-apoptotic load of the cell exceeds its anti-apoptotic buffering capacity, breaching a threshold that then triggers an effector programme capable of running to completion. Perhaps the most important feature of a threshold model is that it is impossible to attribute the ultimate outcome to one specific signal. Elimination of any indivdual component could be sufficient to shift the entire system below the firing threshold. Where thresholds operate, the contributions of individual components are not additive.

mutations, even though *bim* is not induced by p53 (ref. 45). Loss of the Bim protein presumably drops the cell below its apoptotic threshold and allows cell survival in the presence of wild-type p53. Importantly, to bring that cell back to its apoptotic firing threshold it might not be necessary to correct that specific lesion or modulate that specific pathway — adding to the general apoptotic load through other pathways could be equally effective.

Sensing aberrant proliferation

Much of the above discussion has focused on how oncogenic signalling interfaces with the cell death or senescence machineries. However, apoptosis and senescence are not the inevitable outcome of normal cell division, but are mostly confined to aberrantly proliferating cells. The implication is that specific molecular sensors determine whether proliferation is aberrant, implying that concrete criteria must distinguish normal and abnormal cell proliferation. Understanding the nature of such criteria and how they are sensed would provide insight into both the selectivity of tumour suppression and the generic self-organizing rules that craft and maintain normal somatic tissues.

At least two general mechanisms have been identified by which cells and their adjacent tissues might 'sense' which cells are cancerous. One depends upon the obligatory social dependency that somatic cells possess for specific microenvironmental trophic signals, effectively using the orthotopic disposition of cells in tissues as cues of their normalcy. The other appears to involve some kind of internal registry of normal and abnormal proliferative signal strengths, triggering only in response to the latter. Both mechanisms appear to work in concert to limit the transforming potential of mitogenic oncogenes.

Microenvironmental signals

Somatic cells are thought to be continuously dependent upon their neighbours and local microenvironment to provide them with trophic signals that quell their innate suicidal tendencies[74]. One way that activated oncogenes trip the tumour-suppressive failsafe is by super-activating apoptotic and senescence tumour-suppressor programmes, which then overwhelm the limited social buffering capacity of local trophic factors. In addition, oncogene-induced cell expansion forces cells into inhospitable trophic compartments. Consistent with this, cells expressing mitogenic oncogenes such as Myc, E1A and E2F are peculiarly susceptible to induction of apoptosis upon withdrawal of survival factors, such as the insulin-like growth factors I and II (IGF-1, IGF-II) in fibroblasts, or interleukin-3 in myeloid cells[75,76]. In epithelial cells, survival signals are also derived from the association with the extracellular matrix. This is evident in the basal epidermis and intestinal epithelium where obligate survival signals are provided by the basal lamina[77].

Many survival factors prevent apoptosis by triggering receptor tyrosine kinases that ultimately signal through Ras and the phosphatidylinositol-3-OH kinase (PI(3)K) signalling cascade[78]. A key mediator of PI(3)K signalling is the Akt/PKB kinase, which phosphorylates multiple effectors leading to pleiotropic changes in proliferation, metabolism, cell growth and survival. Akt promotes survival by coordinating programmes that directly inhibit apoptotic effectors,

©2004 Nature Publishing Group

suppress transcription of pro-apoptotic genes, and modulate the translation of cell-death regulatory messenger RNAs[78]. Additionally, Akt survival signalling is potentiated by its effects on cellular bioenergetics[79], and its modulation of the mTOR pathway, which controls the cell response to nutrients[79]. Some cytokines also trigger PI(3)K-independent activation of STATs and NF-κB, transcription factors that promote cell survival by modulating the transcription of the Bcl2-related proteins and other anti-apoptotic genes[80].

Because limited trophic support restricts tissue expansion, it is not surprising that mutations that constitutively activate survival-signalling pathways contribute to the neoplastic genotype. Thus, elevated signalling through the IGF pathway occurs in many tumour types[81], and IGF-II availability is required for progression of oncogene-induced insulinomas in mice[82]. Similarly, genetic lesions that activate various elements of the PI(3)K pathway dramatically cooperate with Myc during cancer development[83,84]. Such mutations ameliorate the dependency of incipient tumour cells for their normal somatic compartments as well as acting as generic suppressors of apoptosis that render cells less susceptible to stress and micro-environmental changes[85].

The oncogene checkpoint

Although social circumstances can greatly influence the expansion of normal and pre-neoplastic cells, cells also harbour pre-set and autonomous sensors for aberrant proliferative signalling[20]. Such sensors discern elevated or sustained fluxes of mitogenic signalling, much like stress response 'checkpoints', and generally respond through the p53 pathway. One of the most important of these sensors is ARF, which, as described earlier, is transcriptionally upregulated in response to many oncogenes[20]. ARF is not expressed in normal proliferating tissues, but is rapidly induced in response to aberrant signals such as activated Myc (ref. 86). Thus, ARF expression is buffered against normal mitogenic signalling, becoming active only when some preconfigured signalling threshold is exceeded. This explains why, even though Myc and E2F are activated during the course of cell-cycle progression, ARF is not a cell-cycle regulated gene[20].

Factors that control ARF expression provide clues to the nature of this buffering threshold. In normal cells, the ARF promoter is actively suppressed by E2F3b, a variant of E2F3 that acts as a transcriptional repressor[87]. However, in the presence of E1A or elevated E2F1, E2F3b is displaced from the ARF promoter, allowing the binding of activator E2Fs. What signals this transition remains to be determined, but such observations provide the first clear evidence of an absolute difference in ARF regulation in normal cells versus oncogene-expressing cells. Nonetheless, whereas deletion of E2F3 upregulates ARF in cultured fibroblasts, the same does not occur *in vivo*, implying the existence of additional mechanisms insulating ARF during normal mitogenesis. One probable mechanism involves control of the polycomb group protein Bmi-1 — a chromatin remodelling factor that is an established repressor of the *INK4a/ARF* locus[26]. Perhaps sustained oncogenic signalling suppresses Bmi-1 function, producing a more open chromatin structure that enables activation of the ARF promoter by mitogenic transcription factors such as E2F1.

Deconstructing the network

From the above it is clear that oncogenic mutations can inhibit proliferation through a variety of mechanisms. Although it is possible that each acts to trigger apoptosis or cellular senescence under a specific set of circumstances or in certain cell types, it seems unlikely that evolution would have incorporated so many disparate means to achieve the same end. Instead, a more likely explanation for this mechanistic diversity is that each pathway or signal transducer acts as part of a complex network that coordinates the processes of apoptosis and senescence by targeting each programme at multiple levels. Through this organization, the cell ensures that the process is not dependent on a single event and proceeds efficiently once engaged.

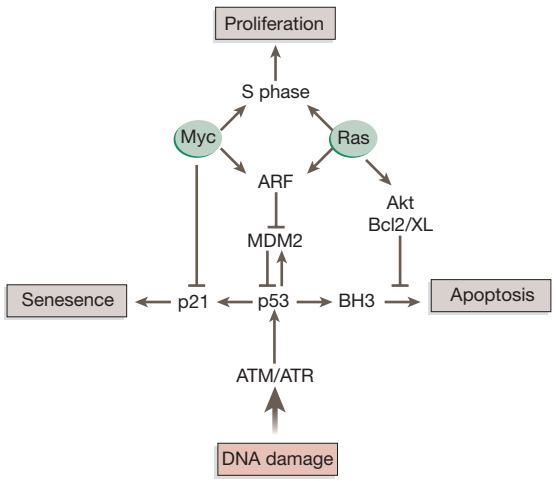

Figure 4 The ARF–p53 circuit in tumour development and therapy. Activation of Myc and Ras can force proliferation or trigger apoptosis or senescence. These oncogenic signals engage the tumour-suppressor network at many points, including through the ARF–p53 circuit shown here. Which components contribute most to tumour suppression depends on context. For example, Myc activates p53 to promote apoptosis while interfering with its ability to induce senescence. Conversely, Ras activates p53 to promote senescence while suppressing apoptosis. This simplified view helps explain why, despite the potential of p53 to control several processes, apoptosis is primarily responsible for p53-mediated tumour suppression in the presence of Myc, and why mutations that disable apoptosis (for example, Bcl2 overexpression) cooperate more effectively with Myc than Ras. As another example, DNA damage and oncogene signalling engage the tumour-suppressor network at different points and, as such, DNA-damage signalling relies more on p53 than on ARF to elicit an anti-proliferative response. Such a model explains why loss of ARF or p53 confer similar advantages during Myc-induced tumorigenesis but not following treatment with DNA-damaging drugs. Here, drug resistance is an unselected trait conferred by p53 mutations that provides a unique advantage as the tumour encounters a new environment (for example, chemotherapy).

By revisiting some of the mechanisms whereby Myc promotes apoptosis, it is possible to envision how an oncogene-triggered tumour-suppressor network might act to coordinate a cell-death programme effectively. By greatly increasing the ratio of pro- to anti-apoptotic Bcl2 proteins, Myc promotes mitochondrial permeabilization and release of cytochrome *c*. Through indirectly activating p53 or E2F, Myc induces Apaf-1, caspases, and the IAP inhibitor Omi/Htra2 (refs 47, 88), and consequently increases the efficiency with which cytochrome *c*, when released, triggers the caspase cascade. Through p53-dependent increases in PTEN, Myc might short-circuit survival signalling, thereby reducing the cell's ability to buffer pro-apoptotic signals. And, by indirectly increasing death receptors and decreasing their antagonists, Myc sensitizes the cell to the actions of death-inducing ligands in the microenvironment. Finally, by upregulating p73, Myc introduces redundancy in the p53-dependent programme, reinforcing many of the processes described above.

Sorting out how individual components of such a complex and multifarious network contribute to the output of each programme networks is a major challenge because, by definition, analysing individual components in isolation cannot provide a complete picture of network dynamics. Biological networks are characterized by multiple feed-forward, feedback, and cross-talk characteristics that compensate for perturbations affecting individual components and lend them great robustness. Consequently, the phenotype caused by disrupting a specific protein might reflect not its normal function but, rather, the net difference between its activity and an opposing compensatory signal. This probably explains the failure of apparently important

©2004 Nature Publishing Group

genes to produce profound phenotypes when deleted in mice, and it will complicate efforts to assign specific roles to certain caspases or IAPs in apoptosis, or Rb-family members in promoting senescence[89–91].

The situation is more complex still given the pivotal importance of cell type and cellular microenvironment in determining the net impact of oncogenic mutations. Cell-type-specific levels of endogenous pro- and anti-apoptotic effectors, together with microenvironmental factors and other oncogenic events, all influence the signalling flux through pathways that contribute to cell proliferation and viability. Consequently, the neoplastic impact of any oncogenic mutation is likely to lead to dramatically different outcomes depending on context. For example, acute activation of Myc in pancreatic β-cells leads to rapid β-cell involution and diabetes[19]. By contrast, activation of Myc in skin triggers proliferation without cell death, probably because of an abundance of local survival factors, resulting in rapid development of papillomatous hyperplasias[73]. In the latter circumstance, anti-apoptotic lesions such as that caused by the loss of p53 exert a selective advantage only when the neoplastic cell moves beyond its normal trophic environment into the dermis[19]. By the same token, mutations that inhibit death-receptor signalling would only enhance viability in environments where death ligands are present.

The multi-functionality of individual signalling molecules adds a final tier of complexity to signalling networks and how they drive tumour evolution. For example, although Myc and Ras both engage the ARF/p53 pathway, they also instigate distinct 'collateral signals' that elicit different outcomes following p53 activation. Although Myc induces apoptosis, it also overrides cell-cycle arrest[92]; thus, subsequent immortalizing mutations provide no further selective advantage. Conversely, Ras promotes senescence yet attenuates apoptosis, rendering subsequent anti-apoptotic mutations mostly redundant[17] (Fig. 4). Thus, both the context and sequence of mutations profoundly influence the trajectory of tumour evolution context, and so determine which lesions end up as most critical for maintenance of the end-stage tumour.

From compliance to autonomy in tumour evolution

One peculiar consequence of all interlocking networks is that any single mutation in such a network can engender adventitious traits that, although having no immediate impact, might confer selective advantages (or disadvantages) later. A pertinent example of such pre-adaptation, or 'exaptation', relates to the impact of p53 mutations on tumours arising in Eμ-Myc transgenic mice[13,18]. In this model, directed mutations that inactivate p53 or PUMA, or that overexpress Bcl2, dramatically accelerate tumorigenesis. However, although tumours that arise in each case are phenotypically similar and display broadly equivalent apoptotic defects, only those with mutant p53 display defects in DNA-damage checkpoints and gross aneuploidy[13,18]. Moreover, Eμ-Myc lymphomas lacking p53 progress to a lethal stage more rapidly than those overexpressing Bcl2, presumably because loss of p53 confers a selective advantage under conditions of checkpoint activation or genomic damage[13,17,18]. Thus, although disruption of Myc's apoptotic function is directly selected during lymphomagenesis, the mechanism by which it occurs influences the future evolution of the tumour as it encounters new stresses or environments. Importantly, what is crucial and what is an evolutionary byproduct will depend on context: different rules are likely to apply to selection against p53 action in, say, suppressing Ras-induced tumorigenesis[17]. Such considerations have important implications for how tumours respond to therapy, both initially and evolutionarily.

Evolving towards drug resistance

The major limitation to conventional cancer therapy is drug resistance, either because the initial tumour fails to respond to therapy or because it acquires resistance during relapse. Most conventional chemotherapeutic agents damage cellular components, and it was long assumed that this damage was directly responsible for the anti-tumour effect. However, damage induced by chemotherapeutic drugs is not invariably lethal but instead actively triggers damage responses (often apoptosis or senescence), and it is these responses that determine the eventual fate of the cell[93]. Ironically, classical cancer therapies unwittingly exploited the very same innate tumour-suppressor networks that suppress aberrant cell proliferation.

The fact that oncogenes and conventional cancer drugs both co-opt the same networks means that mutations that uncouple proliferation from apoptosis and senescence can disable drug responses. In the Eμ-Myc lymphoma model, inactivation of p53 confers an immediate advantage to the tumour by suppressing cell death, and predisposes the tumour to a poor response to chemotherapy[13]. Here, drug resistance is not the directly selected trait, but another example of exaptation. Similar forces could explain the innate drug resistance of some of the more aggressive primary tumours[93], as well as the link between p53 loss and other tumour-promoting mutations resulting in drug resistance in certain human cancers[93].

Although different oncogenes might cause similar phenotypes, drug responses will differ depending on the way these oncogenes affect the cellular signalling network and on network components targeted by different cancer drugs (Fig. 4). For example, mutations in either ARF or p53 are frequent in Eμ-Myc-dependent lymphomagenesis, and targeted disruption of either gene yields accelerated and highly aggressive malignancies. But despite such overt similarities, ARF-null tumours undergo massive apoptosis and are frequently cured following treatment with cyclophosphamide, a DNA-damaging drug, whereas p53-null tumours respond poorly[25]. This can be explained by the way Myc and DNA-damage signals engage the tumour-suppressor network; whereas Myc signalling depends heavily on ARF, DNA damage does not[22,24]. However, p53 is important for the DNA-damage response. Additionally, the genomic instability conferred by p53 loss, less pronounced in ARF-null or Bcl2-overexpressing tumours, might bestow an additional advantage under therapy. Similar principles could contribute to the enormously heterogeneous response to therapy observed in human cancer patients.

Curiously, the very same interrelationship between oncogene signalling and drug action could explain the remarkably selective ability of conventional chemotherapeutic drugs to kill tumour cells. Tumour cells harbour mitogenic lesions that drive their proliferation but also confer a propensity towards apoptosis or senescence. Hence, established tumours reside substantially closer to the threshold at which apoptosis or senescence can be triggered (Fig. 3). By comparison, normal somatic cells, lacking oncogenic mutations and protected by the trophic signals within their orthotopic environments, are far from such thresholds and consequently less susceptible to the cytotoxic and cytostatic effects of therapeutic agents.

In summary, the fact that proliferation is coupled to apoptosis and senescence coerces the evolutionary trajectory of tumours in ways that influence cellular responses to therapy, by promoting drug resistance or, conversely, by increasing the probability that the drug will be effective. By understanding these relationships, the hope is that current cancer therapies can be employed more effectively. A subsidiary question concerns the extent to which similar rules apply to the new targeted therapeutics that target key oncoproteins or their effectors. However, in many instances these novel agents induce apoptosis, raising the possibility that they may act, in part, by hijacking existing tumour-suppressor networks.

Exploiting the Achilles' heel of cancer cells

Re-engaging the disrupted senescence and apoptosis programmes by novel targeted therapeutics in cancer cells offers a compelling general strategy for effective and tumour-cell-specific cancer therapy. However, it will only work if the engines driving apoptosis and senescence persist throughout the lifetime of the tumour. Thus traits selected early on in the neoplastic process might not remain under continuous selection during tumour progression and may even be selected against at later stages. Fortunately, a number of studies using conditional transgenic and knockout mouse models indicate that the

©2004 Nature Publishing Group

initiating oncogenic lesions remain essential for tumour maintenance[19,73,94,95], even when the tumours have evolved to an advanced stage[2,19]. Indeed, even in situations where additional collaborating mutations appear to be required to sustain tumorigenesis[96,97], it seems likely that rational targeting of one or a few pivotal oncogenic lesions would undermine the entire neoplastic edifice.

Another of the remarkable features of such conditional transgenic and knockout mouse models is that dominant oncogenes such as Myc and Ras are capable of driving multiple aspects of advanced tumorigenesis, including angiogenesis and invasion, when unshackled from their inherent apoptosis and senescence programmes. By the same token, the necessity for pre-neoplastic cells to evolve mechanisms to quell their innate predisposition to apoptosis or senescence exposes a critical and exploitable chink in their defences. As well as being addicted to their initiating oncogenic mutations, tumour cells will remain critically dependent on their limited repertoire of anti-apoptotic and anti-senescent mutations; by contrast, normal cells, lacking pro-apoptotic oncogenic lesions and safely ensconced in their stress-free, trophic havens, will not. Consequently, tumour cells appear particularly sensitive to interventions that re-establish pro-apoptotic pathways or disable survival programmes.

Recent *in vivo* studies illustrate that re-engaging apoptotic programmes disabled during tumour evolution can indeed have a profound therapeutic effect[85]. Thus, inhibition of Bcl2, or reactivation of p53, has proven particularly lethal to appropriate tumour types[98,99,100]. Likewise, rapamycin, an inhibitor of the Akt target mTOR, effectively reverses resistance to conventional chemotherapy in Eμ-Myc lymphomas co-expressing Akt (ref. 85). Importantly, rapamycin works selectively only in tumours where the apoptotic fail-safe has been ablated by Akt — not those where apoptosis has been disengaged through lesions that act in parallel to, or downstream of, mTOR (ref. 85). Such studies intimate that the effective use of similar strategies in human patients will require significant insight into the evolution of each individual's neoplastic disease. Nonetheless, there seems no doubt that harnessing the very mutations that cancer cells need to promote their pathological survival and expansion will be the basis of the therapeutic strategy of the future.

Perspective

Cancer has long been considered to be an endlessly adaptable and profoundly complex disease treatable only with blunt approaches that frequently do as much damage to the patient as to the tumour. Contemporary molecular dissection of tumour cells has confirmed the complexity and subtlety of the signalling networks that drive and maintain tumours, but it has also shown us that tumour cells harbour the seeds of their own potential destruction: the very oncogenic mutations that cancer cells need to drive their relentless and pathological expansion possess the potential to unleash powerful tumour-suppressor programmes such as senescence and apoptosis. Cancers arise when the molecular network connecting proliferation and tumour suppression become uncoupled. Even then, however, the underlying tumour-suppressor programmes remain intact, awaiting only adroit human intervention to reconnect them and herald a new era of effective and tumour-specific therapies. □

doi:10.1038/nature03098

1. Evan, G. I. *et al.* Induction of apoptosis in fibroblasts by c-myc protein. *Cell* **69,** 119–128 (1992).
2. Evan, G. I. & Vousden, K. H. Proliferation, cell cycle and apoptosis in cancer. *Nature* **411,** 342–348 (2001).
3. Fridman, J. S. & Lowe, S. W. Control of apoptosis by p53. *Oncogene* **22,** 9030–9040 (2003).
4. Danial, N. N. & Korsmeyer, S. J. Cell death: critical control points. *Cell* **116,** 205–219 (2004).
5. Cory, S., Huang, D. C. & Adams, J. M. The Bcl-2 family: roles in cell survival and oncogenesis. *Oncogene* **22,** 8590–8607 (2003).
6. Green, D. R. & Kroemer, G. The pathophysiology of mitochondrial cell death. *Science* **305,** 626–629 (2004).
7. Peter, M. E. & Krammer, P. H. The CD95(APO-1/Fas) DISC and beyond. *Cell Death Differ.* **10,** 26–35 (2003).
8. Wilkinson, J. C., Cepero, E., Boise, L. H. & Duckett, C. S. Upstream regulatory role for XIAP in receptor-mediated apoptosis. *Mol. Cell. Biol.* **24,** 7003–7014 (2004).
9. Scaffidi, C. *et al.* Two CD95 (APO-1/Fas) signalling pathways. *EMBO J.* **17,** 1675–1687 (1998).
10. Vogelstein, B., Lane, D. & Levine, A. J. Surfing the p53 network. *Nature* **408,** 307–310 (2000).
11. Mihara, M. *et al.* p53 has a direct apoptogenic role at the mitochondria. *Mol. Cell* **11,** 577–590 (2003).
12. White, E. Regulation of the cell cycle and apoptosis by the oncogenes of adenovirus. *Oncogene* **20,** 7836–7846 (2001).
13. Schmitt, C. A., McCurrach, M. E., de Stanchina, E., Wallace-Brodeur, R. R. & Lowe, S. W. INK4a/ARF mutations accelerate lymphomagenesis and promote chemoresistance by disabling p53. *Genes Dev.* **13,** 2670–2677 (1999).
14. Symonds, H. *et al.* p53-dependent apoptosis suppresses tumour growth and progression *in vivo*. *Cell* **78,** 703–711 (1994).
15. Pierce, A. M. *et al.* Increased E2F1 activity induces skin tumours in mice heterozygous and nullizygous for p53. *Proc. Natl Acad. Sci. USA* **95,** 8858–8863 (1998).
16. Yin, C., Knudson, C. M., Korsmeyer, S. J. & Van Dyke, T. Bax suppresses tumorigenesis and stimulates apoptosis *in vivo*. *Nature* **385,** 637–640 (1997).
17. Hemann, M. T. *et al.* Suppression of tumorigenesis by the p53 target PUMA. *Proc. Natl Acad. Sci. USA* **101,** 9333–9338 (2004).
18. Schmitt, C. A. *et al.* Dissecting p53 tumour suppressor functions *in vivo*. *Cancer Cell* **1,** 289–298 (2002).
19. Pelengaris, S., Khan, M. & Evan, G. I. Suppression of Myc-induced apoptosis in beta cells exposes multiple oncogenic properties of Myc and triggers carcinogenic progression. *Cell* **109,** 321–334 (2002).
20. Lowe, S. W. & Sherr, C. J. Tumour suppression by Ink4a-Arf: progress and puzzles. *Curr. Opin. Genet. Dev.* **13,** 77–83 (2003).
21. Sherr, C. J. The INK4a/ARF network in tumour suppression. *Nature Rev. Mol. Cell Biol.* **2,** 731–737 (2001).
22. Zindy, F. *et al.* Myc signalling via the ARF tumour suppressor regulates p53-dependent apoptosis and immortalization. *Genes Dev.* **12,** 2424–2433 (1998).
23. de Stanchina, E. *et al.* E1A signalling to p53 involves the p19(ARF) tumour suppressor. *Genes Dev.* **12,** 2434–2442 (1998).
24. Kamijo, T. *et al.* Tumour suppression at the mouse INK4a locus mediated by the alternative reading frame product p19ARF. *Cell* **91,** 649–659 (1997).
25. Schmitt, C. A. *et al.* A senescence programme controlled by p53 and p16INK4a contributes to the outcome of cancer therapy. *Cell* **109,** 335–346 (2002).
26. Jacobs, J. J. *et al.* Bmi-1 collaborates with c-Myc in tumorigenesis by inhibiting c-Myc-induced apoptosis via INK4a/ARF. *Genes Dev.* **13,** 2678–2690 (1999).
27. Eischen, C. M., Weber, J. D., Roussel, M. F., Sherr, C. J. & Cleveland, J. L. Disruption of the ARF–Mdm2–p53 tumour suppressor pathway in Myc-induced lymphomagenesis. *Genes Dev.* **13,** 2658–2669 (1999).
28. Verschuren, E. W., Klefstrom, J., Evan, G. I. & Jones, N. The oncogenic potential of Kaposi's sarcoma-associated herpesvirus cyclin is exposed by p53 loss *in vitro* and *in vivo*. *Cancer Cell* **2,** 229–241 (2002).
29. Tolbert, D., Lu, X., Yin, C., Tantama, M. & Van Dyke, T. p19(ARF) is dispensable for oncogenic stress-induced p53-mediated apoptosis and tumour suppression *in vivo*. *Mol. Cell. Biol.* **22,** 370–377 (2002).
30. Khan, S. H., Moritsugu, J. & Wahl, G. M. Differential requirement for p19ARF in the p53-dependent arrest induced by DNA damage, microtubule disruption, and ribonucleotide depletion. *Proc. Natl Acad. Sci. USA* **97,** 3266–3271 (2000).
31. Rogoff, H. A. *et al.* Apoptosis associated with deregulated E2F activity is dependent on E2F1 and Atm/Nbs1/Chk2. *Mol. Cell. Biol.* **24,** 2968–2977 (2004).
32. Liao, M. J., Yin, C., Barlow, C., Wynshaw-Boris, A. & van Dyke, T. Atm is dispensable for p53 apoptosis and tumour suppression triggered by cell cycle dysfunction. *Mol. Cell. Biol.* **19,** 3095–3102 (1999).
33. Conn, C. W., Lewellyn, A. L. & Maller, J. L. The DNA damage checkpoint in embryonic cell cycles is dependent on the DNA-to-cytoplasmic ratio. *Dev. Cell* **7,** 275–281 (2004).
34. Flores, E. R. *et al.* p63 and p73 are required for p53-dependent apoptosis in response to DNA damage. *Nature* **416,** 560–564 (2002).
35. Urist, M. & Prives, C. p53 leans on its siblings. *Cancer Cell* **1,** 311–313 (2002).
36. Yang, A., Kaghad, M., Caput, D. & McKeon, F. On the shoulders of giants: p63, p73 and the rise of p53. *Trends Genet.* **18,** 90–95 (2002).
37. Senoo, M., Manis, J. P., Alt, F. W. & McKeon, F. p63 and p73 are not required for the development and p53-dependent apoptosis of T cells. *Cancer Cell* **6,** 85–89 (2004).
38. Irwin, M. S. *et al.* Chemosensitivity linked to p73 function. *Cancer Cell* **3,** 403–410 (2003).
39. Bergamaschi, D. *et al.* p53 polymorphism influences response in cancer chemotherapy via modulation of p73-dependent apoptosis. *Cancer Cell* **3,** 387–402 (2003).
40. Gaiddon, C., Lokshin, M., Ahn, J., Zhang, T. & Prives, C. A subset of tumour-derived mutant forms of p53 down-regulate p63 and p73 through a direct interaction with the p53 core domain. *Mol. Cell. Biol.* **21,** 1874–1887 (2001).
41. Perez, D. & White, E. E1A sensitizes cells to tumour necrosis factor alpha by downregulating c-FLIP S. *J. Virol.* **77,** 2651–2662 (2003).
42. Klefstrom, J., Verschuren, E. W. & Evan, G. c-Myc augments the apoptotic activity of cytosolic death receptor signalling proteins by engaging the mitochondrial apoptotic pathway. *J. Biol. Chem.* **277,** 43224–43232 (2002).
43. Croxton, R., Ma, Y., Song, L., Haura, E. B. & Cress, W. D. Direct repression of the Mcl-1 promoter by E2F1. *Oncogene* **21,** 1359–1369 (2002).
44. Eischen, C. M. *et al.* Bcl-2 is an apoptotic target suppressed by both c-Myc and E2F-1. *Oncogene* **20,** 6983–6993 (2001).
45. Egle, A., Harris, A. W., Bouillet, P. & Cory, S. Bim is a suppressor of Myc-induced mouse B cell leukemia. *Proc. Natl Acad. Sci. USA* **101,** 6164–6169 (2004).
46. Hershko, T. & Ginsberg, D. Up-regulation of Bcl-2 homology 3 (BH3)-only proteins by E2F1 mediates apoptosis. *J. Biol. Chem.* **279,** 8627–8634 (2004).
47. Nahle, Z. *et al.* Direct coupling of the cell cycle and cell death machinery by E2F. *Nature Cell Biol.* **4,** 859–864 (2002).
48. Sears, R., Ohtani, K. & Nevins, J. R. Identification of positively and negatively acting elements regulating expression of the E2F2 gene in response to cell growth signals. *Mol. Cell. Biol.* **17,** 5227–5235 (1997).
49. Matsumura, I., Tanaka, H. & Kanakura, Y. E2F1 and c-Myc in cell growth and death. *Cell Cycle* **2,** 333–338 (2003).

©2004 Nature Publishing Group

50. Leone, G. *et al.* Myc requires distinct E2F activities to induce S phase and apoptosis. *Mol. Cell* **8,** 105–113 (2001).
51. Russell, J. L. *et al.* ARF differentially modulates apoptosis induced by E2F1 and Myc. *Mol. Cell. Biol.* **22,** 1360–1368 (2002).
52. Baudino, T. A. *et al.* Myc-mediated proliferation and lymphomagenesis, but not apoptosis, are compromised by E2f1 loss. *Mol. Cell* **11,** 905–914 (2003).
53. Conner, E. A. *et al.* Dual functions of E2F-1 in a transgenic mouse model of liver carcinogenesis. *Oncogene* **19,** 5054–5062 (2000).
54. Leone, G., DeGregori, J., Sears, R., Jakoi, L. & Nevins, J. R. Myc and Ras collaborate in inducing accumulation of active cyclin E/Cdk2 and E2F. *Nature* **387,** 422–426 (1997).
55. Dimri, G. P., Itahana, K., Acosta, M. & Campisi, J. Regulation of a senescence checkpoint response by the E2F1 transcription factor and p14(ARF) tumour suppressor. *Mol. Cell. Biol.* **20,** 273–285 (2000).
56. Damalas, A., Kahan, S., Shtutman, M., Ben-Ze'ev, A. & Oren, M. Deregulated beta-catenin induces a p53- and ARF-dependent growth arrest and cooperates with Ras in transformation. *EMBO J.* **20,** 4912–4922 (2001).
57. Serrano, M., Lin, A. W., McCurrach, M. E., Beach, D. & Lowe, S. W. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* **88,** 593–602 (1997).
58. Campisi, J. Cellular senescence as a tumour-suppressor mechanism. *Trends Cell Biol.* **11,** S27–S31 (2001).
59. Shay, J. W. & Roninson, I. B. Hallmarks of senescence in carcinogenesis and cancer therapy. *Oncogene* **23,** 2919–2933 (2004).
60. Pearson, M. *et al.* PML regulates p53 acetylation and premature senescence induced by oncogenic Ras. *Nature* **406,** 207–210 (2000).
61. Ferbeyre, G. *et al.* PML is induced by oncogenic ras and promotes premature senescence. *Genes Dev.* **14,** 2015–2027 (2000).
62. Itahana, K. *et al.* Control of the replicative life span of human fibroblasts by p16 and the polycomb protein Bmi-1. *Mol. Cell. Biol.* **23,** 389–401 (2003).
63. Narita, M. *et al.* Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. *Cell* **113,** 703–716 (2003).
64. Paramio, J. M. *et al.* The ink4a/arf tumour suppressors cooperate with p21cip1/waf in the processes of mouse epidermal differentiation, senescence, and carcinogenesis. *J. Biol. Chem.* **276,** 44203–44211 (2001).
65. Elenbaas, B. *et al.* Human breast cancer cells generated by oncogenic transformation of primary mammary epithelial cells. *Genes Dev.* **15,** 50–65 (2001).
66. Horner, S. M., DeFilippis, R. A., Manuelidis, L. & DiMaio, D. Repression of the human papillomavirus E6 gene initiates p53-dependent, telomerase-independent senescence and apoptosis in HeLa cervical carcinoma cells. *J. Virol.* **78,** 4063–4073 (2004).
67. Wu, X. & Levine, A. J. p53 and E2F-1 cooperate to mediate apoptosis. *Proc. Natl Acad. Sci. USA* **91,** 3602–3606 (1994).
68. Benanti, J. A. & Galloway, D. A. Normal human fibroblasts are resistant to RAS-induced senescence. *Mol. Cell. Biol.* **24,** 2842–2852 (2004).
69. Tuveson, D. A. *et al.* Endogenous oncogenic K-ras(G12D) stimulates proliferation and widespread neoplastic and developmental defects. *Cancer Cell* **5,** 375–387 (2004).
70. Guerra, C. *et al.* Tumour induction by an endogenous K-ras oncogene is highly dependent on cellular context. *Cancer Cell* **4,** 111–120 (2003).
71. Lin, A. W. & Lowe, S. W. Oncogenic ras activates the ARF-p53 pathway to suppress epithelial cell transformation. *Proc. Natl Acad. Sci. USA* **98,** 5025–5030 (2001).
72. Kelly-Spratt, K. S., Gurley, K. E., Yasui, Y. & Kemp, C. J. p19(Arf) suppresses growth, progression, and metastasis of Hras-driven carcinomas through p53-dependent and -independent pathways. *PLoS Biol.* **2,** E242 (2004).
73. Pelengaris, S., Littlewood, T., Khan, M., Elia, G. & Evan, G. Reversible activation of c-Myc in skin: induction of a complex neoplastic phenotype by a single oncogenic lesion. *Mol. Cell* **3,** 565–577 (1999).
74. Raff, M. C. Social controls on cell survival and cell death. *Nature* **356,** 397–400 (1992).
75. Askew, D., Ashmun, R., Simmons, B. & Cleveland, J. Constitutive c-*myc* expression in IL-3-dependent myeloid cell line suppresses cycle arrest and accelerates apoptosis. *Oncogene* **6,** 1915–1922 (1991).
76. Harrington, E. A., Bennett, M. R., Fanidi, A. & Evan, G. I. c-Myc-induced apoptosis in fibroblasts is inhibited by specific cytokines. *EMBO J.* **13,** 3286–3295 (1994).
77. Grossmann, J. Molecular mechanisms of 'detachment-induced apoptosis–Anoikis'. *Apoptosis* **7,** 247–260 (2002).
78. Vivanco, I. & Sawyers, C. L. The phosphatidylinositol 3-kinase AKT pathway in human cancer. *Nature Rev. Cancer* **2,** 489–501 (2002).
79. Plas, D. R., Rathmell, J. C. & Thompson, C. B. Homeostatic control of lymphocyte survival: potential origins and implications. *Nature Immunol.* **3,** 515–521 (2002).
80. Grad, J. M., Zeng, X. R. & Boise, L. H. Regulation of Bcl-xL: a little bit of this and a little bit of STAT. *Curr. Opin. Oncol.* **12,** 543–549 (2000).
81. LeRoith, D. & Helman, L. The new kid on the block(ade) of the IGF-1 receptor. *Cancer Cell* **5,** 201–202 (2004).
82. Christofori, G., Naik, P. & Hanahan, D. A second signal supplied by insulin-like growth factor II in oncogene-induced tumorigenesis. *Nature* **369,** 414–418 (1994).
83. Orsulic, S. *et al.* Induction of ovarian cancer by defined multiple genetic changes in a mouse model system. *Cancer Cell* **1,** 53–62 (2002).
84. Kauffmann-Zeh, A. *et al.* Suppression of c-Myc-induced apoptosis by Ras signalling through PI(3)K and PKB. *Nature* **385,** 544–548 (1997).
85. Wendel, H. G. *et al.* Survival signalling by Akt and eIF4E in oncogenesis and cancer therapy. *Nature* **428,** 332–337 (2004).
86. Zindy, F. *et al.* Arf tumour suppressor promoter monitors latent oncogenic signals *in vivo*. *Proc. Natl Acad. Sci. USA* **100,** 15930–15935 (2003).
87. Aslanian, A., Iaquinta, P. J., Verona, R. & Lees, J. A. Repression of the Arf tumour suppressor by E2F3 is required for normal cell cycle kinetics. *Genes Dev.* **18,** 1413–1422 (2004).
88. Jin, S. *et al.* CIAP1 and the serine protease HTRA2 are involved in a novel p53-dependent apoptosis pathway in mammals. *Genes Dev.* **17,** 359–367 (2003).
89. Zheng, T. S. *et al.* Deficiency in caspase-9 or caspase-3 induces compensatory caspase activation. *Nature Med.* **6,** 1241–1247 (2000).
90. Harlin, H., Reffey, S. B., Duckett, C. S., Lindsten, T. & Thompson, C. B. Characterization of XIAP-deficient mice. *Mol. Cell. Biol.* **21,** 3604–3608 (2001).
91. Sage, J., Miller, A. L., Perez-Mancera, P. A., Wysocki, J. M. & Jacks, T. Acute mutation of retinoblastoma gene function is sufficient for cell cycle re-entry. *Nature* **424,** 223–228 (2003).
92. Seoane, J., Le, H. V. & Massague, J. Myc suppression of the p21(Cip1) Cdk inhibitor influences the outcome of the p53 response to DNA damage. *Nature* **419,** 729–734 (2002).
93. Johnstone, R. W., Ruefli, A. A. & Lowe, S. W. Apoptosis: a link between cancer genetics and chemotherapy. *Cell* **108,** 153–164 (2002).
94. Chin, L. *et al.* Essential role for oncogenic Ras in tumour maintenance. *Nature* **400,** 468–472 (1999).
95. Felsher, D. W. & Bishop, J. M. Reversible tumorigenesis by MYC in haematopoietic lineages. *Mol. Cell* **4,** 199–207 (1999).
96. Gunther, E. J. *et al.* Impact of p53 loss on reversal and recurrence of conditional Wnt-induced tumorigenesis. *Genes Dev.* **17,** 488–501 (2003).
97. Moody, S. E. *et al.* Conditional activation of Neu in the mammary epithelium of transgenic mice results in reversible pulmonary metastasis. *Cancer Cell* **2,** 451–461 (2002).
98. Pakunlu, R. I., Cook, T. J. & Minko, T. Simultaneous modulation of multidrug resistance and antiapoptotic cellular defence by MDR1 and BCL-2 targeted antisense oligonucleotides enhances the anticancer efficacy of doxorubicin. *Pharm. Res.* **20,** 351–359 (2003).
99. Bykov, V. J. & Wiman, K. G. Novel cancer therapy by reactivation of the p53 apoptosis pathway. *Ann. Med.* **35,** 458–465 (2003).

Acknowledgements We thank members of the Lowe and Evan laboratories for discussions, and M. Hemann, D. Burgess and M. McCurrach for critical readings of the manuscript. S. L. is supported by an NFCR-AACR research professorship and grants from the National Institutes of Health and the Leukemia and Lymphoma Society, E. C. is supported by a postdoctoral training fellowship from the National Cancer Institute, and G. E. is supported by grants from the NIH, the Juvenile Diabetes Foundation and the Brain Tumor Society.

Competing interests statement The authors declare that they have no competing financial interests.

©2004 Nature Publishing Group