A matter of life and death

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We propose that deregulation of proliferation, together with a reduction in apoptosis, creates a platform that is both necessary and can be sufficient for cancer. The secondary traits of diverse neoplasms are a consequence of cell proliferation, tissue expansion, and other outcomes of this platform.

Introduction: The cause of cancer

An absurd proposal

Much of the general public sees cancer as a modern day plague that culls our nearest and dearest with the capriciousness of the ancient Black Death. Others see its continuing onslaught, in the face of the vast resources directed at its conquest, as the irrefutable evidence of humanity's hubris. However, to the cancer patient, cancer is a terrifying alien entity invading his body and treatable only with medicines of medieval harshness and dubious efficacy. For the oncologist, cancer is a legion of recondite diseases whose diversity complicates therapy to the point where its efficacy can only be expressed by statistics, while to many basic scientists, cancer is a collection of well-established cell lines that conveniently take up foreign DNA and proliferate endlessly. In reality, a mutually agreeable definition of cancer is not possible. For the sake of this review, we will choose to use perhaps the most generally applicable definition of cancer as *the pathological expansion of a tissue resulting in morbidity.*

The possible causes and mechanisms of cancer have provoked much debate and controversy. However, out of this tumult has emerged one widely accepted principle that both the process of carcinogenesis and the tumors in which it results are extremely complex. This conclusion is reinforced by the manifestation of cancer as a bewilderingly diverse panoply of diseases, arising in different organs at different times in different individuals, following unpredictable patterns of progression, and confounding a rational therapy. Surely, treatment of such a diversity of diseases requires painstaking assembly of an equivalent diversity of therapies? Perhaps not.

After all, phenotypic diversity in biology need not necessarily imply corresponding causal complexity. For example, iteration of a common mechanism of cell proliferation has wildly different consequences depending upon cell type, location, and the contingent responses of neighboring tissues. By analogy, all tumors could be driven by a common platform of cell expansion yet nonetheless manifest themselves as diverse and fickle pathologies. In this review, we push this idea to its logical conclusion and propose the outrageous hypothesis that cancer is not complex at all, but a deceptively simple phenomenon (Figure 1). Our absurd proposal is this: *once conditions are met that provide a platform for ineluctable cell expansion, much of the characteristic pathology of cancer arises spontaneously as a consequence of interactions between the expanding mass and its somatic milieu.*

Of course, we would never wish to imply that cell expansion is the totality of tumorigenesis, since any autonomously expanding cell population will encounter shifting selective pressures that further shape and dictate its evolutionary trajectory. However, by stripping away incidental complexities, it is our hope to distill out the common engine of cancer and so identify common and tractable therapeutic targets. Specifically, we propose that rather than arising through the protracted, serial erosion of independent growth restraints, cancers arise from the rare simultaneous acquisition of the two cooperating conditions that permit cell expansion—deregulated cell proliferation and suppressed apoptosis. This, like cancer itself, happens so very rarely because the two processes are obligatorily interdependent—deregulated proliferation on its own triggers expeditious cell death, whereas suppression of apoptosis confers no selective advantage in the absence of cell proliferation—and so have to arise together in the same cell at the same time. This cooperative hypothesis for the emergence of cancer is not only in keeping with the well-characterized synergy exhibited by oncogenes, but also has the great benefit of solving a great conundrum of vertebrate biology: how does the organism achieve facile cell division where needed yet so effectively suppress the emergence of unregulated neoplastic clones? According to our proposal, cells propagate only when in receipt of interlocking signals that simultaneously promote proliferation and suppress the consequent apoptosis (Evan and Littlewood, 1998). This is easily accomplished in the correct somatic context through the connivance of appropriate neighboring cells, whereas simultaneous mutational activation of all requisite cooperating pathways in an individual is an event of comforting rarity.

We will prosecute our case by examining three implications of our hypothesis. First, we will explore why it is normally so difficult to achieve a combined state of proliferation and suppressed cell death by showing that conditions that promote cell proliferation necessarily engage the cell death process. We will then explore how such cell death occurs, so as to understand how it can become suppressed in cancer. Finally, we will consider how these conditions provide an obligate cancer platform that defines targets against which we can target appropriate therapies.

How cancer cells proliferate

Pulling out the stops

Underpinning the relentless and pathological expansion of cancers are lesions that compromise control of the proliferation, survival, differentiation, and migration of tumor cells. Early on in metazoan evolution, somatic cell autonomy became obligatorily restricted, and cell fate subsumed to the needs of the whole

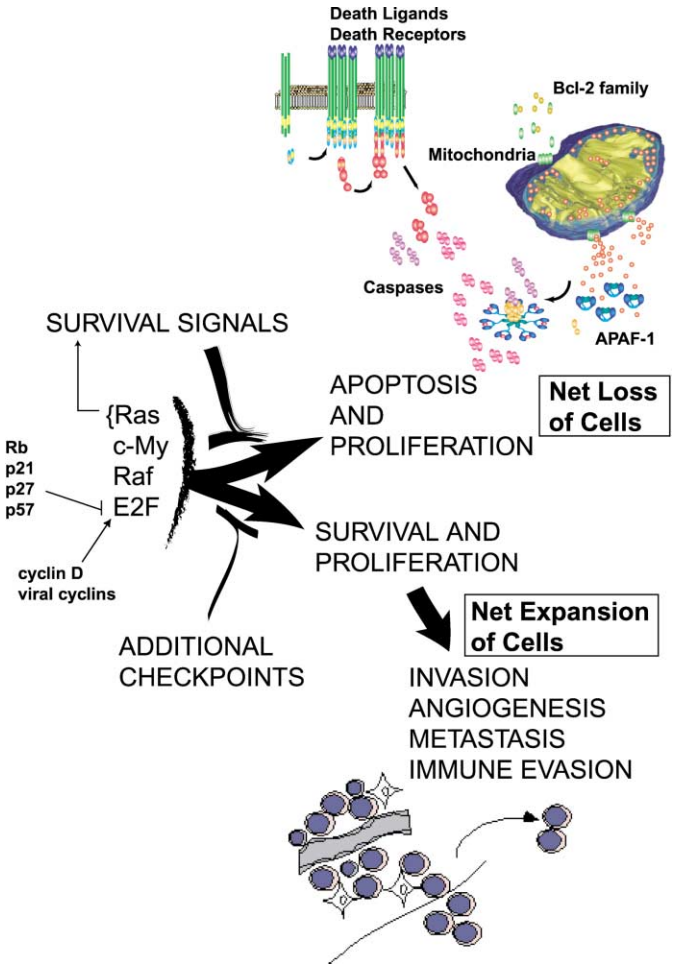

Figure 1. An absurd proposal

Signals that promote proliferation promote apoptosis (this is not an "either/or" situation; both occur simultaneously). The apoptosis pathways so engaged are shown schematically. If apoptosis is blocked by survival signals, expansion occurs, and the manifestation of this expansion is what we see as cancer. This proposal stands in sharp contrast to models in which sequential blocks to transformation are bypassed by mutation and selection to ultimately produce an autonomous cancer.

organism. Cancer is the erosion of this metazoan dowry through the accumulation of genetic damage in somatic cells. In their landmark review "The Hallmarks of Cancer," Hanahan and Weinberg (2000) delineated the principle mechanisms restraining somatic cell proliferative autonomy that must be abrogated for cancers to arise. Normal somatic cell proliferation is shackled both by an absolute need for external mitogens and sensitivity to inhibition by multiple growth-suppressive signals. Consequently, deregulation of cell proliferation requires acquisition of both autonomy from exogenous mitogens and refractoriness to normal growth inhibitory signals. Even then, the capacity to proliferate endlessly is restricted by telomere erosion that must be independently overcome for cancers to emerge. In addition, macroscopic tumor growth requires the capacity to recruit and subvert necessary trophic and vascular support from neighboring tissues, while an acquired ability to spread and metastasize marks the most intractable feature of cancer as a disease. The image is of many independent mechanisms limiting somatic cell autonomy that can be eroded only by the protracted and stochastic accretion of multiple mutations, an accretion reflected in the evident genetic complexity within individual cancer cells. In this view, a series of challenges faces the emerging tumor, and they are met one by one. By having enough of these hurdles in place, most lifetimes run out before cancer becomes an issue. *We suggest that this need not always be the way it works. The Hallmarks are undoubtedly real, but cancer may be rare for another reason.*

Antagonistic pleiotropy—A decidedly two-faced view of the world

More recently, it has become clear that the "altruism" of vertebrate cells is enforced by some remarkably sophisticated evolutionary footwork (Figure 1). The first intimation of this came from the unexpected discovery that oncoproteins like Myc and E1A can act as potent inducers of apoptosis. This led to the proposal that apoptosis might act as an inbuilt failsafe to staunch "inappropriate" proliferation of somatic cells (Harrington et al., 1994b). Many, perhaps all, mechanisms that drive cell proliferation have the potential to trigger, or sensitize a cell to, apoptosis (Evan and Littlewood, 1998). Established examples include activation of the Ras/Raf pathway, deregulated expression of c-Jun, and almost anything leading to promiscuous activity of the E2F G1 progression transcription factors. Moreover, other versions of such antagonistic pleiotropy exist, such as the innate capacities of activated Raf and Ras oncoproteins to trigger permanent growth arrest (Lloyd et al., 1997; Serrano et al., 1997) or the growth inhibitory action of the apoptosis suppressor Bcl-2 (Huang et al., 1997; Linette et al., 1996). A major component of the differential that couples the oppositely spinning wheels of proliferation and growth suppression is the ARF/Mdm-2/p53 tumor suppressor pathway (Sherr, 2001); Ras, E1A, Myc, and E2F all induce ARF, an alternate product of the *INK4a* locus which then binds and inactivates Mdm-2, a key part of the ubiquitin ligase that keeps p53 at bay (Lowe, 1999). However, evidence is also emerging for ARF/p53-independent pathways, underscoring the importance of redundancy in this vitally important tumor suppressive mechanism.

Such hardwiring of growth-suppressive mechanisms into growth-promoting ones is, of course, a very effective way of staunching malignant progression. Unfortunately, since oncoproteins are also part of the normal proliferative machinery, this has the unwanted side effect of inhibiting normal cell growth. Clearly, there must be some way of distinguishing between normal and abnormal proliferation. One possibility is that cells are able specifically to sense "hyperproliferative" signals and respond by activating the failsafe response. However, in the case of Myc at least, prevailing evidence suggests that the cellular decision whether to live or die is determined by the availability of social survival signals which, if present, specifically gate the apoptotic program and so foster net cell proliferation. Consequently, Myc is unable to drive cell expansion without the assistance of cooperating signals; in essence, oncogene cooperation by another name. In addition, by both p53-dependent and -independent mechanisms, Myc sensitizes cells to a diverse range of pro-apoptotic insults, including nutrient deprivation, hypoxia, DNA damage, and death receptor signaling (Alarcon et al., 1996; Evan et al., 1992; Juin et al., 1999), all likely consequences of tumorigenic growth. In this way, Myc, and by inference other oncoproteins, establishes "sentinel" functions that remain dormant in normal cells proliferating in their stress-free orthotopic environments. However, should a cell encounter

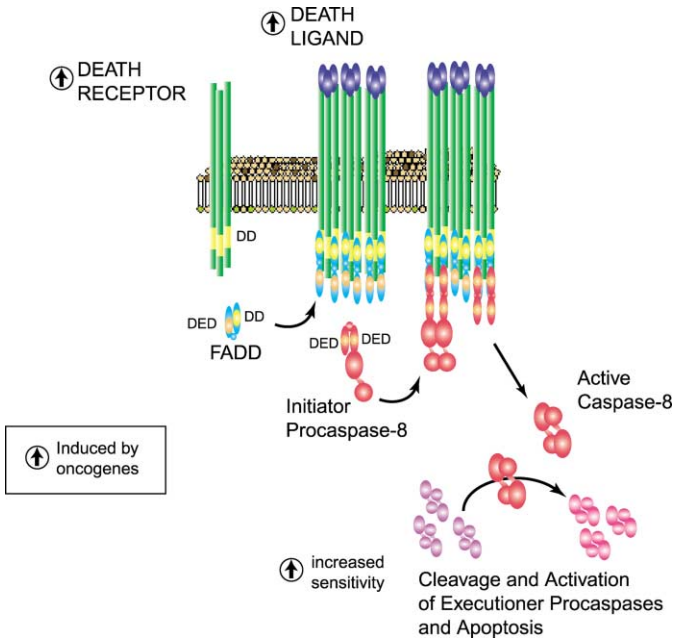

Figure 2. Death by receptor

Trimeric death ligands engage their death receptors (probably also trimeric) to trigger intracellular events leading to apoptosis. The death domains (DD) of the receptor bind to a DD on the adaptor protein FADD, exposing a death effector domain (DED). This binds to a DED in the prodomain of procaspase 8. Bringing two procaspase 8 molecules together results in their auto-activation to a mature initiator caspase, which cleaves and activates executioner caspases. The pathway shown applies to some death receptors (e.g., Fas, Trail-R), while others are more complex (e.g., TNF-R1). Oncogenic signals that promote apoptosis can increase expression of death ligands and/or death receptors, and increase sensitivity to the apoptotic signaling events, downstream of those shown here.

stress or wander into a somatic compartment with inadequate trophic support, then some of these spring into activity to ensure the arrest or timely demise of the cell. The importance of such "sentinel" functions in tumor suppression has long been intimated by the dramatic oncogenic cooperation observed between Myc and anti-apoptotic lesions such as overexpression of Bcl-2 or loss of ARF or p53. With the recent advent of switchable transgenic Myc models, it has become possible to directly observe Myc-induced apoptosis in vivo. Indeed, so potent is the innate tumor suppressive action of Myc-induced apoptosis that its acute activation in vivo in pancreatic β cells triggers their immediate and wholesale ablation (Pelengaris et al., 1999). Such ablation is dramatically transformed into abrupt tumor progression upon suppression of β cell apoptosis by expression of Bcl-x_L.

Importantly, there is nothing inherent in the mechanisms underlying proliferation or cell death that requires them to be connected in this way—rather, antagonistic pleiotropy appears to have arisen from a coevolutionary process to restrict the emergence of cancer.

The induction of apoptosis by growth-deregulating lesions such as Myc, E2F, and loss of Rb has far reaching implications. Perhaps the most fundamental is the idea that the combination of deregulated proliferation and suppressed apoptosis constitutes the minimal platform upon which all neoplasms reside.

How cells die, or not

A handbook of cell survival—Part 1

Since signals that drive entry into the cell cycle also prime the apoptotic machinery, the ensuing fate of the cell is largely determined by the availability of local survival signals which, if present, gate the apoptotic response. Thus, to understand cancer we must first understand cell survival.

Survival signals take many forms, often peculiar to particular cell types. Perhaps the best described are the polypeptide factors such as PDGF, NGF, chemokines, interleukins, and IGFs that act through cognate receptor tyrosine kinases (see later). In addition, the rapid death of many cell types upon matrix detachment (anoikis) indicates that they derive necessary survival signals through their direct contact with neighbors or extracellular matrix, typically mediated by integrins and cell adhesion molecules (Frisch and Screaton, 2001). Other known survival signals for specific cell types include steroids, lysophospholipids, glucose in pancreatic β cells, and synaptic connections for certain neuronal cells. Indeed, the only practical definition of a survival signal is that its withdrawal triggers apoptosis. However, even this pragmatic definition is not without complications, since the same factor can have very different effects in different cell types, or in the same cell under different conditions. For example, EGF is a potent survival signal and mitogen for many epithelial cells, but only a mitogen in fibroblasts. In the case of BAF pro B cells, interleukin-2 (or interleukin-3) and serum together serve to promote survival, whereas exposure to interleukin in the absence of serum triggers rapid upregulation of c-Myc and expeditious apoptosis (Shi et al., 1997).

The ability of survival factors to protect specifically from oncogene-induced apoptosis was first noted with c-Myc, where Myc-induced apoptosis was profoundly inhibited in fibroblasts by serum (Evan et al., 1992) or IGF-1 (Harrington et al., 1994a) and in myeloid cells by IL-3 (Askew et al., 1991). Whether or not cell death occurs, Myc is an equally potent inducer of cell proliferation. However, in the presence of survival factors, cells survive and accumulate. In other cases, activated oncogenes may innately provide both proliferation/apoptosis signals and survival signals, albeit through different effector pathways. Thus, oncogenic Bcr-Abl, the principal lesion underlying chronic myelogenous leukemia, induces expression of c-Myc (Sawyers et al., 1992) but also produces potent anti-apoptotic signals (Amarante-Mendes et al., 1997). Similarly, oncogenic Ras can elicit both apoptosis and survival via its differing downstream effector pathways (Kauffmann-Zeh et al., 1997).

Surviving the lethal insult of oncogene activation is a prerequisite to establish a platform on which the cancer process can build, and therefore the way cell death is controlled is central to an understanding of cancer itself.

Death of a proliferating cell—No great loss

Cell type and tissue of origin have a significant influence on the very earliest stages of tumorigenesis simply because different tissues employ differing strategies to limit runaway cell expansion. Perhaps the most obvious is for a tissue to be postmitotic and its component cells thereby inured to neoplastic mutation. However, this is not a universally appropriate strategy in vertebrates, because many tissues are required to undergo continuous or episodic renewal. More generally, tissues limit exposure of proliferating cells to mutation by restricting self-renewal to a limited cadre of stem cells. Superficial epithelia, such as gut and skin, further curb accumulation of proliferating

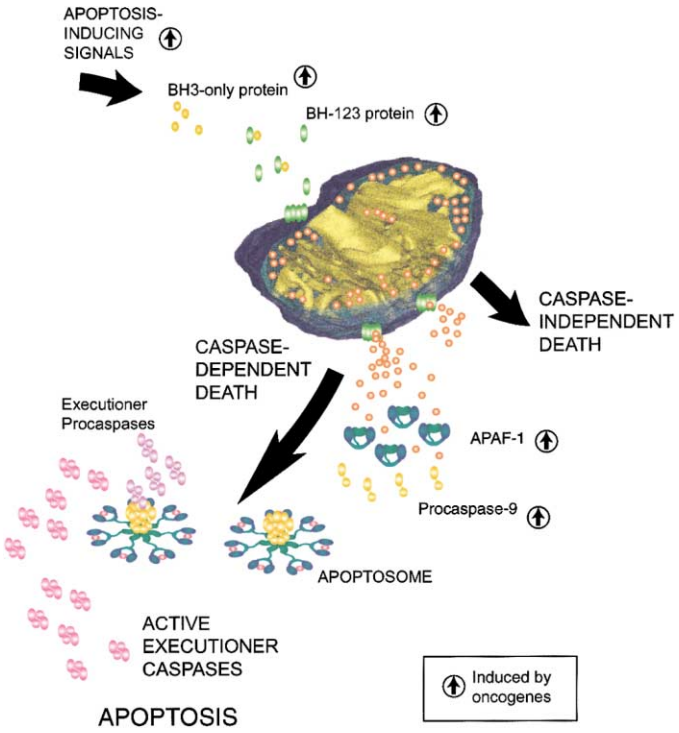

Figure 3. Death by MOMP

Pro-apoptotic signals lead to mitochondrial outer membrane permeabilization (MOMP), releasing proteins that reside in the mitochondrial intermembrane space and disrupting mitochondrial function. Cytochrome c promotes the formation of an apoptosome that activates procaspase 9, which in turn activates executioner caspases to orchestrate apoptosis. Other released proteins, loss of mitochondrial function, and production of reactive oxygen species all promote death that can proceed in a caspase-independent manner. Oncogenic signals the promote apoptosis stimulate events upstream of MOMP and can elevate the levels of the components of the apoptosome. The mitochondrion shown is a tomograph of an organelle that has undergone MOMP in an in vitro system (courtesy of Dr. Don Newmeyer).

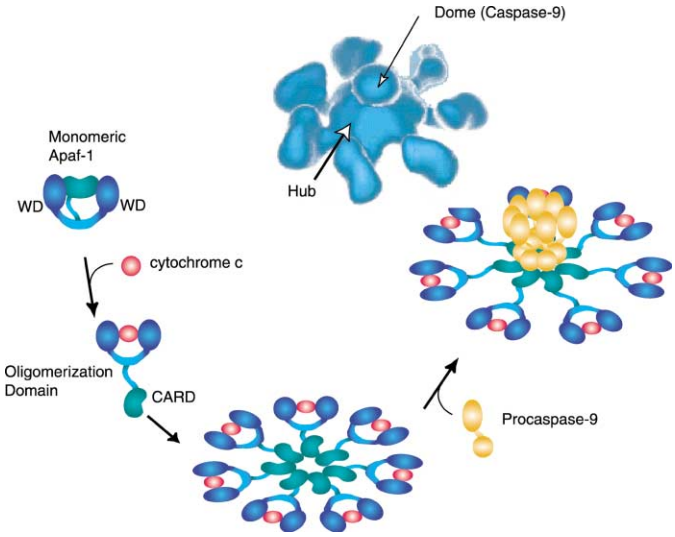

Figure 4. The apoptosome: A wheel of death

Monomeric Apaf-1 probably persists in its inactive form by interaction of its N-terminal region with the WD domains in the C-terminal region. When cytochrome c binds to the WD region, the protein unfolds, exposing an oligomerization domain. Upon stabilization by dATP or ATP, a seven-spoked oligomer forms. Caspase recruitment domains (CARDs) in the hub of the oligomer bind to the CARDs of procaspase 9 molecules to activate them. The activated caspase 9 cleaves and activates executioner caspases to orchestrate apoptosis. (Modified from Acehan et al., 2002).

cells by depositing all progeny on a one-way conveyer belt to the surface, where the cells are then promptly shed. It is axiomatic that derailment of this conveyer is a prerequisite for neoplasia in such a tissue. For most other tissues, apoptosis is the preferred sink for excess cells that must be overcome for tumorigenesis to gain a foothold.

The executioner's scissors

Although any form of regulated cell death is potentially relevant to our thesis, we will focus on that morphologically defined type of active cell death termed "apoptosis" (Wyllie et al., 1980). The principal molecular engines that execute apoptosis, the killing of the cell and its packaging for phagocytic removal, are the caspase proteases that precipitate the apoptotic process by cleaving critical cellular substrates. However, caspase activation is not necessarily consonant with cell death: on the one hand, it is apparent that cells can survive limited caspase activation (Alam et al., 1999) and conversely, that inhibiting caspases will often block the morphological manifestations of apoptosis, but cell death proceeds nevertheless. The existence of such caspase-independent cell death is significant since it intimates that cell death is a highly redundant process that can be thwarted in cancer cells only with great difficulty.

Caspases are highly specific cysteine proteases, most cleaving tetra- or pentapeptide recognition sequences at a P1 aspartate residue. They are synthesized as zymogens and activated by cleavage at aspartates that are themselves in caspase sites to generate the large and small subunits of the mature enzyme. The "initiator" caspases, such as caspases 8 and 9, are typically low abundance zymogens whose extensive prodomains mediate their oligomerization and autoactivation in response to specific upstream signals. The best documented pathways of initiator caspase activation are the assembly of the Death Induced Signaling Complex (DISC) induced by ligation of the TNFR1, CD95/Fas and Trail death receptors (Figure 2), and the formation of the cytosolic apoptosome (Figure 3). The DISC forms when death receptor ligation triggers association of the intracellular adaptor protein FADD with the cytoplasmic tail of the receptor. FADD then recruits procaspase 8 into the DISC, whereupon the procaspase undergoes spontaneous autoactivation (Figure 2). The apoptosome forms when signals trigger release of holocytochrome c from mitochondria, which then triggers assembly of the Apaf-1/caspase 9 holoenzyme (Figures 3 and 4).

Downstream of these initiators are the effector caspases, such as caspases 3 and 7, abundant proteases that cleave cellular substrates and precipitate apoptotic death. Some of the key substrates of these "executioner's scissors" are known. For example, caspase 3 cleavage and inactivation of the nuclease inhibitor iCAD liberates the CAD nuclease to cleave the DNA (Enari et al., 1998), aiding in the subsequent rapid degradation of the cell following its uptake by phagocytes. Caspases also promote the efficient phagocytosis and disposal of apoptotic cells by triggering membrane blebbing through cleavage and activation of several enzymes, including gelsolin, p21-activated kinase, and ROCK-1 (Coleman et al., 2001; Kothakota et al.,

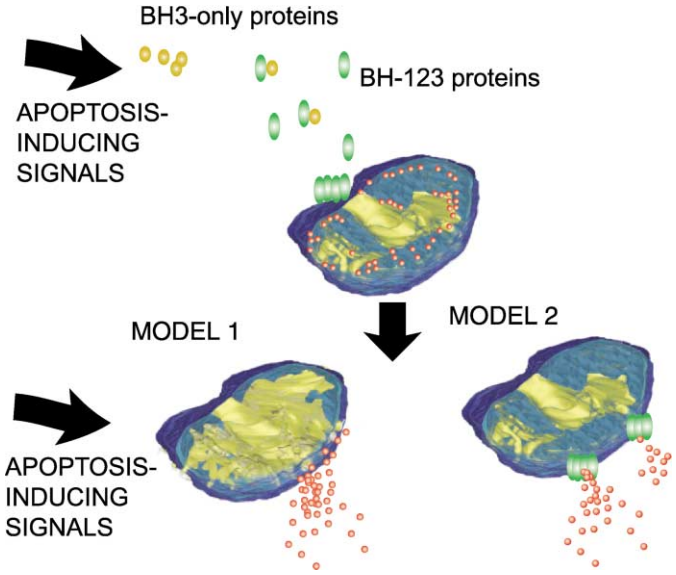

Figure 5. Models of MOMP

Pro-apoptotic BH3-only members of the Bcl-2 family are activated by various signaling events, and these trigger the activation of BH-123 proteins (which include Bax, Bak, and Bok). These oligomerize and insert into the mitochondrial outer membrane. The models to account for MOMP during apoptosis have been reviewed elsewhere (Martinou and Green, 2001). Superficially, in Model 1, this event and various other signals promote MOMP by altering the behavior of the mitochondria by (A) a permeability transition, in which an opening of the adenine nucleotide transporter in the inner membrane causes matrix swelling, leading to outer membrane disruption (Zamzami and Kroemer, 2001), or (B) VDAC closure, in which metabolic signals in the cell trigger a closure of the voltage-dependent anion channel in the outer membrane, resulting in inner membrane perturbations that cause matrix swelling and outer membrane disruption (Vander Heiden and Thompson, 1999). In contrast, in Model 2, the oligomerized BH-123 protein causes the permeabilization of the outer membrane directly, without contributions from mitochondrial processes. This is the pore-forming model in which MOMP is mediated by a pore or other permeability change in the outer membrane, permitting protein release without involving the inner membrane (Waterhouse et al., 2001; Wei et al., 2001). The mitochondria shown are cartoons modified from the tomograph in Figure 4.

1997; Rudel and Bokoch, 1997; Sebbagh et al., 2001), and through their promotion of "eat me" signals, such as the externalization of phosphatidylserine (Fadok et al., 2001).

How activated oncogenes promote apoptosis

Oncogenes and death receptors

Multiple mechanisms underlie the induction of apoptosis by oncogenes. One is via pathways mediated by the death receptors such as TNFR1, Trail, or CD95/Fas. In T lymphocytes, activation and cell cycle entry engages an apoptotic process mediated by expression of Fas-ligand and engagement of Fas sigaling (Sharma et al., 2000). This engagement is dependent on c-Myc (Shi et al., 1992), which induces expression of Fas-ligand expression (Kasibhatla et al., 2000). Induction of apoptosis in rodent fibroblasts by Myc is also dependent upon Fas-Fas-ligand interactions (Hueber et al., 1997), although in this case, the relationship has more to do with Myc expression sensitizing cells to a preexisting Fas signal. Likewise, expression of the adenovirus oncoprotein E1A, which also drives both cell cycle entry and apoptosis, sensitizes cells to apoptosis induced by the death ligands TNF, Fas-ligand, and TRAIL (Routes et al., 2000). E1A-induced apoptosis is p53-dependent (Debbas and White, 1993) and may involve p53-induced upregulation of the TRAIL death receptor, DR5 (Sheikh et al., 1998). However, E1A and p53 seem to induce apoptosis predominantly through the mitochondrial-apoptosome pathway, as discussed below.

Engagement of death receptor pathways by Myc and E1A appears to occur via downstream effector pathways that are distinct and independent from cell cycle progression. This again underscores the fundamental point that the pro-apoptotic actions of activated oncogenes are not merely the consequences of defective proliferation but discrete mechanisms that have evolved in animals with long life spans to ameliorate the oncogenic risk of cell proliferation.

Mitochondria and the activation of caspase-9

Whereas caspase 8 is activated in the death receptor DISC, the initiator caspase 9 is activated in the cytosol through binding to its adaptor, Apaf-1 (apoptotic protease activating factor-1) in a homotypic interaction mediated by their shared CARD domains (Figure 4). This happens when Apaf-1 is activated to oligomerize by holocytochrome c (Zou et al., 1997) and, in turn, Apaf-1 activates caspase 9 without any requirement for caspase 9 cleavage (Stennicke et al., 1999). The active cytochrome c/Apaf-1/caspase 9 complex has been nicknamed the "apoptosome."

Activated oncogenes are known to facilitate apoptosome assembly and activation in various ways. For example, adenovirus E1A induces upregulation of both Apaf-1 and procaspase-9 (Fearnhead et al., 1998). Levels of Apaf-1 in cells may be limiting, so elevation of Apaf-1 would presumably increase sensitivity of apoptosome activation to cytochrome c release. Likewise, E2F1 upregulates expression of Apaf-1 (Moroni et al., 2001). It remains to be seen whether other pro-apoptotic oncogenes such as Myc and Ras similarly upregulate Apaf-1 and/or procaspase-9. However, the most significant and crucial contribution made by activated oncoproteins in the activation of the apoptosome seems to be at the point of holocytochrome c release from mitiochondria.

In any individual cell, the onset of cytochrome c loss from mitochondria is sudden, occurs after a variable time since the apoptotic insult, and involves the rapid and complete release of cytochrome c from all mitochondria (Goldstein et al., 2000). In addition, a variety of other proteins are also released from the mitochondrial intermembrane space at the same time, and many of these have their own discrete roles in promoting apoptosis (see below). Such dramatic discharge of mitochondrial contents is a result of mitochondrial outer membrane permeabilization (MOMP), although significant controversy surrounds the mechanism responsible for MOMP in apoptosis (Martinou and Green, 2001) (Figure 5). Model 1 favors a major role for mitochondrial function in sensing and precipitating MOMP. In contrast, Model 2 views the mitochondrion as a passive lockbox full of deadly apoptogenic factors that are unleashed when pro-apoptotic effectors compromise the integrity of the outer mitochondrial membrane.

MOMP in caspase-dependent and independent cell death

Although cytochrome c is necessary for activation of the Apaf-1/caspase 9 apoptosome, downstream activation of the executioner caspases can nonetheless be forestalled by the IAPs (inhibitor of apoptosis proteins), which act as endogenous caspase inhibitors. X-linked IAP (XIAP), and probably other IAPs, binds to active caspase-9 and inhibits its activity. Furthermore, XIAP (and other IAPs) function as E3-ubiquitin ligases that target active caspases for rapid degradation (Suzuki et al., 2001b).

XIAP, and probably other IAPs, are in turn regulated by at least two polypeptides also released from mitochondria upon MOMP, Smac/DIABLO (Du et al., 2000; Verhagen et al., 2000), and Htra2/Omi (Hegde et al., 2002; Suzuki et al., 2001a; Verhagen et al., 2002). These bind to XIAP via an AVP(I/S) sequence at their N termini and thereby incapacitate IAP inhibition of caspases.

There is also evidence that other proteins within the mitochondrial intermembrane space can promote cell death in caspase-independent ways. One example is Htra2/Omi, which in addition to its ability to block IAPs, appears to promote caspase-independent cell death via its intrinsic serine protease activity (Hegde et al., 2002; Suzuki et al., 2001a). Another is AIF (apoptosis inducing factor), which upon its release translocates to the nucleus where it appears to induce chromatin condensation (Susin et al., 1999). Finally, EndoG nuclease is thought to directly mediate nuclear DNA fragmentation upon its release from the mitochondrial intermembrane space (Li et al., 2001).

Another major consequence of MOMP is degeneration of the electron transport chain that is required for most of the organelle's functions. While this loss occurs rapidly upon caspase activation, it can also be caspase independent. The consequent disruption of ATP generation, which in the absence of caspase activation typically declines within hours or days of MOMP (depending on the cell and the nature of the apoptosis-inducing stimulus) (Goldstein et al., 2000; Waterhouse et al., 2001), ensures the ultimate demise of the cell, although the mode of cell death may more closely resemble necrosis than classical apoptosis. In addition, the breakdown of electron transport promotes the generation of reactive oxygen species which may also hasten cell death.

The relative importance of loss of mitochondrial function, caspase activation, and the caspase-independent actions of other death-promoting proteins in the death of any cell in any instance remains unresolved. However, such diversity and redundancy in cell suicide mechanisms implies an equivalent diversity in the mechanisms that tumor cells are likely to employ in their need to thwart apoptosis. Consequently, identification of the peculiar anti-apoptotic strategy adopted by each tumor type would shed light on the particular pro-apoptotic pathways that have shaped the development of that specific neoplasm and indicate how best to reinstate the defective suicide program.

MOMP and the commitment to cell death

For a survival signal to participate in the process of oncogenesis, it must act before a cell is irrevocably committed to die. Where, in the pathways we have been discussing, does such commitment occur? In those cells in which death receptor signaling is completely dependent on the activation of caspase 8, inhibition of this caspase can confer protection from the lethal consequences of death receptor signaling. In most cases of apoptosis, however, the signals leading to MOMP precede caspase activation and occur in a caspase-independent fashion. When this happens, can inhibition or loss of caspase activation result in cell survival?

We have surprisingly little information on the viability of cells post-MOMP, although factor-deprived neurons have been shown to survive for several days after the release of cytochrome c provided caspase activation is blocked. Upon return of growth factors, the neurons regenerate functional mitochondria and exhibit long-term survival (Deshmukh et al., 2000; Martinou et al., 1999). However, in the case of transformed cells transiently exposed to a variety of pro-apoptotic insults (Myc or E1A oncoproteins, etoposide, UV, staurosporine, actinomycin D), the presence of caspase inhibitors suppresses classical apoptosis but the cells nonetheless exhibit total loss of clonogenicity (Amarante-Mendes et al., 1998; McCarthy et al., 1997). Thus cells may survive but retain zero neoplastic potential.

Mice lacking elements of the postmitochondrial apoptosome (e.g., Apaf-1–/– mice, caspase-9–/– mice, and to a lesser extent, caspase 3–/– mice) show profound developmental defects attributable to inhibition of apoptosis, including craniofacial abnormalities and a grossly enlarged cortex which often extrudes through the skull (Mak and Yeh, 1999). While these developmental effects may support the idea that events downstream of MOMP can act to preserve proliferative capacity of a cell, another possibility exists. Cell death following MOMP proceeds more slowly in the absence than in the presence of caspase activity, and from the period following MOMP until they expire it is possible that cells preserve the ability to produce survival factors for other cells. Those cells that may depend on these will therefore receive more survival factors when their providers die via caspase-independent versus caspase-dependent mechanisms. As a result, more cells accumulate when caspase activation does not occur. We do not know whether or not the cells that amass in these mutant mice have ever undergone (and recovered from) MOMP, or if such alternate processes account for the effect.

From the perspective of cancer, then, the most important question may be this: can inhibition or loss of the apoptotic pathways downstream of MOMP contribute to oncogenesis? Recent reports suggest that the answer may be yes, although not without important reservations. Mouse embryonic fibroblasts lacking Apaf-1 or caspase-9 are reported to be more readily transformed by oncogenes than their wild-type counterparts (Soengas et al., 1999), suggesting that inhibition of apoptotic machinery post-MOMP can indeed contribute to oncogenesis. However, no demonstration of recovery from MOMP (e.g., cells that released cytochrome c but nevertheless recovered and proliferated) was attempted, and no rigorous examination of p53 status in these cells was performed (any loss of p53 function in these cultured cells, which is common, would greatly enhance transformation on its own). Therefore, while provocative, these results are not definitive. However, some support for a role of downstream apoptotic events in oncogenesis comes from studies of human melanoma lines, where the majority of cells show reduced or absent Apaf-1 expression (Soengas et al., 2001). On the other hand, a similar loss of Apaf-1 activity in human ovarian carcinoma lines showed no correlation with susceptibility to apoptosis (Wolf et al., 2001).

We are left with only the possibility that regulation of apoptosis downstream of MOMP can influence cell survival and therefore oncogenesis. For example, the heat shock protein-70 (HSP-70) molecular chaperone is induced by diverse stresses and has been shown to enhance transformation by oncogenes (Beere and Green, 2001). Provocatively, HSP-70 can inhibit activation of caspase-9 by Apaf-1.

MOMP and the Bcl-2 family

While it remains uncertain that cells can survive MOMP, it is nonetheless clear that MOMP is a major player in the determination of loss of cell viability. Recently, the Bcl-2 family proteins have emerged as fundamental regulators of MOMP. The Bcl-2 family members come in three flavors: anti-apoptotic, pro-apoptotic "BH-123" proteins, and the pro-apoptotic "BH3 only" proteins. The known anti-apoptotic members include Bcl-2, Bcl-x_L,

Mcl-1, Bcl-w, and A1, some of which are frequently expressed in primary human cancers. The pro-apoptotic BH-123 proteins share three of the Bcl-2 homology (BH) domains with the anti-apoptotic proteins and include Bax, Bak, and Bok. The pro-apoptotic BH3-only proteins possess only the BH3 domain necessary for their pro-apoptotic activity, and include Bid, Bim, Bik, Bmf, Bad, Hrk, BNIP3, Noxa, and Puma. Bcl-2, Bcl-x_L, Bax, and Bid share similar 3D structures that have an intriguing resemblance to the pore-forming chains of some bacterial toxins. From this has come the idea that they act as pores in the mitochondrial membrane. Indeed, Bcl-2, Bcl-x_L, Bax, and Bid have all been shown to have weak channel-forming activity for small ions through lipid membranes, although whether this is how they regulate apoptosis remains controversial (Martinou and Green, 2001).

The anti-apoptotic proteins Bcl-2 and Bcl-x_L block MOMP in cells and in cell-free systems, while the pro-apoptotic BH-123 members Bax and Bak promote it. Furthermore, cells and isolated mitochondria from mice lacking both Bax and Bak (but not one or the other) exhibit dramatic resistance both to induction of MOMP and to the activation of the mitochondrial pathway of apoptosis (Lindsten et al., 2000), indicating that Bax and Bak fulfill a redundant function intimately (and perhaps directly) involved in MOMP.

In the absence of apoptotic signals, the pro-apoptotic Bax protein exists as an inactive monomer that can be induced to oligomerize and migrate to the mitochondria by various BH3-only proteins (Eskes et al., 2000). Such oligomerization at the mitochondrion correlates with induction of MOMP, hinting that there is a direct connection between the two. One model is that monomeric Bax protein is folded in such a way that its BH3 domain is hidden, but exposed upon binding of an activating BH3-only protein. This activated Bax then interacts with a second Bax monomer, displacing its BH3 domain and so on, creating a chain reaction of pro-apoptotic Bax activation and oligomerization. Oligomerized Bax then either generates a pore or otherwise alters the integrity of the outer mitochondrial membrane. In this scenario, the role of the anti-apoptotic Bcl-2 proteins is to sequester and staunch the activated Bax and/or BH3-only proteins and so prevent or curtail the lethal chain reaction (Cheng et al., 2001). It is unclear if Bax or other BH-123 proteins act on the mitochondrial outer membrane alone or in concert with other proteins in the outer membrane, such as the voltage-dependent anion channel (Shimizu et al., 2001).

The oncogenic consequences of inhibition of MOMP through upregulation of the anti-apoptotic Bcl-2 proteins are most strikingly illustrated in human follicular B cell lymphoma, in which a reciprocal *t(14;18)* translocation deregulates Bcl-2 expression through fusion with the immunoglobulin heavy chain enhancer. The initial result is an indolent tumor whose expansion appears to depend on availability of normal B cell growth-promoting signals. Subsequently, the lymphoma cells accumulate additional mutations (e.g., loss of p53 function and/or expression of c-Myc) and a much more aggressive tumor emerges. This process is thought to be recapitulated in transgenic animal systems, where enforced coexpression of Bcl-2 greatly accelerates Myc-induced B cell lymphomagenesis, presumably because Bcl-2 blocks c-Myc-induced apoptosis while c-Myc overcomes the growth suppressive action of Bcl-2.

Much of the control of apoptosis is determined by the many and diverse BH3-only proteins. However, it is not known if the primary role of BH3-only proteins is to activate the BH-123 killers, inactivate the Bcl-2 protectors, both, or neither. The best guess is that the BH3-only proteins each act as a terminal apoptotic effector, coupling a specific pro-apoptotic signaling pathway to the mitochondria. Consistent with this, the different BH3-only proteins show diverse patterns of tissue expression and transcriptional control. For example, Noxa (Oda et al., 2000) and Puma (Nakano and Vousden, 2001; Yu et al., 2001) are both induced by activated p53 and are presumed to be important mediators of the apoptotic response to genotoxic damage. In addition, however, much of the regulation of BH3-only proteins is posttranscriptional. Thus, Bim (Puthalakath et al., 1999) and Bmf (Puthalakath et al., 2001) are normally associated with the cytoskeleton and are activated by release from this compartment, which may occur upon matrix detachment and underlie the mechanisms of anoikis (although they clearly play other roles as well). Another BH3-only protein, Bid, is activated upon cleavage by a number of different proteases, including caspase 8 (Budihardjo et al., 1999), the cytotoxic lymphocyte protease Granzyme B (Pinkoski et al., 2001), and lysosomal proteases (Stoka et al., 2001). A final example is the BH3-only protein Bad, whose lethal action is blocked only so long as survival factor signaling via the serine/threonine kinase Akt/PKB (and other kinases) keeps it phosphorylated and sequestered by the cytosolic 14-3-3 proteins (Zha et al., 1996).

On the threshold of the abyss

The idea that the various BH3-only proteins serve as terminal effectors of different signaling pathways to trigger MOMP offers an explanation for the remarkable ability of cells to integrate so many diverse pro- and anti-apoptotic influences. In effect, the different BH3-only effectors integrate multiple signaling pathways at the mitochondria and distill them into a binary decision that fires the apoptotic program should some threshold be exceeded.

That apoptosis is triggered when some buffered threshold is exceeded is suggested by the binary nature of the life-death decision. It is also indicated by observations that cells can be subjected to a variety of independently subcritical apoptotic insults that when administered together trigger dramatic apoptosis. This additivity is directly relevant to oncogene-induced apoptosis, which appears highly dependent upon the status of other pro- and anti-apoptotic signaling such as cell type, survival factor availability, death receptor signaling, and DNA damage (Evan et al., 1992; Harrington et al., 1994a; Hueber et al., 1997; Juin et al., 1999). It is the net summation of the action on MOMP of the oncoprotein, together with other existing pro- and anti-apoptotic influences, that determines whether the firing threshold is exceeded and the cell will die. One confusing consequence of this is that it can often appear that there is one particular pathway or BH3-only protein that is pivotal in mediating the cell death by a particular oncogene. In reality, however, that particular pathway may merely be the final straw that breaks the apoptotic camel's back, as with Florida in the 2000 US presidential election, where there was nothing special about that state's vote save that it was the last to be added to the tally. Such considerations probably explain why oncogene-induced apoptosis has been differently attributed to so many disparate critical factors.

The handbook of cell survival—Part 2

While oncogenes and other pro-apoptotic signals combine to erode the threshold for apoptotic firing, their effects are antagonized by a variety of anti-apoptotic mechanisms. As already noted, pivotal determinants of cell survival in vivo are survival

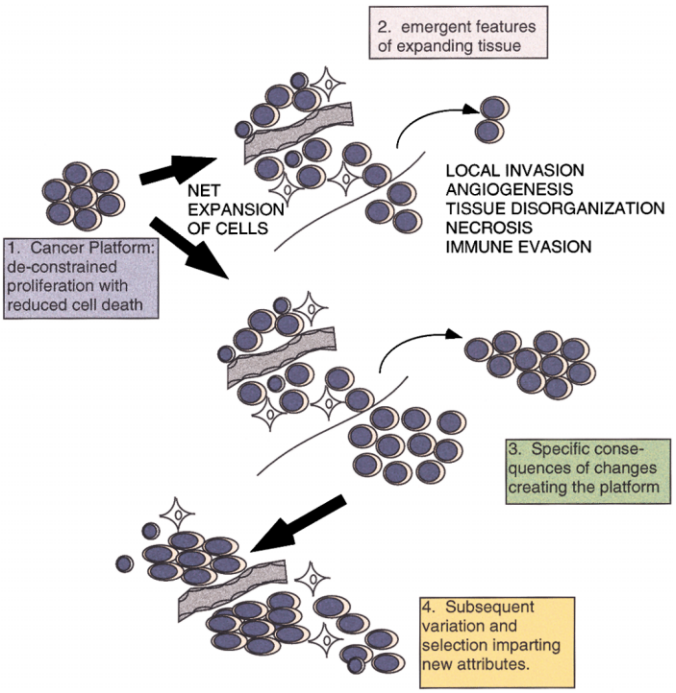

Figure 6. The cancer platform

Cells that have deconstrained proliferation with reduced cell death have attained a cancer platform that can spontaneously yield the features associated with morbidity and mortality. The cancer platform itself results in expansion of the tissue (1). The growing tumor will display features associated with the expansion of the normal tissue, and these features will vary with the cell type (2). These can include recruitment of a blood supply, infiltration into neighboring tissues, and evasion of immune responses, among others. Further, the precise alterations responsible for establishing the cancer platform can have additional effects that manifest in the growing tumor (3). As the expanding tissue alters its environment (or moves into other environments), selection of heritable variants results in the emergence of new features that may be specific to the individual cancer (4).

signals, without which all somatic cells undergo spontaneous suicide (Raff, 1992). Some of the pathways by which survival factors modulate apoptosis are now known, and many are exploited by cancer cells. Perhaps the best-characterized survival signaling pathway is mediated by PI3-kinase, the intracellular enzyme responsible for the generation of phosphoinositide $(3,4,5)P_3$. The resulting PIP3 binds the plecstrin homology domain present in several kinases that foster cell survival and thereby activates them. In this way, the AKT/PKB serine/threonine kinase is activated in response to a variety of upstream signals, including ligation of cytokine receptors or integrins, as well as by oncogenic activation of Ras (Stambolic et al., 1999). Once activated, AKT promotes survival by several distinct mechanisms. One is through phosphorylation of the BH3-only protein Bad which, as outlined above, promotes its sequestration and inactivation. Activated AKT also induces transcription of anti-apoptotic Bcl-2 family members such as Bcl-x_L (Sabbatini and McCormick, 1999) and inhibits the Forkhead transcription factor FKHRL1 (Brunet et al., 1999), which has a number of pro-apoptotic transcriptional targets. AKT has also been shown to promote translocation of the p53 antagonist Mdm2 from the cytoplasm to the nucleus (Mayo and Donner, 2001). In addition, AKT promotes expression of the glucose transporter Glut-1 (Barthel et al., 1999) and activates the glycolytic enzyme hexokinase by direct phosphorylation (Gottlob et al., 2001). Interestingly, AKT-mediated survival is dependent on glucose, whereas survival mediated by Bcl-x_L is not (Gottlob et al., 2001; Plas et al., 2001), raising the possibility that survival factors directly impact on MOMP by sustaining mitochondrial metabolism.

Many tumors bypass the requirement for survival factor signaling by engaging the AKT pathway in various ways. In many instances, AKT is triggered by the relentless action of upstream signaling molecules such as Ras or receptor tyrosine kinases (Testa and Bellacosa, 2001). However, AKT is also overexpressed in some tumors, while activating mutations of the p85 subunit of PI3K are found in others. Finally, a significant number of cancer cells have lost functional PTEN, the phosphatase that dephosphorylates PIP3 to shut down AKT (Cantley and Neel, 1999), and therefore AKT remains active in these cells.

However, not all aspects of cell survival are determined by the PI3-kinase-AKT signaling pathway. For example, the anti-apoptotic effect of Bcr-Abl can be PI3-kinase-independent (Amarante-Mendes et al., 1997). As already noted, at least part of the attachment survival signal of epithelial cells may be mediated by the sequestration of the BH3-only proteins Bim and Bmf within the cytoskeleton (Puthalakath et al., 1999, 2001).

Another important determinant of cell survival is the p65RelA-p50 transcription factor, NF-κB. NF-κB promotes cell survival by antagonizing the apoptotic pathway triggered by death receptors, at least in part through induction of expression of IAP proteins. In addition, NF-κB can induce the expression of anti-apoptotic members of the Bcl-2 family and repress expression of the BH-123 protein, Bax. Like most potentially oncogenic signaling molecules, however, NF-κB also has a paradoxical ability to promote cell death in some instances (Kuhnel et al., 2000; Ryan et al., 2000).

Apoptosis and cancer—Looking for the Achilles' heel

The multiple ways that oncogenes prime the apoptotic program, together with the critical combinatorial role played by other pro- and anti-apoptotic influences in the life/death threshold decision, offers a huge repertoire of mechanisms that tumor cells could exploit in their thirst for survival. Paradoxically, however, oncogenic priming suggests that when tumors first arise, they are actually more prone to induction of apoptosis than their normal counterparts. This innate sensitivity is probably the basis by which most existing anti-cancer therapies act. For example, the traditional rationale for using DNA-damaging agents as cancer therapeutics was the plausible idea that cancer cells cycle more rapidly than normal cells and are therefore more susceptible to anti-proliferative agents. However, it is now evident that this susceptibility has more to do with the potent pro-apoptotic signals induced by DNA damage: signals to which cancer cells are innately more susceptible by virtue of oncogenic priming of their apoptotic program (Evan and Littlewood, 1998). Unfortunately, any initial susceptibility of the genetically plastic tumor cell becomes rapidly eroded under the selective duress of therapy. The unhappy result is the all too common reemergence of a resistant and deadly tumor. Arguably the greatest problem with the conventional therapeutic approach is that it fails to correct the tumor's specific anti-apoptotic lesion(s) and instead employs a cruder strategy of simply loading up the pro-apoptotic side of the equation in the hope of exceeding the apoptotic threshold in the tumor cells before it happens in too many normal ones. If susceptibility to apoptosis really is the Achilles' heel of the cancer cell, we need to know far more about the footwear

that protects it.

One glimpse of the Achilles' heel has been provided by a survey of 60 cancer cell lines treated with a large number of chemotherapeutic agents, in which a number of possible anti- or pro-apoptotic genes were examined (Amundson et al., 2000). Of these, only Bcl-x_L level correlated with resistance to cell death induced by various therapeutics. Some take this correlation as an indication that Bcl-x_L is the major target to be overcome for effective therapy. However, it remains unknown to what extent such upregulation of Bcl-x_L is an adaptation to breast cancer in vivo as opposed to established growth in vitro. Unfortunately, Bcl-x_L is not expressed in all tumors, and so cannot be the lesion responsible for survival of many other tumor cell types. Nonetheless, such data do indicate that tumor evolution favors adoption of specific and restricted pathways of apoptosis suppression. We have little idea of the repertoire of anti-apoptotic mechanisms in human cancer, which is unfortunate, as it may be only by targeting the specific anti-apoptotic lesion in any tumor cell that we will be able to ensure its rapid and specific demise.

When good cells go bad

Building on the cancer platform

Although few would argue with the idea that tumors minimally require deconstrained cell proliferation together with sufficient survival to ensure cell expansion, it is widely assumed that tumor progression involves the stochastic acquisition of additional traits such as the capacity for invasion, metastasis, and angiogenesis. We venture to suggest, however, that many, perhaps most, of these traits emerge spontaneously once the platform for cell expansion has been established (Figure 6). Some may simply be the properties of proliferating cells. For example, proliferating cells differ from their quiescent counterparts metabolically, in their differentiation state, in degrees of cell adhesion and migration, and in basic morphological features such as nuclear:cytoplasmic ratio. Others may be the traits of expanding tissues which, by necessity, elicit complex iterative responses in their surroundings that manifest as angiogenesis, invasion, and evasion of immune responses. Indeed, many of the signature aspects of the "tumor-specific" phenotype—expansion, dysplasia, anaplasia, angiogenesis, and invasion—are also seen in those rare tissues that undergo expansion in the adult, such as trophoblasts, mammary glands, and female reproductive organs. Thus, normal and neoplastic tissues may differ not so much in how they expand but in how that expansion is regulated: in the former expansion stops in response to appropriate cues, while in the latter expansion continues unabated, driven by neoplastic lesions that short-circuit the need for extracellular signals.

In addition, suppression of apoptosis also has the catastrophic potential of promoting mutation and genome instability. Of course, inhibition of apoptosis is not innately mutagenic, but since apoptosis is a major avenue for disposal of mutated or damaged cells, its suppression could permit the survival of cells that would otherwise have been expeditiously deleted. In this regard, not all anti-apoptotic lesions are likely to be equal. For example, evasion of oncogene-induced apoptosis through upregulation of Bcl-x_L might be expected to confer significant resistance to DNA damage-induced apoptosis. However, if the p53 pathway remained intact, the affected cell could still mount a protective response to the insult, perhaps by triggering growth arrest or a MOMP that overwhelms Bcl-x_L protection. In contrast, loss of p53 would dislocate the DNA damage response from the apoptotic machinery while leaving the apoptotic machinery intact. Such a cell would presumably survive and accumulate DNA damage yet remain susceptible to p53-independent triggers of apoptosis.

Any contribution that suppression of apoptosis might make to genome plasticity could have profound consequences for tumor progression. As prototumors evolve, they will confront, and be shaped by, shifting selective pressures that vary in extent, potency, and location. When such selection becomes superimposed on significant genome plasticity, the result will be the evolutionary smorgasbord of heterogeneity and adaptability that makes treatment of advanced cancers seem so intractable. By this stage in somatic evolution, even its description by the single word "cancer" becomes questionable.

A matter of life and death

Notwithstanding this pessimistic assessment, our hypothesis remains that even the most heterogeneous and diverse of cancers share a common and obligate mechanistic ancestry: all teeter precariously on the same platform of deregulated proliferation and reduced cell death. We suspect that cutting the legs from under this platform should topple the supported neoplastic structure. 60 years ago, the doom and gloom surrounding the bewildering diversity of pathologies inflicted by bacteria, multiple species evolving at breathtaking rates, fell prey to the antibiotics that laid bare their common heritage. Now, we want antibiotics for cancer.

Given its mechanistic simplicity, we have only two options to collapse the cancer platform. One is to attack the lesions that drive tumor cell proliferation, which might lead not only to tumor cell arrest but have additional therapeutic benefits if emergent processes like dedifferentiation and angiogenesis are also contingent upon deregulated cell proliferation (Evan and Vousden, 2001). Alternatively, we could reinstate the defective apoptosis, whereupon the tumor cell should die from the apoptotic depredations inflicted on it by its driving oncogenic lesions.

Unfortunately, while this second leg of the platform would seem to offer the most auspicious targets, as already noted, we are enormously handicapped by our comparative ignorance of the diversity and nature of anti-apoptotic mechanisms in cancer. Nonetheless, this may be the principal mechanism by which antibodies to HER2/neu or the EGFR, both of which can provide survival signals, are effective in therapy in vivo. Similarly, STI-571 targeting of Bcr-Abl appears to trigger apoptosis in CML (Gambacorti-Passerini et al., 1997), while antisense inhibition of Bcl-2 has shown some therapeutic efficacy in lymphomas, myelomas, and other hematologic malignancies (Flaherty et al., 2001). Another focus of interest is the p53 pathway, whose reinstatement might trigger the specific demise of cells with damaged genomes or activated oncogenes. Some attempts have been made to reactivate p53 function in tumor cells by gene transfer, through agents that force mutant p53 back into its wild-type configuration, and by inhibiting negative regulators of p53 such as MDM2 in those tumors that retain wild-type p53 (Woods and Vousden, 2001). However, the ultimate clinical utility of such strategies is presently unclear. Another intriguing idea is to convert anti-apoptotic signals or proliferative signals needed by the tumor cells into signals that trigger apoptosis. For example, relocation of anti-apoptotic Bcr-Abl from cytosol to the nucleus appears to cause this oncogenic survival signal to trigger cell death (Vigneri and Wang, 2001). Similarly, inhibition of cyclin/cyclin-dependent kinase 2 has been shown to induce

selective apoptosis of transformed cells by commandeering the innate pro-apoptotic potential of E2F (Chen et al., 1999).

Like antibiotics, all forms of cancer therapy risk selecting for resistance, a problem compounded by the plasticity of the tumor cell genome. The most effective strategy is likely to be combinatorial—a coordinate attack on several targets specific to the cancer. Indeed, in such a strategy, it may not even be necessary for each component to be specific for cancer cells, so long as the combination provides selective ablation. The evolution of such sophisticated forms of combined therapy will undoubtedly proceed by a process that employs both rational and empirical approaches. However, a concerted attack on the lesions supporting the common cancer platform may well provide generic opportunities for effective and specific cancer treatment. By the very nature of the cancer platform, a cancer cell would be most sensitive to the restoration of those apoptotic pathways that is has specifically suppressed. We must find out what those are, and urge a cancer to its own demise.

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