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The Hallmarks of Cancer

Review

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After a quarter century of rapid advances, cancer research has generated a rich and complex body of knowledge, revealing cancer to be a disease involving dynamic changes in the genome. The foundation has been set in the discovery of mutations that produce oncogenes with dominant gain of function and tumor suppressor genes with recessive loss of function; both classes of cancer genes have been identified through their alteration in human and animal cancer cells and by their elicitation of cancer phenotypes in experimental models (Bishop and Weinberg, 1996).

Some would argue that the search for the origin and treatment of this disease will continue over the next quarter century in much the same manner as it has in the recent past, by adding further layers of complexity to a scientific literature that is already complex almost beyond measure. But we anticipate otherwise: those researching the cancer problem will be practicing a dramatically different type of science than we have experienced over the past 25 years. Surely much of this change will be apparent at the technical level. But ultimately, the more fundamental change will be conceptual.

We foresee cancer research developing into a logical science, where the complexities of the disease, described in the laboratory and clinic, will become understandable in terms of a small number of underlying principles. Some of these principles are even now in the midst of being codified. We discuss one set of them in the present essay: rules that govern the transformation of normal human cells into malignant cancers. We suggest that research over the past decades has revealed a small number of molecular, biochemical, and cellular traits—acquired capabilities—shared by most and perhaps all types of human cancer. Our faith in such simplification derives directly from the teachings of cell biology that virtually all mammalian cells carry a similar molecular machinery regulating their proliferation, differentiation, and death.

Several lines of evidence indicate that tumorigenesis in humans is a multistep process and that these steps reflect genetic alterations that drive the progressive transformation of normal human cells into highly malignant derivatives. Many types of cancers are diagnosed in the human population with an age-dependent incidence implicating four to seven rate-limiting, stochastic events (Renan, 1993). Pathological analyses of a number of organ sites reveal lesions that appear to represent evolve progressively from normalcy via a series of premalignant states into invasive cancers (Foulds, 1954).

These observations have been rendered more concrete by a large body of work indicating that the genomes of tumor cells are invariably altered at multiple sites, having suffered disruption through lesions as subtle as point mutations and as obvious as changes in chromosome complement (e.g., Kinzler and Vogelstein, 1996). Transformation of cultured cells is itself a multistep process: rodent cells require at least two introduced genetic changes before they acquire tumorigenic competence, while their human counterparts are more difficult to transform (Hahn et al., 1999). Transgenic models of tumorigenesis have repeatedly supported the conclusion that tumorigenesis in mice involves multiple rate-limiting steps (Bergers et al., 1998; see *Oncogene*, 1999, R. DePinho and T. E. Jacks, volume 18[38], pp. 5248–5362). Taken together, observations of human cancers and animal models argue that tumor development proceeds via a process formally analogous to Darwinian evolution, in which a succession of genetic changes, each conferring one or another type of growth advantage, leads to the progressive conversion of normal human cells into cancer cells (Foulds, 1954; Nowell, 1976).

An Enumeration of the Traits

The barriers to development of cancer are embodied in a teleology: cancer cells have defects in regulatory circuits that govern normal cell proliferation and homeostasis. There are more than 100 distinct types of cancer, and subtypes of tumors can be found within specific organs. This complexity provokes a number of questions. How many distinct regulatory circuits within each type of target cell must be disrupted in order for such a cell to become cancerous? Does the same set of cellular regulatory circuits suffer disruption in the cells of the disparate neoplasms arising in the human body? Which of these circuits operate on a cell-autonomous basis, and which are coupled to the signals that cells receive from their surrounding microenvironment within a tissue? Can the large and diverse collection of cancer-associated genes be tied to the operations of a small group of regulatory circuits?

We suggest that the vast catalog of cancer cell genotypes is a manifestation of six essential alterations in cell physiology that collectively dictate malignant growth (Figure 1): self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. Each of these physiologic changes—novel capabilities acquired during tumor development—represents the successful breaching of an anticancer defense mechanism hardwired into cells and tissues. We propose that these six capabilities are shared in common by most and perhaps all types of human tumors. This multiplicity of defenses may explain why can-

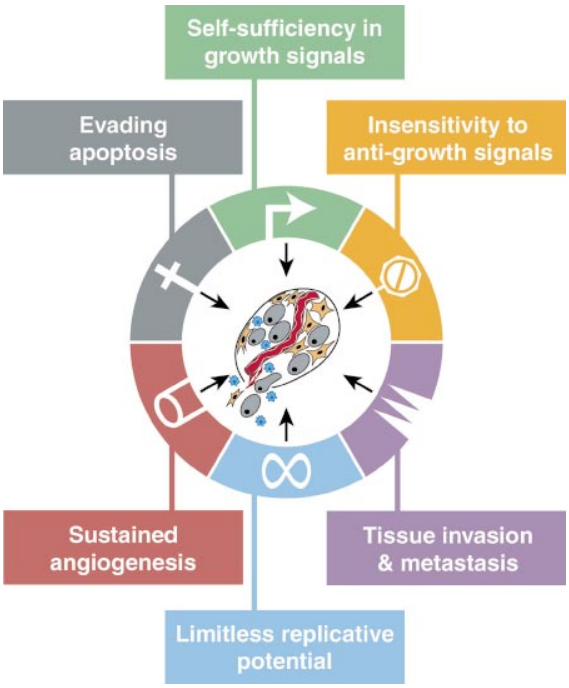

Figure 1. Acquired Capabilities of Cancer

We suggest that most if not all cancers have acquired the same set of functional capabilities during their development, albeit through various mechanistic strategies.

We describe each capability in turn below, illustrate with a few examples its functional importance, and indicate strategies by which it is acquired in human cancers.

Acquired Capability: Self-Sufficiency in Growth Signals

Normal cells require mitogenic growth signals (GS) before they can move from a quiescent state into an active proliferative state. These signals are transmitted into the cell by transmembrane receptors that bind distinctive classes of signaling molecules: diffusible growth factors, extracellular matrix components, and cell-to-cell adhesion/interaction molecules. To our knowledge, no type of normal cell can proliferate in the absence of such stimulatory signals. Many of the oncogenes in the cancer catalog act by mimicking normal growth signaling in one way or another.

Dependence on growth signaling is apparent when propagating normal cells in culture, which typically proliferate only when supplied with appropriate diffusible mitogenic factors and a proper substratum for their integrins. Such behavior contrasts strongly with that of tumor cells, which invariably show a greatly reduced dependence on exogenous growth stimulation. The conclusion is that tumor cells generate many of their own growth signals, thereby reducing their dependence on stimulation from their normal tissue microenvironment. This liberation from dependence on exogenously derived signals disrupts a critically important homeostatic mechanism that normally operates to ensure a proper

Acquired GS autonomy was the first of the six capabilities to be clearly defined by cancer researchers, in large part because of the prevalence of dominant oncogenes that have been found to modulate it. Three common molecular strategies for achieving autonomy are evident, involving alteration of extracellular growth signals, of transcellular transducers of those signals, or of intracellular circuits that translate those signals into action. While most soluble mitogenic growth factors (GFs) are made by one cell type in order to stimulate proliferation of another—the process of heterotypic signaling—many cancer cells acquire the ability to synthesize GFs to which they are responsive, creating a positive feedback signaling loop often termed autocrine stimulation (Fedi et al., 1997). Clearly, the manufacture of a GF by a cancer cell obviates dependence on GFs from other cells within the tissue. The production of PDGF (platelet-derived growth factor) and TGFα (tumor growth factor α) by glioblastomas and sarcomas, respectively, are two illustrative examples (Fedi et al., 1997).

The cell surface receptors that transduce growth-stimulatory signals into the cell interior are themselves targets of deregulation during tumor pathogenesis. GF receptors, often carrying tyrosine kinase activities in their cytoplasmic domains, are overexpressed in many cancers. Receptor overexpression may enable the cancer cell to become hyperresponsive to ambient levels of GF that normally would not trigger proliferation (Fedi et al., 1997). For example, the epidermal GF receptor (EGF-R/*erbB*) is upregulated in stomach, brain, and breast tumors, while the HER2/*neu* receptor is overexpressed in stomach and mammary carcinomas (Slamon et al., 1987; Yarden and Ullrich, 1988). Additionally, gross overexpression of GF receptors can elicit ligand-independent signaling (DiFiore et al., 1987). Ligand-independent signaling can also be achieved through structural alteration of receptors; for example, truncated versions of the EGF receptor lacking much of its cytoplasmic domain fire constitutively (Fedi et al., 1997).

Cancer cells can also switch the types of extracellular matrix receptors (integrins) they express, favoring ones that transmit progrowth signals (Lukashev and Werb, 1998; Giancotti and Ruoslahti, 1999). These bifunctional, heterodimeric cell surface receptors physically link cells to extracellular superstructures known as the extracellular matrix (ECM). Successful binding to specific moieties of the ECM enables the integrin receptors to transduce signals into the cytoplasm that influence cell behavior, ranging from quiescence in normal tissue to motility, resistance to apoptosis, and entrance into the active cell cycle. Conversely, the failure of integrins to forge these extracellular links can impair cell motility, induce apoptosis, or cause cell cycle arrest (Giancotti and Ruoslahti, 1999). Both ligand-activated GF receptors and progrowth integrins engaged to extracellular matrix components can activate the SOS-Ras-Raf-MAP kinase pathway (Aplin et al., 1998; Giancotti and Ruoslahti, 1999).

The most complex mechanisms of acquired GS autonomy derive from alterations in components of the downstream cytoplasmic circuitry that receives and processes the signals emitted by ligand-activated GF receptors and integrins. The SOS-Ras-Raf-MAPK cas-

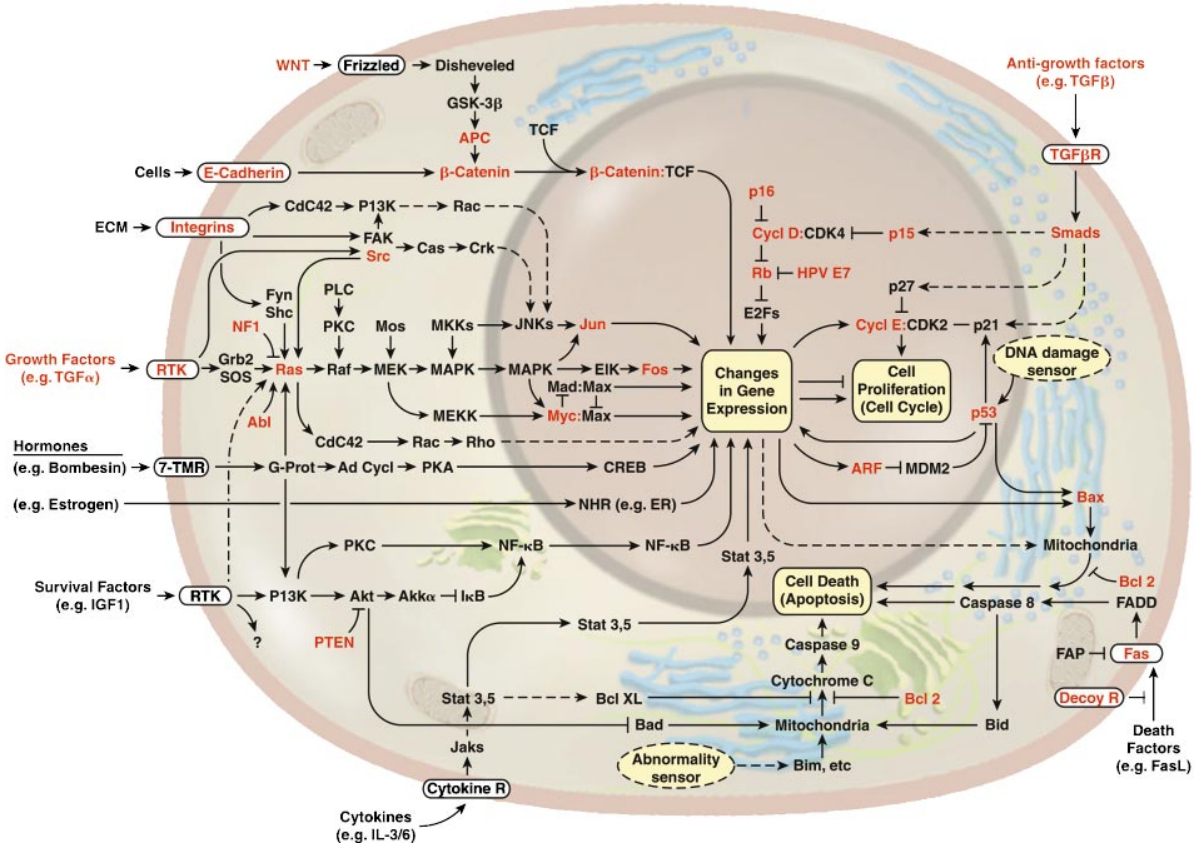

Figure 2. The Emergent Integrated Circuit of the Cell

Progress in dissecting signaling pathways has begun to lay out a circuitry that will likely mimic electronic integrated circuits in complexity and finesse, where transistors are replaced by proteins (e.g., kinases and phosphatases) and the electrons by phosphates and lipids, among others. In addition to the prototypical growth signaling circuit centered around Ras and coupled to a spectrum of extracellular cues, other component circuits transmit antigrowth and differentiation signals or mediate commands to live or die by apoptosis. As for the genetic reprogramming of this integrated circuit in cancer cells, some of the genes known to be functionally altered are highlighted in red.

tumors, Ras proteins are present in structurally altered forms that enable them to release a flux of mitogenic signals into cells, without ongoing stimulation by their normal upstream regulators (Medema and Bos, 1993).

We suspect that growth signaling pathways suffer deregulation in all human tumors. Although this point is hard to prove rigorously at present, the clues are abundant (Hunter, 1997). For example, in the best studied of tumors—human colon carcinomas—about half of the tumors bear mutant *ras* oncogenes (Kinzler and Vogelstein, 1996). We suggest that the remaining colonic tumors carry defects in other components of the growth signaling pathways that phenocopy *ras* oncogene activation. The nature of these alternative, growth-stimulating mechanisms remains elusive.

Under intensive study for two decades, the wiring diagram of the growth signaling circuitry of the mammalian cell is coming into focus (Figure 2). New downstream effector pathways that radiate from the central SOS-Ras-Raf-MAP kinase mitogenic cascade are being discovered with some regularity (Hunter, 1997; Rommel and Hafen, 1998). This cascade is also linked via a variety of cross-talking connections with other pathways; these multiple cell biological effects. For example, the direct interaction of the Ras protein with the survival-promoting PI3 kinase enables growth signals to concurrently evoke survival signals within the cell (Downward, 1998).

While acquisition of growth signaling autonomy by cancer cells is conceptually satisfying, it is also too simplistic. We have traditionally explored tumor growth by focusing our experimental attentions on the genetically deranged cancer cells (Figure 3, left panel). It is, however, increasingly apparent that the growth deregulation within a tumor can only be explained once we understand the contributions of the ancillary cells present in a tumor—the apparently normal bystanders such as fibroblasts and endothelial cells—which must play key roles in driving tumor cell proliferation (Figure 3, right panel). Within normal tissue, cells are largely instructed to grow by their neighbors (paracrine signals) or via systemic (endocrine) signals. Cell-to-cell growth signaling is likely to operate in the vast majority of human tumors as well; virtually all are composed of several distinct cell types that appear to communicate via heterotypic signaling.

Heterotypic signaling between the diverse cell types

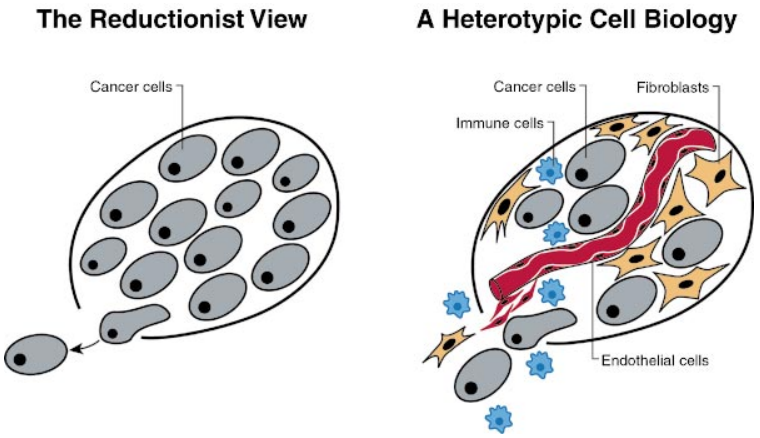

Figure 3. Tumors as Complex Tissues

The field of cancer research has largely been guided by a reductionist focus on cancer cells and the genes within them (left panel)—a focus that has produced an extraordinary body of knowledge. Looking forward in time, we believe that important new inroads will come from regarding tumors as complex tissues in which mutant cancer cells have conscripted and subverted normal cell types to serve as active collaborators in their neoplastic agenda (right panel). The interactions between the genetically altered malignant cells and these supporting coconspirators will prove critical to understanding cancer pathogenesis and to the development of novel, effective therapies.

in explaining tumor cell proliferation as the cancer cell-autonomous mechanisms enumerated above. For example, we suspect that many of the growth signals driving the proliferation of carcinoma cells originate from the stromal cell components of the tumor mass. While difficult to validate at present, such thinking recasts the logic of acquired GS autonomy: successful tumor cells are those that have acquired the ability to co-opt their normal neighbors by inducing them to release abundant fluxes of growth-stimulating signals (Skobe and Fusenig, 1998). Indeed, in some tumors, these cooperating cells may eventually depart from normalcy, coevolving with their malignant neighbors in order to sustain the growth of the latter (Kinzler and Vogelstein, 1998; Olumi et al., 1999). Further, inflammatory cells attracted to sites of neoplasia may promote (rather than eliminate) cancer cells (Cordon-Cardo and Prives, 1999; Coussens et al., 1999; Hudson et al., 1999), another example of normal cells conscripted to enhance tumor growth potential, another means to acquire necessary capabilities.

Acquired Capability: Insensitivity to Antigrowth Signals

Within a normal tissue, multiple antiproliferative signals operate to maintain cellular quiescence and tissue homeostasis; these signals include both soluble growth inhibitors and immobilized inhibitors embedded in the extracellular matrix and on the surfaces of nearby cells. These growth-inhibitory signals, like their positively acting counterparts, are received by transmembrane cell surface receptors coupled to intracellular signaling circuits.

Antigrowth signals can block proliferation by two distinct mechanisms. Cells may be forced out of the active proliferative cycle into the quiescent (G_0) state from which they may reemerge on some future occasion when extracellular signals permit. Alternatively, cells may be induced to permanently relinquish their proliferative potential by being induced to enter into postmitotic states, usually associated with acquisition of specific differentiation-associated traits.

Incipient cancer cells must evade these antiproliferative signals if they are to prosper. Much of the circuitry that enables normal cells to respond to antigrowth signals—the components governing the transit of the cell through the G1 phase of its growth cycle. Cells monitor their external environment during this period and, on the basis of sensed signals, decide whether to proliferate, to be quiescent, or to enter into a postmitotic state. At the molecular level, many and perhaps all antiproliferative signals are funneled through the retinoblastoma protein (pRb) and its two relatives, p107 and p130. When in a hypophosphorylated state, pRb blocks proliferation by sequestering and altering the function of E2F transcription factors that control the expression of banks of genes essential for progression from G1 into S phase (Weinberg, 1995).

Disruption of the pRb pathway liberates E2Fs and thus allows cell proliferation, rendering cells insensitive to antigrowth factors that normally operate along this pathway to block advance through the G1 phase of the cell cycle. The effects of the soluble signaling molecule TGFβ are the best documented, but we envision other antigrowth factors will be found to signal through this pathway as well. TGFβ acts in a number of ways, most still elusive, to prevent the phosphorylation that inactivates pRb; in this fashion, TGFβ blocks advance through G1. In some cell types, TGFβ suppresses expression of the *c-myc* gene, which regulates the G1 cell cycle machinery in still unknown ways (Moses et al., 1990). More directly, TGFβ causes synthesis of the $p15^{INK4B}$ and p21 proteins, which block the cyclin:CDK complexes responsible for pRb phosphorylation (Hannon and Beach, 1994; Datto et al., 1997).

The pRb signaling circuit, as governed by TGFβ and other extrinsic factors, can be disrupted in a variety of ways in different types of human tumors (Fynan and Reiss, 1993). Some lose TGFβ responsiveness through downregulation of their TGFβ receptors, while others display mutant, dysfunctional receptors (Fynan and Reiss, 1993; Markowitz et al., 1995). The cytoplasmic Smad4 protein, which transduces signals from ligand-activated TGFβ receptors to downstream targets, may be eliminated through mutation of its encoding gene (Schutte et al., 1996). The locus encoding $p15^{INK4B}$ may be deleted (Chin et al., 1998). Alternatively, the immediate downstream target of its actions, CDK4, may become unresponsive to the inhibitory actions of $p15^{INK4B}$ be-

in its INK4A/B-interacting domain; the resulting cyclin D:CDK4 complexes are then given a free hand to inactivate pRb by hyperphosphorylation (Zuo et al., 1996). Finally, functional pRb, the end target of this pathway, may be lost through mutation of its gene. Alternatively, in certain DNA virus-induced tumors, notably cervical carcinomas, pRb function is eliminated through sequestration by viral oncoproteins, such as the E7 oncoprotein of human papillomavirus (Dyson et al., 1989). In addition, cancer cells can also turn off expression of integrins and other cell adhesion molecules that send antigrowth signals, favoring instead those that convey progrowth signals; these adherence-based antigrowth signals likely impinge on the pRb circuit as well. The bottom line is that the antigrowth circuit converging onto Rb and the cell division cycle is, one way or another, disrupted in a majority of human cancers, defining the concept and a purpose of tumor suppressor loss in cancer.

Cell proliferation depends on more than an avoidance of cytostatic antigrowth signals. Our tissues also constrain cell multiplication by instructing cells to enter irreversibly into postmitotic, differentiated states, using diverse mechanisms that are incompletely understood; it is apparent that tumor cells use various strategies to avoid this terminal differentiation. One strategy for avoiding differentiation directly involves the *c-myc* oncogene, which encodes a transcription factor. During normal development, the growth-stimulating action of Myc, in association with another factor, Max, can be supplanted by alternative complexes of Max with a group of Mad transcription factors; the Mad–Max complexes elicit differentiation-inducing signals (Foley and Eisenman, 1999). However, overexpression of the c-Myc oncoprotein, as is seen in many tumors, can reverse this process, shifting the balance back to favor Myc–Max complexes, thereby impairing differentiation and promoting growth. During human colon carcinogenesis, inactivation of the APC/β-catenin pathway serves to block the egress of enterocytes in the colonic crypts into a differentiated, postmitotic state (Kinzler and Vogelstein, 1996). Analogously, during the generation of avian erythroblastosis, the *erbA* oncogene acts to prevent irreversible erythrocyte differentiation (Kahn et al., 1986).

While the components and interconnections between the various antigrowth and differentiation-inducing signals and the core cell cycle machinery are still being delineated, the existence of an antigrowth signaling circuitry is clear (Figure 2), as is the necessity for its circumvention by developing cancers.

Acquired Capability: Evading Apoptosis

The ability of tumor cell populations to expand in number is determined not only by the rate of cell proliferation but also by the rate of cell attrition. Programmed cell death—apoptosis—represents a major source of this attrition. The evidence is mounting, principally from studies in mouse models and cultured cells, as well as from descriptive analyses of biopsied stages in human carcinogenesis, that acquired resistance toward apoptosis is a hallmark of most and perhaps all types of cancer.

Observations accumulated over the past decade indi- in virtually all cell types throughout the body. Once triggered by a variety of physiologic signals, this program unfolds in a precisely choreographed series of steps. Cellular membranes are disrupted, the cytoplasmic and nuclear skeletons are broken down, the cytosol is extruded, the chromosomes are degraded, and the nucleus is fragmented, all in a span of 30–120 min. In the end, the shriveled cell corpse is engulfed by nearby cells in a tissue and disappears, typically within 24 hr (Wyllie et al., 1980).

The apoptotic machinery can be broadly divided into two classes of components—sensors and effectors. The sensors are responsible for monitoring the extracellular and intracellular environment for conditions of normality or abnormality that influence whether a cell should live or die. These signals regulate the second class of components, which function as effectors of apoptotic death. The sentinels include cell surface receptors that bind survival or death factors. Examples of these ligand/receptor pairs include survival signals conveyed by IGF-1/IGF-2 through their receptor, IGF-1R, and by IL-3 and its cognate receptor, IL-3R (Lotem and Sachs, 1996; Butt et al., 1999). Death signals are conveyed by the FAS ligand binding the FAS receptor and by TNFα binding TNF-R1 (Ashkenazi and Dixit, 1999). Intracellular sensors monitor the cell's well-being and activate the death pathway in response to detecting abnormalities, including DNA damage, signaling imbalance provoked by oncogene action, survival factor insufficiency, or hypoxia (Evan and Littlewood, 1998). Further, the life of most cells is in part maintained by cell–matrix and cell–cell adherence-based survival signals whose abrogation elicits apoptosis (Ishizaki et al., 1995; Giancotti and Ruoslahti, 1999). Both soluble and immobilized apoptotic regulatory signals likely reflect the needs of tissues to maintain their constituent cells in appropriate architectural configurations.

Many of the signals that elicit apoptosis converge on the mitochondria, which respond to proapoptotic signals by releasing cytochrome C, a potent catalyst of apoptosis (Green and Reed, 1998). Members of the Bcl-2 family of proteins, whose members have either proapoptotic (Bax, Bak, Bid, Bim) or antiapoptotic (Bcl-2, Bcl-XL, Bcl-W) function, act in part by governing mitochondrial death signaling through cytochrome C release. The p53 tumor suppressor protein can elicit apoptosis by upregulating expression of proapoptotic Bax in response to sensing DNA damage; Bax in turn stimulates mitochondria to release cytochrome C.

The ultimate effectors of apoptosis include an array of intracellular proteases termed caspases (Thornberry and Lazebnik, 1998). Two "gatekeeper" caspases, −8 and −9, are activated by death receptors such as FAS or by the cytochrome C released from mitochondria, respectively. These proximal caspases trigger the activation of a dozen or more effector caspases that execute the death program, through selective destruction of subcellular structures and organelles, and of the genome.

The possibility that apoptosis serves as a barrier to cancer was first raised in 1972, when Kerr, Wyllie, and Currie described massive apoptosis in the cells populating rapidly growing, hormone-dependent tumors follow-

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