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Cancer genes and the pathways they control

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The revolution in cancer research can be summed up in a single sentence: cancer is, in essence, a genetic disease. In the last decade, many important genes responsible for the genesis of various cancers have been discovered, their mutations precisely identified, and the pathways through which they act characterized. The purposes of this review are to highlight examples of progress in these areas, indicate where knowledge is scarce and point out fertile grounds for future investigation.

What we know

The cast. Alterations in three types of genes are responsible for tumorigenesis: oncogenes, tumor-suppressor genes and stability genes (**Tables 1** and **2**). Unlike diseases such as cystic fibrosis or muscular dystrophy, wherein mutations in one gene can cause disease, no single gene defect 'causes' cancer. Mammalian cells have multiple safeguards to protect them against the potentially lethal effects of cancer gene mutations, and only when several genes are defective does an invasive cancer develop. Thus it is best to think of mutated cancer genes as contributing to, rather than causing, cancer.

Oncogenes are mutated in ways that render the gene constitutively active or active under conditions in which the wild-type gene is not. Oncogene activations can result from chromosomal translocations, from gene amplifications or from subtle intragenic mutations affecting crucial residues that regulate the activity of the gene product. For example, the most common activating mutation of *BRAF* in human cancers changes a valine to a glutamate at codon 599, a residue within the activation loop of the kinase domain[1]. The activation loop is normally regulated by phosphorylation at adjacent residues (Thr598 and Ser601). This suggests that the glutamate substitution at codon 599 mimics a phosphate group and constitutively activates the enzyme even in the absence of signals that would normally result in phosphorylation of the adjacent threonine or serine residues. The activated BRAF kinase then phosphorylates downstream targets such as extracellular signal–regulated kinase (ERK), leading to aberrant growth[2](**Fig. 1**). A mutation in an oncogene is analogous to a stuck accelerator in an automobile; the car still moves forward even when the driver removes his foot from it. An activating somatic mutation in one allele of an oncogene is generally sufficient to confer a selective growth advantage on the cell.

Tumor-suppressor genes are targeted in the opposite way by genetic alterations: mutations reduce the activity of the gene product. Such inactivations arise from missense mutations at residues that are essential for its activity, from mutations that result in a truncated protein, from deletions or insertions of various sizes, or from epigenetic silencing. A mutation in a tumor-suppressor gene is analogous to a dysfunctional brake in an automobile; the car doesn't stop even when the driver attempts to engage it. Some recently described tumor-suppressor genes have been hypothesized to exert a selective advantage on a cell when only one allele is inactivated and the other remains functional (that is, haploinsufficiency)[3]. However, mutations in both the maternal and paternal alleles of a tumor-suppressor gene are generally required to confer a selective advantage to the cell. This situation commonly arises through the deletion of one allele via a gross chromosomal event—such as loss of an entire chromosome or chromosome arm—coupled with an intragenic mutation of the other allele[4].

Oncogene and tumor-suppressor gene mutations all operate similarly at the physiologic level: they drive the neoplastic process by increasing tumor cell number through the stimulation of cell birth or the inhibition of cell death or cell-cycle arrest. The increase can be caused by activating genes that drive the cell cycle, by inhibiting normal apoptotic processes or by facilitating the provision of nutrients through enhanced angiogenesis (**Figs. 2–4**). A third class of cancer genes, called stability genes or caretakers, promotes tumorigenesis in a completely different way when mutated. This class includes the mismatch repair (MMR), nucleotide-excision repair (NER) and base-excision repair (BER) genes responsible for repairing subtle mistakes made during normal DNA replication or induced by exposure to mutagens (**Table 1**). Other stability genes control processes involving large portions of chromosomes, such as those responsible for mitotic recombination and chromosomal segregation (for example, *BRCA1*, *BLM* and *ATM*; **Table 1**). Stability genes keep genetic alterations to a minimum, and thus when they are inactivated, mutations in other genes occur at a higher rate[5]. All genes are potentially affected by the resultant increased rate of mutation, but only mutations in oncogenes and tumor-suppressor genes affect net cell growth and can thereby confer a selective growth advantage to the mutant cell. As with tumor-suppressor genes, both alleles of stability genes generally must be inactivated for a physiologic effect to result.

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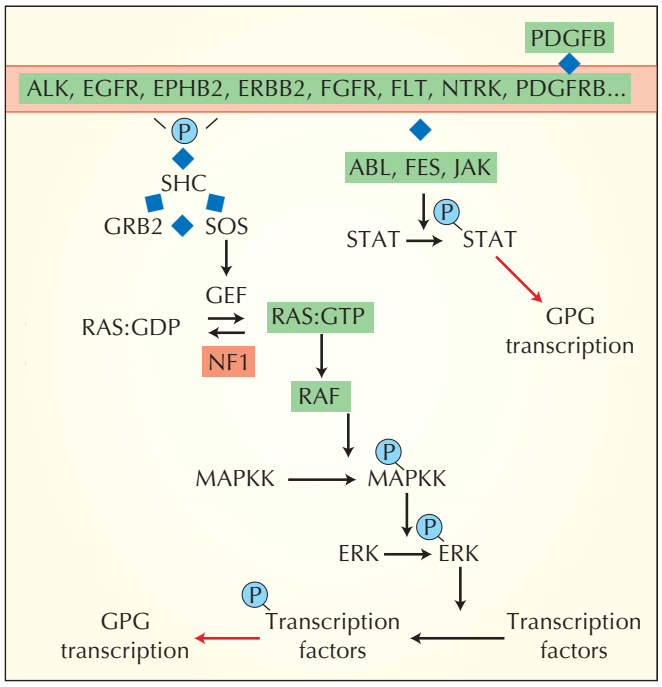

Figure 1 Receptor tyrosine kinase (RTK) pathway. Gene products in red boxes indicate that the corresponding genes are mutated in the germline (and generally also are somatically mutated in nonfamilial tumors; see **Table 1**). Gene products in green boxes indicate that the corresponding gene has been found to be mutated only somatically (see **Table 2**). 'GPG' denotes growth-promoting-genes—that is, genes that stimulate cell proliferation or inhibit the rate of cell death or arrest. Diamonds (◆) indicate protein-protein interactions. Red arrows and red T-bars indicate transcriptional induction and repression, respectively. Small circled 'P', 'OH' and 'Ub' represent covalently attached phosphate, hydroxyl and ubiquitin groups, respectively.

In the analogy to autos, stability genes represent the mechanics and a defective stability gene is akin to an inept mechanic.

Mutations in these three classes of genes can occur in the germline, resulting in hereditary predispositions to cancer (**Table 1**), or in single somatic cells, resulting in sporadic tumors (**Tables 1** and 2). It is important to point out that a mutation is defined as any change in the sequence of the genome. These changes include those affecting single base pairs as well as those creating large or small deletions or insertions, amplifications or translocations. In the germline, the most common mutations are subtle (point mutations or small deletions or insertions), whereas all types of mutation can be found in tumor cells. In fact, cancers represent one of the few disease types in which somatic mutations occurring after birth are pathogenic.

The first somatic mutation in an oncogene or tumor-suppressor gene that causes a clonal expansion initiates the neoplastic process[6]. Subsequent somatic mutations result in additional rounds of clonal expansion (and thus in tumor progression)[7]. Indeed, the best modern definition of a neoplastic cell is one that has clonally expanded as a result of somatic mutations[6,7]. Germline mutations of these genes cause cancer predisposition, not cancer *per se*: people with these mutations have a 'head start' on the neoplastic process, as a mutation that can contribute to cancer is already present in every one of their cells. Such individuals therefore often develop multiple tumors that occur at an earlier age than in individuals whose cancer-gene mutations have all occurred somatically[4]. Examples of hereditary syndromes associated with inherited mutations are listed in **Table 1**. In people with these syndromes, only a very small fraction of the total cells in an at-risk organ become neoplastic because other (somatic) mutations are required to develop a clinically detectable lesion. In nearly all dominantly inherited syndromes caused by tumor-suppressor genes and stability genes, the first somatic mutation affects the normal copy of the gene inherited from the unaffected parent[4]. Interestingly, the most common forms of hereditary cancer predisposition, leading to breast and colon cancers, are caused by inherited mutations of stability genes rather than tumor-suppressor genes or oncogenes (**Table 1**).

The plot. As noted above, cancer-gene mutations enhance net cell growth. As a result of research performed over the past decade, it is clear that there are many fewer pathways than genes. This concept is very familiar to geneticists studying yeast, flies, mice or worms—there are almost always a variety of genes that, when altered, lead to similar phenotypes[8,9]. Application of this concept to cancer has been solidified by elucidation of the biochemical functions of the altered cancer genes, either in cell culture systems, in mice or in other organisms. For example, several cancer genes directly control transitions from a resting stage (G0 or G1) to a replicating phase (S) of the cell cycle (**Fig. 2a**, Rb pathway). The products of these genes include proteins as diverse as cdk4 (a kinase), cyclin D1 (which interacts with and activates cdk4), Rb (a transcription factor) and p16 (which interacts with and inhibits cdk4)[10–12]. The genes encoding Rb and p16 are tumor-suppressor genes inactivated by mutation, whereas those encoding cdk4 and cyclin D1 are oncogenes activated by mutation. In addition to functional studies in model systems, detailed studies of individual tumor types have also provided compelling evidence that these four genes function in a single pathway in human cancers. Such studies have shown that the mutations within this pathway obey an 'exclusivity principle': that is, one and only one of the four genes noted above is generally mutated in any single tumor, exactly as predicted if the functional effect of each mutation was similar[10–13].

Another example of the reason for focusing on pathways rather than individual genes has been provided by studies of the *TP53* tumor-suppressor gene. The p53 protein is a transcription factor that normally inhibits cell growth and stimulates cell death when induced by cellular stress[14–16]. The most common way to disrupt the p53 pathway is through a point mutation that inactivates its capacity to bind specifically to its cognate recognition sequence. However, there are several other ways to achieve the same effect, including amplification of the *MDM2* gene and infection with DNA tumor viruses whose products (such as the E6 protein of human papilloma virus) bind to p53 and functionally inactivate it (**Fig. 2b**, p53 pathway).

One of the most important—and most curious—discoveries of the 1990s was that virtually all DNA tumor viruses that cause tumors in experimental animals or humans encode proteins that inactivate both Rb and p53 (refs. 17–19). Of the hundreds of cancer genes known or remaining to be discovered, why should these two have been singled out as targets for inactivation by all DNA tumor viruses? The answer may be that it is impossible for a tumor of epithelial origin to form unless the p53 and Rb tumor-suppressor gene pathways have been inactivated. This conjecture is supported by studies showing that these two pathways are altered in a large fraction of many types of cancers. We predict that most of the cancers that now appear to be devoid of mutations in these two

Table 1 Cancer predisposition genes

Gene (synonym(s))[a]	Syndrome	Hereditary pattern	Second hit	Pathway[b]	Major heredity tumor types[c]
Tumor-suppressor genes					
APC	FAP	Dominant	Inactivation of WT allele	APC	Colon, thyroid, stomach, intestine
AXIN2	Attenuated polyposis	Dominant	Inactivation of WT allele	APC	Colon
CDH1 (E-cadherin)	Familial gastric carcinoma	Dominant	Inactivation of WT allele	APC	Stomach
GPC3	Simpson-Golabi-Behmel syndrome	X-linked	?	APC	Embryonal
CYLD	Familial cylindromatosis	Dominant	Inactivation of WT allele	APOP	Pilotrichomas
EXT1,2	Hereditary multiple exostoses	Dominant	Inactivation of WT allele	GLI	Bone
PTCH	Gorlin syndrome	Dominant	Inactivation of WT allele	GLI	Skin, medulloblastoma
SUFU	Medulloblastoma predisposition	Dominant	Inactivation of WT allele	GLI	Skin, medulloblastoma
FH	Hereditary leiomyomatosis	Dominant	Inactivation of WT allele	HIF1	Leiomyomas
SDHB, C, D	Familial paraganglioma	Dominant	Inactivation of WT allele	HIF1	Paragangliomas, pheochromocytomas
VHL	Von Hippel–Lindau syndrome	Dominant	Inactivation of WT allele	HIF1	Kidney
TP53 (*p53*)	Li-Fraumeni syndrome	Dominant	Inactivation of WT allele	p53	Breast, sarcoma, adrenal, brain...
WT1	Familial Wilms tumor	Dominant	Inactivation of WT allele	p53	Wilms'
STK11 (*LKB1*)	Peutz-Jeghers syndrome	Dominant	Inactivation of WT allele	PI3K	Intestinal, ovarian, pancreatic
PTEN	Cowden syndrome	Dominant	Inactivation of WT allele	PI3K	Hamartoma, glioma, uterus
TSC1, TSC2	Tuberous sclerosis	Dominant	Inactivation of WT allele	PI3K	Hamartoma, kidney
CDKN2A ($p16^{INK4A}$, $p14^{ARF}$)	Familial malignant melanoma	Dominant	Inactivation of WT allele	RB	Melanoma, pancreas
CDK4	Familial malignant melanoma	Dominant	?	RB	Melanoma
RB1	Hereditary retinoblastoma	Dominant	Inactivation of WT allele	RB	Eye
NF1	Neurofibromatosis type 1	Dominant	Inactivation of WT allele	RTK	Neurofibroma
BMPR1A	Juvenile polyposis	Dominant	Inactivation of WT allele	SMAD	Gastrointestinal
MEN1	Multiple endocrine neoplasia type I	Dominant	Inactivation of WT allele	SMAD	Parathyroid, pituitary, islet cell, carcinoid
SMAD4 (*DPC4*)	Juvenile polyposis	Dominant	Inactivation of WT allele	SMAD	Gastrointestinal
BHD	Birt-Hogg-Dube syndrome	Dominant	Inactivation of WT allele	?	Renal, hair follicle
HRPT2	Hyperparathyroidism Jaw-tumor syndrome.	Dominant	Inactivation of WT allele	?	Parathyroid, jaw fibroma
NF2	Neurofibromatosis type 2	Dominant	Inactivation of WT allele	?	Meningioma, acoustic neuroma
Stability genes					
MUTYH	Attenuated polyposis	Recessive	?	BER	Colon
ATM	Ataxia telangiectasia	Recessive	?	CIN	Leukemias, lymphomas, brain
BLM	Bloom syndrome	Recessive	?	CIN	Leukemias, lymphomas, skin
BRCA1, BRCA2	Hereditary breast cancer	Dominant	Inactivation of WT allele	CIN	Breast, ovary
FANCA, C, D2, E, F,G	Fanconi anemia	Recessive	?	CIN	Leukemias
NBS1	Nijmegen breakage syndrome	Recessive	?	CIN	Lymphomas, brain
RECQL4	Rothmund-Thomson syndrome	Recessive	?	CIN	Bone, skin
WRN	Werner syndrome	Recessive	?	CIN	Bone, brain
MSH2, MLH1, MSH6, PMS2	HNPCC	Dominant	Inactivation of WT allele	MMR	Colon, uterus
XPA, C; ERCC2–5; DDB2	Xeroderma pigmentosum	Recessive	?	NER	Skin
Oncogenes					
KIT	Familial gastrointestinal stromal tumors	Dominant	?	RTK	Gastrointestinal stromal tumors
MET	Hereditary papillary renal cell carcinoma	Dominant	Mutant allele duplication	RTK	Kidney
PDGFRA	Familial gastrointestinal stromal tumors	Dominant	?	RTK	Gastrointestinal stromal tumors
RET	Multiple endocrine neoplasia type II	Dominant	Mutant allele duplication	RTK	Thyroid, parathyroid, adrenal

WT, wild type. [a]Representative genes of all the major pathways and hereditary cancer predisposition types are listed. For a complete list, see ref. 117. Approved gene symbols are provided for each entry, with alternative names in parentheses. [b]In many cases, the gene has been implicated in several pathways. The single pathway that is listed for each gene represents a 'best guess' (when one can be made) and for the reasons noted in the text and in the legend to **Figure 9**, should not be regarded as conclusive. APOP, apoptotic pathway; RTK, receptor tyrosine kinase pathway (see **Fig. 1**). [c]In most cases, the nonfamilial tumor spectrum caused by somatic mutations of the gene includes those occurring in the familial cases plus additional tumor types. For example, mutations of *TP53* and *CDKN2A* are found in many more tumor types than those to which Li-Fraumeni and familial malignant melanoma patients, respectively, are predisposed.

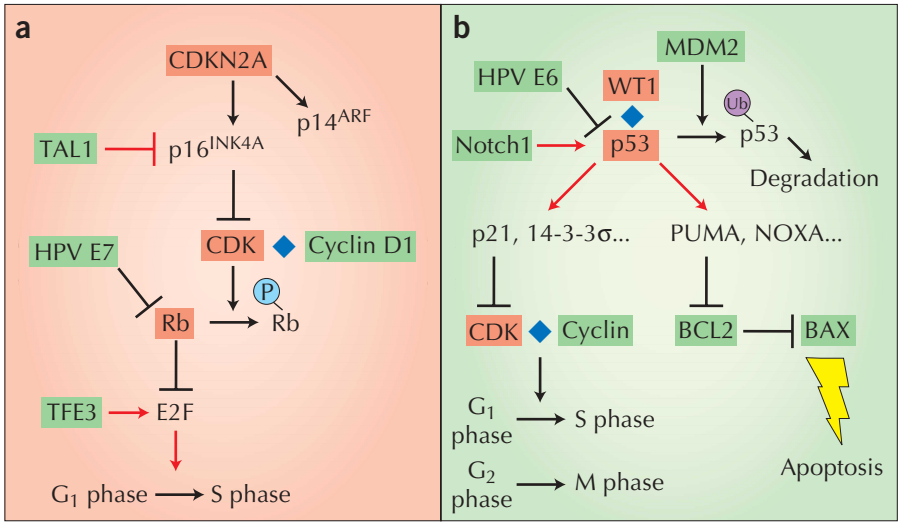

Figure 2 Rb (**a**) and p53 (**b**) pathways. Symbols as in **Figure 1**.

pathways will eventually be shown to contain them. This prediction will become testable once more efficient methods of detecting mutations are developed and all the genes in the pathways become known.

In addition to the Rb and p53 pathways, there are others that have a role in many tumor types, including those involving adenomatous polyposis coil (APC), glioma-associated oncogene (GLI), hypoxia-inducible transcription factor (HIF)-1, phosphoinositide 3-kinase (PI3K), SMADs and receptor tyrosine kinases (RTKs) (**Figs. 1** and **4–8**). In each case, mutations in multiple members of the pathway have been found to be mutated in more than one type of cancer, and in many cases the mutations within a single pathway obey the exclusivity principle noted above. The discoveries of these signal transduction pathways represent major scientific success stories of the last decade[20]. Their delineation has practical as well as theoretical implications. For example, they predict that targeted therapies directed against a particular gene product may be active against a tumor with a mutation of the targeted gene or any gene upstream of the target. Additionally, the fact that defects in a relatively small number of pathways underlie many different tumor types suggests that targeted therapeutics will be effective against a broad range of cancers.

Tumors can be broadly classified as liquid or solid. The former includes leukemias and lymphomas, composed of neoplastic cells whose precursors are normally mobile. Solid tumors are composed of epithelial or mesenchymal cells that normally are immobile. There are numerous other differences between liquid and solid tumors. For example, at least three mutations seem to be required to develop a malignant solid tumor in adults[21]; each of these mutations likely alters one of the pathways described in **Figure 9**. In contrast, only one or two mutations may be required to develop a malignant liquid tumor[6]. Perhaps liquid tumors don't require as many pathways to be inactivated because their precursor cells are already mobile and invasive, key characteristics that solid tumor cells must develop to become malignant. Additionally, oncogene activations caused by chromosome translocation events are the most common genetic alterations observed in liquid tumors[22]. Consistent chromosome translocations are much less common in solid tumors, whereas inactivations of tumor-suppressor genes are ubiquitious[23]. Finally, there are several genes that are uniquely altered in specific subtypes of liquid tumors, seem primarily to affect differentiation, do not obviously participate in the pathways depicted in **Figure 9** and do not occur in hereditary form (**Table 2**). These molecular distinctions add to the cytogenetic, epidemiologic and medical evidence that liquid and solid tumors should be considered separately in terms of their biology, behavior and pathogenesis. As solid tumors are much more common than liquid tumors, most of the examples provided in this review focus on the former.

Supporting cast. That solid tumors are composed of two compartments, one consisting of neoplastic epithelial cells and the other of stromal cells, was pointed out a hundred years ago[24]. The importance of the interactions between stroma and epithelium is becoming increasingly recognized[25,26]. And four discoveries made during the last ten years have propelled one component of the stroma, endothelial cells, into the spotlight.

First, naturally occurring inhibitors of angiogenesis were identified and shown to hinder the growth of experimental tumors[27,28]. Second, synthetic inhibitors of one of the major regulatory pathways of angiogenesis (VEGF) were produced and shown to inhibit

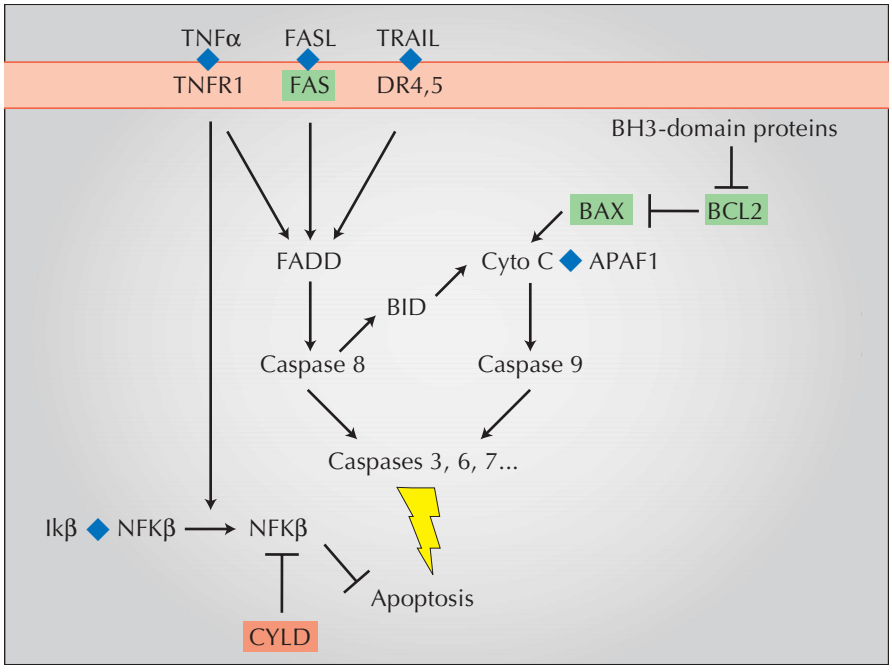

Figure 3 Apoptosis pathway. Symbols as in **Figure 1**.

tumor progression in patients with cancer[29]. Third, inactivation of *VHL*, a classic tumor-suppressor gene, was shown to support renal cell tumor growth through the control of tumor angiogenesis[30]. The protein encoded by VHL is part of a ubiquitin ligase complex that degrades HIF-1α in the presence of oxygen (**Fig. 4**, HIF1 pathway). In the absence of oxygen under normal circumstances or when VHL is mutated in tumors, the HIF-1α transcription factor is stabilized, leading to the expression of cytokines such VEGF and culminating in angiogenesis[31]. And finally, all oncogenes and tumor-suppressor gene pathways have been implicated in angiogenesis, either directly or indirectly, as illustrated by the central position of the HIF1 pathway in **Figure 9**. An understanding of tumor angiogenesis, blood flow, oxygenation and related issues involving the tumor-host relationship is becoming essential to studies of cancer biology as well as to the design of more effective forms of cancer therapy.

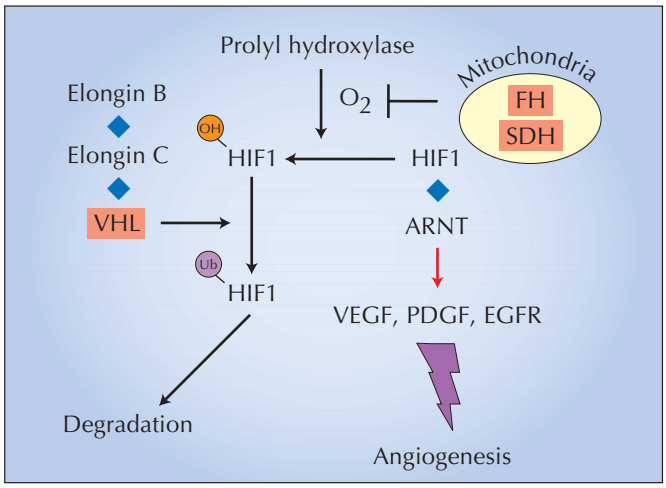

Figure 4 HIF1 pathway. Symbols as in **Figure 1**.

What we don't know

The opening act. The process of tumorigenesis is initiated when a replication-competent cell (stem cell or partially differentiated descendent of a stem cell) acquires a mutation in a 'gatekeeping' pathway that endows it with a selective growth advantage. In some cancers, the gatekeeper has been identified (examples are *RB1*, *APC* and *NF1* in tumors of the eye, colon and nervous system, respectively). In most common tumors, however, the gatekeeper is not known. It is also not known whether cancers of the lung, breast, prostate, bladder or brain can each be initiated through any one of several gatekeeping pathways or through only one. Many of the known gatekeepers were identified through the study of unusual families with predispositions to specific types of cancers[4]. There may be other families that provide clues to the nature of the gatekeepers in the future. We predict, however, that many of these remaining gatekeeping genes will only be identified through more 'brute-force' approaches involving sequence determination of major portions of the cancer cell genome[32]. As gatekeeping mutations provide fundamental insights into the biology and pathogenesis of particular cancers and are of singular importance to future diagnostic and therapeutic strategies, further research on this topic should be a priority.

The producers. As noted above, it appears that the cells of solid tumors must accumulate several rate-limiting mutations in cancer genes to achieve malignant status. If these mutations had to occur simultaneously in a single cell, then the prevalence of cancer would be minimal[33]. The current doctrine is that these mutations occur over time, with each mutation engendering a clonal expansion resulting in a large number of cells that then form a substrate for subsequent mutations[6]. Are normal rates of mutation, coupled with clonal expansions, sufficient to account for the prevalence of cancer, or is some form of genetic instability required for a cell to undergo these multiple, sequential mutations? This issue has been hotly debated for a long time[34,35], but the last 10 years of research have yielded some facts that clarify the issues. First, genetic instability can clearly contribute to cancer, as cogently demonstrated by the hereditary cancer predispositions caused by defects in stability genes (**Table 1**). Second, most cancers do not have a high mutation rate when this rate is measured at the nucleotide level. Accordingly, the number of mutations in a typical cancer cell is ~1 per megabase of DNA, similar to what would be expected in a normal cell that had passed through as many generations and population bottlenecks[36] . These observations argue against a common role for defects in MMR, NER or BER in nonhereditary tumor types. However, there is another form of instability—chromosomal instability (CIN)—that is much more commonly found in cancers, and this is observed at the gross chromosomal rather than the nucleotide level[37]. Though the actual rate of chromosomal changes has only been studied in a small number of cases, the end result of CIN, aneuploidy, is observed in nearly all solid tumors[38]. At the molecular level, chromosome losses are evident as losses of heterozygosity (LOH). An average of 25–30% of the alleles present in normal cells are lost in cancers, and it is not unusual to observe losses of over 75% of the cell's alleles[37]. Classic as well as modern cytogenetic studies are fully consistent with these observations[23,39]. Such wholesale changes in chromosomal content can be advantageous for the cancer cell, allowing the efficient elimination of one allele of a tumor-suppressor gene as well as the production of variants that can rapidly adapt to changing microenvironments. The mechanisms underlying aneuploidy and chromosomal instability in sporadic tumors are still largely unknown, though some candidate genes and pathways, such as those involving cell-cycle checkpoints, telomere crisis or centrosomes, have been proposed[34,40–44]. The role of telomeres in instability is particularly intriguing given the potential importance of telomerase in aging and the age-dependent incidence of most cancers[42,45]. As genetic instabilities

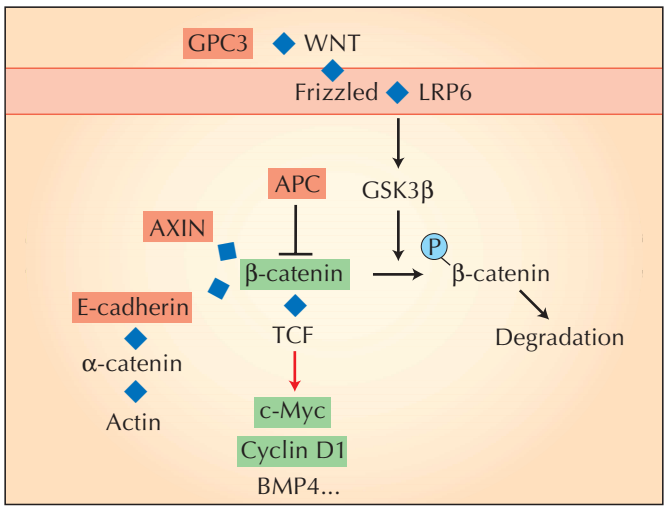

Figure 5 APC pathway. Symbols as in **Figure 1**.

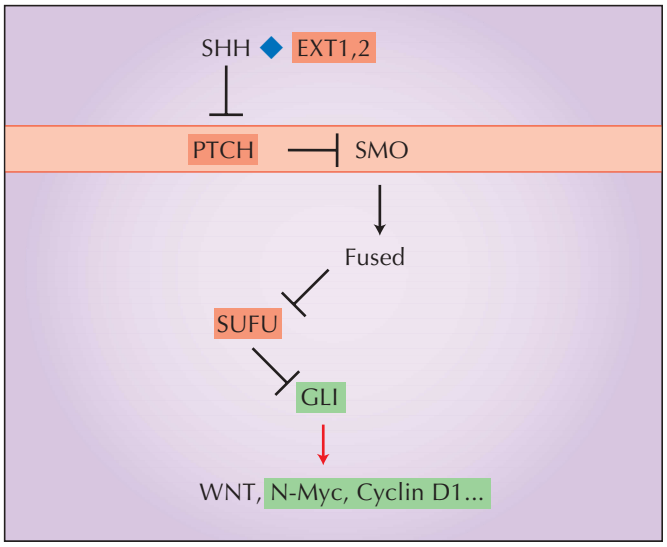

Figure 6 GLI pathway. Symbols as in **Figure 1**.

not only seem to be central to the neoplastic development but also may underlie the development of resistance to chemotherapeutic agents[33,34], the identification of the molecular mechanisms responsible for them is an important area of study.

The ending. Although abnormalities of the cancer genes listed in **Tables 1** and **2** are essential contributors to cancer, most abnormalities in these genes occur relatively early in the disease process and none are known to be specifically associated with the metastatic stage. It is this final stage—the seeding and growth of satellite lesions in other organs—that is ultimately responsible for the great majority of neoplastic deaths[46]. Primary tumors can generally be removed through surgery but widely metastatic lesions cannot be excised and are difficult or impossible to treat with adjuvant therapies.

Some of the biochemical processes involved in the early stages of metastasis, such as increased cell motility and production of matrix-degrading proteases, have been well studied[47]. However, the genetic alterations responsible for endowing cells with these abilities have not been clearly identified. In fact, it has been suggested that metastasis is not dependent at all on new genetic abnormalities that occur after tumors have been established, and that the propensity to metastasize is determined early in the neoplastic process rather than near its end (reviewed in ref. 48). This suggestion is not readily compatible with the evidence that cancer is a genetic disease in which evolution occurs somatically. Just as macroevolution never stops, evolution of the cancer cell does not stop and new variants of tumor cells with potentially greater capacities to invade and metastasize are always being born. This evolution is itself driven by inherent genetic instabilities, as described above. However, this genetic perspective on metastasis cannot be validated—or invalidated—until a better understanding of the metastatic process is in hand.

Same actors in different roles. One might have expected that a specific mutation of a widely expressed gene would have identical or at least similar effects in different mammalian cell types. But this is not in general what is observed. Different effects of the same mutation are not only found in distinct cell types; differences can even be observed in the same cell type, depending on when the mutation occurred during the tumorigenic process. *RAS* gene mutations provide informative examples of these complexities.

i. Cell type specificity: *KRAS2* gene mutations in normal pancreatic duct cells seem to initiate the neoplastic process, eventually leading to the development of pancreatic cancer[49,50]. The same mutations occurring in normal colonic or ovarian epithelial cells lead to self-limiting hyperplastic or borderline lesions that do not progress to malignancy[51–53].

ii. Chronology: In contrast to the effects of *KRAS2* mutations in a normal colonic epithelial cell, a *KRAS2* gene mutation in the same cell type that has already acquired an *APC* mutation results in a clonal expansion that often progresses to cancer[54].

iii. Growth inhibition versus growth promotion: In many human and experimental cancers, *RAS* genes seem to function as oncogenes[55,56]. But *RAS* genes can function as suppressor genes under other circumstances, inhibiting tumorigenesis after administration of carcinogens to mice[57,58].

These and similar observations on other cancer genes are consistent with the emerging general notion that signaling molecules play multiple roles at multiple times, even in the same cell type (for example, see ref. 59). However, the biochemical bases for such variations among cancer cells are almost entirely unknown. One could argue that oncogenes and tumor-suppressor genes are like electronic components whose effects depend on their placement within an electrical circuit. But no such argument can be easily used to explain the cell type specificity of stability gene defects. For example, MMR genes seem to have the identical, nonredundant function in every cell type on the planet: they limit the acquisition of specific types of mutations, such as those in homopolymer tracts[60,61]. Yet inherited mismatch repair defects lead to tumors in the colon and endometrium but spare most other organs, including rapidly dividing, self-renewing tissues such as the small intestine and bone marrow[62–64].

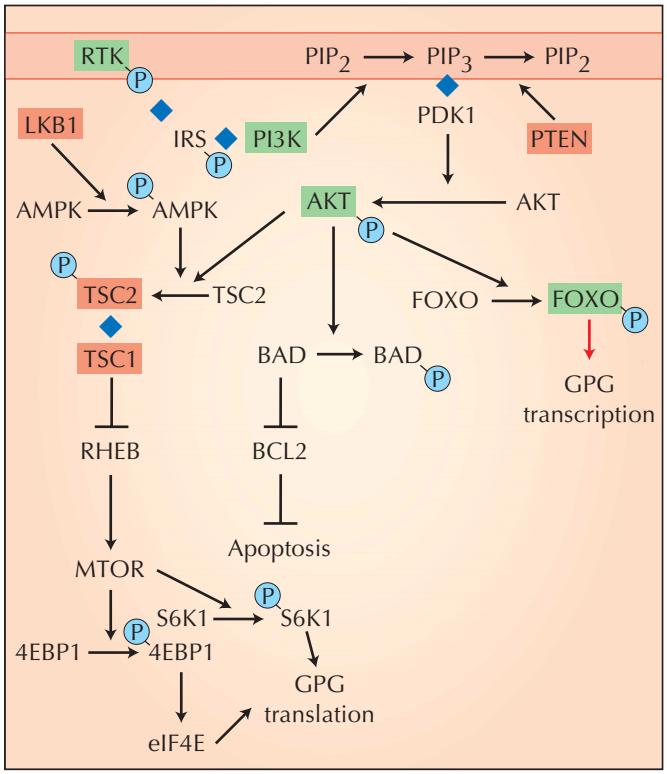

Figure 7 PI3K pathway. Symbols as in **Figure 1**.

It is also notable in this context that many cancer genes affect different organs when mutated in mice than when mutated in humans[8]. An important practical implication of all this complexity is that it is risky to generalize the conclusions of experiments performed in any cell type or to infer the function of a gene in human cancer cells based on studies of the homologous genes in other organisms.

Major cast members or bit players? In addition to the genes that are mutated in a significant portion of cancers of a given type, such as those listed in **Tables 1** and **2**, there are many other genes that have been implicated in neoplasia but not shown to be mutated. These genes have been shown to be expressed at higher or lower levels than expected in normal cells[65,66] and are often associated with 'epigenetic' changes—that is, covalent modifications of DNA or chromatin that are preserved as the cancer cells divide[67,68]. Unlike genetic changes, epigenetic changes identical to those found in cancers are often found in normal cells at some stage of development.

The discovery of such genes is a growth industry now that high-throughput methods for evaluating the genes expressed in cancer cells have been developed[65,66,69–74]. The information gained from these studies has proven extremely promising for the development of diagnostic assays, particularly for prognosis (for example, see ref. 75). But as has been shown for prostate-specific antigen (PSA), the utility of a gene for cancer diagnostics does not necessarily reflect a causative role in the process. It will therefore be essential to determine how genes discovered through expression-based approaches can be elevated from candidate status to culprit status in human neoplasia. When genes are sometimes inactivated by mutation and in other cases inactivated by epigenetic silencing, a cogent case can be made for their involvement in cancer. Relevant examples include the *VHL* and *CDKN2A* tumor-suppressor genes and the *hMLH1* stability gene[67]. In the absence of mutational evidence, functional evidence obtained from studies *in vitro* or in nonhuman species *in vivo* must be used. But because of the issues with cell type, species and chronological specificity noted above, expression and functional studies cannot currently provide the 'smoking guns' that would definitively implicate the gene in human cancers[76,77]. Telomerase provides a cogent example of the difficulties involved in such determinations. There is abundant evidence implicating telomerase in processes characteristic of neoplasia, such as immortalization[45,78] and genetic instability[42,79], and a great deal is known about its biochemical properties[80,81]. Even so, it is unclear at present whether the expression of telomerase in cancers is abnormal, driving the neoplastic process, or simply reflects the fact that cancers are derived from stem cells that normally express this enzyme.

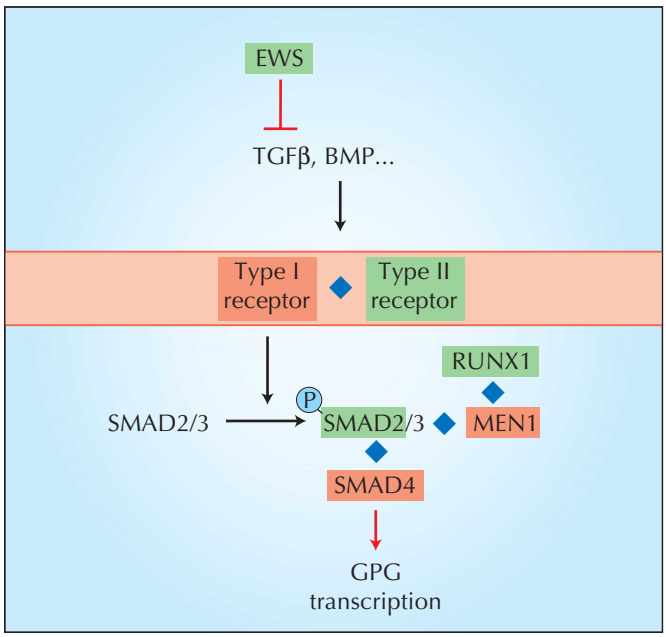

Figure 8 SMAD pathway. Symbols as in **Figure 1**.

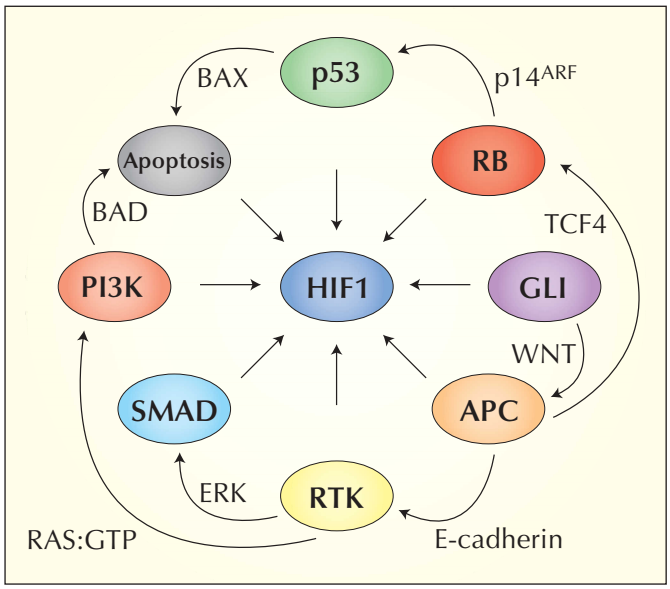

Figure 9 Overview of cancer gene pathways. The major pathways regulating cell birth and cell death are depicted as ovals color-coded to match **Figs. 1–8**. The schematics in **Figs. 1–8** emphasize the genes that have been shown to be genetically altered in human tumors, though many other genes participate in these pathways. Additionally, some of the same genes appear in more than one pathway and there is substantial 'cross-talk' between pathways. Selected mediators of this cross-talk are indicated in the loops that connect the pathways. More detailed information about these pathways can be found in several comprehensive reviews (refs. 11,12,15,31,103–116).

Levels of gene expression are unreliable indicators of causation because disturbance of any network invariably leads to a multitude of such changes only peripherally related to the phenotype[82]. Without better ways to determine whether an unmutated but interesting candidate gene has a causal role in neoplasia, cancer researchers will likely be spending precious time working on genes only peripherally related to the disease they wish to study. One challenge for the future is therefore to develop new model systems. For liquid tumors, innovative systems have indeed been developed, such as those using immunodeficient mice reconstituted with human hematopoietic stem cells[83]. Perhaps analogous humanized models can be developed to explore solid tumors in a more medically meaningful context than is currently possible[84,85].

What makes a box-office smash? Significant gains in cancer therapeutics have also been made in the last decade. Many of these advances have been incremental, but the increments add up. Thus more potent, less toxic derivatives of classic chemotherapeutic agents have been developed, dosing and combination treatments further optimized and side effects ameliorated[86]. However, the

Table 2 Genes that are mutated somatically but not inherited in mutant form

Gene[a] (synonym)	Somatic mutation type[b]	Cancers with mutant gene[c]	Pathway[d]
CTNNB1 (β-catenin)	Activating codon change	Colon, liver, medulloblastomas	APC
BCL2	Translocation	Lymphomas	APOP
TNFRSF6 (*FAS*)	Activating codon change	Lymphomas, testicular germ cell tumors	APOP
BAX	Inactivating codon change	Colon, stomach	APOP
FBXW7 (*CDC4*)	Inactivating codon change	Colon, uterine, ovarian, breast	CIN
GLI	Amplification, translocation	Brain, sarcomas	GLI
HPVE6	HPV infection	Cervical	p53
MDM2	Amplification	Sarcomas	p53
NOTCH1	Translocation	Leukemias	p53
AKT2	Amplification	Ovarian, breast	PI3K
FOXO1A, 3A	Translocation	Rhabdomyosarcomas, leukemias	PI3K
PI3KCA	Activating codon change	Colon, stomach, brain, breast	PI3K
CCND1 (cyclin D1)	Amplification, translocation	Leukemias, breast	RB
HPVE7	HPV infection	Cervical	RB
TAL1	Translocation	Leukemias	RB
TFE3	Translocation	Kidney, sarcomas	RB
ABL1 (*ABL*)	Translocation	Chronic myelogenous leukemia	RTK
ALK	Translocation	Anaplastic large cell lymphoma	RTK
BRAF	Activating codon change	Melanoma, colorectal, thyroid	RTK
EGFR	Amplification, activating codon change	Glioblastomas, non–small cell lung cancers	RTK
EPHB2	Inactivating codon change	Prostate	RTK
ERBB2	Amplification	Breast, ovarian	RTK
FES	Activating codon change	Colon	RTK
FGFR1–3	Translocation	Lymphomas, gastric cancers, bladder cancers	RTK
FLT3, 4	Activating codon change	Leukemias, angiosarcomas	RTK
JAK2	Translocation	Leukemias	RTK
KRAS2, N-RAS	Activating codon change	Colorectal, pancreatic, non–small cell lung cancer	RTK
NTRK1, 3	Translocation, activating codon change	Thyroid, secretory breast, colon	RTK
PDGFB	Translocation	Dermatofibrosarcomas and fibroblastomas	RTK
PDGFRB	Translocation	Leukemias	RTK
EWSR1	Translocation	Ewing's sarcomas, lymphomas, leukemias	SMAD
RUNX1	Translocation	Leukemias	SMAD
SMAD2	Inactivating codon change	Colon, breast	SMAD
TGFBR1, TGFBR2	Inactivating codon change	Colon, stomach, ovarian	SMAD
BCL6	Translocation	Lymphomas	?
EVI1	Translocation	Leukemias	?
HMGA2	Translocation	Lipomas	?
HOXA9, 11, 13; HOXC13, HOXD11, 13; HOX11, HOX11L2	Translocation	Leukemias	?
MAP2K4 (*MKK4*)	Inactivating codon change	Pancreas, breast, colon	?
MLL	Translocation, activating codon change	Leukemias	?
MYC, MYCN, MYCL1	Amplification	Lymphomas, neuroblastomas, small cell lung cancers	?
PTNP1, 11	Activating codon change	Leukemias, colon	?
RARA	Translocation	Promyelocytic leukemia	?
SS18	Translocation	Synovial sarcomas	?

[a]Representative genes of all the major pathways and cancer types are listed. For a complete list, see ref. 76. Approved gene symbols are provided for each entry, with alternative names in parentheses. [b]Activating codon change, intragenic mutation altering one or a small number of base pairs that activates the gene product, indicating that it is an oncogene; inactivating codon change, any mutation (point mutation, small or large deletion, etc.) that inactivates the gene product, indicating that the gene is a tumor suppressor. Amplifications and translocations generally affect oncogenes, though occasional translocations disrupt a gene rather than activate it (such as has been suggested to occur with *RUNX1*; ref. 118). [c]Only representative types of cancers are listed when a gene is mutated in many tumor types, Specific types of leukemias and lymphomas are listed only if a given gene is predominantly mutated in a specific subtype. [d]In many cases, the gene has been implicated in several pathways. The single pathway that is listed for each gene represents a 'best guess' (when one can be made) and for the reasons noted in the text and in the legend to **Figure 9**, should not be regarded as conclusive. APOP, apoptotic pathway; RTK, receptor tyrosine kinase pathway (see **Fig. 9**).

most dramatic therapeutic advances have come from agents targeted against proteins encoded by genes that are mutated in cancers. These include trastuzumab (Herceptin), an antibody against the product of the *ERBB2* gene amplified in some breast cancers[87]; imatinib (Gleevec), an inhibitor of tyrosine kinases altered in chronic myelogenous leukemia (CML)[88] and gastrointestinal stromal tumors (GISTs)[89]; and gefitinib (Iressa), an EGFR kinase inhibitor used to treat lung cancers[90]. Though none of these advances have resulted in cures of many patients with advanced disease, they can substantially improve and prolong lives. One important lesson from the use of these agents is that mutations are more reliable indicators of a good target than is abnormal expression. For example, virtually all GISTs abnormally express high levels of c-kit, but only those tumors with intragenic mutations of *KIT*

respond to therapy with imatinib[91]. Similarly, gefitinib is efficacious only in lung cancers in which the kinase domain of *EGFR* is mutated but not in lung cancers in which *EGFR* is simply overexpressed[92,93].

Interestingly, we still don't know the basis for the selectivity of conventional chemotherapeutic agents. Why cancer cells are more sensitive to antimetabolites, alkylating agents, DNA intercalators and topoisomerase inhibitors than normal replicating cells is enigmatic. The explosion in research on apoptosis in the 1990s has suggested that some tumor cells are less, rather than more, likely to undergo apoptosis after noxious stimuli[94,95]. Differential apoptotic proclivities therefore don't readily explain therapeutic specificities. This is consistent with studies showing that many drugs used in the clinic to treat solid tumors do not function through the induction of apoptosis at the doses achieved in patients[96]. Even with the newer, targeted agents, the basis for selectivity is not generally clear. The ERBB2 and ABL tyrosine kinases, for example, are expressed in many normal cell types; why should targeting these kinases lead to the demise of cancer cells but not normal cells? Concepts such as 'cellular addiction' offer an imaginative answer to this question, but the molecular basis for the postulated addiction is unknown[97]. Similar arguments could be made for 'untargeted' drugs like Taxol; are cancer cells addicted to microtubules? It is interesting that so much work has been done on how cells become resistant to conventional chemotherapeutic agents and so little on why they are sensitive to begin with. Indeed, some studies indicate that it is not the neoplastic cells, but rather the tumor vasculature, that is the actual target of many conventional chemotherapeutic agents[27]. Perhaps if we understood the bases for the responsiveness of cancers to these drugs, we could devise better agents to exploit them.

The sequel. There are at least three major challenges that will occupy most cancer researchers' time over the next 10 years. The first is the discovery of new genes that have a causal role in neoplasia, particularly those that initiate and conclude the process. The second is the delineation of the pathways through which these genes act and the basis for the varying actions in specific cell types. The third is the development of new ways to exploit this knowledge for the benefit of patients.

The first two of these challenges are likely to proceed apace. Technologies for gene discovery are rapidly advancing, the human genome will soon be in finished form, and previous analyses of other genes provide well-traveled road maps for investigators to follow once new genes are in hand. In fact, there are likely to be too many genes, too many interacting proteins and too many potential functions to consider, rather than too few. As noted above, cancer biology has not kept up with cancer molecular genetics and new biological systems are needed to separate wheat from chaff.

The third challenge, involving practical benefits to patients, will be much more difficult to meet. There are hardly any road maps to follow. Eradicating hundreds of billions of cancer cells from a human with metastatic disease is a daunting task. Each of these cells has multiple genetic abnormalities and is capable of rapidly evolving variants to combat any therapeutic onslaught[98,99]. On the other hand, many cancers respond to the somewhat crude weapons that have been developed to date despite their multiple genetic abnormalities. And now that cancer is no longer a 'black box', and is known to be the result of alterations in a limited number of pathways that can in principle be targeted by new generations of drugs, cautious optimism is appropriate.

But will the development of new, more specific therapeutic agents be the best way to minimize cancer morbidity and mortality in the long-term? Most of the increased longevity that Western societies enjoy today has come through better prevention rather than better treatment. It is clear that certain major forms of cancer can be prevented by limiting exposures to carcinogens (such as sunlight and cigarette smoke in skin and lung cancers, respectively). Other forms of cancer can be detected early, thereby limiting morbidity and mortality (for example, tumors of the breast, cervix, prostate and colon). Moreover, it requires 30–40 years for a typical epithelial cell to accumulate the multiple genetic alterations required to progress to metastatic disease[100]. This provides a huge window of opportunity to detect tumors at a stage when they are still curable by conventional surgical methods. Perhaps knowledge of the genes altered in cancer will provide the basis for new generations of diagnostic tests, employing target-specific imaging or analysis of body fluid samples *ex vivo* that will make early detection approaches feasible[101,102]. Though less dramatic than cures, prevention and early detection are perhaps the most promising and feasible means to reduce cancer deaths by the time that *Nature Medicine* celebrates its 20th anniversary.

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COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the *Nature Medicine* website for details).

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