2 Biological hallmarks of cancer

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Overview

An enigma for cancer medicine lies in its complexity and variability, at all levels of consideration. The hallmarks of cancer constitute an organizing principle that provides a conceptual basis for distilling the complexity of this disease in order to better understand it in its diverse presentations. This conceptualization involves eight biological capabilities—the hallmarks of cancer—acquired by cancer cells during the long process of tumor development and malignant progression. Two characteristic traits of cancer cells facilitate the acquisition of these functional capabilities. The eight distinct hallmarks consist of sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, deregulating cellular energetics and metabolism, and avoiding immune destruction. The principal facilitators of their acquisition are genome instability with consequent gene mutation and tumor-promoting inflammation. The integration of these hallmark capabilities involves heterotypic interactions among multiple cell types populating the "tumor microenvironment" (TME), which is composed of cancer cells and a tumor-associated stroma, including three prominent classes of recruited support cells—angiogenic vascular cells (AVC), various subtypes of fibroblasts, and infiltrating immune cells (IIC). In addition, the neoplastic cells populating individual tumors are themselves typically heterogeneous, in that cancer cells can assume a variety of distinctive phenotypic states and undergo genetic diversification during tumor progression. Accordingly, the hallmarks of cancer—this set of necessarily acquired capabilities and their facilitators—constitute a useful heuristic tool for elucidating mechanistic bases and commonalties underlying the pathogenesis of diverse forms of human cancer, with potential applications to cancer therapy.

Distilling the dauntingly complex manifestations of cancer

As outlined in the preceding chapter, and comprehensively described elsewhere in this encyclopedic textbook, the manifestations of cancer are disconcertingly complex and diverse. Cancers affecting different organs vary dramatically, in regard to genetics, histopathology, effects on systemic physiology, prognosis, and response to therapeutic intervention, explaining why the discipline of oncology is largely balkanized into organ-specific specialties, and why the chapters of this textbook are largely aligned as individualistic descriptions of organ-specific cancers.

In the face of this disconcerting diversity and complexity of disease manifestations, one might ask whether there are underlying principles—mechanistic commonalities—masked by the genetic and phenotypic complexities that span the multitude of cancer types and forms. In 2000, and again in 2011, we put forward a hypothesis that the vast complexity of human cancers reflects different solutions to the same set of challenges, namely that the lesions we observe in the forms of symptomatic neoplastic disease have all necessarily acquired, by various strategies, a common set of distinct functional capabilities that enable inappropriately chronic cell proliferation, and the focal or disseminated growth of populations of neoplastic cancer cells. We proposed to call this set of acquired capabilities "hallmarks of cancer."[1,2] We further suggested that two characteristic traits of neoplastic growths—elevated mutability of cancer-cell genomes and inflammation by complex arrays of immune cells—are the key facilitators used by incipient neoplasias to acquire essential hallmark capabilities. Our current conceptualization of the biological hallmarks of cancer incorporates the eight distinct functional capabilities and the two enabling facilitators, these being schematized in Figure 1.

The following sections describe these 10 key aspects of cancer pathophysiology. Then we introduce the observation that cancer cells recruit a variety of normal cell types that contribute in various ways the acquisition of hallmark functionalities. We conclude with a brief discussion on potential clinical implications of the hallmarks concept. For further detail and background, the reader is referred to our initial publications laying out the concept of the hallmarks of cancer,[1,2] as well as to another perspective that expands on the roles of stromal cells in enabling the hallmarks of cancer.[3] Notably, only a few recent publications not cited in these three perspective articles are referenced herein. A textbook on the biology of cancer[4] may provide additional details on many of the mechanisms of cancer pathogenesis described in outline in this chapter.

Acquired functional capabilities embody biological hallmarks of cancer

In our current conceptualization, there are eight hallmark capabilities that are common to many, if not most forms of human cancer (Figure 1). Each capability serves a distinct functional role in supporting the development, progression, and persistence of tumors and their constituent cells, as summarized briefly in the following sections.

Hallmark 1: sustaining proliferative signaling

The defining criterion of cancer as a disease is chronic, inappropriate cell proliferation, which results from corruption of cellular regulatory networks that normally orchestrate (transitory) proliferation of cells during embryonic development, physiological growth, and homeostatic maintenance of tissues throughout the body. Both positive (inductive) and negative (repressive) signals govern cell division and proliferation. Thus, this first hallmark capability embodies a complex set of inductive signals that instruct entry into and progression through the cell growth-and-division cycle to produce daughter

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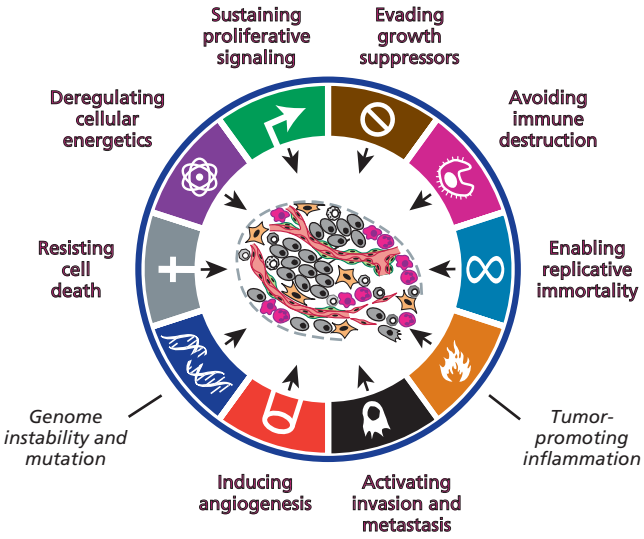

Figure 1 **The biological hallmarks of cancer**. The schematic illustrates what are arguably necessary conditions to manifest malignant disease—the hallmarks of cancer—comprising eight distinct and complementary functional capabilities and two facilitators (in black italics) of their acquisition.[1,2] These hallmark traits may be acquired at different stages in the multistep development of cancer, via markedly distinctive mechanisms in different forms of human cancer. Two aberrant characteristics of cancerous lesions are demonstrably involved in facilitating the acquisition during tumorigenesis of these functional capabilities: genome instability and the resultant mutation of regulatory genes, and the infiltration of immune inflammatory cells endowed by their biology—for example involvement in wound healing—to contribute to one or another hallmark capability. Different forms of cancer may be more or less dependent on a particular hallmark. Thus, adenomatous tumors typically lack the capability for invasion and metastasis. Leukemias may not require angiogenesis or invasive ability, although progression to lymphoma almost certainly requires both. The necessity of evading tumor immunity may be less important for certain cancers but is increasingly appreciated to be widespread.

cells. In the context of cancer, such stimulatory signals are activated and, in contrast to normal situations in which proliferative signaling is transitory, the signals are sustained chronically.

The most well-established and widespread mechanism of sustaining proliferative signaling involves mutational alteration of genes within cancer cells that convert such genes into active drivers of cell proliferation. These activated genes—defined as oncogenes—render otherwise transitory proliferation-promoting signals chronic. Such oncogenes typically encode proteins altered in structure and function or abundance compared to their normal cellular counterparts, which are responsible for receiving proliferative signals from extracellular sources and transmitting the signals through complex regulatory circuits operating within the cell.

Prominent examples of mutated driver oncogenes that sustain proliferative signaling in human cancers include the epidermal growth factor (EGF) receptor and signal transducers in the downstream KRAS–RAF–MEK–MAPK pathway that process and transmit growth-stimulatory signals via a succession of protein phosphorylations to the cell-division machinery operating in the nucleus. Mutations that render one or another of these proteins chronically active are found in many forms of human cancer, including the aforementioned *EGFR* and related receptor tyrosine kinases such as *HER2* and *ALK*; similarly acting mutations result in chronic activation of the downstream signal transducers *KRAS*, *BRAF*, and *MEK*. We note, however, that activation in cancer cells of this central mitogenic pathway does not invariably depend on genetic changes acquired during the course of tumor progression. In certain instances, epigenetic deregulation of autocrine (autostimulatory) and paracrine (cell-to-cell) signaling circuits can also provide cancer cells with chronic growth-promoting signals, doing so in the apparent absence of underlying somatic mutations.

Hallmark 2: evading growth suppressors

The essential counterbalance to proliferative signals in normal cells are braking mechanisms that either overrule the initiation of, or subsequently block, the cell-division process instigated by such signals. The genes encoding these proteins are often termed tumor suppressor genes (TSGs). The most prominent brakes are the direct regulators of progression through the cell growth-and-division cycle, embodied in the retinoblastoma protein (pRb) and several "cyclin-dependent" kinase-inhibitor proteins. The activity of this molecular-braking system is itself normally regulated by the integration of extracellular pro- and antigrowth signals transduced by receptors on the cell surface, along with monitors of the intracellular physiologic state of the cell, in order to regulate tissue homeostasis and orchestrate transitory physiological proliferation.

An intracellular monitoring system, which is centered upon the p53 protein, serves to ensure that cells only advance through their growth-and-division cycles when the physiologic state of the cell is appropriate. Thus, p53 detects unrepaired damage to a cell's genome as well as stressful physiologic imbalances that could impair accurate genome duplication, chromosomal segregation, and cell division. In response to cellular stress alarms, p53 then proceeds to activate inhibitors of the cell-cycle machinery. In cases of severe genomic damage or stressful physiological abnormalities, p53 and its associates can instead induce programmed cell death (see below), an extreme form of putting on the brakes to cell proliferation.

A number of component genes in both of these generic braking mechanisms—the Rb and p53 pathways—are classified as TSGs by virtue of their frequent loss-of-function via deletion or intragenic mutations; alternatively, other mechanisms may achieve the same end by shutting down expression of these genes through epigenetic mechanisms, notably those involving DNA and histone methylation. Thus the p53 gene is mutated in ~40% of all human cancers, and many of the remaining tumors with wild-type p53 instead carry genetic lesions or epigenetic alterations that compromise p53 signaling in other ways.

Genetic profiling of genomes and transcriptomes indicates that a majority of human tumors contain defects—genetic or epigenetic—in the functions of the Rb and p53 tumor-suppressor pathways. Moreover, a large body of functional studies involving manipulation of these pathways in cultured cancer cells and mouse models of tumor initiation, growth, and malignant progression have clearly established the critical importance of TSGs in these pathways as significant barriers to the development of cancer. As such, evasion of growth suppressors is clearly a hallmark capability, necessary to ensure that continuing cancer cell proliferation and consequent tumor growth is not halted by braking mechanisms that, under normal circumstances, limit the extent of cell proliferation in order to maintain tissue homeostasis.

Hallmark 3: resisting cell death

There exists a second, fundamentally distinct barrier to aberrant cell proliferation, which involves intrinsic cellular mechanisms that can

orchestrate the programmed death of cells deemed to be either aberrant or, in the case of normal development and homeostasis, superfluous. The most prominent form of programmed cell death is apoptosis, the genetically programmed fragmentation of a cell destined to die. Included among the situations where normal cells activate their apoptotic program to die are ones where the cell is damaged in various ways, mislocalized, or inappropriately migrating or proliferating. The apoptotic program can be triggered by cell intrinsic and non-cell-autonomous signals that detect different forms of cellular abnormality.

The apoptotic cell-death program involves the directed degradation of the chromosomes and other critical cellular organelles by specialized enzymes (e.g., caspases), the shriveling and fragmentation of the cell, and its engulfment, either by its neighboring cells or by tissue-surveying phagocytes, notably macrophages. The apoptotic cascade is completed in less than an hour in mammalian tissues, explaining why apoptotic cells are often surprisingly rare when visualized in tissue sections, even in a population of cells experiencing apoptosis-inducing environmental conditions, such as cancer cells in tumors subjected to cytotoxic chemotherapy or to acute hypoxia consequent to vascular insufficiency.

The rapid engulfment of apoptotic cell bodies ensures that their death does not release subcellular components that would otherwise provoke an immune response; this "immune silence" contrasts with a second form of programmed cell death: necroptosis. Long known as necrosis and envisioned as the passive dissolution of a dying cell, necrosis can also be an active, programmed process that is governed by cellular regulators and effectors distinct from those regulating apoptosis. Necroptosis can be activated by various conditions, including oxygen and energy deprivation, viral infection, and inflammation.[5] Cells dying by necroptosis (or passive necrosis) rupture, releasing their contents and leaving their carcasses as immunogenic debris that can attract (or exacerbate) an immune inflammatory response, which, as discussed below, can have both tumor-promoting and tumor-antagonizing effects.

A third program capable of inducing cell death, termed autophagy, serves as a recycling system for cellular organelles that can help cells respond to conditions of nutrient deprivation, by degrading nonessential cellular organelles and recycling their component parts. Thus, autophagy generates metabolites and nutrients necessary for survival and growth that cells may be unable to acquire from their surroundings. In addition, while generally a survival system, extreme nutrient deprivation or other acute cellular stresses can lead to a hyperactivation of autophagic recycling that drives a cell to a point-of-no return, in which its complement of organelles falls below the minimum level required for viability; as a consequence, the cell dies via "autophagy-associated" cell death, distinct in its characteristics from both apoptosis and necroptosis. Stated differently, depending on the physiologic state of a neoplastic cell, autophagy may either sustain its survival and facilitate further proliferation or eliminate it via autophagy-associated cell death.[6]

These three distinct mechanisms for triggering cell death must be variably circumvented or attenuated by cancer cells if they and their descendants are to continue their proliferative expansion and phenotypic evolution to states of heightened malignancy.

Hallmark 4: enabling replicative immortality

A third intrinsic barrier to chronic proliferation is integral to the linear structure of mammalian chromosomes: the telomeres at the ends of chromosomes record—by progressive reduction of their length during each cell-division cycle—the number of successive cell generations through which a cell lineage has passed. The telomeres are composed of thousands of tandem copies of a specific hexanucleotide DNA sequence located at the ends of every chromosome that are associated with a specialized set of DNA-binding proteins. Operating together, these nucleoprotein complexes protect the ends of chromosomes both from degradation by the DNA-repair machinery, which is designed to detect DNA damage, and from end-to-end fusions with other chromosomes catalyzed by naked DNA ends.

Notably, when the number of telomere repeats erodes below a certain threshold, a tripwire is triggered, causing cell-cycle arrest or apoptosis mediated by the p53 tumor-suppressor protein, operating in its role to sense DNA damage. Circumventing these p53-induced antiproliferative responses (e.g., by mutational inactivation of the p53 gene) allows cancer cells with eroding telomeres to ignore the short-telomere checkpoint and continue proliferating, but only transiently. Sooner or later, the continuing erosion of telomeric DNA leads to loss of the protective nucleoprotein caps protecting the chromosomal DNA ends, which allows end-to-end fusions of chromosomes, breakage–fusion–bridge cycles during mitosis, and resultant karyotypic chaos that leads to cell death instead of cell division.

The cancer cells in many fully developed tumors circumvent the proliferative barrier presented by telomere erosion and the imminent mitotic catastrophe of telomere dysfunction by activating a system for telomere maintenance and extension that is normally used to preserve the replicative capacity of normal embryonic and tissue stem cells. This system involves expression of the telomere-extending enzyme named telomerase. Less frequently, they engage an alternative interchromosomal recombination-based mechanism for preserving telomere length. Thus, through one strategy or another, cancer cells acquire the capability to maintain their telomeres, avoiding the barrier of intolerably shortened telomeres, thereby enabling the unlimited replicative potential—termed cellular immortality—that is required for continuing expansion of populations of cancer cells.

Hallmark 5: inducing angiogenesis

Like normal organs, tumors require a steady supply of oxygen, glucose, and other nutrients, as well as a means to evacuate metabolic wastes, in order to sustain cell viability and proliferation. The tumor-associated vasculature serves these purposes. The deleterious effect that ischemia has in normal tissue is well established clinically and experimentally: cells die, via one form of programmed cell death or another, causing tissue and organ degradation and dysfunction. Similarly, the growth of developing nests of cancer cells halts when their ability to acquire blood-borne nutrients becomes inadequate, typically when the nearest capillary is more than 200 μ away. Angiogenesis—the formation of new blood vessels—is commonly activated and demonstrably beneficial for many tumor types.

Cells at the diffusion limit from the nearest capillary activate various stress-response systems, of which the most prominent involves the hypoxia-inducible transcription factors (HIF), which regulate hundreds of genes, including ones that directly or indirectly induce angiogenesis and other stress-adaptive capabilities. Much like cells in ischemic tissues, cancer cells lacking sufficient oxygen and glucose will typically die by necrosis/necroptosis, apoptosis, or rampant autophagy. This explains why most vigorously growing tumors are well vascularized with evidence of ongoing angiogenesis.

Of note, the tumor-associated neovasculature is usually aberrant both morphologically and functionally. Tumor blood vessels are tortuous, dilated, and leaky, with erratic flow patterns and "dead zones" in which no blood flow is detectable, in marked contrast to the seamless blood flow operating in the normal

vasculature. Moreover, the degree of vascularity varies widely from one tumor type to another, ranging from intensely vascularized renal carcinomas to poorly vascularized pancreatic ductal adenocarcinomas.

Finally, we note that while chronic angiogenesis is a hallmark of most solid tumors, some may devise an alternative means to acquire access to the vasculature: in certain cases, cancers coopt normal tissue vasculature, by employing the hallmark capability for invasion and metastasis. Thus, particular types of cancer cells can proliferate and grow along normal tissue capillaries, creating sleeves whose outer diameters are dictated by the 200-μ diffusion limit. While vascular cooption is evident in certain cases (e.g., glioblastoma) and in some tumors treated with potent angiogenesis inhibitors, most tumors rely to a considerable extent on chronic angiogenesis to support their expansive growth. Still others may adapt to living in quasi-hypoxic environments where most cancer cells would perish.

Hallmark 6: activating invasion and metastasis

The five hallmarks detailed above stand as logical necessities for the chronic proliferative programs of cancer cells. The sixth is less intuitive: high-grade cancer cells become invasive and migratory. Invasive growth programs enable cancer cells to invade into adjacent tissue as well as into blood and lymphatic vessels (intravasation); these vessels serve thereafter as pipelines for dissemination to nearby and distant anatomical sites. The tissue-draining lymphatic vasculature can transport cancer cells to lymph nodes, where metastatic growths—lymph node metastases—can form; such cell colonies may serve, in turn, as staging areas for further dissemination by entering the bloodstream. Cells entering the bloodstream by direct intravasation within a tumor or indirectly via lymph nodes may soon become lodged in the microvessels of distant organs and extravasate across the vessel walls into the nearby tissue parenchyma. The resulting seeded micrometastases may die or lay dormant in such ectopic tissue locations or, with extremely low efficiency, generate macroscopic metastases—the process of "colonization."

The regulation of the intertwined capabilities for invasion and metastasis is extraordinarily complex, involving both cell-intrinsic programs and assistance from accessory cells in the tissue microenvironment. Prominent among the cancer cell-intrinsic regulatory mechanisms is the activation in epithelial cancer cells (carcinomas) of a developmental program termed the epithelial–mesenchymal transition (EMT),[2,4] which is associated with cell migrations and tissue invasions during normal organogenesis. An interconnected regulatory program induced by the microenvironment in some tumors is the aforementioned hypoxia response system, which triggers the activation of the HIFs, HIF1α and HIF2α, consequently altering expression of hundreds of genes,[7,8] including components of the EMT program. Both transcriptional regulatory systems control genes that can facilitate invasive migration as well as survival in the blood and lymphatic systems, and in ectopic tissue locations.

Notably, the acquisition of this hallmark capability can occur at various points along the pathways of multistep tumor development and progression that lead incrementally from normal cells of origin to those found in aggressive high-grade malignancies. In some cases, the capability for invasion and metastasis arises late, reflecting mutational or epigenetic evolution of the cancer cell, whereby rare subsets of cells populating such primary tumors are enabled to become invasive/metastatic. In other cases, this capability is acquired early, such that many cancer cells within a tumor may already be capable of invasion and metastasis. Moreover, there are indications that the EMT program may in some cases be transiently active and functionally important for dissemination and seeding, but then switched off in macrometastatic colonies.[9,10] It remains unclear whether the acquired traits of invasion and metastasis are beneficial and hence actively selected during the evolution of primary tumors; alternatively, these malignancy-defining capabilities may represent incidental byproducts of activating global regulatory networks (e.g., proliferative signaling, EMT, and HIF) that are initially selected because they facilitate primary tumor formation by contributing to the acquisition of other hallmark functions.

Hallmark 7: deregulating cellular energetics and metabolism

The concept that cancer cells alter their utilization of energy sources—notably glucose—to support their proliferation was introduced almost 90 years ago by Otto Warburg, who observed that certain cultured-cancer cells have enhanced uptake of glucose, which is metabolized via glycolysis, even in the presence of oxygen levels that normally should favor oxidative phosphorylation. The result was counterintuitive, as glycolysis is far less efficient at producing ATP, the primary currency of intracellular energy. However, we now appreciate that the "aerobic glycolysis" described by Warburg produces, in addition to ATP, many of the building blocks for the cellular macromolecules that are required for cell growth and division. Indeed, the metabolism of cancer cells resembles that of actively dividing normal cells rather than being a novel invention of neoplasia. Moreover, it is important to appreciate that there is not a binary switch from oxidative phosphorylation to aerobic glycolysis in cancer cells; rather, cancer cells continue to utilize oxidative phosphorylation in addition to incorporating differing rates of glycolysis, the proportions of which may well prove to be dynamic in time, variable among the cancer cells in different subregions within a tumor and in different tissue microenvironments.

Aerobic glycolysis can be indirectly monitored by positron-emission tomography (PET) using radiolabeled analogs as tracers. PET involving [^{18}F]-fluorodeoxyglucose is commonly used to visualize glycolytic tumors via their elevated expression of glucose transporters and a resulting increase in the uptake of glucose. Although glucose is the primary fuel source used by most cancer cells, glutamine is also emerging as another key blood-borne source of energy and a precursor of lipids and amino acids. In most cases, glutamine likely supplements and enhances glucose in supplying energy and biomaterials for growth and proliferation of cancer cells, although in some cases of glucose insufficiency, glutamine uptake and metabolism may be able to compensate.[11]

A third player in metabolic fueling is lactate. While long considered to be toxic waste that is secreted by cells undergoing aerobic and anaerobic glycolysis, lactate is now appreciated to have diverse tumor-promoting capabilities.[12] In certain cancer cells, particularly those suffering glucose deprivation, extracellular lactate can be imported via specific transporters and used as fuel for generation of ATP and biomaterials. Similarly, some cancer-associated fibroblasts (CAF) can utilize lactate. Hence, metabolic symbioses may be operative in some tumors, involving partnerships between glucose-importing/lactate-exporting cells and lactate-importing cells.[12]

Finally, we note a still unresolved question, about whether this hallmark is significantly independent of the six cited above in terms of its regulatory mechanisms, or conversely is concordantly regulated under the auspices of these other hallmark traits. Thus, oncogenes such as *KRAS* and *cMYC*, as well as the loss of function of TSGs such as *p53*, can serve to reprogram the energy metabolism of

cancer cells. For this reason, the reprogramming of cellular energetics and metabolism was initially defined as an "emerging hallmark."[2] Irrespective of this qualification, it is clearly a crucial property of the neoplastic cell phenotype.[13]

Hallmark 8: avoiding immune destruction

The eighth hallmark has been on the horizon for decades, originally conceived as the proposition that incipient neoplasias must find ways to circumvent active surveillance by the immune system that would otherwise eliminate aberrantly proliferating premalignant cells. While clearly demonstrable in highly antigenic tumors in mouse models, and implicated in virus-induced human cancers, the generality of immune surveillance of incipient cancer as a barrier to neoplastic progression is unresolved. One factor is immune self-tolerance: the vast majority of antigens expressed by spontaneously arising cancer cells are likely shared with those expressed by their cells-of-origin in normal tissues and thus are ignored, reflecting the tolerance of the immune system for self-antigens. Nonetheless, some cancer cells demonstrably express antigens for which the immune system has failed to develop tolerance, including embryonic antigens, and novel antigens produced by rampant mutation of the genome; such antigens can indeed elicit antitumor immune responses and are an increasing focus for strategies aimed to elicit efficacious tumor immunity.

By contrast, the immune response to the ~20% of virus-induced human tumors is clear: oncogenic viruses express foreign antigens (including oncoproteins responsible for driving cell transformation) to which the immune system is not tolerant, resulting in humoral and cellular immune responses that can kill virus-infected precancer cells and thereby eradicate incipient neoplasias. The fact that virus-transformed cells can nevertheless succeed in evading immune elimination to produce overt cancer testifies to immune-evasive capabilities evolved by such tumor viruses or selected for in virus-transformed cancer cells. Nevertheless, the immune system likely serves as a significant barrier to virus-induced tumors, as indicated by the increased rates of cancer in individuals who are immune-compromised for various reasons, including organ-graft recipients and AIDS patients.

Although the incidence of nonvirus-induced human cancers is not markedly increased in the context of immunodeficiency—suggesting a lack of immune surveillance of incipient neoplasias in the other 80% of human cancers—various lines of evidence suggest that some tumor types must indeed deal with immune recognition and attack during later stages of tumor progression and, in response, acquire immune-evasive strategies. Here, histopathological analyses of human tumor biopsies have shed light on the potential role of immune attack and immune evasion. For example, among patients with surgically resected colorectal carcinomas, those whose tumors contained dense infiltrates of cytotoxic T lymphocytes (CTLs) have a better prognosis than patients with tumors of similar grade and size that have comparatively few infiltrating CTLs.[14] Such data implicate the actions of the immune system as a significant obstacle to the progressive growth and dissemination of cancer cells, one that is necessarily blunted or circumvented in some aggressive tumor types.[14] Indeed, immune phenotyping of tumors, including their associated stroma, is being evaluated as a new metric in the diagnosis of tumors that may enable, when combined with traditional criteria, more accurate assessments of prognosis and more effective treatment decisions.[15,16] Accordingly, it is reasonable to view antitumor immune responses as a significant barrier to be circumvented during the lengthy multistage development of many forms of human cancer.

Nevertheless, the rules of immune engagement remain ambiguous when viewing the spectrum of human cancers. Thus, it is generally unclear when during different organ-specific tumor development pathways the attention of the immune system is attracted, or what the precise characteristics and efficacy of resultant immune responses are, or how the genetic constitutions of patients and the tumors that they harbor affect the development of antitumor immunity. Nevertheless, evading immune destruction seems increasingly to be an important mandate for developing tumors and thus an evident hallmark of cancer.

Taken together, we envision that these eight distinct capabilities define a necessary condition for malignancy (Figure 1), along with the two associated facilitators of their acquisition described below. Importantly, however, one cannot ignore the complex mechanisms underlying this conceptual simplicity: different tumors acquire these hallmarks by diverse mechanisms, doing so by coopting and subverting a diverse array of mechanisms normally responsible for cell, tissue, and organismic homeostasis.

Aberrations that enable acquisition of the necessary functional capabilities

The lengthy process of tumor development and malignant progression, long appreciated to involve a succession of rate-limiting steps, reflects the need of evolving neoplastic cells to acquire the eight hallmark capabilities discussed earlier. How then are these functional capabilities acquired? Currently, there are two clearly established means by which the hallmarks are acquired: (1) genome instability and the resulting mutation of hallmark-enabling genes in the overt cancer cells and (2) inflammation by cells of the immune system that help provide these capabilities cooperatively.

Genome instability and the consequent mutation of hallmark-enabling genes

Genome instability and the consequent mutation of hallmark-enabling genes is the primary modality of acquiring hallmark capabilities. The cell genome is subject to routine DNA damage, from a variety of chemically reactive products of normal metabolism, from environmental insults, and from its replication during every cell division. The resulting defects, if left unrepaired, become cell-heritable mutations, explaining the need of an elaborate consortium of proteins that continuously monitor DNA integrity and, in response to damage, undertake repair. Irreparable damage provokes the elimination of cells, a task orchestrated by the p53 TSG, which has for this reason been dubbed the "guardian of the genome."

This highly efficient genome-integrity machine normally keeps the rates of gene mutation and genome rearrangement at low levels, which is likely incompatible with the efficient acquisition of hallmark functions by genetic evolution and phenotypic selection for these necessary capabilities. This dichotomy provides a compelling explanation for the frequent observation of genome instability in cancer cells. Indeed, many tumor types contain neoplastic cells that carry readily identifiable defects in the complex machinery designed to monitor and repair genomic damage. Most apparent are the frequently documented mutant alleles of p53 that have been found in perhaps 40% of all cancers; without p53 on duty, damaged DNA can persist unrepaired, and mutant cells can survive and pass their damaged genomes on to their progeny. Numerous other specialized DNA repair and genome-maintenance enzymes are also found to be defective in many tumors, and inherited familial defects in DNA

repair often lead to elevated risk of cancer development, again by enabling the acquisition of tumor-promoting mutations.

The elevated rates and persistence of proliferation in neoplastic lesions create cell lineages that have undergone far more successive growth-and-division cycles than is typical of cells in normal tissues, further increasing the potential for mutagenic errors occurring during DNA replication. Among these consequences is one that we described earlier: critically shortened and thus dysfunctional, telomeres can trigger chromosomal rearrangements and fusions that can affect gene function in various ways. Mutant cancer cells that survive this karyotypic chaos may have acquired advantageous phenotypes and thus the capability to undergo clonal expansion.

The foundation of cancer in genetic mutation is being further substantiated by the development of high-throughput DNA-sequencing technologies and the consequent ability to systematically analyze large numbers of independently arising cancer-cell genomes. Complemented by other methods for genome scanning, such as comparative genomic hybridization to identify copy number variations and "chromosome painting" to detect translocations, the derangements of the cancer-cell genome are being revealed in unprecedented detail.[17–20] The results substantiate the fact that almost every form of human cancer involves cancer cells whose genomes have been mutated either through chromosomal rearrangements or more localized intragenic mutations or both. The density of genetic alterations varies over many orders of magnitude, from very low numbers detected in certain pediatric cancers to the blizzards of mutations present in the genomes of UV-induced melanomas and tobacco-induced lung cancers. Thus, the aberrations can range from dozens of point mutations to hundreds of thousands, and from quasi-diploid chromosomal karyotypes to widespread aneuploidy, translocations, and multiple large-scale amplifications and deletions.

The data generated by these increasingly high-throughput genomic technologies is presenting a major challenge to clarify which of the plethora of mutational alterations in the cancer-cell genome actually contribute causally to the acquisition of hallmark capabilities. The numbers of mutations that are being cataloged in many cancer cells greatly exceed those that are likely to be important in reshaping cell phenotype. The recurrence of specific mutations in cohorts of patients with the same cancer type or subtype provides one indication of functional involvement. Many other mutations, however, may reflect alternative solutions utilized in one individual's tumor but not another's, and thus are less frequently recurrent. And yet other mutations—often the great majority in a cancer cell's genome—may simply be ancillary consequences of genomic instability, having been carried along for the ride with other function-enabling mutations that do indeed afford selective advantage and thus clonal expansion during tumor growth and progression. Thus, the concept is emerging that cancer cells contain two classes of mutations: drivers and passengers. One future imperative will be to leverage such genome-profiling data to identify the driver mutations and their mechanistic contributions to the acquisition of hallmark capabilities, not only mutations that are frequent in a particular cancer type, but also others that are infrequent but nonetheless functionally important for an individual patient's tumor growth and progression. A second imperative will be to clarify the potential of both recurrent and rare driver mutations as therapeutic targets in different tumor types. An added complexity is that advantageous hallmark traits conferred by driver mutations in some tumors may be acquired in other tumors by changes in the epigenome—the spectrum of cell-heritable changes in chromatin that are not reflected by changes in nucleotide sequence.[21,22] Indeed, it has been argued that all eight of the hallmark capabilities can be conveyed by epigenetic changes in gene regulation, occurring both in the overt cancer cells and in the supporting cells of the tumor-associated stroma.[23] While the prevalence of epigenetic mechanisms as primary orchestrators of tumorigenesis is currently unresolved, genomic instability may prove to play less prominent roles in some tumors, where mutational alterations in DNA may be consequences of hallmark functions rather than causal of them.

The field of cancer genetics is poised for an extraordinary decade during which tens of thousands of cancer-cell genomes will be comprehensively analyzed for multiple parameters, including alterations in DNA sequence and copy number, changes in gene transcription, splicing, and translation, as well as repatterning of histone and DNA methylation (and other modifications) that mediate regional alterations in chromatin structure, thereby governing gene accessibility for transcription. The challenge and the opportunity will be to distill the identity and contributions of specific alterations—genetic and epigenetic—to hallmark-enabling functions from increasingly massive datasets, and to exploit such knowledge for improved detection, evaluation, and informed treatment of human cancers.

Tumor-promoting immune cell infiltration (inflammation)

Tumor-promoting immune cell infiltration (inflammation) is the second important modality through which developing cancers acquire hallmark capabilities. Strikingly, most tumors are infiltrated by a variety of cell types of the immune system (the so-called infiltrating immune cells, or IIC[3]). While the inflammation caused by IIC might reasonably be considered a failed attempt to eradicate a tumor, recent evidence now clearly makes a far more insidious point: IIC help convey in paracrine fashion multiple functional capabilities, encompassing seven of the eight hallmarks.[3] Thus, IIC can variously supply proliferative and survival signals, proangiogenic factors, and facilitate local invasion and blood-borne metastasis. In addition, some of these IIC (T-regulatory cells and myeloid-derived suppressor cells) can actively suppress the cytotoxic T lymphocytes that have been dispatched by the immune system to eradicate cancer cells.

Tumor-promoting IIC are recruited by a variety of means in different tumor types and at various stages of multistep tumorigenesis. The roster of the recruiting signals—including an ensemble of chemokine and cytokine signaling factors—is still incompletely understood. In some cases, the nature of the neoplastic lesion may trigger tissue abnormality or damage signals that attract IIC, represent the adaptive and innate immune systems. In other cases, oncogenic signaling, by activating transcriptional networks, induces expression of cytokines and chemokines that recruit IIC. In early-stage lesions, the recruited IIC can help incipient cancer cells to proliferate, survive, evade antigrowth controls, or activate angiogenesis. At later stages of progression, IIC at the margins of tumors can facilitate invasiveness. Some experiments reveal that IIC can pair with cancer cells as they migrate through the circulation and become established in distant locations.[24] Additionally, certain IIC, such as macrophages, can subject cancer cells to DNA-damaging reactive oxygen species, thereby contributing to the mutational alteration and evolution of the cancer-cell genome.

Most types of solid tumor are associated with tumor-promoting immune infiltrations that range from histologically subtle to the obvious inflammatory responses recognized by pathologists. In addition, the long-appreciated epidemiologic association between chronic inflammation and carcinogenesis supports the proposition that pre-existing inflammatory conditions can be fertile breeding grounds for the inception and progression of certain forms of

cancer. Chronically inflamed tissues share features with wound healing; both involve induction of angiogenesis and stimulation of cell survival, proliferation, and migration/invasion, involving the inflammatory IIC and other cell types (e.g., fibroblasts) that they activate in the affected tissue. These acquired traits represent hallmark capabilities, reinforcing the notion that IIC can inadvertently foster neoplastic initiation and/or progression of incipient cancer cells present in inflammatory tissue microenvironments.

The histopathological complexity of cancer, manifested in tumor microenvironments (TMEs)

Pathologists have long recognized that solid tumors are complex histological structures, incorporating not only cancer cells but also a variety of morphologically distinct cells, recognizable because they are similar to constituents of noncancerous tissues, both normal and affected by conditions such as infections or wound healing. In analogy to the stroma that supports epithelia in many normal tissues, the apparently noncancerous component of tumors has been labeled as the tumor stroma. As in normal tissue stroma, the tumor-associated stroma can be seen to contain blood vessels, assemblages of fibroblastic cells, and in many cases IIC. Historically, a simplistic view of the tumor stroma posited that endothelial cells, through the process of angiogenesis that produced a tumor neovasculature, provided oxygen and nutrients, while cancer-associated fibroblasts (CAFs) were either passengers or provided structural support, and the IIC, discussed earlier, represented ineffectual antitumoral immune responses. As described above, we now appreciate the fact that the diverse stromal cells inside tumors can contribute functionally to the acquisition of seven of the eight hallmarks.[3]

In analogy to normal tissues, tumors are often conceptually compartmentalized into the parenchyma (formed by the cancer cells) and the stroma (formed by the ostensibly normal supporting cells); the assemblage of these two compartments, incorporating as well extracellular material (including extracellular matrix, ECM, and basement membrane, BM) is increasingly referred to as the "tumor microenvironment" (TME), as illustrated in Figure 2; some also refer to the TME exclusively as the noncancerous stromal compartment, although conceptually the microenvironment incorporates the entirety of the tumor, that is, both its neoplastic and stromal compartments.

The three classes of stromal cell—angiogenic vascular cells (AVC), consisting of endothelial cells and supporting pericytes; CAF; and IIC—constitute the bulk of the stromal component of the TME.[3] These simple classifications, however, mask important diversity in cellular phenotypes. Thus, there are a number of CAF subtypes, of which the two most prevalent are derived either from myofibroblasts, mesenchymal stem cells, and tissue stellate cells that all characteristically express alpha-smooth muscle actin, or from connective tissue-derived fibroblasts that do not. Both subtypes of CAF arise via epigenetic reprogramming of their respective normal cells of origin by paracrine signals emanating from the TME; these inductive signals reflect similar signaling circuits used to engage fibroblasts in wound healing or inflammatory responses. A growing number of IIC subtypes are being recognized, each with distinctive functions and characteristics; some may be lineage derived (e.g., expressed by definition in immune-cell progenitors recruited from the bone marrow) and others the result of "local education" by particular inductive signals in the TME. The list of tumor-promoting IIC includes forms (subtypes) of macrophages, neutrophils, partially differentiated myeloid progenitors, and in some cases specialized subtypes of B and T lymphocyte. The endothelial cells and pericytes of the tumor vasculature are comparatively less diverse, although both epitope and gene-expression profiling have revealed tissue and tumor type-specific features of both endothelial cells and pericytes, likely with subtle functional implications in regard to tumor biology. A second distinct class of endothelial cells forms the lymphatic vascular network, which becomes enlarged via lymphangiogenesis proximal to many tumors and is implicated in lymphatic metastasis.

This recent and more nuanced view of stromal cells elevates their importance in understanding disease pathogenesis by virtue of their hallmark-enabling functional contributions.[2,3] CAFs, as an example not discussed earlier, can in different neoplastic contexts secrete proteases, proliferative signaling ligands, and/or other bioactive molecules that contribute to different tumor phenotypes. CAFs have been variously documented to liberate epithelial cells from the growth-suppressive effects imposed by normal tissue architecture, to induce tumor-promoting inflammation, to facilitate both local invasion and metastatic seeding, and to provide cancer cells with metabolic fuel. CAFs can also induce angiogenesis and, remarkably, act in an immune-suppressive manner to blunt the attacks of tumoricidal CTLs.

Looking to the future, an important goal will be to continue mapping the multidimensional landscape of stromal cell types and subtypes operative within different forms of cancer, and at different stages of progression.

Another dimension of the TME involves genetic and functional heterogeneity within populations of cancer cells. Indeed, the cancer cells within individual neoplastic lesions have long been recognized to be morphologically and genetically heterogeneous. Genome-profiling technologies (karyotyping, comparative genomic hybridization, allelic loss analysis, exome (gene) sequencing, and more recently whole-genome sequencing, now at the single-cell level) have documented the mutational evolution of the genome as nascent cancer cells in incipient neoplasias progress to spawn the genetically diverse subpopulations that coexist within high-grade tumors.

A second dimension of intratumoral heterogeneity is evident at the epigenetic level. Thus, in many carcinomas, cancer cells at the margins of invasive tumors are phenotypically distinct, having undergone an EMT that renders them more fibroblastic, with attendant capability for invasion. Others retain various degrees of differentiation characteristic of the cell type from which they originated, for example, squamous epithelia. In addition, the regional variation in histological characteristics seen in various tumor types is now realized to reflect (at least in some cases) genetically distinct clones of cancer cells, the result of mutational alteration of unstable genomes and clonal outgrowth, presumably reflecting different genetic solutions within the same neoplasia to the challenge of acquiring hallmark-enabling capabilities that enable malignant progression.

In addition, most tumors are now appreciated to contain distinct subpopulations—comparatively rare—of cancer cells exhibiting phenotypic similarity, at least superficially, to normal tissue stem cells. These cancer stem-like cells (CSC) typically proliferate comparatively slowly, express cell-surface markers diagnostic of tissue stem cells, and have enhanced capability to form new cancers upon ectopic transplantation of small numbers of cells into appropriate animal hosts, as compared to their more abundant counterparts, who proliferate more rapidly but are inefficient at or incapable of seeding transplant tumors.[9,25] (This latter assay operationally defines such cells as tumor-initiating cells, TIC.) One hypothesis was that the cell of origin of a cancer was a normal tissue stem or progenitor cell, which underwent neoplastic transformation into a CSC that in turn spawned cancer cells much like normal tissue

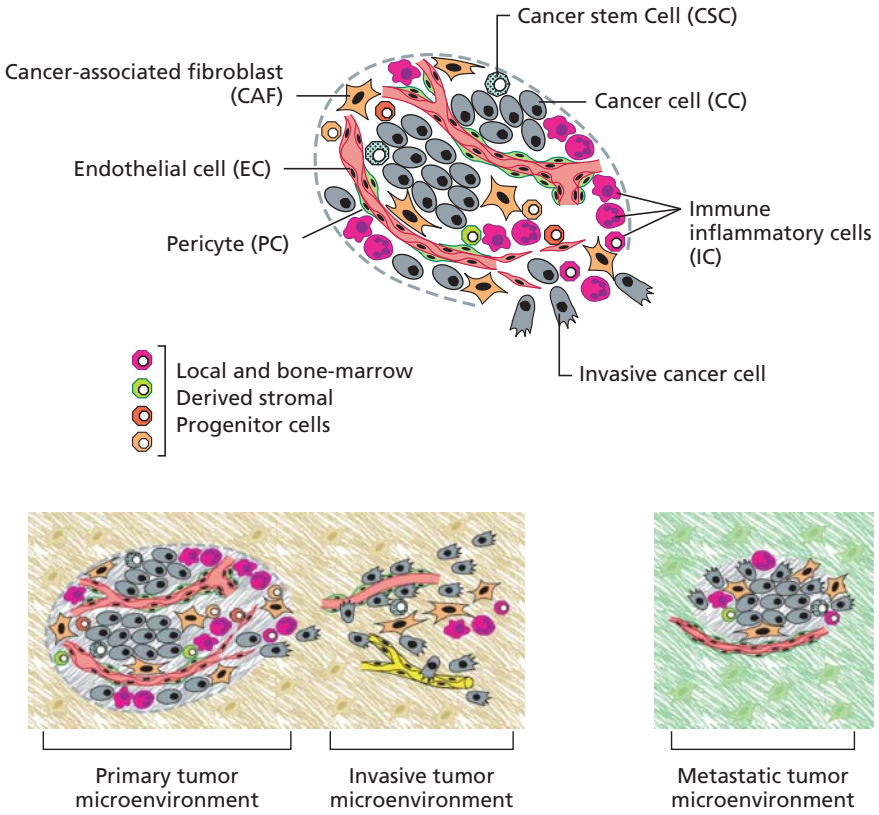

Figure 2 The constitution of the hallmark-enabling tumor microenvironment. An assemblage of distinct cell types constitutes the TME of most solid tumors, involving two distinct compartments—the parenchyma of cancer cells and the stroma of supporting cells. Both compartments contain distinct cell and subcell types that collectively enable tumor growth and progression.[2,3] Notably, the immune inflammatory cells present in tumors can include both tumor-promoting and/or immuno-suppressive as well as tumor-killing subclasses. The lower panels illustrate an important characteristic: the TME is dynamic, varying both in composition and abundance of constituent cell types (and sub-cell types) and in their effects on the histologically distinct stages in multistep tumorigenesis, namely premalignant stages (not shown) and malignant stages, including the cores of primary tumors, invasive margins and frankly invasive lesions, and metastases.

stem cells spawn differentiated cell types, and indeed there are indications of such cases. For example, the CSCs in squamous cell carcinomas of the skin produce partially differentiated cancer cells with features of squamous cells much as normal skin stem cells produce the squamous epithelium. A number of hematopoeitic malignancies evidently also arise from transformation of normal stem/progenitor cells into CSCs. In certain other cases, however, it appears that a dynamic interconversion operates between CSCs and their non-CSC counterparts, whereby CSCs can be converted into non-CSCs and vice versa, such that cancer cells can be converted into CSCs, and vice versa; in some such cases, the EMT appears to switch on the CSC phenotype in cancer cells, while its converse (the mesenchymal-to-epithelial transition, MET) reduces the abundance of CSCs in tumors.[9,25] There are indications that the comparatively less proliferative CSC may be more resistant to some genotoxic anticancer drugs, providing an avenue for drug resistance and clinical relapse. As such, therapeutic targeting of CSCs may be crucial to achieving enduring cancer therapies.

Therapeutic targeting (and cotargeting) of cancer hallmarks

An important question for cancer medicine is whether there are clinical applications of the hallmarks conceptualization? One possible benefit of this concept may come from helping cancer researchers appreciate common principles and thereby rationalize the diverse molecular and cellular mechanisms by which particular forms of human cancer develop and progress to malignancy. A wealth of data is being generated by multiplatform analysis—whole genome sequencing, and genome-wide profiling of RNA transcripts, proteins and phospho-proteins, and DNA and histone methylations—of cancer cells and neoplastic lesions in different tumor types (see, e.g., Ref. 26 and chapters throughout this textbook). Moreover, there will be other extrapolations of these increasingly powerful analytic technologies, including the profiling of lesional stages in tumorigenesis and tumor progression, in particular metastases; additionally, these technologies will likely provide insights into the adaptations that occur in tumors and metastases during the response and relapse phases to mechanism-targeted therapies. The challenge will be to integrate all of this information in order to understand the key determinants of particular carcinogenesis pathways; to identify new therapeutic targets; to identify modes of adaptive resistance to therapy; and then to use the data for diagnosis, prognosis, and treatment decisions. It is possible, although as yet unproven, that the hallmarks of cancer will prove useful in this integration and distillation: perhaps by filtering such cancer "omics" data—of the genome, the transcriptome, the proteome and phosphoproteome, and the methylome—through the growing knowledge base of regulatory pathways, it will be possible to identify the genetic and epigenetic signatures that underlie the acquisition of various hallmarks, potentially informing more precise management of disease.

We also envision that the hallmarks concept will prove useful in the design of future clinical treatment protocols. Notably, there are either approved drugs or drugs in late-stage clinical trials that target each of the eight hallmark capabilities and both of the enabling facilitators of those hallmarks (Figure 3); moreover, for most of the 10, there are multiple distinctive drugs targeting the same mechanistic effectors of these hallmarks. Although this is a provocative development in cancer therapeutics, these mechanism-based therapies targeting individual hallmarks have not in general been transformative for the treatment of late stage, aggressive forms of human cancer. An exception to this rule may be in the exciting ascendance of therapeutic immunomodulation to activate and sustain antitumoral immunity, involving most notably inhibitors of immune checkpoint receptors expressed on T lymphocytes (CTLA4 and PD1). Signaling from these checkpoint receptors can disable cytotoxic T cells, evidently rendering antitumoral immune responses ineffectual, thereby contributing to the hallmark capability for evading immune destruction. Notably, exciting clinical responses are being observed in melanoma and other selected tumors[27,28] treated with therapeutic antibodies that inhibit checkpoint activation, particularly when both checkpoints are cotargeted with therapeutic antibody cocktails.[29] Nevertheless, not all patients respond to such immunotherapies, and the duration of response remains to be ascertained, as does the prevalence of adaptive resistance to such immunotherapies.

For other hallmark-targeting therapies, it is typical, after a period of response, to see adaptive resistance mechanisms kick in, enabling the surviving cancer cells (and cancer stem cells) to circumvent the mechanistic blockade imposed by the treatment and resume progressive growth. Various solutions can be proposed to overcome the failures of currently employed, targeted therapies. We suggest that a fruitful therapeutic strategy might involve applying the concept of functionally distinct hallmarks traits, more specifically by targeting multiple hallmarks concomitantly. This multi-targeting may reduce the likelihood of acquired resistance to treatment, thereby yielding significant improvements in initial responses and in long-term survival.[30] Certainly, an important issue will be effectively managing the toxicities of such combinations. Thus, in addition to simple cocktails, it may be necessary to use hallmark-targeting drugs sequentially, episodically, or in layers, fine-tuned to maximize efficacy while managing toxicity and limiting adaptive resistance. It is further envisioned that refined preclinical mouse models—both genetically engineered *de novo* and patient-derived xenograft (PDX) transplants—will have utility in testing alternative therapeutic trials designs aimed to reduce the matrix of possibilities to clinically feasible numbers, taking the best-performing trial arms from preclinical trials into clinical trials and personalized treatments.[31–33]

In conclusion, the hallmarks of cancer may provide the student of modern oncology with a foundation and a framework for absorbing the subsequent topical chapters of this textbook, and more generally for investigating and interpreting pathogenic mechanisms and applying such knowledge toward the development of more effective diagnosis and treatment of human cancers.

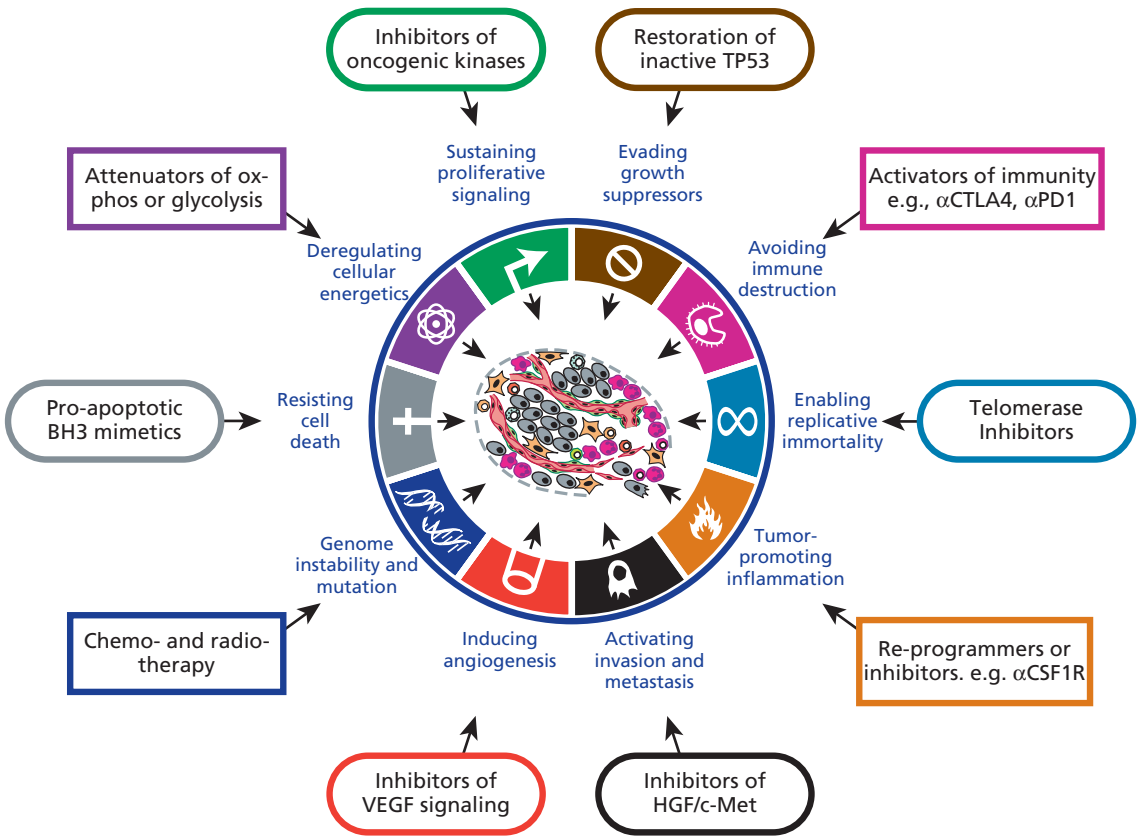

Figure 3 Therapeutic targeting of the hallmarks of cancer. Drugs have been developed that disrupt or interfere with all eight of the hallmark capabilities, and with the two enabling facilitators (genome instability and tumor-promoting inflammation). Some of these hallmark-targeting drugs are approved for clinical use, while others are being tested in late-stage clinical trials; moreover, there is a pipeline full of new hallmark-targeting drugs in development and preclinical evaluation. Recognizing that eventual adaptive resistance during therapeutic treatment is apparent for virtually all of these hallmark-targeting drugs, a hypothesis has emerged: perhaps, by cotargeting multiple independent hallmarks, it will be possible to limit or even prevent the emergence of simultaneous adaptive resistance to independent hallmark-targeting drugs;[30] clinical and preclinical trials are beginning to assess the possibilities.

References

1 Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell*. 2000;**100**:57–70.
2 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;**144**:346–674.
3 Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer Cell*. 2012;**21**:309–322.
4 Weinberg RA. *The Biology of Cancer*. New York: Garland Press; 2013.
5 Vanden Berghe T, Linkermann A, Jouan-Lanhouet S, et al. Regulated necrosis: the expanding network of non-apoptotic cell death pathways. *Nat Rev Mol Cell Biol*. 2014;**15**:135–147.
6 Rebecca VW, Amaravadi RK. Emerging strategies to effectively target autophagy in cancer. *Oncogene*. 2015. doi: 10.1038/onc.2015.99 (Epub ahead of print).
7 Keith B, Johnson RS, Simon MC. HIF1α and HIF2α: sibling rivalry in hypoxic tumour growth and progression. *Nat Rev Cancer*. 2011;**12**:9–22.
8 Semenza GL. Hypoxia-inducible factors: mediators of cancer progression and targets for cancer therapy. *Trends Pharmacol Sci*. 2012;**33**:207–214.
9 Baccelli I, Trumpp A. The evolving concept of cancer and metastasis stem cells. *J Cell Biol*. 2012;**198**:281–293.
10 Savagner P. Epithelial-mesenchymal transitions: from cell plasticity to concept elasticity. *Curr Top Dev Biol*. 2015;**112**:273–300.
11 Daye D, Wellen KE. Metabolic reprogramming in cancer: unraveling the role of glutamine in tumorigenesis. *Semin Cell Dev Biol*. 2012;**23**:362–369.
12 Dhup S, Dadhich RK, Porporato PE, Sonveaux P. Multiple biological activities of lactic acid in cancer: influences on tumor growth, angiogenesis and metastasis. *Curr Pharm Des*. 2012;**18**:1319–1330.
13 Ward PS, Thompson CB. Metabolic reprogramming: a cancer hallmark even Warburg did not anticipate. *Cancer Cell*. 2012;**21**:297–308.
14 Fridman WH, Pagès F, Sautès-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. *Nat Rev Cancer*. 2012;**12**:298–306.
15 Galon J, Mlecnik B, Bindea G, et al. Towards the introduction of the 'Immunoscore' in the classification of malignant tumours. *J Pathol*. 2014;**232**:199–209.
16 Galon J, Angell HK, Bedognetti D, Marincola FM. The continuum of cancer immunosurveillance: prognostic, predictive, and mechanistic signatures. *Immunity*. 2013;**39**:11–26.
17 https://tcga-data.nci.nih.gov/tcga/tcgaHome2.jsp
18 http://www.sanger.ac.uk/research/projects/cancergenome/
19 http://icgc.org/
20 http://www.ncbi.nlm.nih.gov/sky/
21 You JS, Jones PA. Cancer genetics and epigenetics: two sides of the same coin? *Cancer Cell*. 2012;**22**:9–20.
22 Easwaran H, Tsai HC, Baylin SB. Cancer epigenetics: tumor heterogeneity, plasticity of stem-like states, and drug resistance. *Mol Cell*. 2014;**54**:716–727.
23 Azad N, Zahnow CA, Rudin CM, Baylin SB. The future of epigenetic therapy in solid tumours – lessons from the past. *Nat Rev Clin Oncol*. 2013;**10**:256–266.
24 Labelle M, Hynes RO. The initial hours of metastasis: the importance of cooperative host-tumor cell interactions during hematogenous dissemination. *Cancer Discov*. 2012;**2**:1091–1099.
25 Visvader JE, Lindeman GJ. Cancer stem cells: current status and evolving complexities. *Cell Stem Cell*. 2012;**10**:717–728.
26 The Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. *Nature*. 2012;**490**:61–70.
27 Sharma P, Allison JP. The future of immune checkpoint therapy. *Science*. 2015;**348**:56–61.
28 Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. *Cancer Cell*. 2015;**27**:450–461.
29 Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. *Cell*. 2015;**161**:205–214.
30 Hanahan D. Rethinking the war on cancer. *Lancet*. 2013;**383**:558–563.
31 De Palma M, Hanahan D. The biology of personalized cancer medicine: facing individual complexities underlying hallmark capabilities. *Mol Oncol*. 2012;**6**:111–127.
32 Das Thakur M, Pryer NK, Singh M. Mouse tumour models to guide drug development and identify resistance mechanisms. *J Pathol*. 2014;**232**:103–111.
33 Siolas D, Hannon GJ. Patient-derived tumor xenografts: transforming clinical samples into mouse models. *Cancer Res*. 2013;**73**:5315–5319.