SOCIETY OF WELLNESS
NUTRITIONAL SYSTEMS

Functional Herbal Therapy and Cancer: Module 1

Overview and the Hallmarks of Cancer

Professor Kerry Bone's Reading Assignment

Module 1.2 – The Hallmarks of Cancer

1. **The Hallmarks of Cancer**
   Hanahan, D., & Weinberg, R. A. (2000). The hallmarks of cancer. *cell*, 100(1), 57-70.

H and W 2000 Highlights

Many types of cancers are diagnosed in the human population with an age-dependent incidence implicating four to seven rate-limiting, stochastic events

Transformation of cultured cells is itself a multistep process: rodent cells require at least two introduced genetic changes before they acquire tumorigenic competence, while their human counterparts are more difficult to transform.

Taken together, observations of human cancers and animal models argue that tumor development proceeds via a process formally analogous to Darwinian evolution, in which a succession of genetic changes, each conferring one or another type of growth advantage, leads to the progressive conversion of normal human cells into cancer cells.

Each of these physiologic changes—novel capabilities acquired during tumor development—represents the successful breaching of an anticancer defense mechanism hardwired into cells and tissues.

Growth signaling

We suspect that growth signaling pathways suffer deregulation in all human tumors.

While acquisition of growth signaling autonomy by cancer cells is conceptually satisfying, it is also too simplistic..... It is, however, increasingly apparent that the growth deregulation within a tumor can only be explained once we understand the contributions of the ancillary cells present in a tumor—the apparently normal bystanders such as fibroblasts and endothelial cells—which must play key roles in driving tumor cell proliferation.

Evading apoptosis

The ability of tumor cell populations to expand in number is determined not only by the rate of cell proliferation but also by the rate of cell attrition. Programmed cell death—apoptosis—represents a major source of this attrition..... The apoptotic machinery can be broadly divided into two classes of components—sensors and effectors. The sensors are responsible for monitoring the extracellular and intracellular environment for conditions of normality or abnormality that influence whether a cell should live or die.... Examples of these ligand/receptor pairs include survival signals conveyed by IGF-1/IGF-2 through their receptor, IGF-1R, and by IL-3 and its cognate receptor, IL-3R.... Intracellular sensors monitor the cell's well-being and activate the death pathway in response to detecting abnormalities, including DNA damage..... Many of the signals that elicit apoptosis converge on the mitochondria, which respond to proapoptotic signals by releasing cytochrome C, a potent catalyst of apoptosis.....Apoptosis is a major barrier to cancer.

Resistance to apoptosis can be acquired by cancer cells through a variety of strategies. Surely, the most commonly occurring loss of a proapoptotic regulator through mutation involves the p53 tumor suppressor gene. The resulting functional inactivation of its product, the p53 protein, is seen in greater than 50% of human cancers and results in the removal of a key component of the DNA damage sensor that can induce the apoptotic effector cascade. Signals evoked by other abnormalities, including hypoxia and oncogene hyperexpression, are also funneled in part via p53 to the apoptotic machinery; these too are impaired at eliciting apoptosis when p53 function is lost..... We expect that virtually all cancer cells harbor alterations that enable evasion of- apoptosis.

Limitless replicative potential

Three acquired capabilities—growth signal autonomy, insensitivity to antigrowth signals, and resistance to apoptosis—all lead to an uncoupling of a cell's growth program from signals in its environment. In principle, the resulting deregulated proliferation program should suffice to enable the generation of the vast cell populations that constitute macroscopic tumors. However, research performed over the past 30 years indicates that this acquired disruption of cell-to-cell signaling, on its own, does not ensure expansive tumor growth. Many and perhaps all types of mammalian cells carry an intrinsic, cell-autonomous program that limits their multiplication. This program appears to operate independently of the cell-to-cell signaling pathways described above. It too must be disrupted in order for a clone of cells to expand to a size that constitutes a macroscopic, life-threatening tumor.

The early work of Hayflick demonstrated that cells in culture have a finite replicative potential. Once such cell populations have progressed through a certain number of doublings, they stop growing—a process termed senescence..... Provocatively, most types of tumor cells that are propagated in culture appear to be immortalized, suggesting that limitless replicative potential is a phenotype that was acquired in vivo during tumor progression and was essential for the development of their malignant growth state.

The counting device for cell generations has been discovered over the past decade: the ends of chromosomes, telomeres..... The progressive erosion of telomeres through successive cycles of replication eventually causes them to lose their ability to protect the ends of chromosomal DNA..... Telomere maintenance is evident in virtually all types of malignant cells; 85%–90% of them succeed in doing so by upregulating expression of the telomerase enzyme..... while the remainder have invented a way of activating a mechanism, termed ALT, which appears to maintain telomeres....
While telomere maintenance is clearly a key component of the capability for unlimited replication, we remain uncertain about another one, the circumvention of cellular senescence.

Sustained angiogenesis

In order to progress to a larger size, incipient neoplasias must develop angiogenic ability..... Counterbalancing positive and negative signals encourage or block angiogenesis. One class of these signals is conveyed by soluble factors and their receptors, the latter displayed on the surface of endothelial cells; integrins and adhesion molecules mediating cell–matrix and cell–cell association also play critical roles. The angiogenesis-initiating signals are exemplified by vascular endothelial growth factor (VEGF) and acidic and basic fibroblast growth factors (FGF1/2).... The ability to induce and sustain angiogenesis seems to be acquired in a discrete step (or steps) during tumor development, via an "angiogenic switch" from vascular quiescence.

Tissue invasion and metastasis

Sooner or later during the development of most types of human cancer, primary tumor masses spawn pioneer cells that move out, invade adjacent tissues, and thence travel to distant sites where they may succeed in founding new colonies. These distant settlements of tumor cells—metastases—are the cause of 90% of human cancer deaths... Several classes of proteins involved in the tethering of cells to their surroundings in a tissue are altered in cells possessing invasive or metastatic capabilities. The affected proteins include cell–cell adhesion molecules (CAMs)—notably members of the immunoglobulin and calcium-dependent cadherin families, both of which mediate cell-to-cell interactions—and integrins, which link cells to extracellular matrix substrates.
The second general parameter of the invasive and metastatic capability involves extracellular proteases... One imagines that docking of active proteases on the cell surface can facilitate invasion by cancer cells into nearby stroma, across blood vessel walls, and through normal epithelial cell layers.... A further dimension of complexity derives from the multiple cell types involved in protease expression and display. In many types of carcinomas, matrix-degrading proteases are produced not by the epithelial cancer cells but rather by conscripted stromal and inflammatory cells.

Genome instability

The acquisition of the enumerated six capabilities during the course of tumor progression creates a dilemma. The available evidence suggests that most are acquired, directly or indirectly, through changes in the genomes of cancer cells. But mutation of specific genes is an inefficient process, reflecting the unceasing, fastidious maintenance of genomic integrity by a complex array of DNA

monitoring and repair enzymes... Together, these systems ensure that mutations are rare events, indeed so rare that the multiple mutations known to be present in tumor cell genomes are highly unlikely to occur within a human life span.

Yet cancers do appear at substantial frequency in the human population, causing some to argue that the genomes of tumor cells must acquire increased mutability in order for the process of tumor progression to reach completion in several decades time. Malfunction of specific components of these genomic "caretaker" systems has been invoked to explain this increased mutability. The most prominent member of these systems is the p53 tumor suppressor protein, which, in response to DNA damage, elicits either cell cycle arrest to allow DNA repair to take place or apoptosis if the damage is excessive.

Different pathways to cancer

The paths that cells take on their way to becoming malignant are highly variable. Within a given cancer type, mutation of particular target genes such as ras or p53 may be found in only a subset of otherwise histologically identical tumors. Further, mutations in certain oncogenes and tumor suppressor genes can occur early in some tumor progression pathways and late in others. As a consequence, the acquisition of biological capabilities such as resistance to apoptosis, sustained angiogenesis, and unlimited replicative potential can appear at different times during these various progressions.... Nonetheless, we believe that independent of how the steps in these genetic pathways are arranged, the biological endpoints that are ultimately reached—the hallmark capabilities of cancer—will prove to be shared in common by all types of tumors.

2. The Hallmarks of Cancer: The Next Generation

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *cell*, 144(5), 646-674.

H and W 2011 Highlights

The hallmarks of cancer comprise six biological capabilities acquired during the multistep development of human tumors. The hallmarks constitute an organizing principle for rationalizing the complexities of neoplastic disease. They include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis.

Underlying these hallmarks are genome instability, which generates the genetic diversity that expedites their acquisition, and inflammation, which fosters multiple hallmark functions. Conceptual progress in the last decade has added two emerging hallmarks of potential generality to this list—reprogramming of energy metabolism and evading immune destruction. In addition to cancer cells, tumors exhibit another dimension of complexity: they contain a repertoire of recruited, ostensibly normal cells that contribute to the acquisition of hallmark traits by creating the "tumor microenvironment." Recognition of the widespread applicability of these concepts will increasingly affect the development of new means to treat human cancer.

Introduction

We noted (in 2000) as an ancillary proposition that tumors are more than insular masses of proliferating cancer cells. Instead, they are complex tissues composed of multiple distinct cell types that participate in heterotypic interactions with one another. We depicted the recruited normal cells, which form tumor-associated stroma, as active participants in tumorigenesis rather than passive bystanders; as such, these stromal cells contribute to the development and expression of certain hallmark capabilities. During the ensuing decade this notion has been solidified and extended, revealing that the biology of tumors can no longer be understood simply by enumerating the traits of the cancer cells but instead must encompass the contributions of the "tumor microenvironment" to tumorigenesis.

Disruptions of Negative-Feedback Mechanisms that Reduce Growth Signaling

Recent results have highlighted the importance of negative-feedback loops that normally operate to dampen various types of signaling and thereby ensure homeostatic regulation of the flux of signals coursing through the intracellular circuitry. Defects in these feedback mechanisms are capable of enhancing proliferative signaling. The prototype of this type of regulation involves the Ras oncoprotein: the oncogenic effects of Ras do not result from a hyperactivation of its signaling powers; instead, the oncogenic mutations affecting ras genes compromise Ras GTPase activity, which operates as an intrinsic negative-feedback mechanism that normally ensures that active signal transmission is transitory.

Yet another example involves the mTOR kinase, a coordinator of cell growth and metabolism that lies both upstream and downstream of the PI3K pathway. In the circuitry of some cancer cells, mTOR activation results, via negative feedback, in the inhibition of PI3K signaling. Thus, when mTOR is pharmacologically inhibited in such cancer cells (such as by the drug rapamycin), the associated loss of negative feedback results in increased activity of PI3K and its effector Akt/PKB, thereby blunting the antiproliferative effects.

Corruption of the TGF-β Pathway Promotes Malignancy

TGF-β is best known for its antiproliferative effects, and evasion by cancer cells of these effects is now appreciated to be far more elaborate than simple shutdown of its signaling circuitry. In many late-stage tumors, TGF-β signaling is redirected away from suppressing cell proliferation and is found instead to activate a cellular program, termed the epithelial-to-mesenchymal transition (EMT), that confers on cancer cells traits associated with high-grade malignancy.

Autophagy Mediates Both Tumor Cell Survival and Death

Autophagy represents an important cell-physiologic response that, like apoptosis, normally operates at low, basal levels in cells but can be strongly induced in certain states of cellular stress, the most obvious of which is nutrient deficiency. The autophagic program enables cells to break down cellular organelles, such as ribosomes and mitochondria, allowing the resulting catabolites to be recycled and thus used for biosynthesis and energy metabolism. As part of

this program, intracellular vesicles termed autophagosomes envelope intracellular organelles and then fuse with lysosomes wherein degradation occurs. In this fashion, low-molecular-weight metabolites are generated that support survival in the stressed, nutrient-limited environments experienced by many cancer cells.

Perhaps paradoxically, nutrient starvation, radiotherapy, and certain cytotoxic drugs can induce elevated levels of autophagy that are apparently cytoprotective for cancer cells, impairing rather than accentuating the killing actions of these stress-inducing situations. Moreover, severely stressed cancer cells have been shown to shrink via autophagy to a state of reversible dormancy. This survival response may enable the persistence and eventual regrowth of some late-stage tumors following treatment with potent anticancer agents. Thus, in analogy to TGF-β signaling, which can be tumor suppressing at early stages of tumorigenesis and tumor promoting later on, autophagy seems to have conflicting effects on tumor cells and thus tumor progression.

Necrosis Has Proinflammatory and Tumor-Promoting Potential

In contrast to apoptosis, in which a dying cell contracts into an almost-invisible corpse that is soon consumed by neighbors, necrotic cells become bloated and explode, releasing their contents into the local tissue microenvironment. Although necrosis has historically been viewed much like organismic death, as a form of system-wide exhaustion and breakdown, the conceptual landscape is changing: cell death by necrosis is clearly under genetic control in some circumstances, rather than being a random and undirected process.

Perhaps more important, necrotic cell death releases proinflammatory signals into the surrounding tissue microenvironment, in contrast to apoptosis and autophagy, which do not. As a consequence, necrotic cells can recruit inflammatory cells of the immune system, whose dedicated function is to survey the extent of tissue damage and remove associated necrotic debris. In the context of neoplasia, however, multiple lines of evidence indicate that immune inflammatory cells can be actively tumor promoting, given that such cells are capable of fostering angiogenesis, cancer cell proliferation, and invasiveness. Additionally, necrotic cells can release bioactive regulatory factors, such as IL-1α, which can directly stimulate neighboring viable cells to proliferate, with the potential, once again, to facilitate neoplastic progression. Consequently, necrotic cell death, while seemingly beneficial in counterbalancing cancer-associated hyperproliferation, may ultimately do more damage than good. Accordingly, incipient neoplasias and potentially invasive and metastatic tumors may gain an advantage by tolerating some degree of necrotic cell death, doing so in order to recruit tumor-promoting inflammatory cells that bring growth-stimulating factors to the surviving cells within these growths.

Enabling Replicative Immortality

By 2000, it was widely accepted that cancer cells require unlimited replicative potential in order to generate macroscopic tumors. This capability stands in marked contrast to the behavior of the cells in most normal cell lineages in the body, which are able to pass through only a limited number of successive cell growth-and-division cycles. This limitation has been associated with two distinct barriers to proliferation: senescence, a typically irreversible entrance into a

non-proliferative but viable state, and crisis, which involves cell death. Accordingly, when cells are propagated in culture, repeated cycles of cell division lead first to induction of senescence and then, for those cells that succeed in circumventing this barrier, to a crisis phase, in which the great majority of cells in the population die. On rare occasion, cells emerge from a population in crisis and exhibit unlimited replicative potential. This transition has been termed immortalization, a trait that most established cell lines possess by virtue of their ability to proliferate in culture without evidence of either senescence or crisis.

The length of telomeric DNA in a cell dictates how many successive cell generations its progeny can pass through before telomeres are largely eroded and have consequently lost their protective functions, triggering entrance into crisis.

Telomerase, the specialized DNA polymerase that adds telomere repeat segments to the ends of telomeric DNA, is almost absent in non-immortalized cells but expressed at functionally significant levels in the vast majority (~90%) of spontaneously immortalized cells, including human cancer cells.

The two barriers to proliferation—senescence and crisis/apoptosis—have been rationalized as crucial anticancer defenses that are hard-wired into our cells, being deployed to impede the outgrowth of clones of preneoplastic and frankly neoplastic cells. According to this thinking, most incipient neoplasias exhaust their endowment of replicative doublings and are stopped in their tracks by one or the other of these barriers. The eventual immortalization of rare variant cells that proceed to form tumors has been attributed to their ability to maintain telomeric DNA at lengths sufficient to avoid triggering senescence or apoptosis, achieved most commonly by upregulating expression of telomerase or, less frequently, via an alternative recombination-based telomere maintenance mechanism. Hence, telomere shortening has come to be viewed as a clocking device that determines the limited replicative potential of normal cells and thus one that must be overcome by cancer cells.

Angiogenesis

It is now clear that a repertoire of cell types originating in the bone marrow play crucial roles in pathological angiogenesis. These include cells of the innate immune system—notably macrophages, neutrophils, mast cells, and myeloid progenitors—that infiltrate premalignant lesions and progressed tumors and assemble at the margins of such lesions; the peri-tumoral inflammatory cells help to trip the angiogenic switch in previously quiescent tissue and to sustain ongoing angiogenesis associated with tumor growth, in addition to facilitating local invasion...

Activating Invasion and Metastasis

In 2000, the mechanisms underlying invasion and metastasis were largely an enigma. It was clear that as carcinomas arising from epithelial tissues progressed to higher pathological grades of malignancy, reflected in local invasion and distant metastasis, the associated cancer cells typically developed alterations in their shape as well as in their attachment to other cells and to the ex-

tracellular matrix (ECM). The best characterized alteration involved the loss by carcinoma cells of E-cadherin, a key cell-to-cell adhesion molecule. By forming adherens junctions with adjacent epithelial cells, E-cadherin helps to assemble epithelial cell sheets and maintain the quiescence of the cells within these sheets. Increased expression of E-cadherin was well established as an antagonist of invasion and metastasis.

A developmental regulatory program, referred to as the "epithelial-mesenchymal transition" (EMT), has become prominently implicated as a means by which transformed epithelial cells can acquire the abilities to invade, to resist apoptosis, and to disseminate. By co-opting a process involved in various steps of embryonic morphogenesis and wound healing, carcinoma cells can concomitantly acquire multiple attributes that enable invasion and metastasis. This multifaceted EMT program can be activated transiently or stably, and to differing degrees, by carcinoma cells during the course of invasion and metastasis.

Although the evidence is still incomplete, it would appear that EMT-inducing transcription factors are able to orchestrate most steps of the invasion-metastasis cascade save the final step of colonization.
It is increasingly apparent that crosstalk between cancer cells and cells of the neoplastic stroma is involved in the acquired capability for invasive growth and metastasis…Macrophages at the tumor periphery can foster local invasion by supplying matrix-degrading enzymes such as matrix metalloproteinases and cysteine cathepsin proteases.

Observations like these indicate that the phenotypes of high-grade malignancy do not arise in a strictly cell-autonomous manner, and that their manifestation cannot be understood solely through analyses of tumor cell genomes.

Metastasis can be broken down into two major phases: the physical dissemination of cancer cells from the primary tumor to distant tissues, and the adaptation of these cells to foreign tissue microenvironments that results in successful colonization, i.e., the growth of micro-metastases into macroscopic tumors. The multiple steps of dissemination would seem to be in the purview of the EMT and similarly acting migratory programs. Colonization, however, is not strictly coupled with physical dissemination, as evidenced by the presence in many patients of myriad micro-metastases that have successfully disseminated but never progress to macroscopic metastatic tumors.

In some types of cancer, the primary tumor may release systemic suppressor factors that render such micro-metastases dormant, as revealed clinically by explosive metastatic growth soon after resection of the primary growth. In others, however, such as breast cancer and melanoma, macroscopic metastases may erupt decades after a primary tumor has been surgically removed or pharmacologically destroyed; these metastatic tumor growths evidently reflect dormant micro-metastases that have solved, after much trial and error, the complex problem of tissue colonization.

Enabling Characteristics and Emerging Hallmarks

We have defined the hallmarks of cancer as acquired functional capabilities that allow cancer cells to survive, proliferate, and disseminate; these functions are acquired in different tumor types via distinct mechanisms and at various times during the course of multistep tumorigenesis. Their acquisition is made possible by two enabling characteristics. Most prominent is the development of genomic instability in cancer cells, which generates random mutations including chromosomal rearrangements; among these are the rare genetic changes that can orchestrate hallmark capabilities. A second enabling characteristic involves the inflammatory state of premalignant and frankly malignant lesions that is driven by cells of the immune system, some of which serve to promote tumor progression through various means.

Yet other distinct attributes of cancer cells have been proposed to be functionally important for the development of cancer and might therefore be added to the list of core hallmarks. Two such attributes are particularly compelling. The first involves major reprogramming of cellular energy metabolism in order to support continuous cell growth and proliferation, replacing the metabolic program that operates in most normal tissues and fuels the physiological operations of the associated cells. The second involves active evasion by cancer cells from attack and elimination by immune cells; this capability highlights the dichotomous roles of an immune system that both antagonizes and enhances tumor development and progression.

An Enabling Characteristic: Genome Instability and Mutation

A diverse array of defects affecting various components of the DNA-maintenance machinery—often referred to as the "caretakers" of the genome have been documented. The catalog of defects in these caretaker genes includes those whose products are involved in (1) detecting DNA damage and activating the repair machinery, (2) directly repairing damaged DNA, and (3) inactivating or intercepting mutagenic molecules before they have damaged the DNA. From a genetic perspective, these caretaker genes behave much like tumor suppressor genes, in that their functions can be lost during the course of tumor progression, with such losses being achieved either through inactivating mutations or via epigenetic repression.

An Enabling Characteristic: Tumor-Promoting Inflammation

Pathologists have long recognized that some tumors are densely infiltrated by cells of both the innate and adaptive arms of the immune system and thereby mirror inflammatory conditions arising in non-neoplastic tissues.

By 2000, there were already clues that the tumor-associated inflammatory response had the unanticipated, paradoxical effect of enhancing tumorigenesis and progression, in effect helping incipient neoplasias to acquire hallmark capabilities. In the ensuing decade, research on the intersections between inflammation and cancer pathogenesis has blossomed, producing abundant and compelling demonstrations of the functionally important tumor-promoting effects that immune cells—largely of the innate immune system—have on neoplastic progression. Inflammation can contribute to multiple hallmark capabilities by supplying bioactive molecules

to the tumor microenvironment, including growth factors that sustain proliferative signaling, survival factors that limit cell death, proangiogenic factors, extracellular matrix-modifying enzymes that facilitate angiogenesis, invasion, and metastasis, and inductive signals that lead to activation of EMT and other hallmark-facilitating programs.

Importantly, inflammation is in some cases evident at the earliest stages of neoplastic progression and is demonstrably capable of fostering the development of incipient neoplasias into full-blown cancers.

Additionally, inflammatory cells can release chemicals, notably reactive oxygen species, that are actively mutagenic for nearby cancer cells, accelerating their genetic evolution toward states of heightened malignancy.

An Emerging Hallmark: Reprogramming Energy Metabolism

The chronic and often uncontrolled cell proliferation that represents the essence of neoplastic disease involves not only deregulated control of cell proliferation but also corresponding adjustments of energy metabolism in order to fuel cell growth and division. Under aerobic conditions, normal cells process glucose, first to pyruvate via glycolysis in the cytosol and thereafter to carbon dioxide in the mitochondria; under anaerobic conditions, glycolysis is favored and relatively little pyruvate is dispatched to the oxygen-consuming mitochondria. Otto Warburg first observed an anomalous characteristic of cancer cell energy metabolism: even in the presence of oxygen, cancer cells can reprogram their glucose metabolism, and thus their energy production, by limiting their energy metabolism largely to glycolysis, leading to a state that has been termed “aerobic glycolysis.”

The existence of this metabolic switch in cancer cells has been substantiated in the ensuing decades. Such reprogramming of energy metabolism is seemingly counterintuitive, in that cancer cells must compensate for the ~18-fold lower efficiency of ATP production afforded by glycolysis relative to mitochondrial oxidative phosphorylation. They do so in part by upregulating glucose transporters, notably GLUT1, which substantially increases glucose import into the cytoplasm.

Glycolytic fueling has been shown to be associated with activated oncogenes (e.g., *RAS, MYC*) and mutant tumor suppressors (e.g., TP53), whose alterations in tumor cells have been selected primarily for their benefits in conferring the hallmark capabilities of cell proliferation, avoidance of cytostatic controls, and attenuation of apoptosis. This reliance on glycolysis can be further accentuated under the hypoxic conditions that operate within many tumors: the hypoxia response system acts to upregulate in multiple ways glucose transporters and multiple enzymes of the glycolytic pathway.

A functional rationale for the glycolytic switch in cancer cells has been elusive, given the relatively poor efficiency of generating ATP by glycolysis relative to mitochondrial oxidative phosphorylation. According to one long-forgotten, increased glycolysis allows the diversion of glycolytic intermediates into various biosynthetic pathways, including those generating

nucleosides and amino acids; this facilitates, in turn, the biosynthesis of the macromolecules and organelles required for assembling new cells. Moreover, Warburg-like metabolism seems to be present in many rapidly dividing embryonic tissues, once again suggesting a role in supporting the large-scale biosynthetic programs that are required for active cell proliferation.

Interestingly, some tumors have been found to contain two subpopulations of cancer cells that differ in their energy-generating pathways. One subpopulation consists of glucose-dependent ("Warburg-effect") cells that secrete lactate, whereas cells of the second subpopulation preferentially import and utilize the lactate produced by their neighbors as their main energy source, employing part of the citric acid cycle to do so….Additionally, it is becoming apparent that oxygenation, ranging from normoxia to hypoxia, is not necessarily static in tumors but instead fluctuates temporally and regionally, likely as a result of the instability and chaotic organization of the tumor-associated neovasculature.

An Emerging Hallmark: Evading Immune Destruction

A second, still-unresolved issue surrounding tumor formation involves the role that the immune system plays in resisting or eradicating formation and progression of incipient neoplasias, late-stage tumors, and micrometastases. The long-standing theory of immune surveillance proposes that cells and tissues are constantly monitored by an ever-alert immune system, and that such immune surveillance is responsible for recognizing and eliminating the vast majority of incipient cancer cells and thus nascent tumors. According to this logic, solid tumors that do appear have somehow managed to avoid detection by the various arms of the immune system or have been able to limit the extent of immunological killing, thereby evading eradication.
The role of defective immunological monitoring of tumors would seem to be validated by the striking increases of certain cancers in immunocompromised individuals. However, the great majority of these are virus-induced cancers, suggesting that much of the control of this class of cancers normally depends on reducing viral burden in infected individuals, in part through eliminating virus-infected cells. These observations, therefore, seem to shed little light on the possible role of the immune system in limiting formation of the ›80% of tumors of nonviral etiology. In recent years, however, an increasing body of evidence, both from genetically engineered mice and from clinical epidemiology, suggests that the immune system operates as a significant barrier to tumor formation and progression, at least in some forms of non-virus-induced cancer.

Clinical epidemiology also increasingly supports the existence of antitumoral immune responses in some forms of human cancer. For example, patients with colon and ovarian tumors that are heavily infiltrated with cytotoxic T lymphocytes and NK cells have a better prognosis than those that lack such abundant killer lymphocytes.

Still, the epidemiology of chronically immunosuppressed patients does not indicate significantly increased incidences of the major forms of nonviral human cancer, as noted above. This might be taken as an argument against the importance of immune surveillance as an effective barrier to tumorigenesis and tumor progression. We note, however, that HIV and pharmacologically

immunosuppressed patients are predominantly immunodeficient in the T and B cell compartments and thus do not present with the multicomponent immunological deficiencies that have been produced in the genetically engineered mutant mice lacking both NK cells and CTLs; this leaves open the possibility that such patients still have residual capability for an immunological defense against cancer that is mounted by NK and other innate immune cells.

In truth, the above discussions of cancer immunology simplify tumor-host immunological interactions, as highly immunogenic cancer cells may well evade immune destruction by disabling components of the immune system that have been dispatched to eliminate them.

The Tumor Microenvironment

Over the past decade, tumors have increasingly been recognized as organs whose complexity approaches and may even exceed that of normal healthy tissues. When viewed from this perspective, the biology of a tumor can only be understood by studying the individual specialized cell types within it as well as the "tumor microenvironment" that they construct during the course of multistep tumorigenesis.

This depiction contrasts starkly with the earlier, reductionist view of a tumor as nothing more than a collection of relatively homogeneous cancer cells, whose entire biology could be understood by elucidating the cell-autonomous properties of these cells. We enumerate here a set of cell types known to contribute in important ways to the biology of many tumors and discuss the regulatory signaling that controls their individual and collective functions.

Cancer Cells and Cancer Stem Cells

Cancer cells are the foundation of the disease; they initiate tumors and drive tumor progression forward, carrying the oncogenic and tumor suppressor mutations that define cancer as a genetic disease. Traditionally, the cancer cells within tumors have been portrayed as reasonably homogeneous cell populations until relatively late in the course of tumor progression, when hyperproliferation combined with increased genetic instability spawn distinct clonal subpopulations. Reflecting such clonal heterogeneity, many human tumors are histopathologically diverse, containing regions demarcated by various degrees of differentiation, proliferation, vascularity, inflammation, and/or invasiveness. In recent years, however, evidence has accumulated pointing to the existence of a new dimension of intratumor heterogeneity and a hitherto-unappreciated subclass of neoplastic cells within tumors, termed cancer stem cells (CSCs)…. CSCs are defined operationally through their ability to efficiently seed new tumors upon inoculation into recipient host mice.

Recent research has interrelated the acquisition of CSC traits with the EMT transdifferentiation program discussed above…data suggests that the EMT program not only may enable cancer cells to physically disseminate from primary tumors but also can confer on such cells the self-renewal capability that is crucial to their subsequent clonal expansion at sites of dissemination…..An increasing number of human tumors are reported to contain subpopulations with the properties

of CSCs, as defined operationally through their efficient tumor-initiating capabilities upon xenotransplantation into mice. Nevertheless, the importance of CSCs as a distinct phenotypic subclass of neoplastic cells remains a matter of debate, as does their oft-cited rarity within tumors.

These complexities notwithstanding, it is evident that this new dimension of tumor heterogeneity holds important implications for successful cancer therapies. Increasing evidence in a variety of tumor types suggests that cells with properties of CSCs are more resistant to various commonly used chemotherapeutic treatments. Their persistence may help to explain the almost-inevitable disease recurrence following apparently successful debulking of human solid tumors by radiation and various forms of chemotherapy. Indeed, CSCs may well prove to underlie certain forms of tumor dormancy, whereby latent cancer cells persist for years or even decades after surgical resection or radio/chemotherapy, only to suddenly erupt and generate life-threatening disease. Hence, CSCs may represent a double-threat, in that they are more resistant to therapeutic killing and, at the same time, endowed with the ability to regenerate a tumor once therapy has been halted.

The discovery of CSCs and biological plasticity in tumors indicates that a single, genetically homogeneous population of cells within a tumor may nevertheless be phenotypically heterogeneous due to the presence of cells in distinct states of differentiation. However, an equally important source of phenotypic variability may derive from the genetic heterogeneity within a tumor that accumulates as cancer progression proceeds. Thus, elevated genetic instability operating in later stages of tumor progression may drive rampant genetic diversification that outpaces the process of Darwinian selection, generating genetically distinct subpopulations far more rapidly than they can be eliminated.

The authors then go on to discuss the various types of normal (stromal) tissue cells that are found within tumors. These are endothelial cells, pericytes (related to smooth muscle cells), immune inflammatory cells and cancer-associated fibroblasts.

Therapeutic Targeting

The introduction of mechanism-based targeted therapies to treat human cancers has been heralded as one of the fruits of three decades of remarkable progress of research into the mechanisms of cancer pathogenesis. We do not attempt here to enumerate the myriad therapies that are under development or have been introduced of late into the clinic. Instead, we consider how the description of hallmark principles is beginning to inform therapeutic development at present and may increasingly do so in the future.

We note that most of the hallmark-targeting cancer drugs developed to date have been deliberately directed toward specific molecular targets that are involved in one way or another in enabling particular capabilities. Such specificity of action has been considered a virtue, as it presents inhibitory activity against a target while having, in principle, relatively fewer off-target effects and thus less nonspecific toxicity. In fact, resulting clinical responses have generally been transitory, being followed by almost-inevitable relapses.

One interpretation of this history, supported by growing experimental evidence, is that each of the core hallmark capabilities is regulated by partially redundant signaling pathways. Consequently, a targeted therapeutic agent inhibiting one key pathway in a tumor may not completely shut off a hallmark capability, allowing some cancer cells to survive with residual function until they or their progeny eventually adapt to the selective pressure imposed by the therapy being applied. Such adaptation, which can be accomplished by mutation, epigenetic reprogramming, or remodeling of the stromal microenvironment, can reestablish the functional capability, permitting renewed tumor growth and clinical relapse. Given that the number of parallel signaling pathways supporting a given hallmark must be limited, it may become possible to target all of these supporting pathways therapeutically, thereby preventing the development of adaptive resistance.

In response to therapy, cancer cells may also reduce their dependence on a particular hallmark capability, becoming more dependent on another; this represents a quite different form of acquired drug resistance. This concept is exemplified by recent discoveries of unexpected responses to antiangiogenic therapies. Some have anticipated that effective inhibition of angiogenesis would render tumors dormant and might even lead to their dissolution. Instead, the clinical responses to antiangiogenic therapies have been found to be transitory.

Thus, in particular, we can envisage that selective cotargeting of multiple core and emerging hallmark capabilities and enabling characteristics (Figure 6) in mechanism-guided combinations will result in more effective and durable therapies for human cancer.

Conclusion and Future Vision

We have sought here to revisit, refine, and extend the concept of cancer hallmarks, which has provided a useful conceptual framework for understanding the complex biology of cancer. The six acquired capabilities—the hallmarks of cancer—have stood the test of time as being integral components of most forms of cancer. Further refinement of these organizing principles will surely come in the foreseeable future, continuing the remarkable conceptual progress of the last decade.

Looking ahead, we envision significant advances during the coming decade in our understanding of invasion and metastasis. Similarly, the role of aerobic glycolysis in malignant growth will be elucidated, including a resolution of whether this metabolic reprogramming is a discrete capability separable from the core hallmark of chronically sustained proliferation. We remain perplexed as to whether immune surveillance is a barrier that virtually all tumors must circumvent, or only an idiosyncrasy of an especially immunogenic subset of them; this issue too will be resolved in one way or another.

Yet other areas are currently in rapid flux.... Functionally significant epigenetic alterations seem likely to be factors not only in the cancer cells but also in the altered cells of the tumor-associated stroma. It is unclear at present whether an elucidation of these epigenetic mechanisms will materially change our overall understanding of the means by which hallmark capabilities are acquired or simply add additional detail to the regulatory circuitry that is already known to govern them.

Similarly, the discovery of hundreds of distinct regulatory microRNAs has already led to profound changes in our understanding of the genetic control mechanisms that operate in health and disease. By now dozens of microRNAs have been implicated in various tumor phenotypes, and yet these only scratch the surface of the real complexity, as the functions of hundreds of microRNAs known to be present in our cells and altered in expression in different forms of cancer remain total mysteries. Here again, we are unclear as to whether future progress will cause fundamental shifts in our understanding of the pathogenetic mechanisms of cancer or only add detail to the elaborate regulatory circuits that have already been mapped out.

Finally, the circuit diagrams of heterotypic interactions between the multiple distinct cell types that assemble and collaborate to produce different forms and progressively malignant stages of cancer are currently rudimentary. In another decade, we anticipate that the signaling circuitry describing the intercommunication between these various cells within tumors will be charted in far greater detail and clarity, eclipsing our current knowledge. And, as before, we continue to foresee cancer research as an increasingly logical science, in which myriad phenotypic complexities are manifestations of a small set of underlying organizing principles.

Advanced Reading

1. **The Hallmarks of Cancer**
   Hanahan, D., & Weinberg, R. A. (2000). The hallmarks of cancer. *cell*, 100(1), 57-70.

2. **The Hallmarks of Cancer: The Next Generation**
   Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *cell*, 144(5), 646-674.

3. **Rethinking the War on Cancer**
   Hanahan, D. (2014). Rethinking the war on cancer. *The Lancet*, 383(9916), 558-563.