REVIEW

Hallmarks of Cancer: New Dimensions

Douglas Hanahan

ABSTRACT The hallmarks of cancer conceptualization is a heuristic tool for distilling the vast complexity of cancer phenotypes and genotypes into a provisional set of underlying principles. As knowledge of cancer mechanisms has progressed, other facets of the disease have emerged as potential refinements. Herein, the prospect is raised that phenotypic plasticity and disrupted differentiation is a discrete hallmark capability, and that nonmutational epigenetic reprogramming and polymorphic microbiomes both constitute distinctive enabling characteristics that facilitate the acquisition of hallmark capabilities. Additionally, senescent cells, of varying origins, may be added to the roster of functionally important cell types in the tumor microenvironment.

Significance: Cancer is daunting in the breadth and scope of its diversity, spanning genetics, cell and tissue biology, pathology, and response to therapy. Ever more powerful experimental and computational tools and technologies are providing an avalanche of "big data" about the myriad manifestations of the diseases that cancer encompasses. The integrative concept embodied in the hallmarks of cancer is helping to distill this complexity into an increasingly logical science, and the provisional new dimensions presented in this perspective may add value to that endeavor, to more fully understand mechanisms of cancer development and malignant progression, and apply that knowledge to cancer medicine.

INTRODUCTION

The Hallmarks of Cancer were proposed as a set of functional capabilities acquired by human cells as they make their way from normalcy to neoplastic growth states, more specifically capabilities that are crucial for their ability to form malignant tumors. In these articles (1, 2), Bob Weinberg and I enumerated what we imagined were shared commonalities that unite all types of cancer cells at the level of cellular phenotype. The intent was to provide a conceptual scaffold that would make it possible to rationalize the complex phenotypes of diverse human tumor types and variants in terms of a common set of underlying cellular parameters. Initially we envisaged the complementary involvement of six distinct hallmark capabilities and later expanded this number to eight. This formulation was influenced by the recognition that human cancers develop as products of multistep processes, and that the acquisition of these functional capabilities might be mapped in some fashion to the distinguishable steps of tumor pathogenesis. Certainly, the diversity of malignant pathogenesis spanning multiple tumor types and an increasing plethora of subtypes includes various aberrations (and hence acquired capabilities and characteristics) that are the result of tissue-specific barriers necessarily circumvented during particular tumorigenesis pathways. While appreciating that such specialized mechanisms can be instrumental, we limited the hallmarks designation to parameters having broad engagement across the spectrum of human cancers.

The eight hallmarks currently comprise (Fig. 1, left) the acquired capabilities for sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing/accessing vasculature, activating invasion and metastasis, reprogramming cellular metabolism, and avoiding immune destruction. In the most recent elaboration of this concept (2), deregulating cellular metabolism and avoiding immune destruction were segregated as "emerging hallmarks," but now, eleven years later, it is evident that they, much like the original six, can be considered core hallmarks of cancer, and are included as such in the current depiction (Fig. 1, left).

As we noted at the time, these hallmark traits, on their own, fail to address the complexities of cancer pathogenesis, that is, the precise molecular and cellular mechanisms that allow evolving preneoplastic cells to develop and acquire these aberrant phenotypic capabilities in the course of tumor development and malignant progression. Accordingly, we added another concept to the discussion, portrayed as "enabling characteristics," consequences of the aberrant condition of neoplasia that provide means by which cancer cells and tumors can adopt these functional traits. As such, the

Ludwig Institute for Cancer Research – Lausanne Branch, Lausanne, Switzerland. The Swiss Institute for Experimental Cancer Research (ISREC) within the School of Life Sciences at the Swiss Federal Institute of Technology Lausanne (EPFL), Lausanne, Switzerland. The Swiss Cancer Center Leman (SCCL), Lausanne, Switzerland.

Corresponding Author: Douglas Hanahan, Agora Translational Cancer Research Center, Rue du Bugnon 25A, Lausanne CH-1011, Switzerland. Phone: 41-21-545-1119; E-mail: douglas.hanahan@epfl.ch

Cancer Discov 2022;12:31–46

doi: 10.1158/2159-8290.CD-21-1059

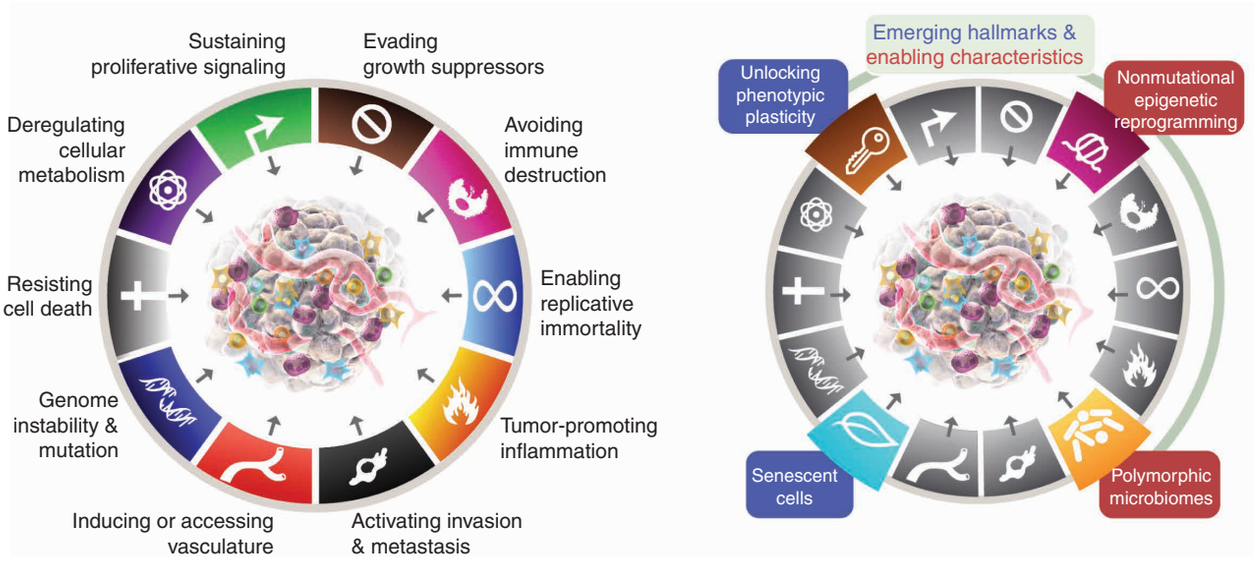

Figure 1. In essence: the Hallmarks of Cancer, circa 2022. Left, the Hallmarks of Cancer currently embody eight hallmark capabilities and two enabling characteristics. In addition to the six acquired capabilities—Hallmarks of Cancer—proposed in 2000 (1), the two provisional "emerging hallmarks" introduced in 2011 (2)—cellular energetics (now described more broadly as "reprogramming cellular metabolism") and "avoiding immune destruction"—have been sufficiently validated to be considered part of the core set. Given the growing appreciation that tumors can become sufficiently vascularized either by switching on angiogenesis or by co-opting normal tissue vessels (128), this hallmark is also more broadly defined as the capability to induce or otherwise access, principally by invasion and metastasis, vasculature that supports tumor growth. The 2011 sequel further incorporated "tumor-promoting inflammation" as a second enabling characteristic, complementing overarching "genome instability and mutation," which together were fundamentally involved in activating the eight hallmark (functional) capabilities necessary for tumor growth and progression. Right, this review incorporates additional proposed emerging hallmarks and enabling characteristics involving "unlocking phenotypic plasticity," "nonmutational epigenetic reprogramming," "polymorphic microbiomes," and "senescent cells." The hallmarks of cancer graphic has been adapted from Hanahan and Weinberg (2).

enabling characteristics reflected upon molecular and cellular mechanisms by which hallmarks are acquired rather than the aforementioned eight capabilities themselves. These two enabling processes were genome instability and tumor-promoting inflammation.

We further recognized that the tumor microenvironment (TME), herein defined to be composed of heterogeneous and interactive populations of cancer cells and cancer stem cells along with a multiplicity of recruited stromal cell types—the transformed parenchyma and the associated stroma—is now widely appreciated to play an integral role in tumorigenesis and malignant progression.

Given the continued interest in these formulations and our enduring intent to encourage ongoing discussion and refinement of the Hallmarks scheme, it is appropriate to consider a frequently posed question: are there additional features of this conceptual model that might be incorporated, respecting the need to ensure that they are broadly applicable across the spectrum of human cancers? Accordingly, I present several prospective new hallmarks and enabling characteristics, ones that might in due course become incorporated as core components of the hallmarks of cancer conceptualization. These parameters are "unlocking phenotypic plasticity," "nonmutational epigenetic reprogramming," "polymorphic microbiomes," and "senescent cells" (Fig. 1, right). Importantly, the examples presented in support of these propositions are illustrative but by no means comprehensive, as there is a growing and increasingly persuasive body of published evidence in support of each vignette.

UNLOCKING PHENOTYPIC PLASTICITY

During organogenesis, the development, determination, and organization of cells into tissues in order to assume homeostatic functions is accompanied by terminal differentiation, whereby progenitor cells—sometimes irrevocably—stop growing upon culmination of these processes. As such, the end result of cellular differentiation is in most cases antiproliferative and constitutes a clear barrier to the continuing proliferation that is necessary for neoplasia. There is increasing evidence that unlocking the normally restricted capability for phenotypic plasticity in order to evade or escape from the state of terminal differentiation is a critical component of cancer pathogenesis (3). This plasticity can operate in several manifestations (Fig. 2). Thus, nascent cancer cells originating from a normal cell that had advanced down a pathway approaching or assuming a fully differentiated state may reverse their course by dedifferentiating back to progenitor-like cell states. Conversely, neoplastic cells arising from a progenitor cell that is destined to follow a pathway leading to end-stage differentiation may short-circuit the process, maintaining the expanding cancer cells in a partially differentiated, progenitor-like state. Alternatively, transdifferentiation may operate, in which cells that were initially committed into one differentiation pathway switch to an entirely different developmental program, thereby acquiring tissue-specific traits that were not preordained by their normal cells-of-origin. The following examples support the argument that differing forms of cellular plasticity,

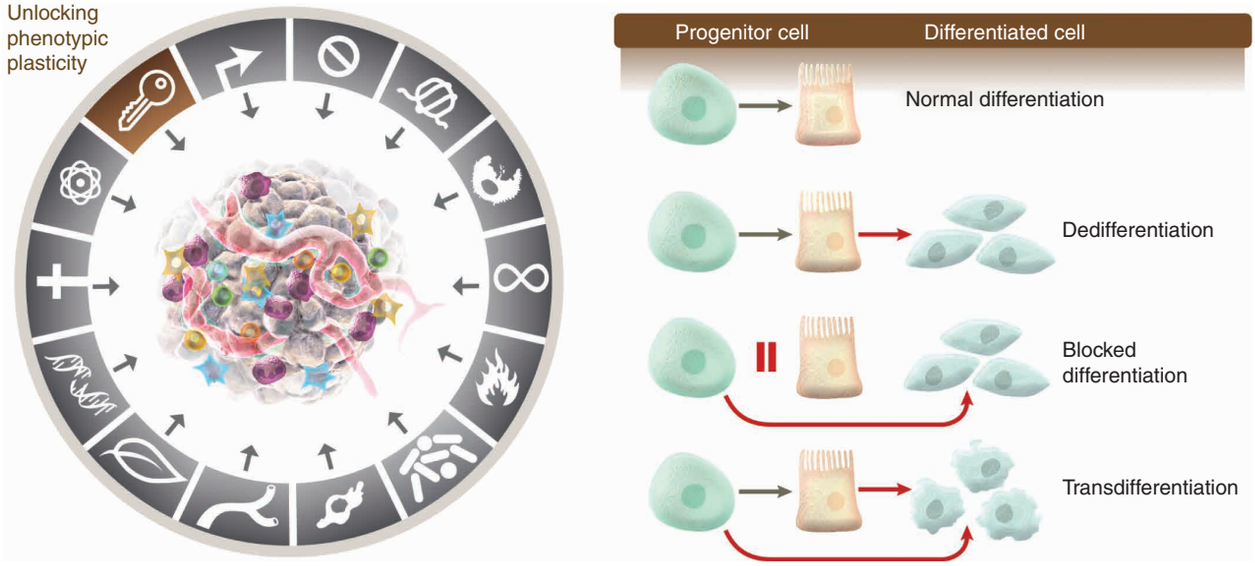

Figure 2. Unlocking phenotypic plasticity. Left, phenotypic plasticity is arguably an acquired hallmark capability that enables various disruptions of cellular differentiation, including (i) dedifferentiation from mature to progenitor states, (ii) blocked (terminal) differentiation from progenitor cell states, and (iii) transdifferentiation into different cell lineages. Right, depicted are three prominent modes of disrupted differentiation integral to cancer pathogenesis. By variously corrupting the normal differentiation of progenitor cells into mature cells in developmental lineages, tumorigenesis and malignant progression arising from cells of origin in such pathways is facilitated. The hallmarks of cancer graphic has been adapted from Hanahan and Weinberg (2).

when taken together, constitute a functionally distinct hallmark capability.

Dedifferentiation

Colon carcinogenesis exemplifies disrupted differentiation, in that there is a teleological necessity for incipient cancer cells to escape from the conveyer belt of terminal differentiation and exfoliation, which could in principle occur via dedifferentiation of not yet irrevocably terminally differentiated colonic epithelial cells, or via blocked differentiation of progenitor/stem cells in the crypts that spawn these differentiating cells. Both differentiated cells and stem cells have been implicated as cell-of-origin for colon cancer (4–6). Two developmental transcription factors (TF), the homeobox protein HOXA5 and SMAD4, the latter involved in BMP signal transmission, are highly expressed in differentiating colonic epithelial cells, and typically lost in advanced colon carcinomas, which characteristically express markers of stem and progenitor cells. Functional perturbations in mouse models have shown that forced expression of HOXA5 in colon cancer cells restores differentiation markers, suppresses stem cell phenotypes, and impairs invasion and metastasis, providing a rationale for its characteristic downregulation (7, 8). SMAD4, by contrast, both enforces differentiation and thereby suppresses proliferation driven by oncogenic WNT signaling, revealed by the engineered loss of SMAD4 expression, providing an explanation for its loss of expression so as to enable dedifferentiation and, subsequently, WNT-driven hyperproliferation (5). Notably, the loss of both of these "differentiation suppressors" with consequent dedifferentiation is associated with acquisition of other hallmark capabilities, as are other hallmark-inducing regulators, which complicates the strict definition of this provisional hallmark as separable and independent.

Another line of evidence involves suppressed expression of the *MITF* master regulator of melanocyte differentiation, which is evidently involved in the genesis of aggressive forms of malignant melanoma. Loss of this developmental TF is associated with the reactivation of neural crest progenitor genes and the downregulation of genes that characterize fully differentiated melanocytes. The reappearance of the neural crest genes indicates that these cells revert to the progenitor state from which melanocytes arise developmentally. Moreover, a lineage tracing study of *BRAF*-induced melanomas established mature pigmented melanocytes as the cells of origin, which undergo dedifferentiation during the course of tumorigenesis (9). Of note, the mutant *BRAF* oncogene, which is found in more than half of cutaneous melanomas, induces hyperproliferation that precedes and hence is mechanistically separable from the subsequent dedifferentiation arising from downregulation of *MITF*. Another study functionally implicated upregulation of the developmental TF *ATF2*, whose characteristic expression in mouse and human melanomas indirectly suppresses *MITF1*, concomitant with malignant progression of the consequently dedifferentiated melanoma cells (10). Conversely, expression in melanomas of mutant forms of *ATF2* that fail to repress *MITF* results in well-differentiated melanomas (11).

Additionally, a recent study (12) has associated lineage dedifferentiation with malignant progression from pancreatic islet cell neoplasias into metastasis-prone carcinomas; these neuroendocrine cells and derivative tumors arise from a developmental lineage that is distinct from the one generating the far larger number of adjacent cells that form the exocrine and pancreas and the ductal adenocarcinomas

that arise therefrom. Notably, the multistep differentiation pathway of islet progenitor cells into mature β cells has been thoroughly characterized (13). Comparative transcriptome profiling reveals that adenoma-like islet tumors are most similar to immature but differentiated insulin-producing β cells, whereas the invasive carcinomas are most similar to embryonic islet cell precursors. The progression toward poorly differentiated carcinomas involves a first step of dedifferentiation that does not initially involve increased proliferation or reduced apoptosis when compared with the well-differentiated adenomas, both of which rather occur later. Thus, the discrete step of dedifferentiation is not driven by observable alterations in the hallmark traits of sustained proliferation and resistance to apoptosis. Rather, upregulation of a miRNA previously implicated in specifying the islet progenitor state, one that is downregulated during terminal differentiation of β cells, has been shown to orchestrate the observed dedifferentiation occurring during malignant progression (12).

Blocked Differentiation

While the above examples illustrate how suppression of differentiation factor expression can facilitate tumorigenesis by enabling more well-differentiated cells to dedifferentiate into progenitors, in other cases incompletely differentiated progenitor cells can suffer regulatory changes that actively block their continued advance into fully differentiated, typically nonproliferative states.

Acute promyelocytic leukemia (APL) has long been documented to result from a chromosomal translocation that fuses the *PML* locus with the gene encoding the retinoic acid α nuclear receptor (RARα). Myeloid progenitor cells bearing such translocations are evidently unable to continue their usual terminal differentiation into granulocytes, resulting in cells trapped in a proliferative, promyelocytic progenitor stage (14). Proof-of-concept of this scheme comes from treating cultured APL cells, mouse models of this disease, as well as afflicted patients, with retinoic acid, the ligand of RARα; this therapeutic treatment causes the neoplastic APL cells to differentiate into ostensibly mature nonproliferating granulocytes, short-circuiting their continuing proliferative expansion (14–16).

A variation on this theme involves another form of acute myeloid leukemia, this one carrying the t(8;21) translocation, which produces the AML1–ETO fusion protein. This protein can, on its own, transform myeloid progenitors, at least in part by blocking their differentiation. Therapeutic intervention in mouse models and in patients with a pharmacologic inhibitor of a chromatin-modifying histone deacetylase (HDAC) causes the myeloid leukemia cells to recommence their differentiation into cells with a more mature myeloid cell morphology. Concomitant with this response is a reduction in proliferative capacity, thereby impairing the progression of this leukemia (17, 18).

A third example, in melanoma, involves a developmental TF, *SOX10*, which is normally downregulated during melanocyte differentiation. Gain- and loss-of-function studies in a zebrafish model of *BRAF*-induced melanoma have demonstrated that aberrantly maintained expression of *SOX10* blocks differentiation of neural progenitor cells into melanocytes, enabling *BRAF*-driven melanomas to form (19).

Other examples of differentiation modulators involve the metabolite alpha-ketoglutarate (αKG), a necessary cofactor for a number of chromatin-modifying enzymes, which is demonstrably involved in stimulating certain differentiated cell states. In pancreas cancer, the tumor suppressor p53 stimulates the production of αKG and maintenance of a more well-differentiated cell state, whereas prototypical loss of p53 function results in reductions in αKG levels and consequent dedifferentiation associated with malignant progression (20). In one form of liver cancer, mutation of an isocitrate dehydrogenase gene (*IDH1/2*) results in the production not of differentiation-inducing αKG but rather a related "oncometabolite," D-2-hydroxygluterate (D2HG), which has been shown to block hepatocyte differentiation from liver progenitor cells by D2HG-mediated repression of a master regulator of hepatocyte differentiation and quiescence, HNF4a. The D2HG-mediated suppression of HNF4a function elicits a proliferative expansion of the hepatocyte progenitor cells in the liver, which become susceptible to oncogenic transformation upon subsequent mutational activation of the *KRAS* oncogene that drives malignant progression to liver cholangiocarcinoma (21). Mutant IDH1/2 and their oncometabolite D2HG are also operative in a variety of myeloid and other solid tumor types, where D2HG inhibits αKG-dependent dioxygenases necessary for histone and DNA methylation events that mediate alterations in chromatin structure during developmental lineage differentiation, thereby freezing incipient cancer cells in a progenitor state (22, 23).

An additional, related concept is "circumvented differentiation," wherein partially or undifferentiated progenitor/stem cells exit the cell cycle and become dormant, residing in protective niches, with the potential to reinitiate proliferative expansion (24), albeit still with the selective pressure to disrupt their programmed differentiation in one way or another.

Transdifferentiation

The concept of transdifferentiation has long been recognized by pathologists in the form of tissue metaplasia, wherein cells of a particular differentiated phenotype markedly change their morphology to become clearly recognizable as elements of another tissue, of which one prominent example is Barrett's esophagus, where chronic inflammation of the stratified squamous epithelium of the esophagus induces transdifferentiation into a simple columnar epithelium that is characteristic of the intestine, thereby facilitating the subsequent development of adenocarcinomas, and not the squamous cell carcinomas that would be anticipated to arise from this squamous epithelium (3). Now, molecular determinants are revealing mechanisms of transdifferentiation in various cancers, both for cases where gross tissue metaplasia is evident and for others where it is rather more subtle, as the following examples illustrate.

One illuminating case for transdifferentiation as a discrete event in tumorigenesis involves pancreatic ductal adenocarcinoma (PDAC), wherein one of the implicated cells of origin, the pancreatic acinar cell, can become transdifferentiated into a ductal cell phenotype during the initiation of neoplastic development. Two TFs—PTF1a and MIST1—govern, via their expression in the context of self-sustaining, "feed-forward" regulatory loops, the specification and

maintenance of the differentiated pancreatic acinar cell state (25). Both of these TFs are frequently downregulated during neoplastic development and malignant progression of human and mouse PDAC. Functional genetic studies in mice and cultured human PDAC cells have demonstrated that experimentally forced expression of *PTF1a* impairs *KRAS*-induced transdifferentiation and proliferation, and can also force the redifferentiation of already neoplastic cells into a quiescent acinar cell phenotype (26). Conversely, suppression of *PTF1a* expression elicits acinar-to-ductal metaplasia, namely transdifferentiation, and thereby sensitizes the duct-like cells to oncogenic *KRAS* transformation, accelerating subsequent development of invasive PDAC (27). Similarly, forced expression of *MIST1* in *KRAS*-expressing pancreas also blocks transdifferentiation and impairs the initiation of pancreatic tumorigenesis otherwise facilitated by the formation of premalignant duct-like (PanIN) lesions, whereas genetic deletion of *MIST1* enhances their formation and the initiation of *KRAS*-driven neoplastic progression (28). Loss of either *PTF1* or *MIST1* expression during tumorigenesis is associated with elevated expression of another developmental regulatory TF, *SOX9*, which is normally operative in the specification of ductal cells (27, 28). Forced upregulation of *SOX9*, obviating the need to downregulate *PTF1a* and *MIST1*, has also been shown to stimulate transdifferentiation of acinar cells into a ductal cell phenotype that is sensitive to *KRAS*-induced neoplasia (29), implicating *SOX9* as a key functional effector of their downregulation in the genesis of human PDAC. Thus, three TFs that regulate pancreatic differentiation can be variously altered to induce a transdifferentiated state that facilitates—in the context of mutational activation of *KRAS*—oncogenic transformation and the initiation of tumorigenesis and malignant progression.

Additional members of the SOX family of chromatin-associated regulatory factors are on the one hand broadly associated both with cell fate specification and lineage switching in development (30), and on the other with multiple tumor-associated phenotypes (31). Another salient example of SOX-mediated transdifferentiation involves a mechanism of therapeutic resistance in prostate carcinomas. In this case, loss of the RB and p53 tumor suppressors—whose absence is characteristic of neuroendocrine tumors—in response to antiandrogen therapy is necessary but not sufficient for the frequently observed conversion of well-differentiated prostate cancer cells into carcinoma cells that have entered a differentiation lineage with molecular and histologic features of neuroendocrine cells, which notably do not express the androgen receptor. In addition to loss of RB and p53, the acquired resistance to antiandrogen therapy requires upregulated expression of the *SOX2* developmental regulatory gene, which is demonstrably instrumental in inducing transdifferentiation of the therapy-responsive adenocarcinoma cells into derivatives that reside in a neuroendocrine cell state that is refractory to the therapy (32).

A third example also reveals transdifferentiation as a strategy employed by carcinoma cells to avoid elimination by a lineage-specific therapy, in this case involving basal cell carcinomas (BCC) of the skin treated with a pharmacologic inhibitor of the Hedgehog-Smoothened (HH/SMO) oncogenic signaling pathway known to drive the neoplastic growth of these cells (33). Drug-resistant cancer cells switch, via broad epigenetic shifts in specific chromatin domains and the altered accessibility of two superenhancers, to a developmentally related but distinct cell type. The newly gained phenotypic state of the BCC cells enables them to sustain expression of the *WNT* oncogenic signaling pathway, which in turn imparts independence from the drug-suppressed *HH/SMO* signaling pathway (34). As might be anticipated from this transdifferentiation, the transcriptome of the cancer cells shifts from a gene signature reflecting the implicated cell-of-origin of BCCs, namely the stem cells of hair follicle bulge, to one indicative of the basal stem cells that populate the interfollicular epidermis. Such transdifferentiation to enable drug resistance is being increasingly documented in different forms of cancer (35).

Developmental lineage plasticity also appears to be prevalent among the major subtypes of lung carcinomas, that is, neuroendocrine carcinomas [small-cell lung cancer (SCLC)] and adenocarcinomas + squamous cell carcinomas [collectively non–small cell lung cancer (NSCLC)]. Single-cell RNA sequencing has revealed remarkably dynamic and heterogeneous interconversion among these subtypes as well as distinct variations thereof during the stages in lung tumorigenesis, subsequent malignant progression, and responses to therapy (36–38). Thus, rather than the simple conceptualization of a pure clonal switch from one lineage into another, these studies paint a much more complex picture, of dynamically interconverting subpopulations of cancer cells exhibiting characteristics of multiple developmental lineages and stages of differentiation, a sobering realization in regard to lineage-based therapeutic targeting of human lung cancer. Regulatory determinants of this dynamic phenotypic plasticity are beginning to be identified (37, 39, 40).

Synopsis

The three classes of mechanism described above highlight selective regulators of cellular plasticity that are separable—at least in part—from core oncogenic drivers and other hallmark capabilities. Beyond these examples lies a considerable body of evidence associating many forms of cancer with disrupted differentiation concomitant with the acquisition of transcriptome signatures and other phenotypes—for example, histologic morphology—associated with progenitor or stem cell stages observed in the corresponding normal tissue-of-origin or in other more distantly related cell types and lineages (41–43). As such, these three subclasses of phenotypic plasticity—dedifferentiation of mature cells back to progenitor states, blocked differentiation to freeze developing cells in progenitor/stem cell states, and transdifferentiation to alternative cell lineages—appear to be operative in multiple cancer types during primary tumor formation, malignant progression, and/or response to therapy. There are, however, two conceptual considerations. First, dedifferentiation and blocked differentiation are likely intertwined, being indistinguishable in many tumor types where the cell-of-origin—differentiated cell or progenitor/stem cell—is either unknown or alternatively involved. Second, the acquisition or maintenance of progenitor cell phenotypes and loss of differentiated

Figure 3. Nonmutational epigenetic reprogramming. Much as during embryogenesis and tissue differentiation and homeostasis, growing evidence makes the case that instrumental gene-regulatory circuits and networks in tumors can be governed by a plethora of corrupted and co-opted mechanisms that are independent from genome instability and gene mutation. The hallmarks of cancer graphic has been adapted from Hanahan and Weinberg (2).

features is in most cases an imprecise reflection of the normal developmental stage, being immersed in a milieu of other hallmark-enabling changes in the cancer cell that are not present in naturally developing cells. In addition, yet another form of phenotypic plasticity involves cell senescence, discussed more generally below, wherein cancer cells induced to undergo ostensibly irreversible senescence are instead able to escape and resume proliferative expansion (44). Finally, as with other hallmark capabilities, cellular plasticity is not a novel invention or aberration of cancer cells, but rather the corruption of latent but activatable capabilities that various normal cells use to support homeostasis, repair, and regeneration (45).

Collectively, these illustrative examples encourage consideration of the proposition that unlocking cellular plasticity to enable various forms of disrupted differentiation constitutes a discrete hallmark capability, distinguishable in regulation and cellular phenotype from the well-validated core hallmarks of cancer (Fig. 2).

NONMUTATIONAL EPIGENETIC REPROGRAMMING

The enabling characteristic of genome (DNA) instability and mutation is a fundamental component of cancer formation and pathogenesis. At present, multiple international consortia are cataloging mutations across the genome of human cancer cells, doing so in virtually every type of human cancer, at different stages of malignant progression, including metastatic lesions, and during the development of adaptive resistance to therapy. One result is the now widespread appreciation that mutations in genes that organize, modulate, and maintain chromatin architecture, and thereby globally regulate gene expression, are increasingly detected and functionally associated with cancer hallmarks (46–48).

There is, in addition, a case to be made for another apparently independent mode of genome reprogramming that involves purely epigenetically regulated changes in gene expression, one that might be termed "nonmutational epigenetic reprogramming" (Fig. 3). Indeed, the proposition of mutation-less cancer evolution and purely epigenetic programming of hallmark cancer phenotypes was raised almost a decade ago (49) and is increasingly discussed (46, 50–52).

The concept of nonmutational epigenetic regulation of gene expression is of course well established as the central mechanism mediating embryonic development, differentiation, and organogenesis (53–55). In the adult, for example, long-term memory involves changes in gene and histone modification, in chromatin structure, and in the triggering of gene expression switches that are stably maintained over time by positive and negative feedback loops (56, 57). Growing evidence supports the proposition that analogous epigenetic alterations can contribute to the acquisition of hallmark capabilities during tumor development and malignant progression. A few examples are presented below in support of this hypothesis.

Microenvironmental Mechanisms of Epigenetic Reprogramming

If not solely by consequence of oncogenic mutations, how then is the cancer cell genome reprogrammed? A growing body of evidence indicates that the aberrant physical properties of the tumor microenvironment can cause broad changes in the epigenome, from which changes beneficial to the phenotypic selection of hallmark capabilities can result in clonal outgrowth of cancer cells with enhanced fitness for proliferative expansion. One common characteristic of tumors (or regions within tumors) is hypoxia, consequent to insufficient vascularization. Hypoxia, for example, reduces the activity of the TET demethylases, resulting in substantive changes in the methylome, in particular hypermethylation (58). Insufficient vascularization likely also limits the bioavailability of critical blood-borne nutrients, and nutrient deprivation has been shown for example to alter translational control and consequently enhance the malignant phenotype of breast cancer cells (59).

A persuasive example of hypoxia-mediated epigenetic regulation involves a form of invariably lethal pediatric ependymoma. Like many embryonic and pediatric tumors, this form lacks recurrent mutations, in particular a dearth of driver mutations in oncogenes and tumor suppressors. Rather, the aberrant growth of these cancer cells is demonstrably governed by a gene regulatory program induced by hypoxia (60, 61). Notably, the putative cell-of-origin of this cancer resides in a hypoxic compartment, likely sensitizing cells resident therein to the initiation of tumorigenesis by as yet unknown cofactors.

Another persuasive line of evidence for microenvironmentally mediated epigenetic regulation involves the invasive growth capability of cancer cells. A classic example involves the reversible induction of invasiveness of cancer cells at the margins of many solid tumors, orchestrated by the developmental regulatory program known as the epithelial-to-mesenchymal transition (EMT; refs. 62–64). Notably, a master regulator of the EMT, *ZEB1*, has been recently shown to induce expression of a histone methyltransferase, *SETD1B*, that in turn sustains *ZEB1* expression in a positive feedback loop that maintains the (invasive) EMT regulatory state (65). A previous study similarly documented that induction of EMT by upregulated expression of a related TF, *SNAIL1*, caused marked alterations in the chromatin landscape consequent to induction of a number of chromatin modifiers, whose activity was demonstrably necessary for the maintenance of the phenotypic state (66). Furthermore, a roster of conditions and factors to which cancer cells at the margins of tumors are exposed, including hypoxia and cytokines secreted by stromal cells, can evidently induce the EMT and in turn invasiveness (67, 68).

A distinctive example of microenvironmental programming of invasiveness, ostensibly unrelated to the EMT program, involves autocrine activation, in pancreas cancer cells and others, via interstitial pressure–driven fluid flow, of a neuronal signaling circuit involving secreted glutamate and its receptor NMDAR (69, 70). Notably, the prototypical stiffness of many solid tumors, embodied in extensive alterations to the extracellular matrix (ECM) that envelop the cells within, has broad effects on the invasive and other phenotypic characteristics of cancer cells. Compared with the normal tissue ECM from which tumors originate, the tumor ECM is typically characterized by increased cross-linking and density, enzymatic modifications, and altered molecular composition, which collectively orchestrate—in part via integrin receptors for ECM motifs—stiffness-induced signaling and gene-expression networks that elicit invasiveness and other hallmark characteristics (71).

In addition to such regulatory mechanisms endowed by the physical tumor microenvironment, paracrine signaling involving soluble factors released into the extracellular milieu by the various cell types populating solid tumors can also contribute to the induction of several morphologically distinct invasive growth programs (72), only one of which—dubbed "mesenchymal"—seems to involve the aforementioned EMT epigenetic regulatory mechanism.

Epigenetic Regulatory Heterogeneity

A growing knowledge base is heightening appreciation of the importance of intratumoral heterogeneity in generating the phenotypic diversity where the fittest cells for proliferative expansion and invasion outgrow their brethren and hence are selected for malignant progression. Certainly, one facet of this phenotypic heterogeneity is founded in chronic or episodic genomic instability and consequent genetic heterogeneity in the cells populating a tumor. In addition, it is increasingly evident that there can be non–mutationally based epigenetic heterogeneity. A salient example involves the linker histone H1.0, which is dynamically expressed and repressed in subpopulations of cancer cells within a number of tumor types, with consequent sequestration or accessibility, respectively, of megabase-sized domains, including ones conveying hallmark capabilities (73). Notably, the population of cancer cells with repressed H1.0 were found to have stem-like characteristics, enhanced tumor-initiating capability, and an association with poor prognosis in patients.

Another example of epigenetically regulated plasticity has been described in human oral squamous cell carcinomas (SCC), wherein cancer cells at the invasive margins adopt a partial EMT (p-EMT) state lacking the aforementioned mesenchymal TFs but expressing other EMT-defining genes that are not expressed in the central core of the tumors (74). The p-EMT cells evidently do not represent a clonal compartmentalization of mutationally altered cells: cultures of primary tumor-derived cancer cells contain dynamic mixtures of both p-EMT^{hi} and p-EMT^{lo} cells, and when p-EMT$^{hi/lo}$ cells were FACS-purified and cultured, both reverted to mixed populations of p-EMT^{hi} and p-EMT^{lo} cells within 4 days. Moreover, although paracrine signals from the adjacent stroma could be envisaged as deterministic for the p-EMT^{hi} state, the stable presence and regeneration of the two epigenetic states in culture argues for a cancer cell–intrinsic mechanism. Notably, this conclusion is supported by analysis of 198 cell lines representing 22 cancer types, including SCC, wherein 12 stably heterogeneous epigenetic states (including the p-EMT in SCC) were variously detected in the cell line models as well as their cognate primary tumors (75). Again, the heterogeneous phenotypic states could not be linked to detectable genetic differences, and in several cases FACS-sorted cells of

a particular state were shown to dynamically reequilibrate upon culture, recapitulating a stable balance among the heterogeneous states seen in the original cell lines.

Additionally, technologies for genome-wide profiling of diverse attributes—beyond DNA sequence and its mutational variation—are illuminating influential elements of the cancer cell genome's annotation and organization that correlate with patient prognosis, and increasingly with hallmark capabilities (76–78). Epigenomic heterogeneity is being revealed by increasingly powerful technologies for profiling genome-wide DNA methylation (79, 80), histone modification (81), chromatin accessibility (82), and posttranscriptional modification and translation of RNA (83, 84). A challenge in regard to the postulate being considered herein will be to ascertain which epigenomic modifications in particular cancer types (i) have regulatory significance and (ii) are representative of purely nonmutational reprogramming, as opposed to being mutation-driven and thus explainable by genome instability.

Epigenetic Regulation of the Stromal Cell Types Populating the Tumor Microenvironment

In general, the accessory cells in the tumor microenvironment that functionally contribute to the acquisition of hallmark capabilities are not thought to suffer genetic instability and mutational reprogramming to enhance their tumor-promoting activities; rather it is inferred that these cells—cancer-associated fibroblasts, innate immune cells, and endothelial cells and pericytes of the tumor vasculature—are epigenetically reprogrammed upon their recruitment by soluble and physical factors that define the solid tumor microenvironment (2, 85). It can be anticipated the multi-omic profiling technologies currently being applied to cancer cells will increasingly be used to interrogate the accessory (stromal) cells in tumors to elucidate how normal cells are corrupted to functionally support tumor development and progression. For example, a recent study (86) suggests that such reprogramming can involve modifications of the epigenome in addition to the inductive interchange of cytokines, chemokines, and growth factors that alter intracellular signaling networks in all of these cell types: when mouse models of metastasis to lung were treated with a combination of a DNA methyltransferase inhibitor (5-azacytidine) and an inhibitor of histone modification (an HDAC), the infiltrating myeloid cells were found to have switched from an immature (tumor-promoting) progenitor state into cells resembling mature interstitial (tumor-antagonizing) macrophages, which, in contrast to their counterparts in untreated tumors, were incapable of supporting the hallmark capabilities necessary for efficient metastatic colonization (86). It can be envisaged that multi-omic profiling and pharmacologic perturbation will serve to elucidate the reprogrammed epigenetic state in such myeloid cells as well as other hallmark-enabling accessory cell types populating tumor microenvironments.

Synopsis

Collectively, these illustrative snapshots support the proposition that nonmutational epigenetic reprograming will come to be accepted as a bona fide enabling characteristic that serves to facilitate the acquisition of hallmark capabilities (Fig. 3), distinct from that of genomic DNA instability and mutation. Notably, it can be anticipated that nonmutational epigenetic reprogramming will prove to be integrally involved in enabling the provisional new hallmark capability of phenotypic plasticity discussed above, in particular being a driving force in the dynamic transcriptomic heterogeneity that is increasingly well documented in cancer cells populating malignant TMEs. The advance of single cell multi-omic profiling technologies is envisaged to illuminate the respective contributions of and interplay between mutation-driven versus nonmutational epigenetic regulation to the evolution of tumors during malignant progression and metastasis.

POLYMORPHIC MICROBIOMES

An expansive frontier in biomedicine is unfolding via illumination of the diversity and variability of the plethora of microorganisms, collectively termed the microbiota, that symbiotically associate with the barrier tissues of the body exposed to the external environment—the epidermis and the internal mucosa, in particular the gastrointestinal tract, as well as the lung, the breast, and the urogenital system. There is growing appreciation that the ecosystems created by resident bacteria and fungi—the microbiomes—have profound impact on health and disease (87), a realization fueled by the capability to audit the populations of microbial species using next-generation sequencing and bioinformatic technologies. For cancer, the evidence is increasingly compelling that polymorphic variability in the microbiomes between individuals in a population can have a profound impact on cancer phenotypes (88, 89). Association studies in human and experimental manipulation in mouse models of cancer are revealing particular microorganisms, principally but not exclusively bacteria, which can have either protective or deleterious effects on cancer development, malignant progression, and response to therapy. So too can the global complexity and constitution of a tissue microbiome at large. Indeed, while the gut microbiome has been the pioneer of this new frontier, multiple tissues and organs have associated microbiomes, which have distinctive characteristics in regard to population dynamics and diversity of microbial species and subspecies. This growing appreciation of the importance of polymorphically variable microbiomes in health and disease posits the question: is the microbiome a discrete enabling characteristic that broadly affects, both positively and negatively, the acquisition of hallmark capabilities for cancer? I reflect on this possibility below, illustrating evidence for some of the prominent tissue microbiomes implicated in cancer hallmarks (Fig. 4), beginning with the most prominent and evidently impactful microbiome, that of the intestinal tract.

Diverse Modulatory Effects of the Gut Microbiome

It has long been recognized that the gut microbiome is fundamentally important for the function of the large intestine (colon) in degrading and importing nutrients into the body as part of metabolic homeostasis, and that distortions in the microbial populations—dysbiosis—in the colon can cause a spectrum of physiologic maladies (87). Among these has been the suspicion that the susceptibility, development, and

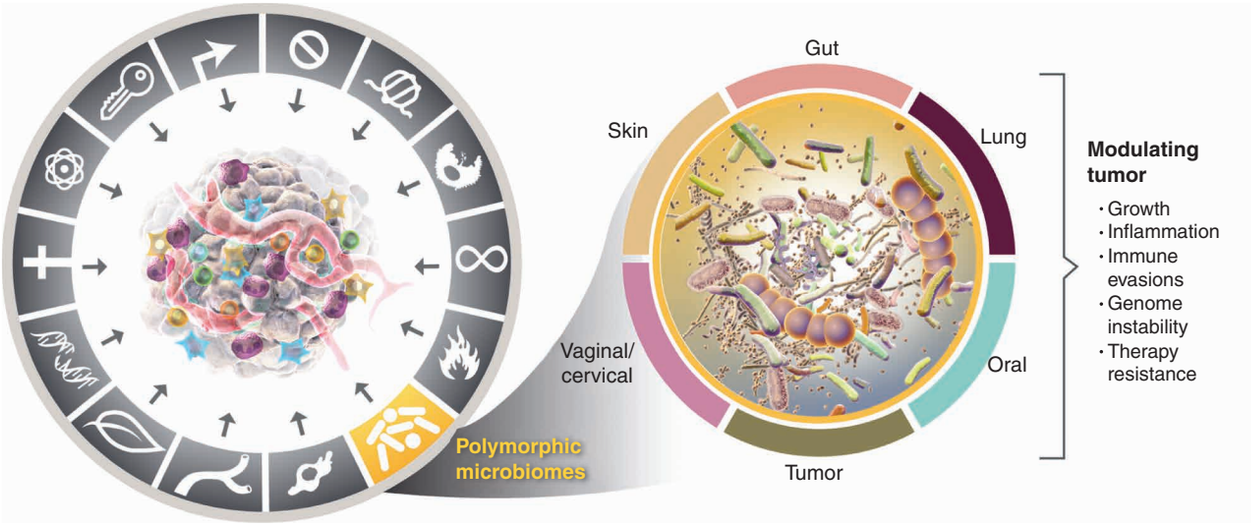

Figure 4. Polymorphic microbiomes. Left, while intersecting with the enabling characteristics of tumor-promoting inflammation and genomic instability and mutation, there is growing reason to conclude that polymorphic microbiomes in one individual versus another, being resident in the colon, other mucosa and connected organs, or in tumors themselves, can diversely influence—by either inducing or inhibiting—many of the hallmark capabilities, and thus are potentially an instrumental and quasi-independent variable in the puzzle of how cancers develop, progress, and respond to therapy. Right, multiple tissue microbiomes are implicated in modulating tumor phenotypes. In addition to the widely studied gut microbiome, other distinctive tissue microbiomes, as well as the tumor microbiome, are implicated in modulating the acquisition—both positively and negatively—of the illustrated hallmark capabilities in certain tumor types. The hallmarks of cancer graphic has been adapted from Hanahan and Weinberg (2).

pathogenesis of colon cancer is influenced by the gut microbiome. In recent years, persuasive functional studies, involving fecal transplants from colon tumor–bearing patients and mice into recipient mice predisposed to develop colon cancer has established a principle: there are both cancer-protective and tumor-promoting microbiomes, involving particular bacterial species, which can modulate the incidence and pathogenesis of colon tumors (90).

The mechanisms by which microbiota impart these modulatory roles are still being elucidated, but two general effects are increasingly well established for tumor-promoting microbiomes and in some cases for specific tumor-promoting bacterial species. The first effect is mutagenesis of the colonic epithelium, consequent to the production of bacterial toxins and other molecules that either damage DNA directly, or disrupt the systems that maintain genomic integrity, or stress cells in other ways that indirectly impair the fidelity of DNA replication and repair. A case in point is *E. coli* carrying the *PKS* locus, which demonstrably mutagenizes the human genome and is implicated in conveying hallmark-enabling mutations (91).

Additionally, bacteria have been reported to bind to the surface of colonic epithelial cells and produce ligand mimetics that stimulate epithelial proliferation, contributing in neoplastic cells to the hallmark capability for proliferative signaling (88). Another mechanism by which specific bacterial species promote tumorigenesis involves butyrate-producing bacteria, whose abundance is elevated in patients with colorectal cancer (92). The production of the metabolite butyrate has complex physiologic effects, including the induction of senescent epithelial and fibroblastic cells. A mouse model of colon carcinogenesis populated with butyrate-producing bacteria developed more tumors than mice lacking such bacteria; the connection between butyrate-induced senescence and enhanced colon tumorigenesis was demonstrated by the use of a senolytic drug that kills senescent cells, which impaired tumor growth (92). In addition, bacterial-produced butyrate has pleiotropic and paradoxical effects on differentiated cells versus undifferentiated (stem) cells in the colonic epithelium in conditions where the intestinal barrier is disrupted (dysbiosis) and the bacteria are invasive, affecting, for example, cellular energetics and metabolism, histone modification, cell-cycle progression, and (tumor-promoting) innate immune inflammation that is immunosuppressive of adaptive immune responses (93).

Indeed, a broad effect of polymorphic microbiomes involves the modulation of the adaptive and innate immune systems via multifarious routes, including the production by bacteria of "immunomodulatory" factors that activate damage sensors on epithelial or resident immune cells, resulting in the expression of a diverse repertoire of chemokines and cytokines that can sculpt the abundance and characteristics of immune cells populating the colonic epithelia and its underlying stroma and draining lymph nodes. In addition, certain bacteria can breach both the protective biofilm and the mucus lining the colonic epithelia and proceed to disrupt the epithelial cell–cell tight junctions that collectively maintain the integrity of the physical barrier that normally compartmentalizes the intestinal microbiome. Upon invading the stroma, bacteria can trigger both innate and adaptive immune responses, eliciting secretion of a repertoire of cytokines and chemokines. One manifestation can be the creation of tumor-promoting or tumor-antagonizing immune microenvironments, consequently protecting against or facilitating tumorigenesis and malignant progression. Concordantly, the modulation by distinctive microbiomes in

individual patients of the intertwined parameters of (i) eliciting (innate) tumor promoting inflammation and (ii) escaping (adaptive) immune destruction can be associated not only with prognosis, but also with responsiveness or resistance to immunotherapies involving immune checkpoint inhibitors and other therapeutic modalities (89, 94–96). Provisional proof-of-concept has come from recent studies demonstrating restored efficacy to immunotherapy following transplants of fecal microbiota from therapy-responsive patients into patients with melanoma who had progressed during prior treatment with immune checkpoint blockade (97, 98).

An ongoing mystery has involved the molecular mechanisms by which particular and variable constituents of the gut microbiome systemically modulate the activity of the adaptive immune system, either enhancing antitumoral immune responses evoked by immune checkpoint blockade, or rather eliciting systemic or local (intratumoral) immunosuppression. A recent study has shed some light: certain strains of *Enterococcus* (and other bacteria) express a peptidoglycan hydrolyase called SagA that releases mucopeptides from the bacterial wall, which can then circulate systemically and activate the NOD2 pattern receptor, which in turn can enhance T-cell responses and the efficacy of checkpoint immunotherapy (99). Other immunoregulatory molecules produced by specific bacterial subspecies are being identified and functionally evaluated, including bacteria-produced inosine, a rate-limiting metabolite for T-cell activity (100). These examples and others are beginning to chart the molecular mechanisms by which polymorphic microbiomes are indirectly and systemically modulating tumor immunobiology, above and beyond immune responses consequent to direct physical interactions of bacteria with the immune system (101, 102).

Beyond the causal links to colon cancer and melanoma, the gut microbiome's demonstrable ability to elicit the expression of immunomodulatory chemokines and cytokines that enter the systemic circulation is evidently also capable of affecting cancer pathogenesis and response to therapy in other organs of the body (94, 95). An illuminating example involves the development of cholangiocarcinomas in the liver: gut dysbiosis allows the entry and transport of bacteria and bacterial products through the portal vein to the liver, where TLR4 expressed on hepatocytes is triggered to induce expression of the chemokine CXCL1, which recruits CXCR2-expressing granulocytic myeloid cells (gMDSC) that serve to suppress natural killer cells so as to evade immune destruction (103), and likely convey other hallmark capabilities (85). As such, the gut microbiome is unambiguously implicated as an enabling characteristic that can alternatively facilitate or protect against multiple forms of cancer.

Beyond the Gut: Implicating Distinctive Microbiomes in Other Barrier Tissues

Virtually all tissues and organs exposed, directly or indirectly, to the outside environment are also repositories for commensal microorganisms (104). Unlike the intestine, where the symbiotic role of the microbiome in metabolism is well recognized, the normal and pathogenic roles of resident microbiota in these diverse locations is still emerging. There are evidently organ/tissue-specific differences in the constitution of the associated microbiomes in homeostasis, aging, and cancer, with both overlapping and distinctive species and abundancies to that of the colon (104, 105). Moreover, association studies are providing increasing evidence that local tumor-antagonizing/protective versus tumor-promoting tissue microbiomes, similarly to the gut microbiome, can modulate susceptibility and pathogenesis to human cancers arising in their associated organs (106–109).

Impact of Intratumoral Microbiota?

Finally, pathologists have long recognized that bacteria can be detected within solid tumors, an observation that has now been substantiated with sophisticated profiling technologies. For example, in a survey of 1,526 tumors encompassing seven human cancer types (bone, brain, breast, lung, melanoma, ovary, and pancreas), each type was characterized by a distinctive microbiome that was largely localized inside cancer cells and immune cells, and within each tumor type, variations in the tumor microbiome could be detected and inferred to be associated with clinicopathologic features (110). Microbiota have been similarly detected in genetically engineered *de novo* mouse models of lung and pancreas cancer, and their absence in germ-free mice and/or their abrogation with antibiotics can demonstrably impair tumorigenesis, functionally implicating the tumor microbiome as an enabler of tumor-promoting inflammation and malignant progression (111, 112). Association studies in human pancreatic ductal adenocarcinoma and functional tests via fecal transplants into tumor-bearing mice have established that variations in the tumor microbiome—and the associated gut microbiome—modulate immune phenotypes and survival (113). An important challenge for the future will be to extend these implications to other tumor types, and to delineate the potentially separable contributions of constitution and variation in the tumor microbiome to that of the gut (and local tissue of origin) microbiome, potentially by identifying specific microbial species that are functionally influential in one location or the other.

Synopsis

Among the fascinating questions for the future is whether microbiota resident in different tissues or populating incipient neoplasias have the capability to contribute to or interfere with the acquisition of other hallmark capabilities beyond immunomodulation and genome mutation, thereby influencing tumor development and progression. There are clues that particular bacterial species can directly stimulate the hallmark of proliferative signaling, for example, in colonic epithelium (88), and modulate growth suppression by altering tumor suppressor activity in different compartments of the intestine (114), whereas direct effects on other hallmark capabilities, such as avoiding cell death, inducing angiogenesis, and stimulating invasion and metastasis, remain obscure, as does the generalizability of these observations to multiple forms of human cancer. Irrespective, there is an increasingly compelling case to be made that polymorphic variation in microbiomes of the intestine and other organs constitutes a distinctive enabling characteristic for the acquisition of hallmark capabilities (Fig. 4), albeit intersecting with and

complementing those of genome instability and mutation, and tumor-promoting inflammation.

SENESCENT CELLS

Cellular senescence is a typically irreversible form of proliferative arrest, likely evolved as a protective mechanism for maintaining tissue homeostasis, ostensibly as a complementary mechanism to programmed cell death that serves to inactivate and in due course remove diseased, dysfunctional, or otherwise unnecessary cells. In addition to shutting down the cell division cycle, the senescence program evokes changes in cell morphology and metabolism and, most profoundly, the activation of a senescence-associated secretory phenotype (SASP) involving the release of a plethora of bioactive proteins, including chemokines, cytokines, and proteases whose identity is dependent on the cell and tissue type from which a senescent cell arises (115–117). Senescence can be induced in cells by a variety of conditions, including microenvironmental stresses such as nutrient deprivation and DNA damage, as well as damage to organelles and cellular infrastructure, and imbalances in cellular signaling networks (115, 117), all of which have been associated with the observed increase in the abundance of senescent cells in various organs during aging (118, 119).

Cellular senescence has long been viewed as a protective mechanism against neoplasia, whereby cancerous cells are induced to undergo senescence (120). Most of the aforementioned instigators of the senescent program are associated with malignancy, in particular DNA damage as a consequence of aberrant hyperproliferation, so-called oncogene-induced senescence due to hyperactivated signaling, and therapy-induced senescence consequent to cellular and genomic damage caused by chemotherapy and radiotherapy. Indeed, there are well-established examples of the protective benefits of senescence in limiting malignant progression (118, 119). To the contrary, however, an increasing body of evidence reveals quite the opposite: in certain contexts, senescent cells variously stimulate tumor development and malignant progression (119, 121). In one illuminating case study, senescent cells were pharmacologically ablated in aging mice, in particular depleting senescent cells characteristically expressing the cell-cycle inhibitor $p16^{-INK4a}$: in addition to delaying multiple age-related symptoms, the depletion of senescent cells in aging mice resulted in reduced incidences of spontaneous tumorigenesis and cancer-associated death (122).

The principal mechanism by which senescent cells promote tumor phenotypes is thought to be the SASP, which is demonstrably capable of conveying, in paracrine fashion to viable cancer cells in proximity, as well as to other cells in the TME, signaling molecules (and proteases that activate and/or desequester them) so as to convey hallmark capabilities. Thus, in different experimental systems, senescent cancer cells have been shown to variously contribute to proliferative signaling, avoiding apoptosis, inducing angiogenesis, stimulating invasion and metastasis, and suppressing tumor immunity (116, 118, 120, 121).

Yet another facet to the effects of senescent cancer cells on cancer phenotypes involves transitory, reversible senescent cell states, whereby senescent cancer cells can escape from their SASP-expressing, nonproliferative condition, and resume cell proliferation and manifestation of the associated capabilities of fully viable oncogenic cells (44). Such transitory senescence is most well documented in cases of therapy resistance (44), representing a form of dormancy that circumvents therapeutic targeting of proliferating cancer cells, but may well prove to be more broadly operative in other stages of tumor development, malignant progression, and metastasis.

Moreover, the hallmark-promoting capabilities of senescent cells are not limited to senescent cancer cells. Cancer-associated fibroblasts (CAF) in tumors have been shown to undergo senescence, creating senescent CAFs that are demonstrably tumor-promoting by virtue of conveying hallmark capabilities to cancer cells in the TME (115, 116, 121). Moreover, senescent fibroblasts in normal tissues produced in part by natural aging or environmental insults have similarly been implicated in remodeling tissue microenvironments via their SASP so as to provide paracrine support for local invasion (so-called "field effects") and distant metastasis (116) of neoplasias developing in proximity. Additionally, senescent fibroblasts in aging skin have been shown to recruit—via their SASP—innate immune cells that are both immunosuppressive of adaptive antitumoral immune responses anchored by CD8 T cells, and stimulatory of skin tumor growth (123), with the latter effect potentially reflecting paracrine contributions of such innate immune cells (myeloid cells, neutrophils, and macrophages) to other hallmark capabilities.

While less well established, it seems likely that other abundant stromal cells populating particular tumor microenvironments will prove to undergo senescence, and thereby modulate cancer hallmarks and consequent tumor phenotypes. For example, therapy-induced senescent tumor endothelial cells can enhance proliferation, invasion, and metastasis in breast cancer models (124, 125).

Certainly, such clues warrant investigation in other tumor types to assess generality of fibroblastic, endothelial, and other stromal cell senescence as a driving force in tumor evolution. Also currently unresolved are the regulatory mechanisms and functional determinants through which a particular senescent cell type in a given TME evokes a tumor-promoting versus a tumor-antagonizing SASP, which can seeming be alternatively induced in the same senescing cell type, perhaps by different instigators when immersed in distinctive physiologic and neoplastic microenvironments.

Synopsis

The concept that tumors are composed of genetically transformed cancer cells interacting with and benefiting from recruited and epigenetically/phenotypically corrupted accessory (stromal) cells is well established as instrumental to the pathogenesis of cancer. The considerations discussed above and described in the reviews and reports cited herein (and elsewhere) make a persuasive case for the proposition that senescent cells (of whatever cellular origin) should be considered for addition to the roster of functionally significant cells in the tumor microenvironment (Fig. 5). As such, senescent cells warrant being factored into the quest for deep knowledge of cancer mechanisms. Furthermore, the realization of

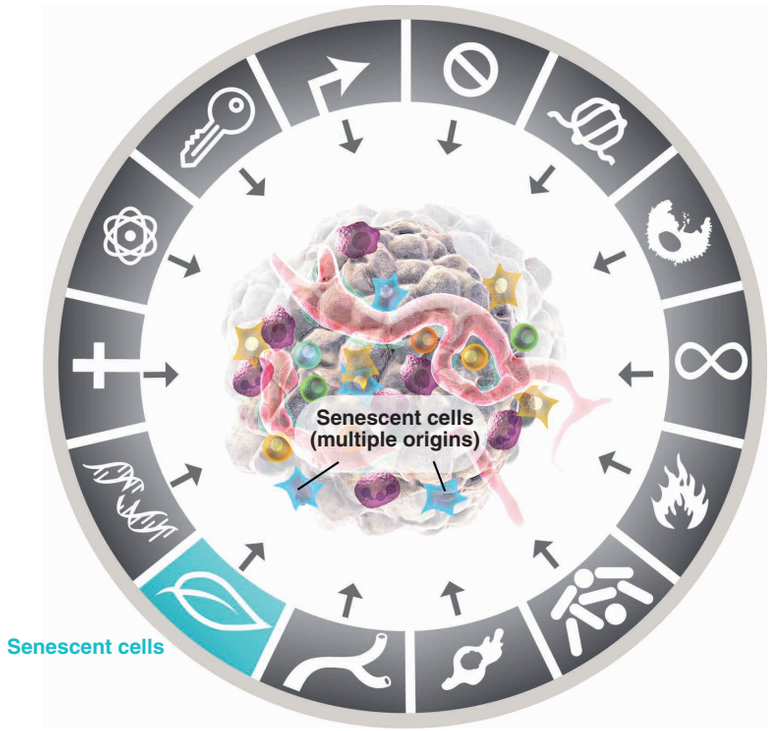

Figure 5. Senescent cells. Heterogeneous cancer cell subtypes as well as stromal cell types and subtypes are functionally integrated into the manifestations of tumors as outlaw organs. Clues are increasingly implicating senescent cell derivatives of many of these cellular constituents of the TME, and their variable SASPs, in modulating hallmark capabilities and consequent tumor phenotypes. The hallmarks of cancer graphic has been adapted from Hanahan and Weinberg (2).

their importance motivates the ancillary goal to therapeutically target tumor-promoting senescent cells of all constitutions, be it by pharmacologic or immunologic ablation, or by reprogramming the SASP into tumor-antagonizing variants (115, 121, 126).

CONCLUDING REMARKS

While the eight hallmarks of cancer and their two enabling characteristics have proved of enduring heuristic value in the conceptualization of cancer, the considerations presented above suggest that there may be new facets of some generality and hence of relevance to more fully understanding the complexities, mechanisms, and manifestations of the disease. By applying the metric of discernable if not complete independence from the 10 core attributes, it is arguable that these four parameters may well—pursuant to further validation and generalization beyond the case studies presented—become integrated into the hallmarks of cancer schematic (Fig. 6). Thus, cellular plasticity may come to be added to the roster of hallmark capabilities. Notably, while the eight core and this nouveau capability are each, by their definition as a hallmark, conceptually distinguishable, aspects of their regulation are at least partially interconnected in some and perhaps many cancers. For example, multiple hallmarks are coordinately modulated in some tumor types by canonical oncogenic drivers, including

(i) *KRAS* (https://cancer.sanger.ac.uk/cosmic/census-page/KRAS),
(ii) *MYC* (https://cancer.sanger.ac.uk/cosmic/census-page/MYC),
(iii) *NOTCH* (https://cancer.sanger.ac.uk/cosmic/census-page/NOTCH1; ref. 127), and
(iv) *TP53* (https://cancer.sanger.ac.uk/cosmic/census-page/TP53),

highlighting the important challenge to more fully elucidate the regulatory networks governing these acquired capabilities.

In addition to adding cellular plasticity to the roster, nonmutational epigenetic reprogramming and polymorphic variations in organ/tissue microbiomes may come to be incorporated as mechanistic determinants—enabling characteristics—by which hallmark capabilities are acquired, along with tumor-promoting inflammation (itself partially interconnected to the microbiome), above and beyond the mutations and other aberrations that manifest the afore-mentioned oncogenic drivers.

Finally, senescent cells of different origins—including cancer cells and various stromal cells—that functionally contribute to the development and malignant progression of cancer, albeit in markedly distinctive ways to those of their nonsenescent brethren, may become incorporated as generic components of the TME. In conclusion, it is envisaged that raising these provisional "trial balloons" will stimulate debate, discussion, and continuing experimental investigation in the cancer research community about the defining conceptual parameters of cancer biology, genetics, and pathogenesis.

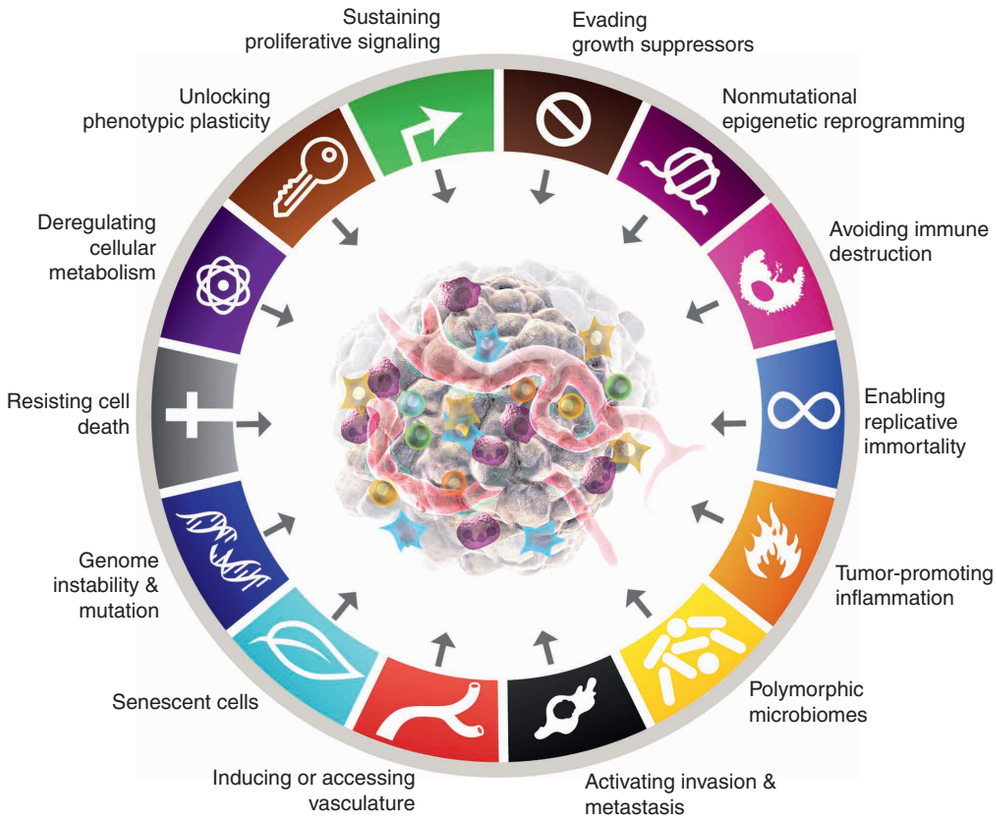

Figure 6. Hallmarks of Cancer—new additions. Depicted are the canonical and prospective new additions to the "Hallmarks of Cancer." This treatise raises the possibility, aiming to stimulate debate, discussion, and experimental elaboration, that some or all of the four new parameters will come to be appreciated as generic to multiple forms of human cancer and hence appropriate to incorporate into the core conceptualization of the hallmarks of cancer. The hallmarks of cancer graphic has been adapted from Hanahan and Weinberg (2).

Author's Disclosures

No disclosures were reported.

Acknowledgments

First and foremost, I profoundly thank Bob Weinberg for an exceptional tradition of insightful and formative discussions, and for excellent comments and suggestions to the first vignette of this manuscript. Additionally, I wish to thank: Ben Stanger; Bradley Bernstein, Giovanni Ciriello, and William Flavahan; Jennifer Wargo; and Sheila Stewart for their valuable comments and suggestions on the four vignettes, respectively, and SayoStudio for assistance in crafting the figures.

Received August 4, 2021; revised November 17, 2021; accepted November 18, 2021; published first January 12, 2022.

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