Hallmarks of Cancer: Molecular Underpinnings

1

Dhananjay A. Chitale

Abstract

Cancer, a genetic disease, is an autonomous organ composed of heterogeneous cancer cell clones supported by its microenvironment. The cancer cells gain a set of properties and characteristics called hallmarks of cancer and enabling characteristics, respectively, that highjack the normal cellular mechanisms for their purpose. These are thought to be acquired in a stepwise fashion, in no particular order, giving cancer cells a survival advantage over normal cells, making them self-sustainable, invade and metastasize to distant locations. This chapter provides a brief overview and key concepts on the molecular underpinnings of cancer cells composed of eight hallmarks of cancer and two enabling characteristics.

Learning Objectives

- To understand the molecular genomic alterations in primary cancers conceptually as Hallmarks of Cancer as the basis for cancer metastasis
- Based on the understanding of genomic underpinnings of cancers, learn diagnostic, prognostic, and predictive therapeutic strategies

1.1 Introduction

Cancer is a genetic disease underpinned by mutations, either somatic or rarely genetically inherited, layered with epigenetic alterations that lead to an autonomous organ that is under its own control, defying normal cellular signaling processes. The molecular genetic characteristics of cancer impart tumor cells a set of properties called *hallmarks of cancer* that Hanahan and Weinberg initially published in their seminal paper published in 2000 [1]. They initially described six such properties, viz. self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. These properties are acquired by the cancer cell with an inherent assumption that normal cells progressively transform into a neoplastic state through stepwise gain of mutations that confer survival advantage and disseminate at distant sites. The stages or steps of these changes are necessarily not linear, and different tumors may have different sequential gains in these properties. After a decade or so, with additional accumulation of molecular literature and understanding of cancer, in 2011, Hanahan and Weinberg revised the original hallmarks and added two new ones to the original six, and expanded on the functional roles and contributions made by the tumor microenvironment [2].

In this chapter, using Hanahan and Weinberg's publications as the backbone [1, 2], we focus on detailed descriptions of these properties that produce the cellular phenotype and briefly elucidate the molecular underpinnings to gain such properties in the primary tumors with some examples. The details of underlying molecular mechanisms such as point mutations, gene translocations/fusions, deletions, insertions, amplification, chromosomal aneuploidy, transcriptional changes (mRNA profiles), epigenetic changes, MicroRNA profiles, and regulating DNA structural regulatory changes are described in the next chapter titled "Unifying Concept of Genomic Changes: The Mutational Landscape of Cancers."

1.2 Sustaining Proliferative Signaling

Normal tissues regulate the proliferation of cells tightly controlled through growth factor-induced cell signaling, thus controlling the tissue architecture and function. This process is orchestrated through a growth factor binding to its specific

D. A. Chitale (✉)
Department of Pathology and Laboratory Medicine, Henry Ford Health System, Detroit, MI, USA
e-mail: dchital1@hfhs.org

S. P. Leong et al. (eds.), *Cancer Metastasis Through the Lymphovascular System*,
https://doi.org/10.1007/978-3-030-93084-4_1

receptor transiently, activating a cascade of several downstream cytoplasmic signal-transduction proteins transmitting the signal to the nucleus. This, in turn, induces and activates transcription factors and epigenetic alterations initiating DNA transcription. Genes encoding proteins that promote cell cycle progression ultimately result in cell division. The expression of other genes at the same time also leads to changes in metabolic activity (energy metabolism) and proteins supporting cell survival that are needed for optimal growth. The knowledge about the proliferative growth factor signaling through the paracrine mechanism remains limited [2]. However, in cancer cells, the most fundamental trait is their ability to sustain proliferative signals, many through tyrosine kinase activity, even in the absence of growth factors. This self-sustaining property is achieved through several molecular mechanisms involving one or multiple parts of the cell signaling pathways. Primary mechanisms include **mutations in the oncogenes** that constitutively activate cell signaling pathways and **deregulation of inhibitory feedback loops,** enhancing cell signaling.

1.2.1 Mutation in Oncogenes

Normal cellular genes that promote cell proliferation called proto-oncogenes are mutated or overexpressed autonomously in many cancers, which are then termed as oncogenes. An array of mechanisms alters the function of the oncogenes by constitutive activation and resistance to control by normal external signals free from checkpoints. The molecular underpinnings of this hallmark of cancer can involve growth factors, growth factor receptors, proteins involved in signal transduction, nuclear regulatory proteins, and cell cycle regulator.

One pathway is through **increasing growth factor production**. The cancer cells may produce growth factors themselves (autocrine capability); for example, glioblastomas overexpress platelet-derived growth factor (PDGF) and PDGF receptor (PDGFR) [3]. Tumor cells may send signals to stimulate the normal cells in the tumor microenvironment, which supply the growth factors (paracrine function). Amplification of FGF3 growth factor is observed in many cancers (stomach, bladder, breast) [4]. Another mechanism is producing high levels of receptor proteins, such as *Her2 (ERB1B2)* gene amplification in breast cancers. In this case, receptor signaling can be deregulated and sensitized even in the presence of normal amounts of ligand [5–8]. In lung adenocarcinomas, discovery of **activating hot spot mutations** in the tyrosine kinase domain encoded by exon 19 and 21 of *ERB1B1 (EGFR)* receptor has revolutionized the way lung adenocarcinomas are treated using small molecular targeted therapies (tyrosine kinase inhibitors-TKI). Another well-known means of cancer signaling upregulation is achieved through constitutive activation of components of the signaling pathways downstream of the receptors and independent of growth factor ligand activation. Many recurrent **somatic mutations** have been detected by high-throughput DNA sequencing that predicts the **constitutive activation of signal transduction**. Recurrent activating mutations in the *BRAF* gene, usually due to point mutations, have been noted in many solid tumors [9], including 40% of malignant melanomas [10]. These mutations lead to constitutively active signaling through mitogen-activated protein kinase(MAPK) pathway that regulates a wide variety of cellular processes such as proliferation, differentiation, apoptosis, and stress responses [11]. Another canonical signaling pathway that is frequently upregulated due to mutations in the catalytic subunits of phosphoinositide 3-kinase (PI3K) is involved in cellular functions such as cell growth, proliferation, differentiation, motility, survival, and intracellular trafficking [12, 13]. The tumor cells can also attain self-sufficiency due to the **deregulation of nuclear regulatory proteins**. Transcriptional activators, e.g., MYC, a master transcription factor that regulates genes needed for rapid cell growth, are overexpressed either through translocation (Burkitt's lymphoma) [14] or through amplification (breast carcinoma, lung adenocarcinoma, colon adenocarcinoma, prostate adenocarcinoma) [9] in aggressive tumors. **Dysregulation of cell cycle regulators** such as cyclins often occurs in some tumors. For example, cyclin D1 (CCDN1) is upregulated due to translocation (CCND1/IGH) in mantle cell lymphoma, plasma cell myeloma, making the tumor cells proliferate constitutively.

1.2.2 Deregulation of Negative Feedback Loops

Normal cellular mechanisms of cell signaling include negative feedback loops that inactivate the activated signaling to maintain homeostasis. In tumors, the genes controlling this negative feedback are downregulated in diverse tumor types, thus enhancing the mitogenic and proliferative signals. For example, many carcinomas of solid organs such as the colon, pancreas, lung etc., harbor activating mutations in the *KRAS* gene [9]. The effects on downstream signaling are not due to direct activation. But the constitutive activation of *RAS* protein through these mutations leads to loss of intrinsic GTPas activity of RAS, thus disrupting the autoregulatory negative-feedback mechanism [2]. Another example is heterogeneous groups of enzymes called Dual-specificity phosphatases (DUSP) that regulate diverse cellular signaling cascades, including much-researched MAPK phosphatases. Dysregulation of DUSPs has been implicated as major modulators of critical signaling pathways in various tumors [15].

Another well-known example is phosphatase and tensin homolog (PTEN), which degrades phosphatidylinositol 3,4,5-phosphate (PIP3), thus dampening PI3K signaling. Loss of PTEN function due to either somatic mutations such as deletion or gene silencing by hypermethylation leads to amplification of the PI3K signaling in many solid tumors [12, 13]. Germline mutations in *PTEN* in Cowden syndrome, an autosomal dominant inherited condition, predispose individuals to many benign and malignant tumors of the skin, breast, thyroid, endometrium, colorectum, and kidney [16].

1.3 Insensitivity to Growth Suppressors

In the normal cellular proliferation machinery, set of genes categorized as tumor suppressors control the checkpoints to prevent uncontrolled cell growth. These antiproliferative mechanisms lead to quiescence or permanent cell cycle arrest, depending on the scenario. Several tumor suppressor genes are part of this regulatory network limiting cell proliferation or survival responding to activated signaling (Table 1.1). A similar phenomenon occurs in cancer cells with overactive cell signaling that leads to a nonproliferative state called oncogene-induced senescence [18]. Like mitogenic signaling, growth inhibitory signals also arise outside of the cells (paracrine) orchestrated through receptors, signal transducers, and nuclear transcription regulators. Cancer cells evade these checkpoints, thus gaining survival advantage and cell proliferation. These can be categorized based on the molecular underpinnings, viz. genes encoding inhibitors of mitogenic signaling pathways, inhibitors of cell cycle progression, inhibitors of pro-growth programs of metabolism and angiogenesis, inhibitors of invasion and metastasis, enablers of genomic stability, DNA repair factors, and some with unknown mechanisms [17]. It is thought that tumor suppressors have a broader inhibitory effect on many hallmarks of cancer. The key prototypical tumor suppressor proteins include *RB1* and *TP53,* both playing a central role as checkpoint controls for a cell to proliferate or activate growth arrest, senescence, or activate apoptosis. These are described in detail below.

Much of our understanding and concepts regarding tumor suppressor genes and their function are derived from studies on the first tumor suppression gene discovered, the retinoblastoma (*RB1*) gene, the so-called Governor of The Cell Cycle. Based on observations between hereditary retinoblastomas vs. sporadic, Knudson proposed the "two-hit" hypothesis of oncogenesis that states that both the alleles (one from mother and one from father) need to be inactivated by similar or different molecular mechanisms to cause malignancies. In inherited germline *RB1* mutation, the second normal allele is inactivated by somatic mutation silencing the gene function. While in sporadic retinoblastomas, both the alleles are inactivated by somatic mutations [19]. Large-scale genomic sequencing has identified similar somatic *RB1* gene mutations in subsets of the lung, breast, bladder carcinoma, and glioblastoma [9], and among the top 5 somatically altered genes in metastatic tumors [20]. The current model is that loss of normal cell cycle control occurs due to dysregulation of at least one of the four key regulators: *CDKN2A, CCND1, CDK4*, and *RB1* in many human cancers [17]. The loss of tumor suppressor function of *RB1* occurs directly due to mutation in the *RB1* gene or indirectly by the gain of function CDK/Cyclin D activity keeping *RB1* protein in the hyperphosphorylated state, thus inhibiting the binding and sequestering of E2F transcription factors. Release of E2F leads to transcriptional activation and cell cycle progression.

TP53, also known as Guardian of The Genome, is also a tumor suppressor gene regulating cell cycle progression, DNA repair, cellular senescence, and apoptosis. *TP53* is mutated in 50% of all cancers, including lung, colon, breast, ovary, and glioblastoma. Unlike RB1, the loss of function is primarily somatic and rarely seen as germline inheritable genetic mutation seen in Li-Fraumeni syndrome. Like RB1, the loss of function occurs by somatic mutations in both the alleles or in cases of germline mutation, and other allele is inactivated by somatic mutation. Another analogy to *RB1* loss of function is that the functional loss of p53 protein can occur in the absence of TP53 mutations. *MDM2* gene amplification observed in 33% of human sarcomas leads to p53 deficiency due to accelerated degradation. Transforming proteins of several DNA viruses bind to p53 and promote degradation. With the loss of p53 protein function, DNA damage and, therefore, driver mutations accumulate in oncogenes, ultimately leading to the malignant transformation of cells.

RB protein transduces growth-inhibitory signals originating externally and internally and decides whether a cell should proceed through its growth-and-division cycle. In comparison, *TP53* receives inputs from stress and abnormality sensors intracellular signaling. Depending on the degree of genomic damage, the levels of nucleotide pools, growth-promoting signals, glucose, or oxygenation, TP53 can halt cell cycle progression until normalization. Alternatively, if there is irreparable damage to cellular subsystems, TP53 can trigger apoptosis [2]. In the absence of both these proteins, the cell proliferating characteristic of cancer cells remains unimpeded.

1.3.1 Evasion of Contact Inhibition

Normal cells stop proliferating upon contact with other cells. This contact inhibition that is part of tissue homeostasis is lost in tumors. E-cadherin encoded by the *CDH1* gene is a cell adhesion molecule that plays a vital role in

Table 1.1 Selected tumor suppressor genes and associated familial syndrome and cancers grouped by cancer hallmarks are listed in this table

Gene	Protein	Function	Familial syndromes	Sporadic cancers
Inhibitors of mitogenic signaling pathways				
APC	Adenomatous polyposis coli protein	Inhibitor of WNT signaling	Familial colonic polyps and carcinomas	Carcinomas of stomach, colon, pancreas; melanoma
NF1	Neurofibromin-1	Inhibitor of RAS/MAPK signaling	Neurofibromatosis type 1 (neurofibromas and malignant peripheral nerve sheath tumors)	Neuroblastoma, juvenile myeloid leukemia
NF2	Merlin	Cytoskeletal stability, hippo pathway signaling	Neurofibromatosis type 2 (acoustic schwannoma and meningioma)	Schwannoma, meningioma
PTCH	Patched	Inhibitor of hedgehog signaling	Gorlin syndrome (basal cell carcinoma, medulloblastoma, several benign tumors)	Basal cell carcinoma, medulloblastoma
PTEN	Phosphatase and tensin homologue	Inhibitor of PI3K/AKT signaling	Cowden syndrome (variety of benign skin, GI, and CNS growths; breast, endometrial, and thyroid carcinoma)	Diverse cancers, particularly carcinomas and lymphoid tumors
SMAD2, SMAD4	SMAD2, SMAD4	Component of the TGF-β signaling pathway, repressors of MYC and CDK4 expression, inducers of CDK inhibitor expression	Juvenile polyposis	Frequently mutated (along with other components of the TGF-β signaling pathway) in colonic and pancreatic carcinoma
Inhibitors of cell cycle progression				
RB	Retinoblastoma (RB) protein	Inhibitor of G_1/S transition during cell cycle progression	Familial retinoblastoma syndrome (retinoblastoma, osteosarcoma, other sarcomas)	Retinoblastoma; osteosarcoma; carcinomas of breast, colon, lung
CDKN2A	p16/INK4a and p14/ARF	p16: Negative regulator of cyclin-dependent kinases; p14, an indirect activator of p53	Familial melanoma	Pancreatic, breast, and esophageal carcinoma; melanoma; certain leukemias
Inhibitors of pro-growth programs of metabolism and angiogenesis				
VHL	von Hippel–Lindau (VHL) protein	Inhibitor of hypoxia-induced transcription factors (e.g., HIF1α)	von Hippel–Lindau syndrome (cerebellar hemangioblastoma, retinal angioma, renal cell carcinoma)	Renal cell carcinoma
STK11	Liver kinase B1 (LKB1) or STK11	Activator of AMPK family of kinases; suppresses cell growth when cell nutrient and energy levels are low	Peutz-Jeghers syndrome (GI polyps, GI cancers, pancreatic carcinoma, and other carcinomas)	Diverse carcinomas (5%–20% of cases, depending on the type)
SDHB, SDHD	Succinate dehydrogenase complex subunits B and D	TCA cycle, oxidative phosphorylation	Familial paraganglioma, familial pheochromocytoma	Paraganglioma
Inhibitors of invasion and metastasis				
CDH1	E-cadherin	Cell adhesion, inhibition of cell motility	Familial gastric cancer	Gastric carcinoma, lobular breast carcinoma
Enablers of genomic stability				
TP53	p53 protein	Cell cycle arrest and apoptosis in response to DNA damage	Li-Fraumeni syndrome (diverse cancers)	Most human cancers
DNA repair factors				
BRCA1, BRCA2	Breast cancer-1 and breast cancer-2 (BRCA1 and BRCA2)	Repair of double-stranded breaks in DNA	Familial breast and ovarian carcinoma; carcinomas of the male breast; chronic lymphocytic leukemia (BRCA2)	Rare
MSH2, MLH1, MSH6	MSH1, MLH1, MSH6	DNA mismatch repair	Hereditary nonpolyposis colon carcinoma	Colonic and endometrial carcinoma
Unknown mechanisms				
WT1	Wilms tumor-1 (WT1)	Transcription factor	Familial Wilms tumor	Wilms tumor, certain leukemias
MEN1	Menin	Transcription factor	Multiple endocrine neoplasia-1 (MEN1) (pituitary, parathyroid, and pancreatic endocrine tumors)	Pituitary, parathyroid, and pancreatic endocrine tumors

CNS Central nervous system, *GI* Gastrointestinal, *TCA* Tricarboxylic acid

Adapted from Robbins and Cotran Pathologic Basis of Diseases, tenth Edition, Kumar, V., Abbas, A. K., Aster, J. C., Turner, J. R., Robbins, S. L., & Cotran, R. S., Page 292, Table 7.7, Copyright Elsevier, 2021 [17]

contact-mediated growth inhibition of epithelial cells. Disruption of E-cadherin is noted in many solid tumors such as gastric signet ring cell carcinomas and invasive lobular carcinoma of the breast. This allows cells to easily disaggregate due to loss of "stickiness" and promotes distant metastasis. Another function of E-cadherin is that it binds and sequesters β-catenin. Disruption of this complex leads to the translocation of β-catenin to the nucleus, like the WNT pathway that promotes proliferation.

Another mechanism by which cells regulate proliferation is through the NF2 gene. Merlin, the NF2 gene product, promotes contact inhibition by coupling cell-surface adhesion molecules such as E-cadherin to transmembrane receptor tyrosine kinase such as EGFR. This adhesion strengthening also limits the emission of mitogenic signals [21, 22].

One of the complex underpinnings enhancing the tumor suppressor pathway in tumors (e.g., colon, pancreas) is dysregulation of the WNT signaling pathway. WNT pathway activation facilitates β-catenin activity by blocking the formation of a "destruction complex" composed of APC and other proteins. β-catenin, in turn, regulates cell proliferation by modulating transcription factors such as MYC and cyclin D1. β-Catenin activity is kept in check by this destruction complex composed of APC and other proteins. Mutations in the *APC* gene occur in 70–80% of early colonic adenomas, and carcinomas lead to unchecked activity of β-catenin.

1.3.2 Downregulation of Cell Proliferation Inhibitor—Transforming Growth Factor β (TGF-β) Pathway

In many cancers such as colon, stomach, and endometrium, there are loss-of-function mutations in TGF-β receptors leading to loss of inhibitory control on cellular proliferation. There is downregulation of SMAD genes in pancreatic tumors, downstream signal transducers in TGF-β, thus corrupting the TGF-β pathway. In addition to promoting tumor growth, depending on the cellular context, altered TGF-β signaling is found to activate epithelial-mesenchymal transition (EMT) that makes the cancer cell more aggressive [23, 24].

1.4 Resisting Cell Death

Apoptosis, programmed cell death dismantling cells into components, is activated in various cellular conditions orchestrated by many signaling circuitry, including TP53 and RB1 checkpoints. The signaling circuitry governing this program is triggered by physiologic stresses such as exaggerated oncogenic signaling, DNA damage due to chemotherapy, radiation, or hyperproliferative state of the cancer cell. The activation of the apoptotic program is thus achieved through two significant circuits—extrinsic to cell (death receptor—e.g., Fas ligand / Fas receptor) and intrinsic (mitochondrial) cellular programs, and also perforin/granzyme pathway. Intrinsic and extrinsic pathways activate a proteolytic cascade of caspases (caspase 8, 9, and 3) that destroy the cell in an organized manner to form apoptotic bodies observed and reported in pathology literature many decades. Perforin / Granzyme pathway is activated by cytotoxic T cells either by activating caspase 10 or directly lead to DNA cleavage. In the intrinsic pathway, there is disruption of mitochondrial membrane activating BAX/BAK pro-apoptotic proteins that increase the permeability of mitochondrial membrane that allows cytochrome c to leak into the cytoplasm where it activates caspase-9 through APAF-1 binding [25].

Abrogation of the apoptotic program in cancer cells is possible by two central mechanisms and is associated with high-grade tumors and developing resistance to therapies. The tumor cells counterbalance pro-apoptotic signaling by upregulating anti-apoptotic proteins such as Bcl-2 family of proteins [26, 27]. For example, in follicular lymphoma, the molecular underpinning is the t(14;18)(q32;q21) translocation leading to constitutive activation of Bcl2 by transcriptionally active IGH promotor region. The IGH-Bcl2 fusion help escape apoptosis. Another mechanism of escape from apoptosis is achieved through the loss of TP53 function. Loss of TP53 prevents upregulation of a BH3-only protein called PUMA (BH3 proteins neutralize actions of Bcl2 related anti-apoptotic proteins). The evasion of apoptotic circuitry is complex in different tumors and different mechanisms are derailed to evade apoptosis.

1.4.1 Autophagy

In a state of severe nutrient deficiency, cells activate signaling circuits that either arrest cell growth or even cannibalize their own organelles, proteins, and membranes for energy production. In a rapidly growing tumor, the cells may become dormant activating autophagy due to scarcity of nutrients. A similar phenomenon may be used by cancer cells under therapeutic pressure, thus making tumor resistant to therapies and leading to therapeutic failures. The role of autophagy in cancer is still highly debatable and is based on the state of the tumor cell; it can be viewed as tumor protective in certain circumstances and adverse in others [28].

1.5 Enabling Replicative Immortality: The Stem-Cell-Like Properties

Over the last two decades, literature on cancer stem cells has significantly evolved through intense cancer research, albeit still under debate. The first descriptions of cancer stem cells

were published on acute myeloid leukemia by Bonnet and Dick in 1997 [29]. The basic property of a cancer as having replicative immortality is conceptualized through its ability to self-replicate and differentiate through cancer stem-cell, a cell that is thought to be like a normal stem-cell counterpart. The debate continues whether the "stemness" arises through the transformation of a normal stem cell or through the ability of the cancer cells to transform into stem-like state through the acquisition of mutation through the selection process. In either pathway, the molecular underpinnings are thought to be by evading senescence and mitotic crisis and self-renewal ability.

Normal cells typically divide 50 to 70 times ("Hayflick limit") [30, 31], after which the cells become senescent, i.e., lose their ability to divide again. But cancer cells gain ability to evade senescence in part by abnormalities in RB and TP53 proteins. Maintaining RB protein in hypophosphorylated (active) state and downregulation of TP53 leads to disruption of G1/S cell cycle checkpoint, thus keeping the cell in a proliferative mode.

Another mechanism by which cancer cells achieve senescence evasion is through the maintenance of telomerase, which restores/"replenishes" the telomere sequences. Normally replicative capacity of a cell is lost after each cell division due to the shortening of the telomere, a repetitive nucleotide sequence at each end of the chromosome protecting the chromosomal deterioration and truncation. Eventually, the cell loses its replicative capacity completely and becomes senescent. But in many of tumors, human telomerase reverse transcriptase (TERT) upregulation leads to overexpression of telomerase enzyme, thus defying mitotic crisis and senescence [32, 33]. The gain of telomerase function may be induced due to cellular mitotic crisis from oncogenic program activation in cancer cells, thus maintaining the replicative potential and the damage to oncogenes and tumor suppressor genes. The telomerase activity is also thought to be upregulated in cancer stem cells.

Tissue stem cells and germ cells retain the capability to divide indefinitely through numerous cell divisions. By expressing telomerase, they resist mitotic crises and the accumulation of mutations. As stem cells divide, during embryogenesis or when a cell is under stress, either both (symmetric) or one (asymmetric) remains as daughter cells [34]. Cancer stem cells are thought to retain their limitless proliferative capacity and immortality, although debate continues as to the mechanism and number of cancer stem cells in different types of cancers. In summary, a small proportion of cancer cells retain or acquire stem-like properties that inactivate senescence signals, reactivate telomerase, and enable replicative immortality.

1.6 Angiogenesis

For tumors to grow and sustain proliferative advantage, an adequate supply of oxygen, nutrients, and metabolic waste management needs an adequate blood supply. Cancers stimulate neoangiogenesis to sustain its growth. The angiogenesis is observed under the microscope in some tumors extensive vascular network, for example, primary and metastatic renal cell carcinoma [35], while in some other tumors, few blood vessels such as scirrhous invasive ductal carcinoma of the breast (Fig. 1.1). The current understanding of angiogenesis is controlled by a balance between promoters and inhibitors of vascular proliferation [36, 37]. In the physiologic states, such as female endometrial cycling and wound healing, "angiogenic switch" is triggered transiently. However, in cancers as the tumor grows in size, the quiescent stage is terminated, and vascular proliferation is initiated by increased local production of angiogenic factors and/or loss

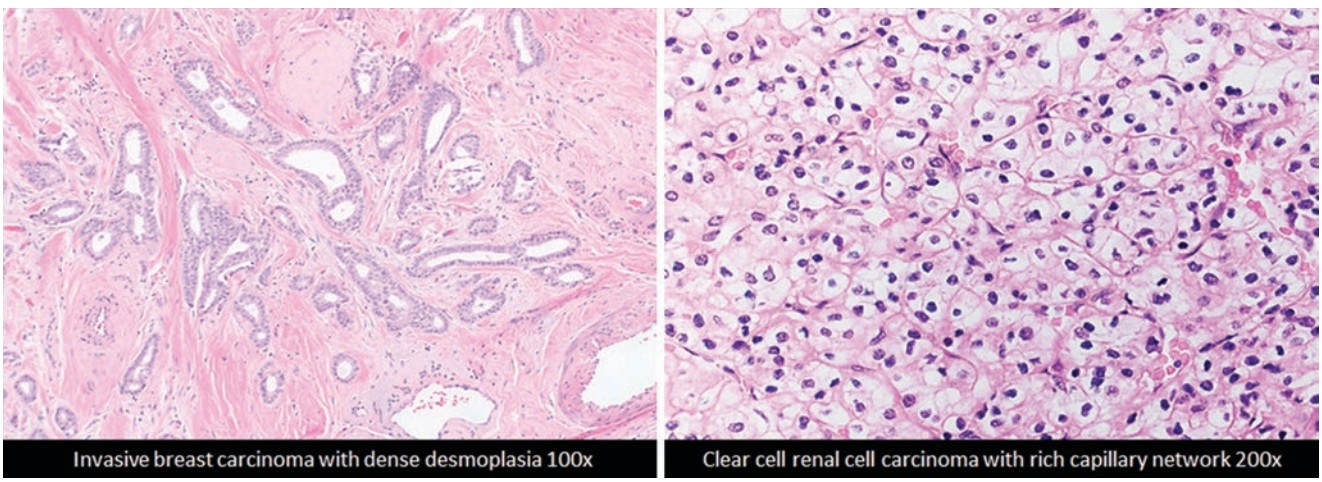

Fig. 1.1 Invasive breast carcinoma with dense desmoplasia and clear cell carcinoma with rich capillary network

of angiogenic inhibitors [17]. The angiogenic factors are locally produced by tumor cells, inflammatory cells, or stromal cells. Some of the inflammatory cells and, in particular, endothelial progenitor cells are thought to be bone marrow-derived in some cases [38]. The molecular underpinnings of angiogenesis in tumor cells include upregulation of many genes, the key genes include hypoxia-inducible transcription factor 1α (HIF1α), vascular endothelial growth factor (VEGF), as angiogenesis inducers, and thrombospondin-1 (TSP-1) as an inhibitor. The pro-angiogenic factors include: Hypoxia led stabilization of HIF1α that upregulates VEGF gene expression. VEGF is also upregulated by oncogene signaling. This stimulates endothelial growth towards the tumor. Normally, TP53 upregulates TSP-1 expression that inhibits angiogenesis by repressing VEGF. TP53 mutations lead to p53 protein loss that permits angiogenesis.

Earlier it was thought that angiogenesis sets in fully when the tumors proliferate. But recent literature has shown that the histologic changes of angiogenesis, such as microvessel density, are noted in the pre-malignant stages of neoplasms in various organs, including squamous cell lesions in the oral mucosa, skin, uterine cervix, vulva, and anal canal [39, 40]. The fundamental properties of pre-malignant conditions for prevention were first described in 1976 by Sporn [41]. Using the knowledge of the molecular basis of hallmarks of cancer, Ryan and Faupel-Badger correlated these concepts with these properties of pre-malignant conditions [42], further supporting the acquisition of many of the hallmarks of cancers in the early stages of development.

1.7 Invasion and Metastasis

One of the most aggressive properties of cancer cells is the invasion and destruction of local tissues and metastasis. The original concept of a pattern of cancer metastasis was described by an English surgeon Stephen Paget in 1889 published as "seed and soil" hypothesis [43] [44]. Paget introduced the concept of the spread of tumor cells through interactions between the cancer cells (seed) and the host organ (soil). Even after a century, this concept is upheld by extensive research with "seed" now identified as the cancer stem cell [45]. It has been shown that this hallmark of cancer occurs through a complex multistep progression called the invasion-metastasis cascade.

The major steps in this process include invasion of the extracellular matrix (ECM), angiolymphatic dissemination, extravasation and tissue homing, and colonization. From primary site to metastatic site, the journey of cancer cells includes breaching the supporting basement membrane, navigating the interstitial connective tissues, and penetrating the vascular wall to gain access to vessels. During homing and colonization, this process is reversed. In the first step, the cells become dis-cohesive and reduce interactions ("Loosening up" of tumor cells). This is followed by degradation of extracellular matrix (ECM), attachment to "remodeled" ECM components, migration, and invasion of tumor cells. In the first step, the well-known alteration in cancer cells is loss of adhesion molecule E-cadherin encoded by the *CDH1* gene through a process called epithelial-mesenchymal transition (EMT). EMT, thought to be an integral part of metastasis, especially in breast and prostate cancers, is controlled by a combination of Snail, Slug, Twist, and Zeb1/2 transcription factors, favoring promigratory properties. The next step is the degradation of the basement membrane and interstitial connective tissues. Through autocrine or paracrine secretions, proteolytic enzymes such as matrix metalloproteinase (MMP), cathepsin D, and urokinase plasminogen activator help cancer cells invade. The final step of invasion is the migration of cancer cells by locomotion through basement membranes and areas of proteolysis by the contractile actin cytoskeleton. Finally, through vascular dissemination, homing, and colonization, a few cancer cells are successful in distant metastasis. Some tumors metastasize to specific sites, thus showing organ tropism. The underlying molecular mechanism is thought to be through the expression of adhesion or chemokine receptors, whose ligands are expressed by endothelial cells at the metastatic site [46].

The details of molecular underpinnings of metastasis are described in a separate chapter in this section, and only high-level key discussion points are described above.

1.8 Emerging Hallmarks and Enabling Characteristics

With mounting literature support, two additional hallmarks of cancer, including **Deregulating Cellular Energetics** and **Avoiding Immune Destruction** and enabling characteristics **Genomic Instability** and **Mutation and Tumor-promoting Inflammation** were introduced [2]. Each of these are described below.

1.9 Growth Promoting Altered Cellular Metabolism (Deregulating Cellular Energetics)

To support the autonomous growth of cancer cells and get oxygen and nutrients through neoangiogenesis, cancer cells reprogram the energy metabolism, which is recently described as a key hallmark of cancer [2]. Cancer metabolism research in cancer biology predates the discovery of oncogenes and tumor suppressor genes [47]. There is overwhelming research support that cancer cells reprogram metabolism to improve cellular sustainability and resilience

to gain selective advantage. The cancer cells change their metabolic pathway to the glycolytic pathway as the preferred source of energy where glucose is converted to lactose to generate ATP, even under aerobic conditions. This was first described by Otto Warburg in 1930 and is now known as the Warburg effect or aerobic glycolysis [48]. Although ATP generation is lower than the mitochondrial oxidative metabolic pathway through the tricarboxylic acid (TCA) cycle, this pathway is preferred as its metabolic intermediates are used by the rapidly proliferating cells to synthesize cellular components to fulfill the biosynthetic requirement. Biosynthesis of macromolecules or anabolic pathways is an integral part of cancer metabolism to support growth.

The molecular underpinnings of the altered energy metabolism include upregulation of oncogenes in receptor tyrosine kinase/PI3K-AKT-mTOR signaling that enhances the activity of glucose transporters and multiple glycolytic enzymes promoting lipid, protein, and nucleic acid biosynthesis. Overexpression of transcription factor MYC leads to upregulation of genes involved in anabolic metabolism through glycolytic enzymes and glutaminase. On the tumor suppressor side, STK11, an antagonist of oxidative glycolysis, is downregulated to promote cell growth.

As described above, autophagy is activated in nutrient-deficient conditions where cellular organelles and other components are digested and reused. Depending on the scenario, the tumor inactivates autophagy signaling to promote growth. On the other side, tumor cells may use autophagy to go into a "hibernation" state due to external pressures, such as chemotherapy, to become dormant and prevent cell death.

Recent sequencing data in certain tumors such as gliomas, acute myeloid leukemia, intrahepatic cholangiocarcinoma, and central chondrosarcoma has shown somatic mutations in Krebs cycle (TCA) genes, viz. isocitrate dehydrogenase genes-1 and 2 (*IDH1, IDH2*). Point mutations in *IDH1* or *IDH2* genes lead to mutant *IDH* protein, which loses its normal function and converts α-ketoglutarate to 2-hydrozyglutarate. This protein is considered an oncometabolite which acts as an inhibitor of epigenetic regulatory enzymes leading to cancer gene signaling pathway activation. Similarly, other enzymes from the TCA cycle that are mutated in other cancers include succinate dehydrogenase (SDH), fumarate hydratase (FH), and L-2-hydroxyglutarate dehydrogenase. These oncometabolites present a potential therapeutic target [49].

1.10 Evasion of Host Defense (Avoiding Immune Destruction)

Paul Ehrlich first hypothesized the role of host defense by the immune system in eliminating the tumor cells by recognizing them as "foreign." Lewis Thomas introduced and Macfarlane Burnet then consolidated this hypothesis and termed it as "immune surveillance." This phenomenon is thought to serve as a normal function of the immune system by constantly scanning the body for tumor cells and eliminating them [17, 50]. This concept was tested by several experiments and observations and was initially abundant due to a lack of sufficient evidence. Burnet suggested that the cancer cells' expression of tumor-specific neo-antigens induced an immunological response, thus eliminating such cells before clinical manifestations. We now know that certain tumors are heavily infiltrated by lymphocytes and other immune cells. Such tumors often harbor numerous driver as well as passenger mutations that have been discovered through high-throughput whole-genome sequencing. This "hyper mutator" genotype results in the production of neo-antigens that induces a robust host CD8+ cytotoxic T-cell cell response, as reported in many malignant melanomas, many colorectal cancers, lung cancers, among others [51] (Fig. 1.2). Direct demonstration of tumor-specific T cells and antibodies in patients with cancers was noted in a few. Some experiments challenged the hypothesis of immune surveillance, which showed that not all the immunodeficient or immunosuppressed patients develop cancers. However, in immunocompromised patients (acquired or hereditary), viral associated tumors are prevalent (HPV, EBV, HHV8), and so are nonviral-associated solid tumors [52].

Dunn and Schreiber developed a concept of "cancer immunoediting," which includes three stages of "sculpting of tumor" by the immune system. The immune surveillance hypothesis was proposed to be a general part of the cancer editing process and is considered the first stage called the **elimination** of tumor cells. The next step is called the **equilibrium** phase, which appears to be the longest that leads to the formation of new variants in the cancer cells with different mutations that resist the immune pressure. Different molecular mechanisms are triggered when cancer cells go into quiescence or the slow-cycling phase described above. Cancer cells likely activate senescence gene signaling to avoid cell death induced by immune cells. From this stage, some clines emerge and **escape** this immune surveillance and evade immune destruction. The molecular underpinnings include selective outgrowth of antigen-negative variants, loss or reduced expression of major histocompatibility (MHC) class I molecules escaping an attack from CD8+ cytotoxic T cells (CTL). Another mechanism that cancer cells use to evade immune destruction is by inhibiting T cell activation. The tumor cells upregulate inhibitory receptor CTLA-4 on tumor-specific T cells preventing sensitization and extended escape from the tumor-specific T cells. Another mechanism includes overexpression of Programmed death ligand (PD-L)-1 and PD-L2 cell surface proteins that inactivates CD8+ CTL by docking with PD-1 receptor. Thus, tumor cells avoid detection for elimination and escape

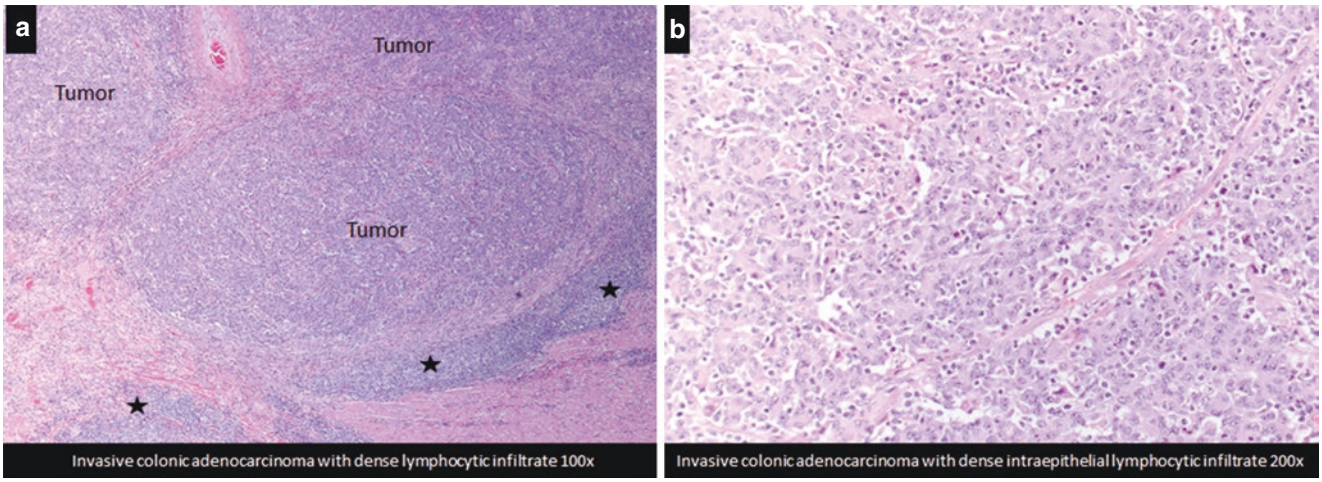

Fig. 1.2 Invasive colonic adenocarcinoma with dense Crohn's-like inflammatory response, (**a**) stars marking peritumoral dense lymphocytic infiltrate (100×), (**b**) depicting intra-epithelial lymphocytes (200×)

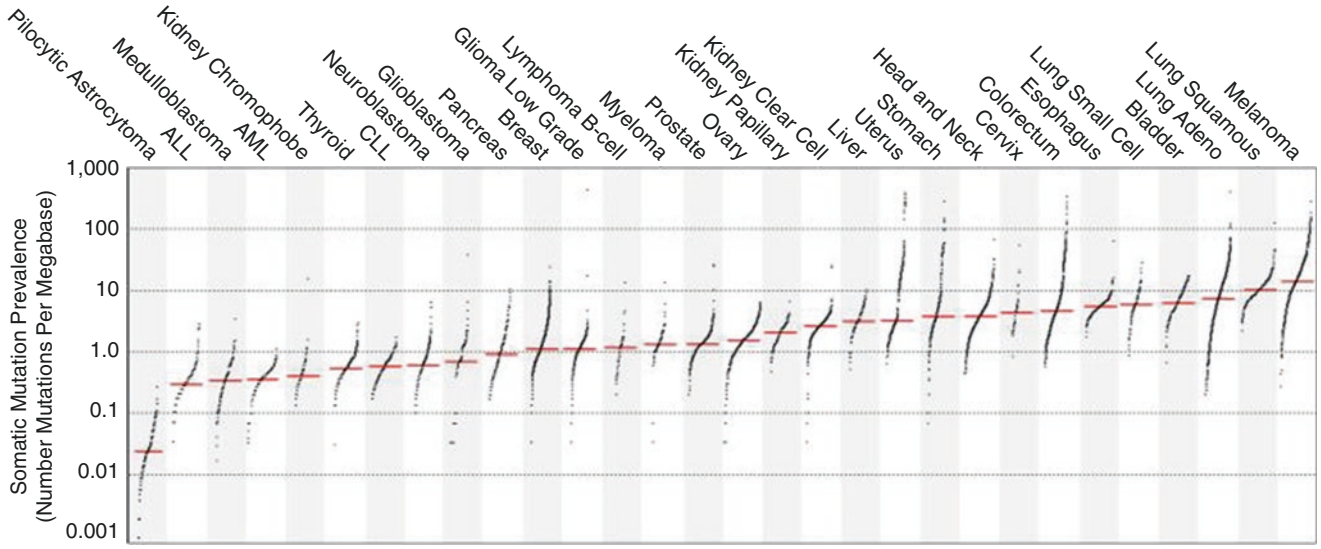

Fig. 1.3 Prevalence of somatic mutations per megabase across human cancer types in ascending order from left to right [53]

immune surveillance. The anti-PD-L1 and anti-CTLA-4 therapies block this inhibition in some solid tumors that overexpress PD-L1 protein (melanoma, lung cancers) and renders them vulnerable to the CD8+ CTL. These therapies (antibodies) that overcome immune evasion by the tumors are called immune checkpoint inhibitors (Fig. 1.3).

Detailed molecular underpinnings of this process is described in the immune microenvironment chapter of this section.

1.11 Genomic Instability

Genomic instability as a distinct enabling characteristics was added to the original six hallmarks in 2011 [2]. The success of all the hallmarks of cancer depends profoundly on the underlying genomic alterations, directly or indirectly. Through multistep gain of mutations, the cancer cell clones get a survival advantage to become more autonomous. This phenomenon is nurtured and amplified by inactivation of

genome maintenance system to detect and resolve these defects, either due to somatic alterations (e.g., epigenetic alterations in DNA mutation and histone modifications) or inherited mutations (e.g., TP53 (Li-Fraumeni syndrome) or Lynch (HNPCC) syndrome due to mutations in the mismatch repair (MMR) genes). Broad categories that are part of this genome maintenance system include genes that produce proteins involved in detecting DNA damage and activating the repair machinery, proteins directly repairing damaged DNA, and proteins inactivating or intercepting mutagenic molecules before they have damaged the DNA [54, 55].

Genomic instability is acquired due to the variable loss of these functions. TP53 gene pauses the cell function when there is DNA damage to allow DNA repair to occur. TP53 gene mutations are most frequently observed in many tumors described in the sections above, leading to further accumulation of mutations. DNA MMR deficiency is another mechanism that leads to the accumulation of numerous mutations (hypermutator phenotype) producing many tumors in many organs (e.g., proximal colonic adenocarcinomas, endometrial carcinomas). Loss-of-function mutations in genes encoding nucleoside excision repair system lead to inability to repair UV radiation induced cross-linking of pyrimidine residues thwarting replication, as seen in xeroderma pigmentosum. Defects in the homologous recombination DNA repair system constitute a group of disorders that includes Bloom syndrome, ataxia-telangiectasia, and Fanconi anemia. BRCA1 and BRCA2 involved in DNA repair are mutated in familial breast and ovarian cancers, prostate cancers to name a few. DNA polymerase mutations lead to loss of proofreading capabilities that lead to genomic instability, as noted in some colonic and endometrial carcinomas. Finally, many lymphoid neoplasms show a high level of genomic instability due to errors occurring during immunoglobulin and T cell receptor gene rearrangements (RAG1, RAG2, activation-induced cytosine deaminase).

1.12 Cancer-Enabling Inflammation

In pathology literature, observations of dense inflammatory infiltrate in certain solid tumors have been described for many decades. The density of the infiltrate, from dense to very few noticeable cells, as well as the composition of cells—acute vs. chronic inflammatory cells (innate and adaptive), vary in different morphologic subtypes. Such immune response was thought to be a favorable phenomenon of the immune system attempting to eliminate tumor cells, as described above, shaping the newer clones of tumor cells that eventually escape destruction. But there is enough evidence that the inflammatory response may have a paradoxical effect of enabling cancer progression [2]. Inflammation enables several hallmarks of cancer by having a direct effect on the tumor cells as well as modifying the tumor microenvironment described in the latter part of this section. Few key characteristics include the release of factors that promote proliferation, removal of growth suppressors, enhanced resistance to cell death, inducing angiogenesis, activating invasion and metastasis, and evading immune destruction [17].

1.13 Conclusion

Cancer development is a complex biological process that is underpinned by the multistep acquisition of molecular genetic aberrations imparting the above-described eight hallmarks of cancers and two enabling traits. These are gained either through small changes such as single nucleotide polymorphisms, insertions/deletions, and amplification or through large structural genetic changes such as translocations, aneuploidy, and genomewide methylation, to name a few. These changes confer a survival advantage and ability to invade, recur, and metastasize to distant sites. Several molecularly targeted therapies are currently available that are the standard of practice, while some molecular markers part of AJCC cancer staging. Understanding the molecular basis of cancer will further help healthcare professionals, researchers design assays for diagnostic, prognostic, and predictive purposes and the pharmaceutical industry to discover newer targeted therapeutics to pursue personalized precision medicine.

References

1. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100(1):57–70. https://doi.org/10.1016/s0092-8674(00)81683-9.
2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74. https://doi.org/10.1016/j.cell.2011.02.013.
3. Westermark B. Platelet-derived growth factor in glioblastoma-driver or biomarker? Ups J Med Sci. 2014;119(4):298–305. https://doi.org/10.3109/03009734.2014.970304.
4. Xie Y, Su N, Yang J, Tan Q, Huang S, Jin M, et al. FGF/FGFR signaling in health and disease. Signal Transduct Target Ther. 2020;5(1):181. https://doi.org/10.1038/s41392-020-00222-7.
5. Hynes NE, MacDonald G. ErbB receptors and signaling pathways in cancer. Curr Opin Cell Biol. 2009;21(2):177–84. https://doi.org/10.1016/j.ceb.2008.12.010.
6. Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell. 2010;141(7):1117–34. https://doi.org/10.1016/j.cell.2010.06.011.
7. Perona R. Cell signalling: growth factors and tyrosine kinase receptors. Clin Transl Oncol. 2006;8(2):77–82. https://doi.org/10.1007/s12094-006-0162-1.
8. Witsch E, Sela M, Yarden Y. Roles for growth factors in cancer progression. Physiology (Bethesda). 2010;25(2):85–101. https://doi.org/10.1152/physiol.00045.2009.
9. Consortium, A. P. G. AACR project GENIE: powering precision medicine through an international consortium. Cancer

Discov. 2017;7(8):818–31. https://doi.org/10.1158/2159-8290.CD-17-0151.
10. Davies MA, Samuels Y. Analysis of the genome to personalize therapy for melanoma. Oncogene. 2010;29(41):5545–55. https://doi.org/10.1038/onc.2010.323.
11. Guo YJ, Pan WW, Liu SB, Shen ZF, Xu Y, Hu LL. ERK/MAPK signalling pathway and tumorigenesis. Exp Ther Med. 2020;19(3):1997–2007. https://doi.org/10.3892/etm.2020.8454.
12. Osaki M, Oshimura M, Ito H. PI3K-Akt pathway: its functions and alterations in human cancer. Apoptosis. 2004;9(6):667–76. https://doi.org/10.1023/B:APPT.0000045801.15585.dd.
13. Yuan TL, Cantley LC. PI3K pathway alterations in cancer: variations on a theme. Oncogene. 2008;27(41):5497–510. https://doi.org/10.1038/onc.2008.245.
14. Magrath I. Epidemiology: clues to the pathogenesis of Burkitt lymphoma. Br J Haematol. 2012;156(6):744–56. https://doi.org/10.1111/j.1365-2141.2011.09013.x.
15. Patterson KI, Brummer T, O'Brien PM, Daly RJ. Dual-specificity phosphatases: critical regulators with diverse cellular targets. Biochem J. 2009;418(3):475–89. https://doi.org/10.1042/bj20082234.
16. Tan MH, Mester JL, Ngeow J, Rybicki LA, Orloff MS, Eng C. Lifetime cancer risks in individuals with germline PTEN mutations. Clin Cancer Res. 2012;18(2):400–7. https://doi.org/10.1158/1078-0432.CCR-11-2283.
17. Kumar V, Abbas AK, Aster JC, Turner JR, Robbins SL, Cotran RS. Robbins & Cotran pathologic basis of disease; 2021.
18. Collado M, Serrano M. Senescence in tumours: evidence from mice and humans. Nat Rev Cancer. 2010;10(1):51–7. https://doi.org/10.1038/nrc2772.
19. Knudson AG. Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci. 1971;68(4):820–3. https://doi.org/10.1073/pnas.68.4.820.
20. Robinson DR, Wu YM, Lonigro RJ, Vats P, Cobain E, Everett J, et al. Integrative clinical genomics of metastatic cancer. Nature. 2017;548(7667):297–303. https://doi.org/10.1038/nature23306.
21. Curto M, Cole BK, Lallemand D, Liu CH, McClatchey AI. Contact-dependent inhibition of EGFR signaling by Nf2/Merlin. J Cell Biol. 2007;177(5):893–903. https://doi.org/10.1083/jcb.200703010.
22. Okada T, Lopez-Lago M, Giancotti FG. Merlin/NF-2 mediates contact inhibition of growth by suppressing recruitment of Rac to the plasma membrane. J Cell Biol. 2005;171(2):361–71. https://doi.org/10.1083/jcb.200503165.
23. Ikushima H, Miyazono K. TGFbeta signalling: a complex web in cancer progression. Nat Rev Cancer. 2010;10(6):415–24. https://doi.org/10.1038/nrc2853.
24. Massague J. TGFbeta in cancer. Cell. 2008;134(2):215–30. https://doi.org/10.1016/j.cell.2008.07.001.
25. Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol. 2007;35(4):495–516. https://doi.org/10.1080/01926230701320337.
26. Adams JM, Cory S. The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene. 2007;26(9):1324–37. https://doi.org/10.1038/sj.onc.1210220.
27. Lowe SW, Cepero E, Evan G. Intrinsic tumour suppression. Nature. 2004;432(7015):307–15. https://doi.org/10.1038/nature03098.
28. Saha S, Panigrahi DP, Patil S, Bhutia SK. Autophagy in health and disease: a comprehensive review. Biomed Pharmacother. 2018;104:485–95. https://doi.org/10.1016/j.biopha.2018.05.007.
29. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med. 1997;3(7):730–7. https://doi.org/10.1038/nm0797-730.
30. Burnet Sir M. Intrinsic mutagenesis: a genetic approach to ageing. Dordrecht: Springer Netherlands; 2012.
31. Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. Exp Cell Res. 1961;25:585–621. https://doi.org/10.1016/0014-4827(61)90192-6.
32. Heidenreich B, Rachakonda PS, Hemminki K, Kumar R. TERT promoter mutations in cancer development. Curr Opin Genet Dev. 2014;24:30–7. https://doi.org/10.1016/j.gde.2013.11.005.
33. Maciejowski J, de Lange T. Telomeres in cancer: tumour suppression and genome instability. Nat Rev Mol Cell Biol. 2017;18(3):175–86. https://doi.org/10.1038/nrm.2016.171.
34. Batlle E, Clevers H. Cancer stem cells revisited. Nat Med. 2017;23(10):1124–34. https://doi.org/10.1038/nm.4409.
35. Zee YK, O'Connor JP, Parker GJ, Jackson A, Clamp AR, Taylor MB, et al. Imaging angiogenesis of genitourinary tumors. Nat Rev Urol. 2010;7(2):69–82. https://doi.org/10.1038/nrurol.2009.262.
36. Baeriswyl V, Christofori G. The angiogenic switch in carcinogenesis. Semin Cancer Biol. 2009;19(5):329–37. https://doi.org/10.1016/j.semcancer.2009.05.003.
37. Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. Nat Rev Cancer. 2003;3(6):401–10. https://doi.org/10.1038/nrc1093.
38. Patenaude A, Parker J, Karsan A. Involvement of endothelial progenitor cells in tumor vascularization. Microvasc Res. 2010;79(3):217–23. https://doi.org/10.1016/j.mvr.2010.01.007.
39. Menakuru SR, Brown NJ, Staton CA, Reed MW. Angiogenesis in pre-malignant conditions. Br J Cancer. 2008;99(12):1961–6. https://doi.org/10.1038/sj.bjc.6604733.
40. Raica M, Cimpean AM, Ribatti D. Angiogenesis in pre-malignant conditions. Eur J Cancer. 2009;45(11):1924–34. https://doi.org/10.1016/j.ejca.2009.04.007.
41. Sporn MB. Approaches to prevention of epithelial cancer during the preneoplastic period. Cancer Res. 1976;36(7 PT 2):2699–702. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1277177.
42. Ryan BM, Faupel-Badger JM. The hallmarks of premalignant conditions: a molecular basis for cancer prevention. Semin Oncol. 2016;43(1):22–35. https://doi.org/10.1053/j.seminoncol.2015.09.007.
43. Talmadge JE, Fidler IJ. AACR centennial series: the biology of cancer metastasis: historical perspective. Cancer Res. 2010;70(14):5649–69. https://doi.org/10.1158/0008-5472.CAN-10-1040.
44. Akhtar M, Haider A, Rashid S, Al-Nabet A. Paget's "seed and soil" theory of cancer metastasis: an idea whose time has come. Adv Anat Pathol. 2019;26(1):69–74. https://doi.org/10.1097/PAP.0000000000000219.
45. Langley RR, Fidler IJ. Tumor cell-organ microenvironment interactions in the pathogenesis of cancer metastasis. Endocr Rev. 2007;28(3):297–321. https://doi.org/10.1210/er.2006-0027.
46. Riggi N, Aguet M, Stamenkovic I. Cancer metastasis: a reappraisal of its underlying mechanisms and their relevance to treatment. Annu Rev Pathol. 2018;13:117–40. https://doi.org/10.1146/annurev-pathol-020117-044127.
47. DeBerardinis RJ, Chandel NS. Fundamentals of cancer metabolism. Sci Adv. 2016;2(5):e1600200. https://doi.org/10.1126/sciadv.1600200.
48. Koppenol WH, Bounds PL, Dang CV. Otto Warburg's contributions to current concepts of cancer metabolism. Nat Rev Cancer. 2011;11(5):325–37. https://doi.org/10.1038/nrc3038.
49. Collins RRJ, Patel K, Putnam WC, Kapur P, Rakheja D. Oncometabolites: a new paradigm for oncology, metabolism, and the clinical laboratory. Clin Chem. 2017;63(12):1812–20. https://doi.org/10.1373/clinchem.2016.267666.
50. Ribatti D. The concept of immune surveillance against tumors. The first theories. Oncotarget. 2017;8(4):7175–80. https://doi.org/10.18632/oncotarget.12739.

51. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science. 2015;348(6230):69–74. https://doi.org/10.1126/science.aaa4971.
52. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3(11):991–8. https://doi.org/10.1038/ni1102-991.
53. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature. 2013;500(7463):415–21. https://doi.org/10.1038/nature12477.
54. Ciccia A, Elledge SJ. The DNA damage response: making it safe to play with knives. Mol Cell. 2010;40(2):179–204. https://doi.org/10.1016/j.molcel.2010.09.019.
55. Negrini S, Gorgoulis VG, Halazonetis TD. Genomic instability—an evolving hallmark of cancer. Nat Rev Mol Cell Biol. 2010;11(3):220–8. https://doi.org/10.1038/nrm2858.