An Update to Hallmarks of Cancer

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Abstract
In the last decade, there has been remarkable progress in research toward understanding and refining the hallmarks of cancer. In this review, we propose a new hallmark - "pro-survival autophagy." The importance of pro-survival autophagy is well established in tumorigenesis, as it is related to multiple steps in cancer progression and vital for some cancers. Autophagy is a potential anti-cancer therapeutic target. For this reason, autophagy is a good candidate as a new hallmark of cancer. We describe two enabling characteristics that play a major role in enabling cells to acquire the hallmarks of cancer - "tumor-promoting microenvironment and macroenvironment" and "cancer epigenetics, genome instability and mutation." We also discuss the recent updates, therapeutic and prognostic implications of the eight hallmarks of cancer described by Hanahan et al. in 2011. Understanding these hallmarks and enabling characteristics is key not only to developing new ways to treat cancer efficiently but also to exploring options to overcome cancer resistance to treatment.

Introduction And Background
The transformation of a normal cell into a neoplasm is a complex process. Hanahan et al. summarized hallmarks of cancer including six core hallmarks, two emerging hallmarks and two enabling characteristics [1]. The bidirectional communication between a cancer cell and tumor microenvironment (TME) and macroenvironment creates a tumor-supportive environment and premetastatic niche, which helps cancer cell to develop, grow and metastasize [2]. In the last decade, there has been tremendous progress in research to understand the hallmarks of cancer. Hanahan et al. emphasized the importance of autophagy, independently or in association with apoptosis, as a barrier that needs to be avoided by cancer cells to reach efficient tumorigenesis, as part of resisting cell death hallmark of cancer [1]. Although they call attention to the cytoprotective effect of autophagy on cancer cells in the face of nutrient starvation and toxic effects of radiotherapy and chemotherapy, possibly leading them to a state of dormancy [1], little was known at that time about the role of autophagy in tumor progression and if this could be seen as another hallmark of cancer. Since then, evidence had emerged on the pro-survival role of autophagy in cancer cells. In this review, we first proposed a new hallmark - pro-survival autophagy. Then, we modified two enabling characteristics that play a major role in enabling cells to acquire the hallmarks of cancer and discussed the recent updates, therapeutic and prognostic implications of the eight hallmarks of cancer described by Hanahan et al. in 2011 (Figure 1) [1].
Review

New hallmark of cancer

Pro-survival Autophagy

Autophagy is an important process in cell death, conservation of protein homeostasis and maintenance of normal organelle function, as it removes damaged structures under cell environment stress conditions. Autophagy can occur in three different pathways: chaperone-mediated autophagy (with the participation of intermediate ligand chaperone proteins like HSP70s), microautophagy (direct engulfment of small cytoplasmic structures by lysosomes), and macroautophagy (where cytoplasmic components are sequestered into the autophagosome and degraded on lysosomes) [3].

Autophagy is essential for oncogenic K-Ras-induced malignant cell transformation in human breast epithelial cells, as the mRNA protein levels of ATG5 and ATG7 (autophagy-specific genes) were increased in cells overexpressing K-Ras and that targeted suppression of these genes inhibited cell growth and tumor formation [4]. Tan and colleagues observed that autophagy was increased in hypoxic regions of tumors in three different human tumor cell lines and hypoxia-induced cell death was more rapid in their autophagy-deficient variants with shRNA knockdown of the genes ATG7 and BECLIN1 [5]. Amid situations of metabolic...
stress in tumors, autophagy is required to maintain the pool of functioning mitochondria through recycling damaged or nonfunctional intracellular material. This process ensures the supply of nutrients (like amino acids and fatty acids) and ATP, needed for tumor growth [6]. Deficiency in autophagy leads to the accumulation of abnormal mitochondria, lack of ATP, and key tricarboxylic acid (TCA)-cycle intermediates, resulting in mitochondrial dysfunction. Mitochondrial dysfunction leads to the generation of toxic reactive oxygen species (ROS) and mitochondrial damage [7] and is an important process in pancreatic tumor growth [8], as well as liver tumor formation in ATG5 and ATG7 deficient mice [9].

Although not totally clear, some studies suggest a positive correlation between autophagy and epithelial-mesenchymal transition (EMT), which is needed in cancer progression and metastasis, probably via p62 (an autophagy adaptor protein) and tumor growth factor β (TGF-β), which is the most important regulator of EMT in human cancers [10]. Knockdown of BECLIN1, an important autophagy activator, was shown to suppress EMT in colon cancer [11], and its activation via phosphorylation by ULK2 promotes EMT in lung cancer cells [12].

Because autophagy facilitates cancer cells' survival in an environment with hypoxia and metabolic stress, it reduces tumor necrosis and consequently the infiltration of macrophages in the primary tumor, that is a required step for metastasis [10]. Also, autophagy provides tumor cells the ability to avoid anoikis, a process of detachment-induced programmed cell death, resulting from loss of, or inappropriate cell adhesion from the extracellular matrix (ECM) via RNA-like endoplasmic reticulum kinase (PERK), that promotes autophagy and ROS detoxication in mammary epithelial cells [13], upregulation of BNIP3 mediated by ERK3/HIF-1α pathway induced autophagy by suppressing mTOR/S6K1 [14] and deficiency of miRNA-30a, a tumor suppressor that targets Beclin-1 and ATG5 in hepatocellular carcinoma [15]. Anoikis resistance also occurs via the regulation of SDCBP/MDA-9/Syntenin in gliomas [16].

Cancer stem cells (CSCs) that play an important role in tumor recurrence and resistance to anti-neoplastic treatment strategies, seem to have a role in tumor maintenance and function related to autophagy. Some autophagy markers like ATG5, ATG12, and LC3B were found to be overexpressed in dormant stem cell-like breast cancer cells [17] and BECLIN1 staining areas were found to be surrounded by actively proliferating cells [18]. In pancreatic cancer, HIF-1α and autophagy regulate the balance between CSCs and non-CSCs [19]. A subset of CSCs showed resistance to chemotherapy and was associated with a high autophagy activity [20].

Autophagy, as an important survival mechanism against various cellular stresses, can induce resistance to various anti-cancer therapeutic agents (cytotoxic chemotherapy, radiotherapy, molecular target agents and antiangiogenic agents) by reducing ROS damage, blocking apoptosis, and maintaining the CSC pool [10]. Some therapeutic agents like imatinib and paclitaxel have already known mechanisms of resistance related to autophagy [21,22].

The better comprehension of pro-survival autophagy leads to studies assessing classes of therapeutic anti-cancer targets in this field, most of them with preclinical evidence, but some are still ongoing clinical trials. One of them are class III isoform of phosphoinositide 3 kinase (PI3K), that induces autophagy by generating PI3-phosphate, needed for the formation of autophagosome membrane [23]. Wortmannin, a covalent irreversible binding inhibitor of PI3K have demonstrated enhanced response when associated to cisplatin in uterine cancer [24], and spautin-1, which blocks autophagy by deubiquitinating Beclin-1, improved imatinib-induced apoptosis in chronic myeloid leukemia [25]. The ULK1 inhibitor SBI-0206965 attenuated cell survival in non-small cell lung cancer tumor cells [26], reduced tumor growth and metastasis in neuroblastoma cells [27] and exhibits a potential anticancer effect against clear cell renal carcinoma [28]. ATG4 inhibitors NSC185058 and Ticonazoloe, respectively, exhibit anti-tumor effect in osteosarcoma tumor models in vivo [29] and enhance the cytotoxicity of chemotherapeutic drugs, suppressing tumor viability [30]. Hydroxychloroquine (HCQ) attenuates lysosomal acidification, resulting in obstruction of the activity of the lysosomal degradative enzyme and inhibiting autophagy, but at a high concentration with greater cytotoxicity [31]. Lys05, a potent autophagy inhibitor derivative of HCQ demonstrated anti-tumor activity [32]. Bafilomycin-A1 specifically obstructs V-ATPases and blocks autophagy flux by inhibiting lysosomal acidification, facilitating cell cycle arrest and caspase-dependent apoptotic cell death in colon cancer [33].

The importance of pro-survival autophagy is well established in tumorigenesis, as it is related with a lot of events in cancer progression and critical for some of them in various types of cancers. Besides that, autophagy is a potential anti-cancer therapeutic target. For this reason, autophagy is a good candidate for a new hallmark of cancer.

**Enabling characteristics**

_Tumor Promoting Microenvironment and Macromenvironment_

Hanahan et al included tumor-promoting inflammation as one of the enabling characteristics of cancer [1]. Most tumors trigger a tumor-promoting inflammatory response [34] which is recognized as the seventh hallmark of cancer [35]. Inflammation plays a major role in every step of cancer development [34,36].
Tumor initiation: The inflammatory microenvironment increases mutations and genetic instability either by producing ROS and reactive nitrogen intermediates or via cytokines that stimulate ROS [34,36]. Inflammatory cytokines express activation-induced cytidine deaminase and inhibit p53 thereby increasing genetic instability and mutations [34]. Inflammation promotes stem cell expansion [34].

Tumor promotion: Inflammatory response induces genes promoting cell proliferation and survival [34]. Tumor associated macrophages in response to hypoxia produce vascular endothelial growth factor (VEGF) promoting neo-angiogenesis [34,36].

Tumor metastasis and invasion: Inflammatory myeloid cells and cancer cells produce tumor growth factor β that helps in EMT and metastasis [34]. Inflammatory cells produce proteases to facilitate proteolysis, that is required for invasion [34,36]. Survival of metastatic seeds is assisted by cytokines [34]. Inflammation also upregulates adhesion molecules that facilitate the attachment of metastatic cells in target organs [34].

The role of several inflammatory cytokines as prognostic markers is being studied. Higher levels of IL1 β, IL1Ra, IL18, and IL1α in breast cancer tissues and significantly higher IL1 β levels in stage II, III or IV breast cancers were reported [37]. Higher levels of IL-17+ and IL-22+ T lymphocytes are associated with the progression of basal-cell and squamous cell skin cancers [38]. Targeting these inflammatory cytokines might have a potential therapeutic effect in these cancers.

The inflammatory cells acquire pro Vs antitumor capabilities depending on the interaction with TME and the macroenvironment. In addition to tumor-promoting inflammation, there are several studies in the last decade highlighting the bidirectional interaction between a cancer cell and microenvironment as well as macroenvironment that is key for a cancer cell to develop, survive, progress, and invade. Tumor exosomes play a major role in communication with TME and the macroenvironment to promote tumor growth and metastasis. Tumor exosomes modify stroma and immune cells creating a metastatic niche supporting the seeding of tumor cells [39].

Tumor macroenvironment also referred to as tumor organismal environment includes metabolic, endocrine, lymphatic, hematopoietic, immunologic, microbiotic and neurogenic environments [40]. Metabolic imbalance in the macroenvironment seen in two major metabolic disorders, diabetes and obesity, is shown to be associated with promoting tumor development [40]. Hormonal changes in the endocrine macroenvironment impact tumor growth and tumor-promoting inflammation [2]. Tumors also control the endocrine environment via paraneoplastic syndromes [41]. The lymphatic environment helps tumors communicate with systemic circulation [40]. Hematopoietic and immunologic environments influence bone marrow thereby facilitating tumors to suppress antitumor immunity [40]. Recent studies demonstrated the role of gut microbiota in inflammation-promoting tumorigenesis. Gut microbiota promotes tumor progression via the production of certain inflammatory cytokines such as TNFα, IL-6, and IL-17 [2]. Gut microbiota dysbiosis was reportedly related not only to local inflammation-promoting tumor development but also distant cancer such as hepatocellular carcinoma and breast carcinoma and liver metastases in lymphoma [40]. Further studies are required to investigate the effect of modifying gut microbiota dysbiosis in promoting antitumor immunity. The neurogenic environment is particularly gaining interest recently. Zhao et al demonstrated that denervation suppresses gastric tumorigenesis [42]. Furthermore, the concept that adrenergic nerves drive tumor angiogenesis was recently described [43]. Tumor exosomes are reported to control tumor-promoting neurogenesis and glumatine released from local neurites is reported to facilitate breast to brain metastases [40]. Sympathetic and parasympathetic signaling showed a role in the growth of prostate cancer [40].

Cancer Epigenetics, Genome Instability and Mutation

Epigenetics is defined as heritable modifications in gene expression induced via changes in chromatin structure barring adjustments of DNA sequence [44,45]. Accumulating evidence indicates that, without genetic alterations, epigenetic mechanisms are implicated in the acquisition of malignant phenotype [46]. Epigenetic alterations interact at all levels of the development of most cancers, working collectively to promote cancer progression [47]. The genetic foundation of cancer is widely accepted; however, recent research proposes that epigenetic changes may also be a key initiating events in some types of cancer [48].

Epigenetic mechanisms that regulate chromatin structure can be divided into four essential categories - DNA methylation, covalent histone modifications, non-covalent mechanisms such as the incorporation of histone variants and nucleosome remodeling and non-coding RNAs together with microRNAs (miRNAs) [49,50].

The altered epigenetics of most cancers cells suggests that epigenetic therapies should have a fundamental clinical impact [47]. The main question remains if we can use our understanding of the epigenetic regulators to find so-called synthetic vulnerabilities that would give us new therapeutic possibilities in the treatment of cancer?

In 2011, Hanahan and Weinberg added genome instability into their list of fundamental characteristics of
cancer, particularly as an enabling characteristic [1]. The result of the failure of crucial teams of proteins that protect the DNA of the genome from being mutationally corrupted, rearranged, and re-programmed is 'oncogenic' mutations that convey on cancer cells various hallmark capabilities. Very recently, Pan-cancer Analysis of Whole Genomes (PCAWG), an international collaboration to identify common patterns of mutation, published an analysis of 2,658 whole cancer genomes across 38 tumor types (https://www.nature.com/collections/afdejfafdb/Feb2020). One of the major studies of this project shows that each tumor had four or five driver mutation on average and at least one driver mutation was found in about 95% of the tumor samples compared with just 67% with exome sequencing [51]. Cancer develops through a process of somatic evolution [52]. Early oncogenesis is characterized by mutations in a constrained set of driver genes and specific copy number gains [52]. However, the mutational spectrum changes significantly throughout tumor evolution in 40% of samples. New patterns of mutations were reported that result from environmental exposures such as tobacco smoke [53]. Endogenous sources of mutation and epigenomic features promote genomic instability during cancer evolution [54]. Recent work has shown that even in repair-sufficient cells, endogenous and oncogenic stress can occasionally overwhelm the normal genome maintenance pathway [54].

Solid tumors of epithelial origin with extreme levels of genomic instability are associated with a potentially better prognosis compared with intermediate level [55]. Also, cancers with extreme levels of genomic instability may be teetering on the brink of a threshold where so much of their genome is adversely altered that cells rarely replicate successfully [5] these cancers are more immunogenic than other cancers with a less extensive burden of genetic aberrations. Pua et al. reviewed studies that established a link between inflammation and the pathophysiology of cancer, and this stems largely from the genomic instability that results from inflammatory cytokines and signaling [56]. Inflammation leads to suppression of cell cycle arrest and apoptosis, further allowing the proliferation of mutant cells.

Recent fast progress in CRISP/Cas9 base editing technology has made it technically highly feasible to generate site specific nucleotide substitutions of DNA by manipulating highly intricate DNA repair pathway [57].

### An update to 2011 hallmarks of cancer

Sustaining Proliferative Signaling

One primary hallmark described by Hanahan et al is the cell’s ability to become auto-sufficient and enable signals to sustain a continuous proliferative pathway [1]. Some messages can be extracellular growth signals, which bind to transmembrane receptors or transcellular and intracellular stimuli [1]. The mitogenic signals and regulation are complex, and the activity seems to be controlled from one cell to the others as paracrine signaling [1]. In cancer cells, this process is better comprehended, and mutant cells obtain the ability to replicate in many ways [58-61].

The mitogenic stimulus can be generated by an upstream receptor pathway or by downstream and intracellular circuits.

Upstream circuits: Tumor cells may induce the surrounding cells to support their growth with various growth factors [62,63]. The cancer cell may have the ability to increase the number of displayed transmembrane receptors. It will make them hyper-responsive to GF ligands and increase the proliferative rate. These receptors usually have intracellular tyrosine kinase actions. For example, EGF-R/erbB in Breast, stomach, and brain tumors. A structural modification can make these ligand-independent receptors easily firing. For example, some truncated versions of the EFG missing part of their cytoplasmic domain fire continuously [61].

Downstream cytoplasmic pathways may be GF independent and result in continuous activation of proliferation.

a. Somatic mutations trigger more downstream circuits: As shown by Davies and Samuels, 40% of melanomas have some mutations in B-Raf protein result in continuous signaling generated by the activation of Raf to MAP- kinase (mitogen-activated protein). Mutations in phosphoinositide 3-Kinase (PI3-kinase) isoforms may hyper-stimulate the signaling pathway [64,65].

b. Disturbances of Negative-Feedback mechanisms that attenuate proliferative signaling: Antiproliferative signals control and maintain cell homeostasis by inhibition of proliferation [66-69]. Failure in a negative feedback mechanism will allow the cell to replicate indefinitely. For example, the Ras oncoprotein: does not increase the activity of the signal. Instead, it compromises an enzyme action, the Ras GTPase, which is responsible for transitory signal in the Ras pathway. The mutation compromises the negative feedback and results in continuous activation and signal transduction. The PTEN phosphatase counter-acts PI3K Kinase. If PTEN presents a loss-of-function mutation, it may result in the amplification of PI3K signaling, leading to tumorigenesis. mTOR kinase results in negative feedback and inhibition of PI3K signal. The repression of mTOR will result in increased PI3K activity and its effector Akt/PKB, causing dampening in the antimitotic
Many critical cell proliferation pathways involve miRNAs, dysregulation of which is responsible for evading growth suppressors. Micro-RNAs (miRNAs) are known to regulate the expression of genes in breast cancer and prostate cancer cells and expression of BRCA 1 and BRCA 2 in breast cancer cells. Melatonin was found to induce phosphorylation of p53 at Ser-15 causing proliferation inhibition and cell cycle arrest at the G1 phase, up-regulation of nuclear p21, and hypophosphorylation of Rb. Several other mechanisms, directly or not linked to pRb and p53 pathways, positively or negatively related to antiproliferative control, emerged in the last years and confirm the evasion of growth suppressors as an actual hallmark of cancer, helping to understand widely the complexity of this system, which can be a potential therapeutic target in many types of cancers.

Evading Growth Suppressors

Cancer cell needs to avoid antigrowth signals to thrive. Growth suppression resides centrally in pRb and p53, which operate as part of a large network that is inter-ligated in a way that favors redundancy and more efficient control. Tumor cells escape these antiproliferative control by loss of function of these genes or alterations in response to their close regulators as TGFβ or immediate downstream targets of its action, as CDK4 and its inhibitory proteins and contact inhibition of cell growth, like NF2 / Merlin and LKB1 epithelial polarity protein, that when disrupted can also facilitate uncontrolled proliferation as seen in cancer tissues.

Alternative Reading Frame (ARF)

ARF is a tumor suppressor protein encoded in the INK4b/ARF/INK4a gene locus located on chromosome 9p21 in humans that activate p53 in response to oncogenic signals, such as c- MYC. Although transcriptional regulation of ARF has been known since the late 1990s, most of its post-translational regulation have emerged after 2004, that this protein could be ubiquitinated and degraded via proteasomal degradation and the enzymes involved in this process like ULF, SIVA1 and MKRN1 were discovered only after 2010. Besides that, ARF can also suffer lysosomal degradation mediated by CHIP and HSP90. Inhibition of HSP90 by geldanamycin can induce cell growth retardation and cellular senescence in human normal cells and mouse embryonic fibroblasts. De-ubiquitination of ARF by USP10, which results in stabilization of ARF and promotes cellular senescence.

The low expression of ARF mRNA is frequently observed in human cancers and is usually caused by hypermethylation on the CpG island of the ARF promoter or deletion of the genetic region and has been described in breast, bladder, colon, liver, gastric, lung, oral, prostate and brain cancers and has emerged as a predictor of poor prognosis in breast, head and neck, colon and bladder carcinomas.

Galectins

Glycosylation changes had emerged as an important process in cancer progression and among the glucan-binding proteins that deciphers the information encrypted by the glycoma, galectins had great importance. Gal7 showed to be a proapoptotic Galectin induced by TP53 in colorectal and urothelial cancer. Gal7 expression can be silenced by methylation of CpG islands in the LGALS7 gene and hypermethylation at a region of the exon2 that is predicted to be a TP53-binding. Gal7 expression can be silenced by methylation of CpG islands in the LGALS7 gene and hypermethylation at a region of the exon2 that is predicted to be a TP53-binding. Nevertheless, the overexpression of Gal7 had a tumor-promoting behavior in thymic lymphoma and breast cancer, a paradoxical effect that could be explained by the observation that both NF-κB-binding and TP53 transcription factors can control its expression. In breast cancer cell lines, WT and mutant TP53 increased NF-κB activity and up-regulated Gal7 expression. Conversely, in a p53-null cell line, with high NF-κB activity, Gal7 undetectable, suggesting NF-κB-TF53 complex to be required to the LGALS7 promoter activation. Reciprocal regulation between Gal7 and TP53 was also proposed when observed that Gal7 was able to impede TP53 translocation to the nucleus. Another galectin, Gal3, was shown to be repressed by TP53, with increased expression of Gal3 been observed in p-53 mutant tumors. In human prostate cancer cells, knockdown of Gal3 promoted a cell cycle arrest at the G1 phase, up-regulation of nuclear p21, and hypophosphorylation of Rb.

Melatonin

Melatonin was found to induce phosphorylation of p53 at Ser-15 causing proliferation inhibition and prevention of DNA damage accumulation. The treatment with melatonin resulted in increased p53 expression in breast cancer and prostate cancer cells and expression of BRCA 1 and BRCA 2 in breast cancer cells.

Micro-RNAs (miRNAs)

Many critical cell proliferation pathways involve miRNAs, dysregulation of which is responsible for evading
The ability of tumoral cells to achieve replicative immortality, allowing subsequently macroscopic growth, developed through analysis of apoptotic signaling pathways and apoptosis resistance mechanisms.

In conclusion, new drug targets can be identified and target selective therapeutic methods could be developed through analysis of apoptotic signaling pathways and apoptosis resistance mechanisms.

Enabling Replicative Immortality

The ability of tumoral cells to achieve replicative immortality, allowing subsequently macroscopic growth,
has been widely accepted as a hallmark of cancer [1]. Several studies point out the central role of telomeres maintenance necessary to bypass the natural cell aging process [118,119]. The gradual telomere shortening, which occurs after multiple cell divisions, regulates the life span of the cells and their capacity for replication [120]. When a telomere becomes too short and reaches a critical length, usually after 50 to 60 cell cycles, the cell senescence is induced [119,121]. Subsequently, genes like p53, p21 and Rb/p16 INK4A that are associated with growth cell arrest and apoptosis, are activated. When a short telomere is not recognized by the cell cycle arrest checkpoint, it will undergo further shortening and DNA damage proteins will be activated, leading to homologous recombination or non-homologous end joining of the chromosome, generating thereby aberrant chromosomes. Such a cell with unstable DNA is defined in the crisis phase and normally undergoes apoptosis. This process of telomere shortening inducing senescence or crisis/apoptosis in normal cells is considered a sort of natural tumor-suppressing mechanism.

In carcinogenesis, cells can activate mechanisms of telomere maintenance to overcome the cell senescence or apoptosis caused by telomere shortening. Several mechanisms of telomere maintenance have been identified and include telomerase gene hTERT promoter mutations [122], telomerase reactivation, oncogenes and tumoral suppressor genes mutations, alternative lengthening of telomeres (ALT) - telomerase independent mechanism [123].

According to data, in 85%-90% of cancer cells, the mechanism of telomere stabilization is reached by telomerase activation and only 5%-15% exhibit an ALT pathway [124-126].

Despite the upregulation of telomere reverse transcriptase (TERT) expression via promoter mutation [127-132] and telomerase activation in most malignant cells, telomeres in cancer are shorter than those in normal tissues: as several studies show, 90% of cancer cells contain short telomeres and high levels of telomerase activity. In fact, it seems the shorter the telomere is, the higher the cancer aggressiveness, and the poorer the prognosis [132,133]. For example, 75% of oral carcinomas, 80% of lung cancers, 84% of prostate cancers, 85% of liver cancers, 93% of breast cancers, 94% of neuroblastomas, 95% of colorectal cancers, and 98% of bladder cancers have detectable telomerase activity [134,135]. Some cancer cell lines keep noticeably short telomeres (prostate PC-3, stomach MRN74 and breast HBC-4 cancer cells) and elongate their telomeres by TERT over-expression [136,137]. Patients with glioblastoma with isocitrate dehydrogenase 1 (IDH1) mutation and ALT activation, which is associated with longer telomeres, showed better clinical outcome than those with ALT negative tumor [135,134,138-140]. Interestingly, TERT or even telomerase activity is inversely correlated with telomere length [135,141].

Huang et al. showed that upon mitogen stimulation, not all but only a small subpopulation of T- cells reactivate telomerase and preferentially elongate short telomeres [142]. It is possible that there are various cell subpopulations with long to short telomeres during cancer development [142]. Furthermore, genomic instability elicited by shortened telomeres might be advantageous to cancer evolution [143]. In fact, induction of chromosomal instability via the telomeric DNA damage response followed by end-to-end fusions promotes oncogenic transformation [144,145]. Telomerase activation confers immortality but not neoplastic properties to cancer cells [135,146], given that malignant transformation requires a multi-step process [135,147].

Nevertheless, should the precise moment of telomeres shortening triggering telomerase activation be known, this could then have major effectiveness in stopping the tumor. This remains a challenge for future researchers [126].

**Inducing Angiogenesis**

Angiogenesis is a physiological process, that determines the formation of new vessels from preexisting ones. Angiogenesis is involved not only in embryonic development but also in damage and recovery. This process is tightly regulated and controlled by different mechanisms. By contrast, in pathological conditions (like cancer) angiogenesis is dysregulated and hyperactivated. The relationship between angiogenesis and cancer was first described in 1968 when several proangiogenic factors were discovered [148,149].

Tumor angiogenesis is a multistep process, and its main generator is hypoxia in tumor cells due to inadequate blood supply. It causes the production of angiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), angiogenin, transforming growth factor α, TGF-β, tumor necrosis factor (TNF)-α etc. by cancer cells, which bind to endothelial cell receptors of the vessels and initiate above mentioned process. Whenever the endothelial cells are stimulated, the secretion of matrix metalloproteinases (MMPs) is prompted, which causes degradation of the basal membrane. This process allows endothelial cells to invade surrounding tissues and start forming new vessels. In addition, factors such as angiotensin-1, -2, and their receptor Tie-2c are needed for the stabilization of newly formed vessels [150,151].

Angiogenesis plays a key role in cancer and according to many studies level of angiogenic factors is correlated with tumor aggressiveness and has a strong predictive role [1,152].
Activating Invasion and Metastasis

Metastasis is a hallmark of cancer and the cause of most cancer-related deaths [1]. It is a multistep process by which tumor cells leave the primary tumor, travel to a distant site, and establish secondary tumors in distant organs (Figure 2) [1,153].

There are a variety of determining factors that govern the flexibility of a primary tumor to metastasize to different organs. These include genetic disorders to growth factors within the environment of the first tumor, the flexibility of tumor cells to detach from neighboring cells, the flexibility of tumor cells to digest the ECM and invade the vasculature. The tumor cells reorganize their cytoskeleton, express adhesion molecules on their surface to acknowledge adhesion sites, and chemotaxis or become migratory and motile resulting in loss of contact inhibition, and ultimately migrate to inappropriate locations giving rise to metastatic dissemination.

In vivo as well as in vitro studies showed that metastatic tumor cells migrate individually [154]. However, in humans, seeding requires the joint action of a cluster of cancer cells moving together, which brings EMT into the picture [155]. EMT program is believed to be a spectrum of transitional stages between the epithelial and mesenchymal phenotypes, in contrast to a progression that includes a binary choice between full-epithelial and full-mesenchymal phenotypes [156].

In recent years, there has been a vital debate on whether EMT features a central role in cancer metastasis and resistance to chemotherapy [157]. Some studies in pancreatic and lung cancers showed that EMT is not essential for metastasis, but it does contribute to chemoresistance. More evidence is required to completely elucidate the role of EMT in cancer progression and the metastatic process [158-160].

Another important thing that researchers are looking at is ECM. ECM is a dynamic and complex system that is composed of a wide spectrum of matrikines and cells that take part in invasion and metastasis [161].

Our deep understanding of the dynamics of this hallmark will help us identify targets for molecular therapies which will halt or possibly reverse cancer growth and metastasis.

Reprogramming of Energy Metabolism

Abnormally high metabolic rates by cancer cells alter anti-tumor immunity by changing TME in the metabolic mechanism of glycolysis or amino acid metabolism. There are studies discussing the crosstalk between energy reprogramming in cancer cells and its association with antitumor immunity, and therefore suggest intervention of cancer metabolic agents provide an add-on benefit to cancer immunotherapy [162].

Glycolysis and Lactate Production

The Warburg effect shows that tumors and cancer cells have increased rates of glucose uptake and lactate production, even in the presence of sufficient oxygen and low rate of oxidative phosphorylation [163].
Metabolic reprogramming in tumor cells causes changes in TME by competing metabolic environment in neighboring T cells, that lead to T cell metabolic exhaustion. Notably, several clinical studies have revealed that aerobic glycolytic activities in humans is negatively correlated with intrinsic antitumor immunity. For example, LDHA (lactic dehydrogenase A)-associated lactic acid accumulation in melanomas inhibits tumor surveillance by T and NK cells, and LDHA mediated lactate production suppresses IFN-γ expression in both tumor-infiltrating and immune evasion murine models [164]. Pyruvate kinase muscle isozyme M2 (PKM2) has a crucial role in sustaining nutrients demands in cancer cell proliferation[165]. Recently, PKM2 has been reported to promote PD-L1 (programmed cell death 1 ligand 1) expression in tumor, and PKM2 activators might synergize with PD-1 (programmed cell death 1)/PD-L1 checkpoint inhibitors to provide suppressive roles to tumor immune escape [166].

Amino Acids Metabolism

Tryptophan (Trp) and arginine (Arg) amino acids are considered to provide key nutrients in TME. Trp attenuates antitumor immunity in primary tumors and the neighboring tumor lymph nodes and Arg catalolism has been linked to suppression of antitumor immunity.

Indoleamine 2,3-dioxygenase 1 (IDO1) enzyme is a rate-limiting enzyme in the metabolism of Trp in the peripheral tissues and IDO inhibitors inhibit the first step of Trp catabolism [167]. IDO1 is overexpressed in human cancer cells, suppresses effector T cell function, and promotes regulatory T cells [168]. Tumor or stromal cells in malignant lesions catabolizing Trp and/or Arg suppress CD8+ effector T cells and stabilize Treg cells to protect tumor cells [169].

PD-1 ligation and activation impairs metabolic reprogramming, including glycolysis and amino acid metabolism in T cells by inducing the expression of carnitine palmitoyl transferase 1A (CPT1A), a rate limiting enzyme of the fatty acid oxidation (FAO) pathway, and conversely, CTLA-4 inhibits glycolysis without augmenting FAO [170]. Arginine inhibitor, INC001158 combined with immune checkpoint inhibitor pembrolizumab, is being studied in advanced or metastatic solid tumors (ClinicalTrials.gov Identifier: NCT02903914). Epacadostat, an IDO1 inhibitor, is being studied in combination with pembrolizumab in patients with metastatic and/or locally advanced sarcoma (NCT05414229). Anti-IDO-1 agent (LY3381916) is also tested in combination with anti- PD-L1 checkpoint antibody (LY3300054) in solid tumors (NCT05345613). Additionally, a phase 2 study is ongoing to evaluate the activity of PD-1 inhibitor, nivolumab alone with and without IDO-inhibitor, BMS-986205, in patients with recurrent or persistent endometrial carcinoma or endometrial carcinosarcoma (NCT04106414). Given great and extensive interests of IDO inhibitors and other metabolic agents, it is expected there will be more clinical studies underway in addressing metabolic intervention in TME to aid on immunotherapy.

Evading Immune Suppression

Evading antitumor immunity plays a major role in tumor progression and survival and must be considered as one of the hallmarks of cancer. Tumor escapes immune destruction by several mechanisms. TGFβ plays a significant role in inhibiting T helper cell differentiation and promoting antitumor immunity [171,172]. Tumor derived factors convert immature myeloid cells into myeloid derived suppressor cells (MDSC) that suppress antitumor immune response [2]. Tumor recruits and educate immune cells such as NK cells, regulatory T cells, dendritic cells, granulocytes, macrophages, and MDSC thereby creating permissive microenvironment [173]. Tumor modulates spleen and bone marrow via exosomes creating permissive macroenvironment [173]. Tumor exosomes not only impair T cell function, but also produce monocytic MDSC that impairs tumor recognition by immune cells [39]. ECM can impair antigen presenting cells and inhibit T-cell activation suppressing T-cell function against tumor [174]. Commensal microbiota is also reported to have a role in impairing antitumor immunity [40].

In this era of immunotherapy, several studies are underway to improve anticancer therapy based on these mechanisms of evading immune suppression. Commensal microbiota was reported to have a role in improving anti-tumor response to immunotherapy and chemotherapy in extraintestinal tumors [2]. Blocking TGFβ signaling could potentially promote antitumor immunity [171], which is proved in advanced colorectal cancer using TGFβ inhibitors [172].

Conclusions

In conclusion, we summarized the hallmarks of cancer. Pro-survival autophagy is described as a new hallmark of cancer given its important role in tumorigenesis and potentially an anti-cancer therapeutic target. The bidirectional interaction between a cancer cell and microenvironment as well as macroenvironment is key for a cancer cell to develop, survive, progress, and invade. In addition, cancer epigenetics, genome instability and mutation play a major role in enabling cells to acquire the hallmarks of cancer. Despite the advanced understanding, we have so far on hallmarks of cancer, there is much more to uncover in this field of research. Future research in this field is necessary to develop better ways to treat cancer.
Additional Information

Disclosures

Conflicts of interest: In compliance with the ICMJE uniform disclosure form, all authors declare the following: Payment/services info: All authors have declared that no financial support was received from any organization for the submitted work. Financial relationships: All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. Other relationships: All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

References

1. Hanahan D, Weinberg RA: Hallmarks of cancer: the next generation. Cell. 2011, 144:646-74. 10.1016/j.cell.2011.02.015
2. Rutkowski MR, Svoronos N, Perales-Puchalt A, Conejo-Garcia JR: The tumor macroenvironment: Cancer-promoting networks beyond tumor beds. Adv Cancer Res. 2015, 128:235-62. 10.1016/bs.acr.2015.04.011
3. Das CK, Banerjee I, Mandal M: Pro-survival autophagy: an emerging candidate of tumor progression through maintaining hallmarks of cancer. Semin Cancer Biol. 2020, 66:59-74. 10.1016/j.semcancer.2019.08.020
4. Kim MJ, Woo SJ, Yoon CH, et al.: Involvement of autophagy in oncogenic K-Ras-induced malignant cell transformation. J Biol Chem. 2011, 286:12924-32. 10.1074/jbc.M110.138958
5. Tan Q, Wang M, Yu M, Zhang J, Bristow RG, Hill RP, Tanmook IF.: Role of autophagy as a survival mechanism for hypoxic cells in tumors. Neoplasia. 2016, 18:547-55. 10.1016/j.nen.2016.04.005
6. Guo JY, White E: Autophagy is required for mitochondrial function, lipid metabolism, growth, and fate of KRAS(G12D)-driven lung tumors. Autophagy. 2015, 9:1656-8. 10.4161/auto.26125
7. Rabinowitch JD, White E: Autophagy and metabolism. Science. 2010, 330:1344-8. 10.1126/science.1195497
8. Yang S, Wang X, Contino G, et al.: Pancreatic cancers require autophagy for tumor growth. Genes Dev. 2011, 25:717-29. 10.1101/gad.2016111
9. Taslima A, Komatsu M, Hara T, et al.: Autophagy-deficient mice develop multiple liver tumors. Genes Dev. 2011, 25:795-800. 10.1101/gad.2016211
10. Huang T, Song X, Yang Y, et al.: Autophagy and hallmarks of cancer. Crit Rev Oncog. 2018, 23:247-67. 10.1615/CritRevOncog.2018027913
11. Shen H, Yin L, Deng G, et al.: Knockdown of beclin-1 impairs epithelial-mesenchymal transition of colon cancer cells. J Cell Biochem. 2018, 119:7022-31. 10.1002/jcb.26912
12. Kim YH, Baek SH, Kim EK, et al.: Uncoordinated 51-like kinase 2 signaling pathway regulates epithelial-mesenchymal transition in AS49 lung cancer cells. FEBS Lett. 2016, 590:1365-74. 10.1002/1356-3246.12172
13. Avivar-Valderas A, Salas E, Bobrovnikova-Marjon E, Diehl JA, Nagi G, Debubh I, Aguirre-Ghiso JA.: PERK integrates autophagy and oxidative stress responses to promote survival during extracellular matrix detachment. Mol Cell Biol. 2011, 31:5616-29. 10.1128/MCB.05164-11
14. Sun L, Li T, Wei Q, Zhang Y, Jia X, Wan Z, Han L.: Upregulation of BNIP3 mediated by ERK/HIF-1α pathway induces autophagy and contributes to anoikis resistance of hepatocellular carcinoma cells. Future Oncol. 2014, 10:1387-98. 10.2217/fon.14.170
15. Fu XT, Shi YH, Zhou J, et al.: MicroRNA-30a suppresses autophagy-mediated anoikis resistance and metastasis in hepatocellular carcinoma. Cancer Lett. 2018, 412:108-17. 10.1016/j.canlet.2017.10.012
16. Talukdar S, Pradhan AK, Bhoopathi P, et al.: Regulation of protective autophagy in anoxia-resistant glioma stem cells by SDCBP/MDA-9/Syntenin. Autophagy. 2018, 14:1845-6. 10.1080/15548627.2018.1502564
17. Chatterjee M, van Golen KL.: Breast cancer stem cells survive periods of farnesyl-transferase inhibitor-induced dormancy by undergoing autophagy. Bone Marrow Res. 2011, 2011:562958. 10.1155/2011/562958
18. Sanchez CG, Penfornis P, Oskowitz AZ, et al.: Activation of autophagy in mesenchymal stem cells provides tumor stromal support. Carcinogenesis. 2011, 32:964-72. 10.1093/carcin/bgr029
19. Zhu H, Wang D, Liu Y, et al.: Role of the Hypoxia-inducible factor-1 alpha induced autophagy in the conversion of non-stem pancreatic cancer cells into CD133+ pancreatic cancer stem-like cells. Cancer Cell Int. 2013, 13:119. 10.1186/1475-2867-13-119
20. Olja R, Iha V, Singh SK, Bhattacharyya S: Autophagy inhibition suppresses the tumorigenic potential of cancer stem cell enriched side population in bladder cancer. Biochim Biophys Acta. 2014, 1842:2073-86. 10.1016/j.bbaddis.2014.07.007
21. Chen K, Shi W: Autophagy regulates resistance of non-small cell lung cancer cells to paclitaxel. Tumour Biol. 2016, 37:10359-44. 10.1007/s13277-016-4929-x
22. Gupta A, Roy S, Lazar AJ, et al.: Autophagy inhibition and antimarials promote cell death in gastrointestinal stromal tumor (GIST). Proc Natl Acad Sci U S A. 2010, 107:14535-8. 10.1073/pnas.100348107
23. Vanhaesebroeck B, Guillermot-Gulbert I, Graupera M, Bilanges B: The emerging mechanisms of isoform-specific PI3K signalling. Nat Rev Mol Cell Biol. 2010, 11:529-41. 10.1038/nrm2882
24. Olja R, Singh SK, Bhattacharyya S, Dhanda RS, Rakha A, Mandal AK, Iha V.: Inhibition of grade dependent autophagy in uterine carcinoma increases cell death under nutritional limiting condition and potentiates the cytotoxicity of chemotherapeutic agent. J Urol. 2014, 191:1889-98. 10.1016/j.juro.2014.01.006
25. Shao S, Li S, Qin Y, et al.: Spautin-1, a novel autophagy inhibitor, enhances imatinib-induced apoptosis in chronic myeloid leukemia. Int J Oncol. 2014, 44:1661-8. 10.3892/ijo.2014.2515
26. Egan DF, Chun MG, Yamos M, et al.: Small molecule inhibition of the autophagy kinase ULK1 and identification of ULK1 substrates. Mol Cell. 2015, 59:285-97. 10.1016/j.molcel.2015.05.051
27. Dowler CM, Bhat N, Gebur MT, Chen L, Wills CA, Miller BA, Wang HG: Targeted inhibition of ULK1 promotes apoptosis and suppresses tumor growth and metastasis in neuroblastoma. Mol Cancer Ther. 2018, 17:2565-76. 10.1158/1535-7163.MCT-18-0176
62
60
58
57
56
55
54
53
52
50
49
48
46
45
44
43
42
41
39
38
37
36
33
32
31
30
29
28
20
212x77] in invasion enhance hepatocyte growth factor signaling in mammary carcinoma cells to promote scattering and

Cheng N, Chytil A, Shyr Y, Joly A, Moses HL: 10.1007/s12094-006-0162-1

Perona R: 21:177-84.

Hynes NE, MacDonald G: 25:85-101.

10.1016/j.cell.2010.06.011

Lemmon MA, Schlessinger J: 2014, 159:440-55.

Platt RJ, Chen S, Zhou Y, et al.:

Dis. 2020, 1:1-9.

2017, 77:2179-85.

Nature. 2020, 578:94-101.

Gerstung M, Jolly C, Leshchiner I, et al.:

Nature. 2020, 578:82-93.

ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium:

10.3109/15376516.2011.562671

Brait M, Sidransky D:

10.1007/978-1-4939-1804-1_1

Kanwal R, Gupta K, Gupta S:

2006, 7:21-33.

Feinberg AP, Ohlsson R, Henikoff S:

Jones PA, Laird PW:

Taby R, Issa JP:

Reprod Med. 2009, 27:351-7.

Dupont C, Armant DR, Brenner CA:

Cell signalling: growth factors and tyrosine kinase receptors

10.1016/j.cell.2017.01.002

ErbB receptors and signaling pathways in cancer

10.1007/978-1-4939-1804-1_1

negative impact on osteosarcoma tumors

10.1016/j.ebiom.2018.07.034

28. Lu J, Zhu L, Zheng LP, et al.: Overexpression of ULK1 represents a potential diagnostic marker for clear cell renal carcinoma and the antitumor effects of SBI-0206965. EBioMedicine. 2018, 34:85-93.

10.1016/j.ebiom.2018.07.034

Akin D, Wang SK, Habibzadeh-Tari P, et al.: A novel ATG4B antagonist inhibits autophagy and has a negative impact on osteosarcoma tumors. Autophagy. 2014, 10:2021-55. 10.4161/auto.32229

Liu PF, Tsai KL, Hsu CJ, et al.: Drug repurposing screening identifies tioconazole as an Atg4 inhibitor that suppresses autophagy and sensitizes cancer cells to chemotherapy. Theranostics. 2018, 8:830-45. 10.7150/thno.22012

32. Seitz C, Hugle M, Cristofanoff S, Tchougahdjian A, Fulda S: The dual PI3K/mTOR inhibitor NVP-BEZ235 and chloroquine synergize to trigger apoptosis via mitochondrial-lysosomal cross-talk. Int J Cancer, 2013, 152:2682-93. 10.1002/ijc.27935

30. Liu, Pei, Tsai, K.L., Hsu, C.J., et al.: Drug repurposing screening identifies tioconazole as an Atg4 inhibitor that suppresses autophagy and sensitizes cancer cells to chemotherapy. Theranostics. 2018, 8:830-45. 10.7150/thno.22012

31. Lafortune P, Wang W, Wang W, et al.: Transforming growth factor-beta signaling-deficient fibroblasts enhance hepatocyte growth factor signaling in mammary carcinoma cells to promote scattering and invasion. Mol Cancer Res. 2008, 6:1521-33. 10.1158/1541-7786.MCR-07-2203

56. Platt RJ, Chen S, Zhou Y, et al.:

CRISPR-Cas9 knockin mice for genome editing and cancer modeling. Semin Cancer Biol. 2019, 65:197-206. 10.1016/j.semcancer.2019.12.021

49. A novel ATG4B antagonist inhibits autophagy and has a negative impact on osteosarcoma tumors. Autophagy. 2014, 10:2021-55. 10.4161/auto.32229

Liu PF, Tsai KL, Hsu CJ, et al.: Drug repurposing screening identifies tioconazole as an Atg4 inhibitor that suppresses autophagy and sensitizes cancer cells to chemotherapy. Theranostics. 2018, 8:830-45. 10.7150/thno.22012

32. Seitz C, Hugle M, Cristofanoff S, Tchougahdjian A, Fulda S: The dual PI3K/mTOR inhibitor NVP-BEZ235 and chloroquine synergize to trigger apoptosis via mitochondrial-lysosomal cross-talk. Int J Cancer, 2013, 152:2682-93. 10.1002/ijc.27935

30. Liu, Pei, Tsai, K.L., Hsu, C.J., et al.: Drug repurposing screening identifies tioconazole as an Atg4 inhibitor that suppresses autophagy and sensitizes cancer cells to chemotherapy. Theranostics. 2018, 8:830-45. 10.7150/thno.22012

31. Lafortune P, Wang W, Wang W, et al.: Transforming growth factor-beta signaling-deficient fibroblasts enhance hepatocyte growth factor signaling in mammary carcinoma cells to promote scattering and invasion. Mol Cancer Res. 2008, 6:1521-33. 10.1158/1541-7786.MCR-07-2203
transcription factors

Woods K, Thomson JM, Hammond SM:
10.1371/journal.pgen.0030146

Coller HA, Forman JJ, Legesse-Miller A:
Pharmacol. 2010, 14:365-9.

Kim CH, Yoo YM:
DNA damage accumulation

Santoro R, Marani M, Blandino G, Muti P, Strano S:
Wang Y, Nangia-Makker P, Tait L, Balan V, Hogan V, Pienta KJ, Raz A:
Grosset AA, Labrie M, Gagné D, Vladoiu MC, Gaboury L, Doucet N, St-Pierre Y:
mature p53
Campion CG, Labrie M, Lavoie G, St-Pierre Y:
galectin-7, a myoepithelial cell marker, enhances spontaneous metastasis of breast cancer cells
Demers M, Rose AA, Grosset AA, Biron-Pain K, Gaboury L, Siegel PM, St-Pierre Y:
regulating MMP-9 gene expression

Kim SW, Park KC, Jeon SM, Ohn TB, Kim TI, Kim WH, Cheon JH:
urothelial cancer to cisplatin through the accumulation of intracellular reactive oxygen species
Matsui Y, Ueda S, Watanabe J, Kuwabara I, Ogawa O, Nishiyama H:
Polyak K, Xia Y, Zweier JL, Kinzler KW, Vogelstein B:
Girotti MR, Salatino M, Dalotto- Moreno T, Rabinovich GA:
10.14348/molcells.2018.0100
Ko A, Han SY, Choi CH, et al.:
mediated p53 responses
Chen D, Shan J, Zhu WG, Qin J, Gu W:
of the ARF tumor suppressor
Kuo ML, den Besten W, Bertwistle D, Roussel MF, Sherr CJ:
10.1038/nrc2772

The hallmarks of cancer

Senescence in tumours: evidence from mice and humans . Nat Rev Cancer. 2010, 10:51-7.

Collado M, Serrano M: and activates Akt. Cancer Res. 2006, 66:1500-8. 10.1158/0008-5472.CAN-05-2925

Kuo ML, den Besten W, Bertwistle D, Roussel MF, Sherr CJ: N-terminal polyubiquitination and degradation of the ARF tumor suppressor. Genes Dev. 2004, 18:1862-74. 10.1101/gad.1213904

Chen D, Shan J, Zhu WG, Qin J, Gu W: Transcription-independent ARF regulation in oncogenic stress-mediated p53 responses. Nature. 2010, 464:624-7. 10.1038/nature08820

Ko A, Shin JY, Seo J, et al.: Acceleration of gastric tumorigenesis through MKRN1-mediated posttranslational regulation of p14ARF. J Natl Cancer Inst. 2012, 104:1660-72. 10.1093/jnci/djs424

Wang X, Zha M, Zhao X, et al.: Siva inhibits p53 function by acting as an ARF E3 ubiquitin ligase . Nat Commun. 2013, 4:1551. 10.1038/ncomms2535

Han SY, Ko A, Kitano H, et al.: Molecular chaperone Hsp90 is necessary to prevent cellular senescence via lysosomal degradation of p14ARF. Cancer Res. 2017, 77:543-54. 10.1158/0008-5472.CAN-16-0613

Ko A, Han SY, Song J: Regulatory network of ARF in cancer development . Mol Cells. 2018, 41:581-9. 10.14548/molecules.2018.0100

Girotti MR, Salatino M, Dalotto- Moreno T, Rabinovich GA: Sweetening the hallmarks of cancer: galectins as multifunctional mediators of tumor progression. J Exp Med. 2020, 217:20182041. 10.1087jem.20182041

Poleyak K, Xia Y, Zweier JL, Kinzler KW, Vogelstein B: A model for p53-induced apoptosis . Nature. 1997, 389:500-5. 10.1038/389500a0

Matsui Y, Ueda S, Watanabe J, Kuwabara I, Ogawa O, Nishiyama H: Sensitizing effect of galectin-7 in urothelial cancer to cisplatin through the accumulation of intracellular reactive oxygen species. Cancer Res. 2007, 67:1212-20. 10.1158/0008-5472.CAN-06-3283

Kim SW, Park KC, Jeon SM, Ohn TB, Kim TI, Kim WH, Cheon JH: Abrogation of galectin-4 expression promotes tumorigenesis in colorectal cancer. Cell Oncol (Dordr). 2013, 36:1619-78. 10.1007/s13402-013-0124-x

Demers M, Magnaldo T, St-Pierre Y: A novel function for galectin-7: promoting tumorigenesis by up-regulating MMP-9 gene expression. Cancer Res. 2005, 65:5205-10. 10.1158/0008-5472.CAN-05-0134

Demers M, Rose AA, Grosset AA, Biron-Pain K, Gaboury L, Siegel PM, St-Pierre Y: Overexpression of galectin-7, a myoepithelial cell marker, enhances spontaneous metastasis of breast cancer cells. Am J Pathol. 2010, 176:3025-31. 10.2353/ajpath.2010.090876

Campion CG, Labrie M, Lavoie G, St-Pierre Y: Expression of galectin-7 is induced in breast cancer cells by mutant p53. PLoS One. 2013, 8:e72468. 10.1371/journal.pone.0072468

Grosset AA, Labrie M, Gagné D, Vlaudio MC, Gaboury L, Doucet N, St-Pierre Y: Cytosolic galectin-7 impairs p53 functions and induces chemoresistance in breast cancer cells. BMC Cancer. 2014, 14:801. 10.1186/1471-2407-14-801

Stiasny A, Freier CP, Kuhn C, et al.: The involvement of E6, p53, p16, MDM2 and Gal-3 in the clinical outcome of patients with cervical cancer. Oncol Lett. 2017, 14:4467-76. 10.3892/ol.2017.7677

Chen Y, Nagia-Malke P, Tulia L, Balan V, Hogan V, Pienta KJ, Raz A: Regulation of prostate cancer progression by galectin-3. Am J Pathol. 2009, 174:1515-23. 10.2353/ajpath.2009.080816

Santoro R, Marani M, Blandino G, Muti P, Strano S: Melatonin triggers p53/58 phosphorylation and prevents DNA damage accumulation. Oncogene. 2012, 31:2931-42. 10.1038/onc.2011.469

Hill SM, Frasch T, Xiang S, Yuan L, Duplessis T, Mao L: Molecular mechanisms of melatonin anticaner effects. Intcge Cancer Ther. 2009, 8:337-46. 10.11711/15375540953352

Kim CH, Yoo YM: Melatonin induces apoptotic cell death via p53 in LNCaP cells . Korean J Physiol Pharmacol. 2010, 14:565-9. 10.4196/kjpp.2010.14.6.365

Coller HA, Forman JJJ, Legoeree-Miller A: 'Myc'ed messages': myc induces transcription of EZF1 while inhibiting its translation via a microRNA polycistrion. PLoS Genet. 2007, 3:e146. 10.1371/journal.pgen.0030146

Woods K, Thomson JM, Hammond SM: Direct regulation of an oncogenic micro-RNA cluster by EZF transcription factors. J Biol Chem. 2007, 282:2130-4. 10.1074/jbc.C00252200
19. He L, Thomson JM, Hemann MT, et al.: A microRNA polycistron as a potential human oncoprotein. Nature. 2005, 435:828-33. 10.1038/nature03552

20. Gilles JK, Lorimer IA: Regulation of p27Kip1 by miRNA 221/222 in glioblastoma. Cell Cycle. 2007, 6:2005-9. 10.4161/cc.6.16.4526

21. le Sage C, Nagar R, Egan DA, et al.: Regulation of the p27(Kip1) tumor suppressor by miR-221 and miR-222 promotes cancer cell proliferation. EMBO J. 2007, 26:5699-708. 10.1038/sj.emboj.7601790

22. Du B, Wang Z, Zhang X, Feng S, Wang G, He J, Zhang B: MicroRNA-545 suppresses cell proliferation by targeting cyclin D1 and CDK4 in lung cancer cells. PLoS One. 2014, 9:e88022. 10.1371/journal.pone.0088022

23. de Oliveira JC, Oliveira LC, Mathias C, et al.: Long non-coding RNAs in cancer: another layer of complexity. J Gene Med. 2019, 21: e5065. 10.1002/jgm.3065

24. Du Y, Xiong G, You X, et al.: Elevation of highly up-regulated in liver cancer (HULC) by hepatitis B virus X protein promotes hepatoma cell proliferation via down-regulating p18. J Biol Chem. 2012, 287:26502-11. 10.1074/bc.2011.342113

25. Tang SS, Zheng BY, Xiong XD: LincRNA-p21: implications in human diseases. Int J Mol Sci. 2015, 16:18732-40. 10.3390/ijms160818732

26. Liu J, Xing Y, Xu L, Chen W, Cao W, Zhang C: Decreased expression of pseudogene PTENP1 promotes malignant behaviours and is associated with the poor survival of patients with HNSCC. Sci Rep. 2017, 7:41179. 10.1038/srep41179

27. Yang F, Zhang L, Lue HS, et al.: Long noncoding RNA high expression in hepatocellular carcinoma facilitates tumor growth through enhancement of zeste homolog 2 in humans. Hepatology. 2011, 54:1679-89. 10.1002/hep.24565

28. Naemura M, Murasaki K, Inoue Y, Okamoto H, Kotake Y: Long noncoding RNA Arr3 regulates proliferation of non-small cell lung cancer and cervical. Cancer Cells Anticancer Res. 2015, 35:5317-22.

29. Jacobson MD, Weil M, Raif MC: Programmed cell death in animal development. Cell. 1997, 88:547-54. 10.1016/S0092-8674(00)81875-3

30. Zörnig M, Hueber A, Baum W, Evan G: Apoptosis regulators and their role in tumorigenesis. Biochim Biophys Acta. 2001, 1551:1. 10.1016/S0167-4838(01)00051-2

31. Daniel NN, Korsmeyer SJ: Cell death: critical control points. Cell. 2004, 116:205-19. 10.1016/S0092-8674(04)00846-7

32. Beereser C, Cadiou H, Vieira HL, et al.: Bel-2 and Bax regulate the channel activity of the mitochondrial adenine nucleotide translocator. Oncogene. 2000, 19:529-36. 10.1038/sj.onc.1203298

33. Chen ZX, Pervaiz S: Bel-2 induces pro-oxidant state by engaging mitochondrial respiration in tumor cells. Cell Death Differ. 2007, 14:1617-27. 10.1038/sj.cdd.4402165

34. Huang HL, Fang LW, Lu SP, Chou CK, Luh TY, Lai MZ: DNA-damaging reagents induce apoptosis through reactive oxygen species-dependent Fas aggregation. Oncogene. 2003, 22:8168-77. 10.1038/sj.onc.1206979

35. Oda E, Okiki R, Murasawa H, et al.: Noxa, a Bcl-3 only member of the Bel-2 family and candidate mediator of p53-induced apoptosis. Science. 2000, 288:1053-8. 10.1126/science.288.5468.1053

36. Schuler M, Bossy-Wetzel E, Goldstein JC, Fitzgerald P, Green DR: p53 induces apoptosis by caspase activation through mitochondrial cytochrome c release. J Biol Chem. 2000, 275:7337-42. 10.1074/jbc.275.73377

37. Chipuk JE, Maurer U, Green DR, Schuler M: Pharmacologic activation of p53 elicits Bax-dependent apoptosis in the absence of transcription. Cancer Cell. 2005, 4:571-81. 10.1016/S1535-6108(05)00272-1

38. Chipuk JE, Kuwana T, Boucher-Hayes L, Droin NM, Newmeyer DD, Schuler M, Green DR: Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. Science. 2004, 305:1010-4. 10.1126/science.1092754

39. Blasco MA: Telomeres and human disease: ageing, cancer and beyond. Nat Rev Genet. 2005, 6:611-22. 10.1038/nrg1565

40. Shay JW, Wright WE: Hayflick, his limit, and cellular ageing. Nat Rev Mol Cell Biol. 2000, 1:72-6. 10.1038/35036093

41. Hayfllick L, Moorhead PS: The serial cultivation of human diploid cell strains. Exp Cell Res. 1961, 25:585-621. 10.1016/0014-4827(61)90192-6

42. Reddel RR: The role of senescence and immortalization in carcinogenesis. Carcinogenesis. 2000, 21:477-84. 10.1093/carcin/21.3.477

43. Bell RJ, Rube HT, Kreig A, et al.: The transcription factor GABP selectively binds and activates the mutant TERT promoter in cancer. Science. 2015, 348:1036-9. 10.1126/science.aaa6015

44. Chin YE, Kitagawa M, Su WC, You ZH, Huamot Y, Fu XY: Cell growth arrest and induction of cyclin-dependent kinase inhibitor p21 WAF1/CIP1 mediated by STAT1. Science. 1996, 272:719-22. 10.1126/science.272.5262.719

45. Shay JW, Bacchetti S: A survey of telomerase activity in human cancer. Eur J Cancer. 1997, 33:787-91. 10.1016/S0959-8049(97)00062-2

46. Heaphy CM, Subbawong AP, Hong SM, et al.: Prevalence of the alternative lengthening of telomeres telomere maintenance mechanism in human cancer subtypes. Ann J Pathol. 2011, 179:1668-15. 10.1016/j.ajpath.2011.06.018

47. Jafri MA, Ansari SA, Alqahthani MI, Shay JW: Roles of telomeres and telomerase in cancer, and advances in telomerase-targeted therapies. Genome Med. 2016, 8:69. 10.1186/s13073-016-0304-7

48. Horn S, Figl A, Rachakonda PS, et al.: TERT promoter mutations in familial and sporadic melanoma. Science. 2015, 353:959-61. 10.1126/science.aab0015

49. Huang FW, Hodis E, Xu ML, Kryukov GV, Chiu L, Garraway LA: Highly recurrent TERT promoter mutations in human melanoma. Science. 2013, 339:957-9. 10.1126/science.1229259

50. Borah S, Xu L, Zaug AJ, et al.: Cancer. TERT promoter mutations and telomerase reactivation in urothelial cancer. Science. 2015, 347:1006-10. 10.1126/science.1260200

51. Killila PJ, Reitman ZJ, Jiao Y, et al.: TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. Proc Natl Acad Sci U S A. 2015, 110:6021-6. 10.1073/pnas.1503607110
Immunol. 2017, 8:1300.

M2 is required for the expression of the immune checkpoint PD-L1 in immune cells and tumors

Li X, Wenes M, Romero P, Huang SC, Fendt SM, Ho PC:

Lu P, Weaver VM, Werb Z:

Diepenbruck M, Christofori G:

Zheng X, Carstens JL, Kim J, et al.:

De Craene B, Berx G:

Nieto MA, Huang RY, Jackson RA, Thiery JP:

Ye X, Weinberg RA:

Clark AG, Vignjevic DM:

Talmadge JE, Fidler IJ:

Rajabi M, Mousa SA:

Tonini T, Rossi F, Claudio PP:

Hahn WC, Weinberg RA:

Blasco MA, Lee HW, Harde MP, et al.:

Nishida N, Yano H, Nishida T, Kamura T, Kojiri M: Angiogenesis in cancer. Vasc Health Risk Manag. 2006, 2:215-9. 10.2147/vhrm.2006.2.3.213

Tonini T, Rossi F, Claudio PP: Molecular basis of angiogenesis and cancer. Oncogene. 2003, 22:6549-56. 10.1038/sj.onc.1206811

Fouad YA, Aanei C: Revisiting the hallmarks of cancer. Am J Cancer Res. 2017, 7:1016-36.

Rajabi M, Moussa SA: The role of angiogenesis in cancer treatment. Biomedicines. 2017, 5:34. 10.3390/biomedicines5020034

Butler KS, Hines WC, Heaphy CM, Griffith JK: The persistence of the Warburg effect and the plasticity of cancer metabolism. Trends Cell Biol. 2016, 36:13-22. 10.1016/j.tcb.2015.07.012

Derycke LD, Bracke ME: Differentiation and the role of the microenvironment. Cell. 2016, 166:21-45. 10.1016/j.cell.2016.06.028

Li X, Weinberg RA: Epithelial-mesenchymal plasticity: a central regulator of cancer progression. Trends Cell Biol. 2015, 25:675-86. 10.1016/j.tcb.2015.07.012

Nieto MA, Huang RY, Jackson RA, Thiery JP: EMT. Cell. 2016, 166:21-45. 10.1016/j.cell.2016.06.028

De Craene B, Berx G: Regulatory networks defining EMT during cancer initiation and progression. Nat Rev Cancer. 2013, 13:97-110. 10.1038/nrc3447

Fischer RR, Durrans A, Lee S, et al.: Epithelial-to-mesenchymal transition is not required for lung metastasis but contributes to chemoresistance. Nature. 2015, 527:472-6. 10.1038/nature15748

Zheng X, Carstens JL, Kim J, et al.: Epithelial-to-mesenchymal transition is dispensable for metastasis but induces chemoresistance in pancreatic cancer. Nature. 2015, 527:525-30. 10.1038/nature16064

Diepenbruck M, Christofori G: Epithelial-mesenchymal transition (EMT) and metastasis: yes, no, maybe?. Curr Opin Cell Biol. 2016, 43:7-15. 10.1016/j.ceb.2016.06.002

Lu P, Weaver VM, Werb Z: The extracellular matrix: a dynamic niche in cancer progression. J Cell Biol. 2012, 196:395-406. 10.1083/jcb.201102147

Li X, Wenner M, Romero P, Huang SC, Fendt SM, Ho PC: Navigating metabolic pathways to enhance antitumor immunity and immunotherapy. Nat Rev Clin Oncol. 2016, 13:425-41. 10.1038/nrclinonc.2016.80

Warburg O: The metabolism of carcinoma cells. Cancer Res. 1925, 9:138-63. 10.1158/jcr.1925.148

Brand A, Singer K, Koehl GE, et al.: p16INK4a-associated lactic acid production blunts tumor immunosurveillance by T and NK cells. Cell Metab. 2016, 24:657-71. 10.1016/j.cmet.2016.08.011

Dong G, Mao Q, Xia W, Xu Y, Wang J, Xu L, Jiang F: PRK2 and cancer: the function of PRK2 beyond glycolysis. Oncol Lett. 2016, 11:980-6. 10.3892/ol.2016.4168

Palsson-McDermott EM, Dyck L, Zaslona Z, Menon D, Gettrick AF, Mills KH, O'Neill LA: Pyruvate kinase M2 is required for the expression of the immune checkpoint PD-L1 in immune cells and tumors. Front Immunol. 2017, 8:1300. 10.3389/fimmu.2017.01300

Lemos H, Huang L, Prendergast GC, Mellor AL: Immune control by amino acid catabolism during...
168. Komiyah T, Huang CH: Updates in the clinical development of epacadostat and other indoleamine 2,3-dioxygenase 1 inhibitors (IDO1) for human cancers. Front Oncol. 2018, 8:425. 10.3389/fonc.2018.00423

169. Platten M, Wick W, Van den Eynde BJ: Tryptophan catabolism in cancer: beyond IDO and tryptophan depletion. Cancer Res. 2012, 72:5455-40. 10.1158/0008-5472.CAN-12-0669

170. Patsoukis N, Bardhan K, Chatterjee P, et al.: PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. Nat Commun. 2015, 6:6692. 10.1038/ncomms7692

171. Gorelik L, Flavell RA: Immune-mediated eradication of tumors through the blockade of transforming growth factor-beta signaling in T cells. Nat Med. 2001, 7:1118-22. 10.1038/nn1001-1118

172. Tauriello DV, Palomo-Ponce S, Stork D, et al.: TGFβ drives immune evasion in genetically reconstituted colon cancer metastasis. Nature. 2018, 554:538-43. 10.1038/nature25492

173. Becker JC: Tumor-educated myeloid cells: impact the micro- and macroenvironment. Exp Dermatol. 2014, 23:157-8. 10.1111/exd.12241

174. Pickup MW, Mouw JK, Weaver VM: The extracellular matrix modulates the hallmarks of cancer. EMBO Rep. 2014, 15:1243-53. 10.15252/embr.201459246