## *Review Article*
# Epigenetic mechanisms and the hallmarks of cancer: an intimate affair

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**Abstract:** Epigenetic mechanisms comprising DNA methylation, histone modifications, and noncoding RNAs affect chromatin structure and regulate gene expression. These mechanisms control normal embryonic development and adult life and their deregulation contributes to several diseases including cancer. The process of tumorigenesis is complex and results from the evolution of different "hallmarks of cancer". Hanahan and Weinberg presented in 2000 and 2011 seminal contributions in the cancer field, first the six hallmarks of cancer and a decade later two additional hallmarks and two enabling characteristics were added. Here, we surmise that epigenetic mechanisms regulate and contribute to every single hallmark in cancer, and thus represent the hallmark of hallmarks in tumorigenesis. Focusing on epigenetics as a major hallmark in cancer formation has profound preventive, therapeutic, and clinical implications.

## Introduction

Cancer is one of the most prominent causes of mortality. It is estimated that 8.2 million deaths occurred worldwide in 2012 [1] and more than six hundred thousand cancer deaths are predicted to follow just in the United States in 2020 [2]. Cancer is a multifaceted and diverse disease in which cancer cells share common mechanisms of tumor formation, progression, and ability to grow beyond their natural environment. Scientists proposed several theories throughout the years to explain the origins of cancer [3]. However, no theories or features of cancer cells were more discussed and adopted then the "hallmarks of cancer" as proposed by Hanahan and Weinberg [4, 5]. Hanahan and Weinberg have defined the hallmarks of cancer "as acquired functional capabilities that allow cancer cells to survive, proliferate, and disseminate" [6]. Within a decade, eight hallmarks were characterized namely "the ability of cancer cells to sustaining proliferative signaling, evading growth suppressors, resisting cell death, inducing angiogenesis, activating invasion and metastasis, enabling replicative immortality, deregulating cellular energetics, and avoiding immune destruction". In addition, "genome instability and mutation and tumor-promoting inflammation" were added as two enabling characteristics in the achievement of hallmarks [5].

All the different hallmarks and enabling characteristics describe functional properties of normal cells that are acquired during the process of tumor formation and are "the driving forces of tumorigenesis" [7]. Horne *at al*. re-evaluated the hallmarks and unified them under the umbrella of cancer evolution rather than being separated into discrete categories [8]. Some of these hallmarks may have overlapping properties such as avoiding growth suppressors and resisting cell death which will in turn sustain the proliferative signaling of tumor cells. Other hallmarks may be needed at different phases of tumor development, for instance activating invasion and metastasis occurs later in tumorigenesis. Furthermore, the contribution and chronology of the hallmarks may vary according to the different cancers, tissue of origin, composition of the tumor, and the contributions of the microenvironment and immune system.

Historically cancer was mostly considered a genetic disease, however the "somatic muta-

tion theory" did not explain the origin of most cancers [9]. Tumor cells are mostly characterized by abnormalities in responding to internal and external signals, cellular identity, and deregulation of gene expression [10-12]. In fact, epigenetic mechanisms are tightly controlled and regulate embryonic development and adult life and their deregulation has been involved in many disorders including cancer [13, 14]. Large-scale cancer genome sequencing efforts have indicated that almost half of human cancers bear mutations in chromatin proteins [15, 16]. In addition, malignant cells show CpG islands hypermethylation, mostly in tumor suppressor genes, a reduction of total DNA methylation, and progressive histone modifications changes [17]. Recently, noncoding RNAs (ncRNAs) whether small ncRNA (< 200 nt) namely microRNAs (miRNAs) and long ncRNAs (lncRNAs) were observed to be deregulated in cancer and to majorly contribute to tumorigenesis [18-20]. Recently, Flavahan *et al*. concluded that abnormal epigenetic mechanisms and chromatin structures give rise to oncogenic properties that manifest themselves in all the hallmarks of cancer [21]. The focus of this review is to present evidence showing that epigenetic mechanisms regulate and contribute to every single hallmark in cancer and that perhaps present themselves as hallmark of hallmarks. The role of mutations cannot be overlooked in tumor formation as there is a major crosstalk between genetic and epigenetic modifications but will not be the focus of this is review [22].

**Epigenetic mechanisms and the hallmarks of cancer**

Epigenetics is "defined as the study of mitotically and meiotically heritable changes in gene function that are not dependent on DNA sequence" [23]. It was recently postulated that epigenetic aberrations and chromatin states may cause extensive oncogenic gain of function properties and may satisfy all of the hallmarks of cancer [21]. External and internal signals may enhance or reduce chromatin resistance leading to a "restrictive state that blocks differentiation programs" or epigenetic plasticity, respectively [21]. Epigenetic plasticity in turn provides a permissive environment for premalignant and malignant cells to stimulate different gene regulatory pathways resulting in abnormal cell fates. Some of these acquired

changes may be "passengers" or "drivers" to the process of tumorigenesis. These driver epigenetic conditions may be fixed during cell proliferation by various mechanisms including DNA methylation, histone modifications, and ncRNA contributions resulting in tumor suppressor gene inhibition and oncogene activation.

Here we will focus on the most investigated epigenetic mechanisms in tumorigenesis namely DNA methylation, histone modifications, miRNA, and lncRNA deregulation in cancer and their contributions to the different hallmarks. We will select few examples that justify our selection of epigenetic mechanism contributions to all of cancer's hallmarks. For further examples and details, the reader is referred to excellent comprehensive reviews [18-22].

**Sustaining proliferative signaling**

Sustaining proliferative signaling is considered as one of the utmost crucial properties of tumor cells [6]. Several players and signaling pathways ensure the coordinated regulation of cell division during normal embryonic development and adult life. Cancer cells obstruct these signals by a variety of mechanisms ranging from secreting their own growth factors that function in autocrine fashion. These growth factors in turn bind to receptors commonly endowed with tyrosine kinase activities that regulate cell cycle progression. Cancer cell proliferation may be also fueled by signals received from cells in the stroma that may in turn be affected by the tumor cells themselves. Alternatively, cancer cells may upregulate the receptors that receive the mitogenic signal or may acquire mutations in receptors that result in their ligand-independent activation.

Proto-oncogenes are commonly hypomethylated in several cancers [24]. It is well established that the epidermal growth factor (EGF) and c-myc are hypermethylated in liver cancer [25]. In addition, negative feedback signals that inhibit cell proliferation or cell cycle progression may be impaired. For instance, PTEN counteracts the oncogenic PI3K signaling by degrading its product PIP. *PTEN* promoter is methylated in some cancers resulting in shut down of its transcription and loss of PTEN expression [26].

Genetic and epigenetic mechanisms are interrelated. For instance in gliomas and other tumors isocitrate dehydrogenase (*IDH*) muta-

tions are common initiating events [27, 28]. This results in the formation of the 2-hydroxy-glutarate oncometabolite which obstructs hydroxylases which are involved in DNA demethylation, resulting in hypermethylated DNA. The DNA binding protein CTCF is involved in insulating chromatin loops from excessive enhancer stimulation and thus its function is reduced in *IDH* mutants [21]. Consequently, in gliomas, the oncogene *PDGFRA*, that encodes the platelet-derived growth factor receptor A, is due to loss of CTCF gene insulation and results in hyperproliferation of glioma cells. The loss of insulator function is conserved through cell proliferation due to the stability of DNA methylation.

The histone code is commonly deregulated in cancer [17]. Several proto-oncogenes are abnormally expressed by aberrations in histone modifications. For instance, fibroblast growth factor receptor 2 (FGFR2) is implicated in breast cancer susceptibility. High levels of FGFR2 in cancer cells have been linked to polymorphic sequences with constitutively acetylated histones [29]. Other patterns of histone post-translational modifications such as methylation, phosphorylation, and ubiquitination are commonly deregulated in cancer in several genes impacting cell proliferation [30].

Cell proliferation is tightly controlled by proto-oncogene products as well as by ncRNAs. The expression of coding and noncoding genes is affected by ncRNAs. Recently, ncRNAs, epigenetics, and cancer were shown to be regulating each other [18]. ncRNAs affect epigenetic mechanisms which in turn control ncRNAs [31] and these mechanisms are aberrant in cancer [18]. miRNAs control gene expression by abrogating transcript translation or reducing its stability. miRNAs are crucial for the control of cell growth and survival [32]. These miRNAs can be amplified at certain loci or lost in deleted chromosomal segments [33]. miRNAs are deregulated in most cancers and affect proto-oncogenes or tumor suppressor genes and, therefore, function as tumor suppressors or oncomiRs [34]. miR-17 cluster encodes six miRNAs that function as oncomiRs due to their upregulated expression by c-myc, resulting in cell cycle progression and regulation of E2F [35]. miR-124a represses the oncogene CDK6 and is commonly silenced in colon cancer, re-

sulting in retinoblastoma (Rb) phosphorylation and inactivation of [36].

lncRNAs are a heterogeneous group of ncRNAs that exceed 200 nucleotides in length and encode less than 100 amino acids in their open reading frame [37]. lncRNAs are deregulated in cancer and affect most of its hallmarks [20]. For instance, lncRNAs sustain proliferative signaling through the sex steroid hormones. Sex steroidal hormones in female and males control female mammary glands, uterus, and ovary or prostate gland and testis, respectively. They function by binding to their intracellular receptors activating or repressing gene expression through co-activators and co-repressors, respectively. SRA, the steroid receptor RNA activator, functions as a lncRNA [38]. SRA with SRC-1 is a constituent of an RNA and protein complex that regulates the nuclear receptors through their AF-1 domain. SRA levels are elevated in breast tumors which might result in altered ER/PR action observed in breast tumorigenesis [39]. The situation is even more complex as SRA was found to produce a protein that functions as a corepressor and activator of nuclear receptors [40]. There are also other examples of lncRNAs than SRA that play a role in cell proliferation. More than hundred differentially expressed lncRNAs were found by RNA-Sequencing to be differentially regulated in different prostate benign and malignant tissues [41]. PCAT-1 (prostate cancer associated transcript-1) was one characterized lncRNA to be overexpressed in aggressive and advanced prostate tumors. PCAT silencing in prostate cell lines reduced cell growth and resulted in more than two hundred upregulated genes. Gene ontology showed the enrichment of these latter genes in cell cycle control and mitosis. Interestingly, the promoter region of cell cycle genes were shown to encode more than 200 lncRNAs, underscoring the importance of these RNA molecules in cell cycle and cell proliferation control [42].

**Evading growth suppressors**

In addition to sustaining proliferation in tumor formation, cancer cells need to evade negative growth regulators. The most prominent brakes to cell division and cell cycle progression are the Rb protein, the p53 pathway, and the cyclin-dependent kinase inhibitors (CDKIs) [43,

44]. The Rb protein is responsive to external and internal cues to dictate whether cells should continue cell division or halt cell cycle progression. The p53 protein on the other hand is mostly responsive to internal cues and would halt cell cycle progression if the amount of stress or DNA damage can be repaired. If the damage is excessive and irreparable then p53 will activate cell death signaling pathways, mostly apoptotic ones.

In cancer the tumor suppressor genes have their promoter regions commonly hypermethylated [45]. The *Rb* promoter is regulated by CTCF which is in turn controls promoter stability. CTCF binding to promoter sequences is methylation-dependent and in case of hypermethylated DNA, commonly observed in human cancers, the *Rb* promoter is silenced [46]. *p53* is the most prevalently mutated and inactivated gene in cancer [47]. *p53* promoter methylation is frequent in various cancers including neuroblastomas and melanomas [48, 49]. p53 gene expression is tightly regulated by the activator p14ARF and the repressor Mdm2. *p14-ARF* promoter region is commonly methylated in many cancers resulting in reduced levels of p53. Finally, the CDKIs are also tumor suppressor genes commonly inactivated or reduced in cancer. A prominent and first example is the promoter methylation of *p16* [50].

Histone code aberrations are also identified in tumor suppressor genes that negatively control cell division. The transcriptional factor Rb inhibits proliferation by downregulating the expression of several genes needed for cell cycle progression through the assembly of a multi-protein complex including repressors such as histone deacetylases (HDACs). The fine-tuning of HDACs and histone acetyltransferases (HATs) is tightly regulated in normal cells but unchecked in cancer cells. HDAC type I levels are upregulated in some tumors that are of high grades and may result in transcriptional repression of some genes needed for proliferation and cell cycle control [51].

p53 and signaling network are regulated by miRNAs at various levels. miRNAs can directly target p53 or indirectly through its regulators [52]. So far more than 20 miRNAs were listed to directly target p53 and to reduce its expression. Many of these latter miRNAs are elevated in human tumors resulting in decreased p53

levels [53]. p53 repressors such as Mdm2 and Mdm4 can be targeted by other miRNAs. More than nine miRNAs can directly target MDM2, reducing its expression, thus activating p53. Interestingly, some of these miRNAs are transcriptionally regulated by p53 and, therefore, constitute a positive feedback loop. In conclusion, these findings indicate that p53 expression and activity are controlled by miRNAs and aberration of these miRNAs in cancer may result in the loss of p53.

Several lncRNAs were also demonstrated to control p53 levels and activity [54]. 7SL is a lncRNA that is overexpressed in several cancers and silencing of 7SL reduces cell proliferation, induces senescence, and autophagy [55]. On the other hand, overexpressing 7SL enhances DNA replication and cell growth. RNA pull-down experiments showed that 7SL interacts with p53 transcripts and reduces its translation [55]. Tumor cells have also utilized lncRNAs in avoiding growth suppressors such as CDKIs. For instance, the lncRNA ANRIL induces the proliferation of non-small cell lung cancer (NSCLC) and cervical cancer cells via the silencing of the *p15* and *p16* genes [56].

### Resisting cell death

Aberrant cells are eliminated in multicellular organisms by cell death, such as apoptosis, to preserve the organism. It is therefore crucial for tumor cells to reduce or eliminate this mechanism during their development [57]. Apoptosis is a regulated process and is divided into extrinsic or intrinsic programs according to external or internal cell death signals, respectively. Deregulation of intrinsic apoptosis is more commonly involved in cancer formation [6].

The Bcl-2 family members are major regulators of intrinsic apoptosis transferring signals between apoptotic stimuli and effector players [58]. They are functionally divided as prosurvival members such as Bcl-2 and its close members, such as Bcl-w, Bcl-$x_L$, A1, Mcl-1, and apoptotic ones namely Bak and Bax and the BH3 only members (Bim, Bid, Puma, Bad, Noxa...). Bax and Bak interrupt the mitochondrial membrane potential and result in the burst of cytochrome c and Apaf-1 and formation of the apoptosome complex that activates caspase 9 [59]. Apoptosis is tightly controlled by the balance between the pro-and anti-apop-

totic members. Although Bcl-2 chromosomal translocations are commonly observed in hematological malignancies, however other overexpression mechanisms have been observed. In melanomas, EZH2, the histone methyltransferase enhancer of zeste homolog 2, shows aberrant activity resulting in its reduced binding to the promoter region of *Bcl-2*, resulting in its activation and contributing to apoptosis resistance in melanoma aggressiveness [60]. The *Bcl-2* gene was observed to be hypomethylated in B-cell chronic lymphocytic leukemia, resulting in high expression levels [61]. Deregulated methylation of *Bcl-2* and *bax* is observed in glioblastoma multiform resulting in apoptosis disruption [62]. Interestingly, HDAC inhibitors result in reduced Bcl-2 levels and apoptosis induction in t(14;18) lymphomas [63].

The Bcl-2 family members are also under post-transcriptional control by miRNAs [64]. More than 35 miRNAs reduce the levels of the survival Bcl-2 members of which 12 were demonstrated to target directly Bcl-2 including miR-15/16. This latter cluster is one of the first discovered tumor suppressor miRNA to be silenced or excised in chronic lymphocytic leukemia (CLL) [65]. miR-206 directly targets Bcl-2 and its reduced expression enhanced Bcl-2 levels in glioblastoma tissues and correlated with disease progression [66]. Also lncRNA contributed to the regulation of Bcl-2 expression levels. The lncRNA HOTTIP was shown to upregulate Bcl-2 levels and to associate with chemoresistance in SCLC [67].

A key sensor in DNA damage is the "guardian of the genome" p53. Several epigenetic mechanisms were previously listed to reduce p53 levels and to result in reduced DNA repair checkpoints [68]. However, other mechanisms may contribute to the inactivation of the DNA damage response such as dysfunction of the DNA methyltransferase DNMT3A in acute myeloid leukemia and mutations in DNMT3A are linked to poor disease prognosis [69].

### Enabling replicative immortality

Cells acquire replicative immortality to form tumors, in contrast to normal cells that have a limited replicative capability. Unlimited proliferation is linked to the property of cancer cells to protect and keep elongated the ends of telo-

meric DNA [70]. Nonimmortalized cells have limited telomerase activity as opposed to elevated levels detected in immortalized and cancer cells. Telomeric shortening is recognized as a tumor suppressive mechanism in normal cells and leads to senescence and/or apoptosis [71].

Evidence indicates that the catalytic subunit of human telomerase hTERT (human telomerase transcriptase) can be epigenetically regulated. Expression of *hTERT* is regulated by DNA methylation, histone modifications, and ncRNAs [72]. Usually hypermethylation at promoter sequences is correlated with gene silencing, however the situation differs on the *hTERT* promoter. The hypermethylated *hTERT* promoter inhibits its repressors such as CTCF from binding [73]. On the other hand, *hTERT* promoter is stimulated when the activators SP1 and c-myc bind to unmethylated CpG sites at positions 11, 12, 19, and 27 [74]. The *hTERT* promoter displays elevated DNA methylation levels, and lower methylation correlates with enhanced telomerase expression and activity [75]. In hepatocellular carcinoma, histone modification and DNA methylation regulate *hTERT*.

Histones organize chromatin in the cell nucleus and modification of their charges affects their affinity to DNA. Histone tail post-translational modifications include acetylation, methylation, ubiquitination, and phosphorylation [76]. Active gene transcription is usually associated with hyperacetylated histones and H3K4 (lysine 4 on histone 3) methylation. However, inactive gene expression is linked to hypoacetylated histones and methylation at both lysine 9 and 27 of histone 3. The highest levels of acetylated histones H3 and H4 and methylation at lysine 4 of histone 3 correlate with elevated levels of *hTERT* expression in cancer cells [77].

The *hTERT* promoter is also regulated by miRNAs [72]. For instance, miR-491-5p inhibits *hTERT* and its expression is reduced in cervical cancer [78]. It is considered a tumor suppressor miRNA that targets *hTERT* in cervical cancer as its enforced expression inhibits the growth of tumor cells. In several cancers, specific miRNAs have been detected to regulate *hTERT* by a diversity of mechanisms. The most common mechanism of these *hTERT*-specific miRNAs is their interaction with *hTERT*-3'UTR to downregulate its expression. In gastric cancer, miR-

1207-5p and miR-1266 levels are reduced resulting in elevated levels of hTERT [79]. Other miRNAs bind to the ORF of *hTERT* transcripts and inhibit their translation as observed for miR-1182 [80]. These latter miRNAs were shown to be downregulated in gastric cancer resulting in elevated levels of hTERT. Interestingly, a new mechanism of *hTERT* regulation by lncRNA was noted through its sponging effect of specific miRNAs that target *hTERT*. The lncRNA BC032469 is elevated in gastric cells and upregulates hTERT by buffering its specific miR-1207-5p [81]. In addition, the lncRNA H19 was observed to target hTERT and to negatively correlate with hTERT expression levels in acute promyelocytic leukemia [82].

Recently, the nucleoprotein structure of telomeres was noted to be aberrant in neoplastic transformation [83]. Tumors can rely on alternative mechanism for telomere lengthening (ALT) than telomerase activation to elongate their telomeres. Telomeric DNA is in a heterochromatin hypoacetylated form due to the inclusion of histones H3.3 and the function of sirtuin deacetylases. ALT mechanism and telomeric nucleoprotein are deregulated in cancer due to H3.3 mutations resulting in an oncohistone and sirtuin deacetylase functions destabilizing the chromatin landscape [84].

### Inducing angiogenesis

Tumors are not able to grow beyond 1-2 mm in size without inducing angiogenesis. These new blood vessels, sprouting from preexisting ones, are needed by tumor cells for the supply of oxygen and nutrients and for the disposal of carbon dioxide and waste. The angiogenic process is under strict control within the tumor microenvironment due to the opposing actions of angiogenic and anti-angiogenic factors such as VEGF and their receptors (VEGFR) and thrombospondin-1, respectively [85].

The epigenome methylation status has been implicated recently in tumor angiogenesis [86]. VEGF-A represents one of the strongest inducers of angiogenesis and its expression is upregulated in several tumors [87]. Recurrent bladder transitional cell carcinoma (TCC) with muscle invasive properties is characterized by aggressive angiogenesis and bad prognosis. VEGF-A levels are elevated in TCC and methylation status of VEGFA differed between low-grade

TCC and high-grade TCC where promoter hypermethylation characterizes the former one [88]. VEGFRs are also elevated in tumors and methylation status of their promoters regulates their expression levels [89].

The von Hippel-Lindau (VHL) is a tumor suppressor gene that reduces tumor growth and down-regulates many angiogenic factors [90]. The loss of VHL in several tumors causes extensive tumor vascularization [91]. The *VHL* gene encodes pVHL which is a constituent of E3 ubiquitin ligase that adds ubiquitin residues and targets the hypoxia-inducible factor (HIF) for proteasomal-mediated degradation under normoxic conditions. HIF is a transcription factor that controls the expression of *VEGF*. Loss of pVHL by genetic or epigenetic mechanisms causes accumulation of HIF and consequently VEGF overproduction resulting in marked angiogenesis. Hypermethylation of VHL promoter commonly occurs in several cancers [92].

Histone-methylating enzymes such as EZH2 constitute the catalytic subunit of the Polycomb Repressive Complex 2. EZH2 causes histone H3 lysine 27 trimethylation which is a marker of gene silencing. EZH2 regulates angiogenesis and its silencing abrogates capillary tube formation in cultured cells. *In vivo,* EZH2 promotes tumor growth, metastasis, and angiogenesis as it silences anti-angiogenic genes [93].

Recently, miRNAs and lncRNAs were shown to regulate angiogenesis and to contribute to neoplastic transformation [94]. Several miRNAs such as "miR-17-92 cluster, miR-378, miR-296, let-7f, miR-27b, miR-130, and miR-126" were demonstrated to have angiogenic activities [95]. More than 40 miRNAs have been implicated in VEGF regulation [19]. The miR-17-92 induces angiogenesis in several solid tumors [96]. c-myc is a powerful inducer of angiogenesis by reducing the expression of Tsp1 which is also down-regulated by miR-17-92 [96]. miR-378 is elevated in several cancers and regulates VEGF expression [97]. miR-125a binds to VEGF-3'UTR to degrade it, miR-378 binds and competes to the same region, thus inhibiting VEGF silencing. Another study demonstrated that miR-378 is an oncogene, not only through induction of angiogenesis but also by promoting growth and tumor survival through targeting Fus-1 and Sufu tumor suppressor genes [98].

LncRNAs impact tumor formation through angiogenesis and have been quoted as "angio-LncRNAs" [99]. LncRNAs can function as natural antisense transcripts that can impact the proteins or can be located between protein-coding genes [100]. Hepatocellular carcinomas overexpress lncRNA MVIH which promotes angiogenesis and microvessel formation through abrogation of phosphoglycerate kinase 1 secretion [101]. Studies have indicated that lncRNAs can regulate VEGF signaling pathway [102]. MALATI "Metastasis-associated lung adenocarcinoma transcript 1" is a lncRNA that impacts tumor angiogenesis by enhancing endothelial cell proliferation [103]. MALATI is overexpressed in several tumors and stimulates invasion and metastasis [104]. The loss of MALAT1 reduces the proliferation of tumor cells and their ability to undergo angiogenesis as this lncRNA was shown to regulate cell-cycle related endothelial factors [104]. Other lncRNAs were also demonstrated to regulate angiogenesis such as HOXD-AS1, HIF-1A-AS2, and MEG3 among others by regulating angiogenic factors or hypoxia inducible factor 1-α (HIF-1α) or through other unknown mechanisms [94].

## Activating invasion and metastasis

Activating invasion and metastasis is the property of all hallmarks that contributes the most to malignancy and cancer-related death. Invasion and metastasis lead to an intertwined cascade that starts with tumor cell invasion, intravasation, extravasation, and successful growth of metastatic colonies [105]. Several listed epigenetic players in the regulation of angiogenesis also impact invasion and metastasis. Cell-cell and cell to extracellular matrix adhesion molecules are altered in invasion and metastasis such as the loss of E-cadherin and replacement by N-cadherin. However, the "epithelial-to-mesenchymal transition" (EMT) mostly regulates invasion and metastasis of carcinoma cells [106]. Genetic and most recently epigenetic mechanisms have been involved in the activation of EMT [107]. EMT is a normal process of embryogenesis and organogenesis, however this process is majorly deregulated in invasion and metastasis. EMT is a dedifferentiation program that involves a wide array of transcriptional factors that impact broadly epigenetic mechanisms and gene regulation.

ATP-chromatin dependent remodeling complexes play major roles in chromatin relaxation and compaction and impact gene regulation, DNA replication, DNA repair, chromosome segregation, and recombination. Chromatin remodeling complexes of which there are four major families: SWI/SNIF, CHD, ISWI, and INO80, contain an ATPase domain that hydrolyzes ATP to modify histones and affect nucleosome structure [108]. These chromatin remodeling complexes are deranged during malignant progression [109]. MTA1/2 is a member of the CHD family and its overexpression is involved with the invasiveness of several cancers. However, MTA3 is another member of CHD family that inhibits transcription of SNAI1 and inhibits EMT, invasion, and metastasis in breast cancer [110]. In addition, mutations are commonly observed in the SWI/SNIF remodeling complex where at least one member is mutated in 20% of human tumors [111]. DNA methylation also contributes to EMT where the DNA binding proteins MeCP2 and MBD1/2 recognize and attach to the methylated CpG in *E-cadherin* promoter resulting in gene silencing in tumor cells [112].

The EMT results in wide range transcriptional silencing of epithelial genes and activation of mesenchymal ones which is directed by histone modifications. In breast cancer cells, the HAT hMOF catalyzes H4K16 acetylation to preserve the expression of EMT tumor suppressors, namely E-cadherin and TSM1 [113]. Furthermore, the nuclear factor HNF3 collaborates with p300/CBP on *E-cadherin* promoter to antagonize EMT and metastasis of breast cancer cells [114]. Histone deacetylases are also implicated in the regulation of E-cadherin. The EMT transcriptional factor ZEB1 recruits HDAC1/2 to the *E-cadherin* promoter to decrease its expression and to induce EMT and invasion in pancreatic cancer [115]. However, ZEB1 recruits SIRT1 for the silencing of *E-cadherin* and to induce EMT and metastasis in prostate cancer cells [116]. Other epithelial genes, such as EPCAM and ESRP1, are involved in ZEB1-induced repression of EMT and induction of metastasis. Histone methylation may play repressive functions in EMT. Symmetric demethylation on H3K9 mainly by G9a results in transcriptionally repressed genes. For instance, G9a dimethylates H3K9 in the promoter of the cell adhesion EPCAM and results in EMT and metastasis in lung cancer cells [117].

Regulators RNAs such as miRNAs and lncRNAs are also involved in the regulation of EMT and their deregulation was observed in numerous

cancers [107]. Several of the previously listed miRNAs and lncRNAs that affect cell proliferation and angiogenesis also impact invasion and metastasis. For instance, miR-133a inhibits cell growth and invasion in NSCLC [118]. miR-223 directly silences PARP1 and enhances migration and invasion in esophageal adenocarcinoma [119].

ZEB1/2 transcripts are targeted at their 3'UTR by a dozen of miRNAs in several types of cancer cells [120] of which five miR-200 family members regulate differentiation and epithelial properties of several cell types and tissues [121]. ZEB1/2 and miR-200 and miR-205 reciprocally bind to suppress each other's expression to retain epithelial properties. In particular, the latter miRNAs inhibit ZEB1/2 expression and induction of epithelial phenotype [122]. Therefore, the delicate balance between miR-200/205 and ZEB1/2 constitutes a "feedback regulatory loop" which controls EMT and metastasis [123].

Several lncRNAs were demonstrated to control invasion and metastasis by several mechanisms including transcriptional and translational control, scaffolding structure, decoy for other miRNAs, and miRNA sponging effect [124]. In metastatic breast cancer, the lncRNA treRNA is overexpressed. TreRNA inhibits the translation of the E-cadherin epithelial marker by sequestering the RNA-binding proteins hnRNPK, PUF60, RXR1, and FXR2 and, therefore, promoting EMT and metastasis [125]. The lncRNA HOTAIR is overexpressed in several cancers where it acts as a scaffold needed for instance for the assembly of c-myc transcription initiation complex. In breast cancer cells, the oncoprotein HBXIP recruits c-myc to HOTAIR resulting in c-myc transcriptional activation of genes involved in metastasis [126]. Furthermore, HOTAIR overexpression retargets the polycomb repressive complex 2 (PRC2) to several genomic regions impacting colorectal and breast cancer metastasis [127, 128]. HOTAIR enhances invasion and migration and its silencing reduces cell growth and metastasis in colorectal cancer cells [129]. HOTAIR can also sponge miR-148a which reactivates Snail2 resulting in EMT in esophageal carcinoma cells [130]. The lncRNA ZFAS1 is an oncogene in hepatocellular carcinoma with elevated expression in tumor *versus* normal tissues [131]. The oncogenic

function of ZFAS1 is due to the sponging of miR-150, resulting in the reactivation of the metalloproteinases (MMP) 14 and 16 and ZEB1 and subsequent metastasis [131]. The lncRNA HNF1A-AS1 is overexpressed in adenocarcinomas where it directly attaches to DNMT1 facilitating its association with the *E-cadherin* promoter and subsequent EMT suppression [132]. HNF1A-AS1 downregulation upregulates E-cadherin while downregulating N-cadherin and β-catenin expression.

## Reprogramming energy metabolism

Tumor cells boost their cellular and energy metabolism in order to keep up with the sustained proliferation and to adjust to the challenging tumor microenvironment [133, 134]. For almost a century, it has been noted that cancer cell metabolism differs from normal ones due to their rewiring of energy metabolism to keep up with the hyperproliferative state [135]. The "Warburg effect" ensures that tumor cells utilize glucose by aerobic glycolysis instead of oxidative phosphorylation to ensure rapid energy demand and provides intermediates for anabolic reactions. It was not until the last decade that cancer metabolism was added as one of the cancer hallmarks [5] and the benefits of its therapeutic targeting became obvious [136].

Pavlova and Thompson classified deregulated cancer metabolism into six hallmarks as listed by verbatim: "(1) deregulated uptake of glucose and amino acids, (2) use of opportunistic modes of nutrient acquisition, (3) use of glycolysis/TCA cycle intermediates for biosynthesis and NADPH production, (4) increased demand for nitrogen, (5) alterations in metabolite-driven gene regulation, and (6) metabolic interactions with the microenvironment" [137]. In general, tumor cells acquire most of these hallmarks while few gain all of them. Oncogenes and tumor suppressor genes regulate cell proliferation and may achieve this by redirecting tumor cell metabolism [138, 139]. In particular, the effects of k-ras, c-myc, HIF1α, and mTOR on cancer metabolism may be counteracted by p53, pRB, and PTEN. Oncogenic mutations in PIK3CA, AKT, PTEN, and k-ras can substantially induce glycolysis by upregulating the mTOR-AKT signaling, the rate limiting steps in glycolysis as well as the GLUT1, glucose transporter

[140, 141]. On the other hand, p53 inhibits glycolysis and promotes oxidative phosphorylation while pRB reduces glutamine utilization in the TCA cycle and downregulates the glutamine transporter ASCT2 [142, 143].

Epigenetic mechanisms link nutritional status to changes in gene expression. Tumor cells may express a proliferative gene expression profile by highjacking the epigenome. In fact, metabolic alterations and epigenetic mechanisms are intimately intertwined in cancer cells [144, 145]. Cancer metabolism and epigenetics crosstalk resulting in the metabolic rewiring that provides cofactors needed for epigenetic enzymes and production of oncometabolites that regulate epigenetic mechanisms.

The Warburg effect may be observed due to the differential DNA methylation detected in the glycolytic phenotype in tumor cells. *VHL* hypermethylation results in constitutive expression of HIF1α and increased glycolysis in renal cell carcinoma [146]. The glucose transporter GLUT1 is downregulated by Derlin-3-proteasomal-mediated degradation. *Derlin-3* is epigenetically silenced by DNA hypermethylation, resulting in increased transport of glucose to tumor cells and enhanced Warburg effect [147]. The rate limiting steps in gluconeogenesis, fructose 1,6-biphosphatase 1 and fructose 1,6-biphosphatase 2, that antagonize glycolysis are due to promoter hypermethylation in gastric cancer cells [148, 149]. This results in enhanced glycolysis needed for anabolic metabolism and ATP production. Tumor progression and enhanced glycolysis have been observed by genetic silencing of *BRCA1* by DNA hypermethylation in breast cancer cells [150]. Therefore, differential DNA methylation regulates the Warburg effect in tumor cells.

The Sirtuins (SIRTs) are family members of HDAC inhibitors that can either operate as oncogenes or tumor suppressor genes [151]. They are the most studied histone modifying enzymes in tumor metabolism. SIRT6 can function as a tumor suppressor by deacetylating and reducing HIF-induced glycolysis and c-myc-related glutaminolysis [152]. Frequent deletions of SIRT6 are observed in several human cancers. In addition, SIRT7 directly interacts with c-myc and represses its metabolic aberrations [152]. Further studies are required to study the effect of other histone modifications on the metabolic alterations in tumor cells.

ncRNAs can regulate metabolism of cancer cells at different metabolic pathways. miRNAs control gene expression of several players in cancer metabolism [153]. They can contribute to the Warburg effect by enhancing glycolysis and downregulating oxidative phosphorylation through the TCA cycle. miRNAs also regulate common tumor oncogenic pathways such as c-myc, HIF1α, and k-ras that may impact cancer metabolism [154]. miRNAs also impact the genes involved in glucose uptake, namely miR-1291 for GLUT1 [155], miR-195-5p for GLUT3 [156], and miR-93 for GLUT4 [157]. miRNAs also affect key glycolytic enzymes such as miR-143 and miR-155 for hexokinase 2 [157] and miR-326 for pyruvate kinase M2 [158]. Glutaminase targeting by miR23a reduces glutaminolysis in cancer cells [159].

LncRNAs regulate glucose, lipid, and amino acid metabolism [160]. LncRNAs were shown to regulate glucose metabolism by binding to their GLUTs transporters. ANRIL is a large antisense lncRNA that transcribes an RNA in antisense INK4B-ARF-INK4A cluster [161]. ANRIL is increased and promotes nasopharyngeal carcinoma development where it activates the AKT/mTOR pathway resulting in elevated GLUT1 and lactate dehydrogenase expression [162]. LncRNAs also regulate glycolysis by binding to vital enzymes such as 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2 in cancer-associated fibroblasts to promote invasion in ovarian cancer [163]. The lncRNA ceruloplasmin upregulates the key glycolytic enzyme glucose-6-phosphate isomerase causing glycolysis and tumor progression [164]. The lncRNA UCA1 is elevated in bladder carcinoma and enhances glycolysis by upregulating its first regulatory enzyme hexokinase [165]. A variety of these lncRNAs are deregulated in a several solid and hematological malignancies [160] and understanding of their metabolic roles will shed light on cancer prognostics and therapeutics.

### Evading immune destruction

The immune response plays a double-edge sword in cancer as it can fight tumor cells or can generate an inflammatory tumor microenvironment that enhances tumor progression. Immune checkpoints are mechanisms that keep in check the immune response and control autoimmunity. Tumor cells have managed to avoid tumor surveillance and evading

immune destruction by "hijacking" immune checkpoints [166]. Recent successes in generating drugs for targeting and silencing these immune checkpoints and subsequent activation of tumor immunity provided hope for previously untreatable advanced malignancies [167]. The most investigated immune checkpoint inhibitors are focused on inhibitors of programmed death protein-1 (PD-1) and its ligands PD-L1 and PD-L2, in addition to inhibitors of cytotoxic T lymphocyte associated antigen 4 (CTLA-4). PD-1 is present on B cells, activated T cells (CD4 and CD8), natural killer (NK) cells, and dendritic cells. While CTLA-4 controls the T-cell activity in the initial phase of immune reaction, PD-1 regulates the late activity of the immune phase in the tumor microenvironment.

The epigenetic modulation of immune checkpoints has revealed crucial mechanisms [168]. An elevated risk of gastric cancer was correlated with enhanced hypermethylation of *CTLA-4* promoter [169]. The cytotoxic activity of NK cells is epigenetically regulated by methylation of the killer cell immunoglobulin-like receptors and the DNMT inhibitor azacytidine restores the function of NK cells [170]. PD-1, PD-L1, and PD-L2 expression are also regulated by promoter methylation where the use of the demethylating agent decitabine results in their upregulation in myelodysplastic syndromes [171]. Clinical trials are studying the efficacy of combining immune checkpoint inhibitors and DNA demethylating agents [172].

The *CTLA-4* promoter is controlled by histone acetylation and nuclear factor of activated T-cell binding (NFAT) [173]. PD-1 expression on CD8 T cells is rich with enhancer sequences containing acetylation of H3K27 and monomethylation of H3K4 which are markers of activated transcription [174]. In response to several cytokines, the T helper (TH)-1 genes *TBET* and *INFγ* show elevated expression due to promoter acetylation [175]. Several clinical trials are evaluating the promise of combining immune checkpoint inhibitors and HDAC inhibitors [168].

Numerous studies point out to the link between miRNAs and immune checkpoints regulation. PD-1 is regulated in cancer by miR-138-5p [176]. Several miRNAs target PD-L1 such as miR-34a-5p, miR-200, miR-513a-5p, and miR-570-3p [19]. CTLA-4 is directly targeted by miR-

138 and has shown efficacy in anti-glioma therapy [177]. A great number of miRNAs loci display CpG rich islands indicating that DNA methylation is a key regulatory mechanism [178]. In lung cancer, p53 was demonstrated to regulate tumor evasion through miR-34 which targets PD-L1 [179]. Several miRNAs regulate epigenetically immune checkpoints and, therefore, present potential therapeutic strategies in targeting these miRNAs either alone or in combination with checkpoint inhibitors.

Several lncRNAs were recently shown to regulate cancer immunity [180]. The lncRNA APOC1P1-3 is overexpressed in breast cancer, due to hypomethylation of its promoter region, and is related to tumor size [181]. APOC1P1-3 binds directly to tubulin resulting in its decreased acetylation, inactivation of caspase-3, and apoptosis inhibition. T cell activity in the tumor microenvironment affects tumor development. Several reports point out to the crucial role of lncRNA in mediating T cell functions. Some lncRNAs regulate Treg differentiation and others abrogate cytotoxic functions [182]. The lncRNA HOTAIR induces retinoic acid-mediated differentiation of myeloid cells to granulocytes [183]. The lncRNA lnc-DC controls dentritic cells differentiation by binding directly to STAT3 and activating it [184]. These few listed examples indicate that lncRNAs can be used as targets for anticancer therapies.

Hanahan and Weinberg have defined two enabling characteristics which facilitate the acquisition of the different hallmarks [5]. In particular, the contributions of the genome instability that lead to random mutations and gross chromosomal abnormalities leading to the different hallmarks. In addition, the role of tumor-promoting inflammation in tumorigenesis starting from the pre-tumorigenic to the benign then malignant phases is well-established [185]. Epigenetic mechanisms and these two enabling characteristics crosstalk and impact each other as discussed next.

### How epigenetic mechanisms contribute to genome instability and mutations

Genome instability leads to the buildup of point-mutations and gross chromosomal changes throughout the genome. These in turn lead to the formation of oncogenes and the loss of tumor suppressor genes that favor tumor development. Although genetic mutations are

crucial for tumor formation, however, epigenetic mechanisms are as essential. Epigenetic mechanisms modify chromatin structure and can lead to global DNA mutations. Also, epigenetic mechanisms can affect concomitantly the expression of hundreds of genes that affect tumor progression.

The DNA methylation pattern of the genome of cancer cells differs from that of normal ones. Cancer genomes are typically hypermethylated at CpG islands at specific genes and hypomethylated at repetitive DNA elements [186]. For instance the hypermethylation of CpG islands occurs early in approximately 25% of lung and colorectal cancer tissues [187, 188]. Several DNA repair genes are silenced by DNA hypermethylation in many tumor types leading to further accumulation of DNA mutations and genome instability. The DNA repair gene *MGMT* is hypermethylated and silenced in more than 40% of colorectal and brain tumor tissues [189]. The *MLH1* gene is hypermethylated and silenced in colorectal, ovarian, and endometrial cancers which results in malfunctioning of the DNA mismatch repair complex and aberrations in microsatellite repeat stability [190-192]. The *BRAC1* gene is hypermethylated in medullary breast carcinomas, sporadic mucinous breast carcinomas, and sporadic ovarian carcinomas by 67%, 55%, and 31%, respectively [193, 194]. Alternatively global DNA hypomethylation leads to the activation of oncogenes and of chromosomal and centromeric instability [195, 196]. Global DNA hypomethylated was suggested to cause chromosomal instability in the early steps of hepatocellular carcinoma [197].

Chromatin modifiers are crucial for the maintenance of chromatin, packing into nucleosomes, and regulated gene expression and their dysregulation is observed in early and advanced phases of neoplastic transformation. This balance is carefully maintained by enzymes that add or remove acetyl groups (HATs *versus* HDACs), methyl groups (HMTs *versus* HDMs), and phosphoryl groups (histone kinases *versus* histone phosphatases). Mutations are detected in cancers in genes encoding HATs, HMTs, and HDMs in particular on lysine residues [198]. For instance, NSD1 and NSD3, two lysine methyl transferases, are subject to chromosomal translocation in acute myeloid leukemia while the lysine HDMs 5A is mutated in this leuke-

mia [199]. HAT mutations are commonly detected in SCLC and B cell lymphomas [200, 201]. Several HDACs are overexpressed in cancer and result in silencing of tumor suppressor genes. The following HDACs are upregulated in cancers such HDAC1 in breast, prostate, colorectal, esophageal, and gastric cancers, HDAC2 in gastric, cervical, and colorectal cancer, HDAC3 in colorectal, gastric, and prostate cancer, and HDAC6 in breast cancer [202, 203]. The overexpression of HDACs in several cancers is due to the interplay between transcription factors and epigenetic modulators on their promoter regions. HDAC1 and HDAC2 are overexpressed in colorectal cancer due to the binding of the transcriptional factors, Sp1/Sp3, HAT p300, and histone H3K4 methyltransferase SET1 [204]. These results show that the use of HDAC inhibitors may be a therapeutic strategy in reactivating tumor suppressor genes in HDACs overexpressing cancers.

miRNAs are also controlled by epigenetic mechanisms and are commonly altered in human cancers [205]. Several miRNAs, miR-9, miR-124, miR-137, miR-148, and miR-512 are silenced by CpG hypermethylation in some cancers. Some specific miRNAs called "epi-miRNAs" were demonstrated to target epigenetic regulators [206] and their deregulation contributes to genomic instability [207]. miRNA-induced genomic instability may be due to loss of control of the cell cycle checkpoints, DNA repair mechanism, and mitotic separation. miR-372 is an oncomiR that targets p53 and deregulates p53-mediated CDKI and cell cycle regulation through its inhibition of LATS2 [208]. The stability of the genome was also shown to be guarded by p53 and LATS2 to prevent tetraploidization [209]. Therefore, deregulation of miR-372 promotes oncogenic transformation and genomic instability. The oncomiR miR-155 is overexpressed in several cancers and can result in lymphoblastic leukemia [210]. Studies indicated that DNA mismatch genes (MLH1, MSH2, and MSH6) are controlled by miR-155. Colorectal cancer cells overexpressing miR-155 reduced the levels of MLH1, MSH2, and MSH6 and showed microsatellite instability [211]. In addition, miR-155 was shown to damage telomeres by reducing the levels of the telomere binding factor TERF1 leading to genomic instability and telomere fragility in breast cancer [212]. LncRNA can affect chromatin remodel-

ing and gene expression and, therefore, regulate genome stability [213]. It remains to be determined whether lncRNA aberrations by epigenetic mechanisms lead to genome instability.

## Tumor-promoting inflammation and epigenetic mechanisms

It is well-established from epidemiological studies that inflammatory conditions enhance cancer incidence [214]. Epidemiological studies estimate that chronic inflammation is linked to 15% of cancer incidence [215]. In particular, the following chronic inflammatory conditions make individuals susceptible to cancer namely infection by *H. pylori* is linked to high incidence of gastric cancer while hepatitis C virus is associated with liver cancer; inflammatory bowel condition with colorectal cancer; and inflammation due to chronic ultraviolet exposure with skin cancer. Inflammation and cancer are closely linked. While chronic inflammation increases the incidence of cancer and consumption of drugs that reduce inflammation decreases cancer incidence and death [216].

Although acute inflammation may have beneficial effects in fighting infections and repairing tissue damage, however chronic infection by viruses, environmental exposures to irritating agents such as asbestos, ultraviolet radiation, and air pollutants cause chronic inflammation that predisposes individuals to certain cancers. Several signaling pathways are activated due to reactive oxygen species (ROS), inflammatory interleukins (ILs), and cytokine secretions (IL-6, IL-1β, TNF-α), and survival signaling pathways (NF-κB, STAT3, MAPK). In turn, the inflammatory microenvironment accentuates tumor progression by the release of protumorigenic cytokines and growth factors by inflammatory and immune cells.

Epidemiological, *in vivo*, and *in vitro* studies show a connection between chronic inflammation and deregulated epigenetic mechanisms in cancer formation [214]. *H. pylori* infection causes gastric cancer. It was also demonstrated that epigenetic alterations are involved in gastric cancer [217]. Patients infected with *H. pylori* display a high incidence of *E-cadherin* promoter methylation and silencing in comparison with non-infected individuals [218]. *E-cadherin* silencing was detected by more than 50% as early as in intestinal metaplasia

and was further increased in tumor invasion and metastasis. In addition, several CpG islands containing genes were hypermethylated in *H. pylori*-infected mucosa [219]. Epigenetic alterations are also detected in other inflammatory conditions such as ulcerative colitis, that predispose individuals to colorectal cancer, have shown increased methylation of the CDKI *p16* [220].

The association studies between inflammatory conditions and epigenetic mechanisms were causally linked in animal studies. Suppression of *H. pylori* infection in animal model of gastric cancer resulted in reversal of infection-causing methylation changes in gastric mucosa of infected animals [221]. In precancerous colorectal tissues of mice treated with azoxymethane- and dextran sulfate sodium show elevated levels and activity of HDAC and depletion of acetylated H3K27 [222]. Histone hypoacetylation was reversed with aspirin treatment, a nonsteroidal inflammatory drug. The literature is rich in providing more examples relating chronic inflammation and epigenetic mechanisms in early and late phases of tumor formation *in vivo* and *in vitro* models [214].

Some of the listed molecular mechanisms that link cancer-causing chronic inflammation and epigenetic mechanisms are alterations of cellular metabolism and cofactors namely S-adenosyl methionine (SAM) needed for several epigenetic enzymes such as DNMTs and HMTs and the cofactor acetyl CoA needed for HAT activity [223]. Also inflammation may affect epigenetic mechanisms through DNA damage. ROS generation in the tumor microenvironment by inflammatory cells can damage DNA. In colorectal cancer, the tumor suppressor gene caudal type homeobox-1 (*CDX1*) is hypermethylated and silenced due to hydrogen peroxide [224]. DNA is susceptible to ROS-induced DNA damage particularly at guanine bases [225]. Also ROS hinder the ability of DNMTs to bind to hemimethylated DNA resulting in global DNA hypomethylation and genomic instability [226].

Several reports have indicated that inflammation alters miRNA profile in epithelial cells of the colon and, therefore, linking miRNAs to tumor development [227]. There are 11 differentially regulated miRNAs that affected inflammatory chemokine production such as macrophage inflammatory peptide (MIP)-2 alpha that is secreted by the epithelial cells. miRNA profile

in epithelial cells can be modified by various mechanisms during inflammation including NF-κB activation, IL-6 induced STAT3 phosphorylation, or cytokine secretion [227, 228]. One of these modified during inflammation is the tumor-suppressor miR-7 which targets EGFR. In a mouse model of inflammation-induced gastric cancer, 40 miRNAs were regulated during the different phases of inflammation-induced cancer [229]. Activated macrophages where shown to inhibit miR-7 and contribute to gastric cancer formation. The Let-7 family of miRNAs consists of 12 members which target c-myc and ras [230, 231] and their genomic positions are commonly deleted in colorectal cancer and other tumors [232]. miRNA genes are commonly observed at fragile sites of chromosomes and common chromosomal breakpoints. More than 50% of 186 investigated miRNAs were demonstrated to map at fragile sites and in cancer-linked chromosomal regions [232]. miR-31 expression was shown to increase in inflammatory bowel to cancer which targets the negative regulator of HIF-1α and consequently upregulating HIF-1α activity [233]. The miRNAs profile observed in cancer inflammatory tissues can be present in epithelial or immune cells.

Recently, lncRNA profile was shown through a genome profiling to be modified during different phases of tumor progression in cholangiocarcinoma [234]. Several of these lncRNAs regulate genes involved in inflammation and oxidative stress signaling. Another lncRNA lincRNA-Cox2 was shown to control the body inflammatory reactions to injury and infections. In *lincRNA-Cox2* knockout animal models, macrophages and mouse tissues had deregulated expression of inflammatory genes [235]. Deletion of *lincRNA-Cox2* reduced the expression of cyclooxygenase-2 which is a major enzyme in prostaglandin synthesis. LincRNA-Cox2 functions as an enhancer RNA for the gene *Ptgs2* that encodes cyclooxygenase-2.

### Conclusions

The hallmarks of cancer by Hanahan and Weinberg have shaped and focused research in cancer biology for the last two decades [4, 5]. Although we agree with most of the hallmarks in shaping the chronic process of neoplastic transformation, however, we propose that aberrations of epigenetic mechanisms lie at their hearts or so called "hallmark of hallmarks"

(**Figure 1**). Some of these hallmarks may have overlapping functions such as supporting proliferative signaling, escaping growth suppressors, and counteracting cell death, however each one individually contributes to cancer formation.

The chronology of the different hallmarks is not crucial in orchestrating the transformation process but what matters is that epigenetic mechanisms are early events in the transformation process and that genomic instability and mutation and tumor promoting inflammation assist in the acquisition of these hallmarks. Previous reports have perceived the different hallmarks as continuous evolutionary events in cancer formation [8, 236]. The hallmarks of cancer are shaped by genomic instability and tumor-promoting inflammation that in turn contribute to abnormalities in epigenetic mechanisms. A couple of years before Hanahan and Weinberg suggested that tumor-promoting inflammation is an enabling characteristic of cancer hallmarks, Colotta *et al.* proposed that "cancer-related inflammation, is the seventh hallmark of cancer and it links to genetic instability" [185].

Ageing and environmental factors collaborate with epigenetic mechanisms to contribute to the different hallmarks. It was observed that hypomethylation-associated ageing may be a contributing factor of cancer and chronic inflammation as it increases the immunogenicity of DNA [237]. Epigenetic damage results from ageing where genome methylation status of individuals resembles general genome hypomethylation observed in cancer genomes. Environmental factors encompass toxicants, diets, and alcohol intake that affect the epigenome [238]. The effects of diets such as energy intake, folate consumption, and polyphenol content may contribute to changes in the epigenome [239]. Some of these alterations may be subtle and occurring earlier than any carcinogenic effect detection. In fact epigenetic deregulation occurs very early such as DNA methylation changes are identified in normal tissues and increase steadily with age [240]. For instance, the *EN1* gene expresses engrailed-1 homeobox protein which controls pattern formation during embryogenesis and is widely expressed in the cerebellum [241]. *EN1* promoter hypermethylation has been detected in
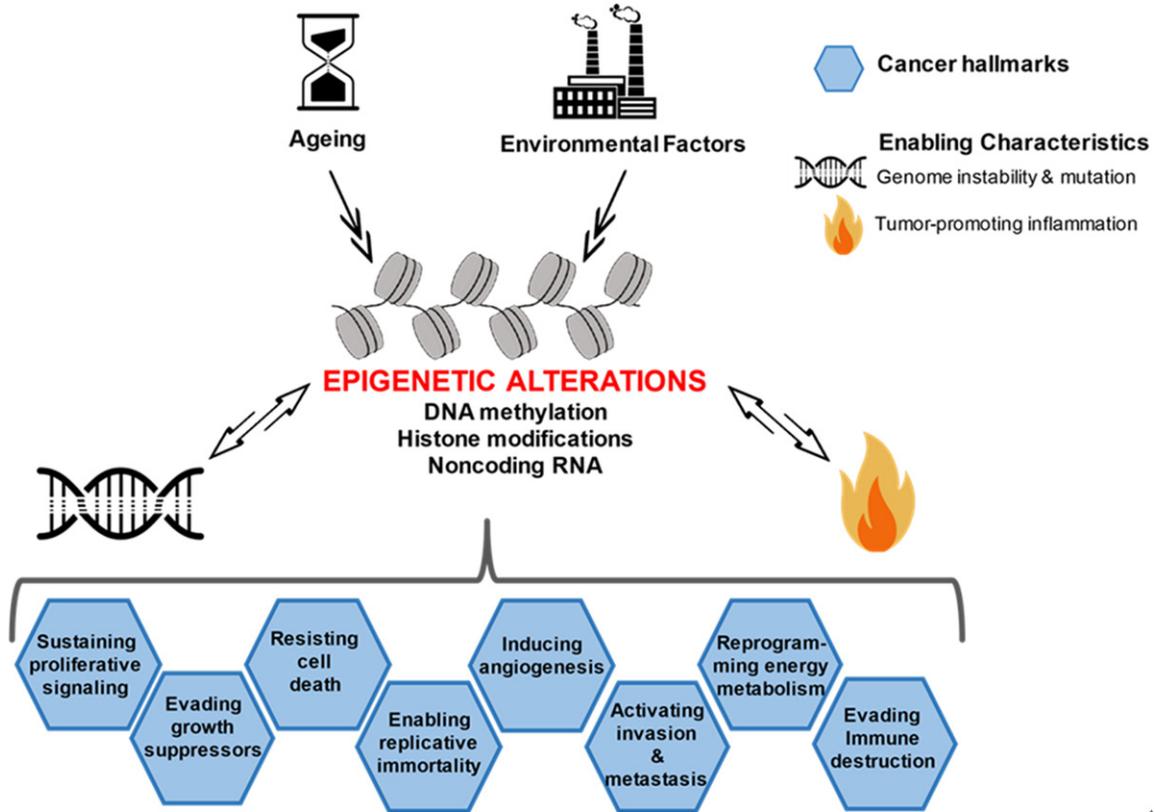
**Figure 1.** The central role of epigenetic mechanisms in the hallmarks of cancer. Epigenetic alterations comprise DNA methylation, histone modifications, and noncoding RNAs. Ageing and environmental factors collaborate with epigenetic mechanisms to contribute to the different hallmarks. The hallmarks of cancer are shaped by the enabling characteristics: "genomic instability and mutation and tumor-promoting inflammation" that in turn lead to abnormalities in epigenetic mechanisms.

several solid tumors including colorectal [242] and prostate cancers [243]. Hypermethylation was associated with tumor grading and patient clinical outcome as 30% reduction in the 5-year survival rate was observed in colorectal cancer patients compared to those without *EN1* hypermethylation [242]. These findings stipulate that aberrant methylation of certain biomarkers such as *EN1* gene can be used for early cancer detection.

Loss of imprinting (LOI) of insulin-growth factor 2 (*IGF2*) may happen early in colorectal cancer cell transformation and may be used as an early marker of cancer. *IGF2* LOI results in increased cell proliferation due to activation of the second silent allele resulting in doubling of gene expression of this mitogen [244]. In Barrett's esophagus, chronic acid reflux results in inflammatory reactions and epigenetic changes well before cancer formation [245]. Epigenetic modifications and DNA methylation

changes are common pre-tumorigenic events for decades earlier than cancer appearance. This underscores the usefulness of detecting epigenetic changes early in the carcinogenic process and thus may have chemopreventive and therapeutic implications.

The significance of epigenetic mechanisms in the hallmarks of cancer is their reversibility and, therefore, therapeutic implications. The exciting aspect of epigenetics model of cancer is its central role in the cancer hallmarks and that its dysregulation may act as a "common driver through cancer progression" [22]. Some of these epigenetic mechanisms directly impact the hallmarks of cancer and others may have indirect effects through genomic instability, mutations, and tumor promoting environment as listed in this review. The original hallmarks of cancer are very much designed by the mutation theory, and thus reversibility and therapeutic targeting may not be easily accom-

plished, hence the crucial role of epigenetic mechanisms in chemoprevention and therapeutic tumor targeting.

Finally, by targeting epigenetic mechanisms we will be aiming at several hallmarks of cancer. This represents a great advantage of "epidrugs" as they aim reversible processes. Unfortunately, attempts to use "epidrugs" has been linked to several genome-wide effects. The use of general DNA methylase inhibitors generated genome instability [246] and the use of general HDAC inhibitors sometimes promoted tumor growth [247]. We have not deciphered yet most functions of ncRNAs. Further research will uncover the crucial role ncRNAs play in the various steps of tumor formation and may contribute substantially to understanding the role of epigenetic mechanisms as biomarkers and therapeutic targets of cancer [18, 20].

### Disclosure of conflict of interest

None.

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