Al-Rafidain J Med Sci. 2023;5:1-13.
DOI: https://doi.org/10.54133/ajms.v5i.128

AJMS
Online ISSN (2789-3219)

Review Article

Therapeutic Targeting of the Regulators of Cancer Epigenomes

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Received: 24 May 2023; Revised: 22 June 2023; Accepted: 25 June 2023

Abstract

Aim: To assess the value of targeting the various molecules that regulate the epigenome in the management of cancer. ***Method***: Peer-reviewed articles were examined in PubMed, Google Scholar and ResearchGate search tools using keywords given in the manuscript. ***Main points***: Three major epigenomic modifications namely DNA methylation, histone methylation and histone acetylation attracted most research interest and led to a few globally approved drugs for the treatment of various malignancies. The DNA methylation profiles of cancer have been successfully employed in many aspects of the management of this disease. ***Conclusion***: Epigenomic profiling of cancer specimens has already been successfully employed in the management of cancer in a handful of specialized clinics and this application could be extended further following more in-depth investigations in this field.

Keywords: Epigenetics and cancer, Epigenomic and cancer, Cancer epigenome and epidrugs.

الاستهداف العلاجي لمنظمات المورثات السرطانية

الخلاصة

الهدف: تقييم قيمة استهداف الجزيئات المختلفة التي تنظم الإبيجينوم في إدارة السرطان. **الطريقة**: تم فحص المقالات التي تمت مراجعتها من قبل المقيمين في أدوات البحث PubMed و Google Scholar و ResearchGate باستخدام الكلمات الرئيسية الواردة في المخطوطة. **النقاط الرئيسية**: جذبت ثلاثة تعديلات جينومية رئيسية وهي مثيلة الحمض النووي ومثيلة الهيستون وأيسثلة الهستون معظم الاهتمام البحثي وأدت إلى تصميم عدد قليل من الأدوية المعتمدة عالميا لعلاج الأورام الخبيثة المختلفة. تم استخدام ملامح مثيلة الحمض النووي للسرطان بنجاح في العديد من جوانب إدارة هذا المرض. **الأستنتاج**: تم بالفعل استخدام التنميط فوق الجيني لعينات السرطان بنجاح في علاج السرطان في عدد قليل من المراكز العلاجية المتخصصة ويمكن توسيع هذا التطبيق بشكل أكبر بعد إجراء المزيد من الأبحاث المعمقة في هذا المجال.

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Article citation: Al-Janabi II. Therapeutic targeting of the regulators of cancer epigenomes. *Al-Rafidain J Med Sci*. 2023;5:1-13. doi: https://doi.org/10.54133/ajms.v5i.128

INTRODUCTION

Cancer is not exclusively a disease caused by alteration of DNA sequences. The initiation and progression of this disease are also dependent on other factors including its microenvironment and epigenetics [1-3]. During the last 30 years, an increasing number of investigations have concluded that epigenomic alterations can be as critical as genetic mutations in the dysregulation of cellular pathways that contribute to tumorigenesis [4]. The field of epigenetics can be considered as a second layer of genomic information encoded in the DNA and its associated proteins to further guide gene expression and bridge the gap between the genome and environmental signals [5]. The tuning of gene expression through epigenomic modifications is a crucial process in cell differentiation and embryogenesis with mounting evidence of its importance in carcinogenesis [6]. The term epigenetics was originally coined by Waddington in 1942 although its definition remains both contentious and ambiguous [7,8]. At the whole genome level, epigenomics and epigenome (in contrast to epigenetics and genes) are considered more appropriate to use as they encompass modifications to the whole of the genome. Epigenome is defined as heritable and reversible modifications that can occur at the epigenome level without changing the sequence of the DNA bases

but can change the DNA conformation and therefore alter gene expression [9]. To understand the epigenome, we must imagine how the vastly long DNA chain is stored in the very small space within the nucleus of the cell. Specialized proteins called histones are responsible for compacting the DNA within the nucleus and the resulting structure of the DNA wrapped around these proteins is called chromatin. The compact nature of the chromatin, although useful in holding a large amount of material in a limited space, leads to the inevitable denial of accessibility to the DNA. Access to the DNA by various proteins and factors is required and crucial for the control of gene expression. This creates a default situation of gene inactivity unless the DNA is unwrapped and unfolded allowing interactions with regulatory factors. To achieve this, various enzymes are provided to remodel the chromatin and allow contextual access to the DNA molecule. There are many families of histone proteins but those that are associated with the DNA molecule are known as H1, H2A, H2B, H3 and H4. The amino acid sequences of these histones are highly conserved in different organisms indicating their critical functions [10]. Each histone protein consists of a globular domain and a tail and is subject to posttranslational modifications among them, methylation and acetylation being the most extensively studied. Two molecules each of H2A, H2B, H3 and H4 come together to form an octamer around which 1.65 turns of DNA (145-147 base pairs (bp)) are wrapped. This forms the basic repeating unit of the chromatin which is called a nucleosome. The nucleosomes are joined by a linker DNA chain that runs between them for an average of 20 bp [11]. The presence of one molecule of histone H1 can wrap a further 20 bp of DNA (producing a full two turns of the DNA) and results in a structure termed a chromatosome [12]. The protein and DNA assembly, the chromatin, can assume two major configurations: a) heterochromatin which is highly condensed leading to gene inactivity and b) euchromatin which is a more open configuration supporting most of the active genes. The ability of this chromatin assembly to adapt and respond, through epigenomic modifications, to various environmental changes during the development and the lifetime of an organism is a major cornerstone of existence (Figure 1) [13]. The link between the epigenome and cancer stems from the wide recognition that cancer is a complex disease driven not only by genetic alterations but also by epigenomic modifications. The association between the dysregulation of the epigenome and cancer is a feature of many cancers [14-17]. Sequencing of tumours from many patients revealed mutations in various proteins that function to regulate the epigenome [18]. These epigenomic regulators are enzymes that are relatively easy to target, providing a good opportunity to develop therapeutic agents to treat cancer.

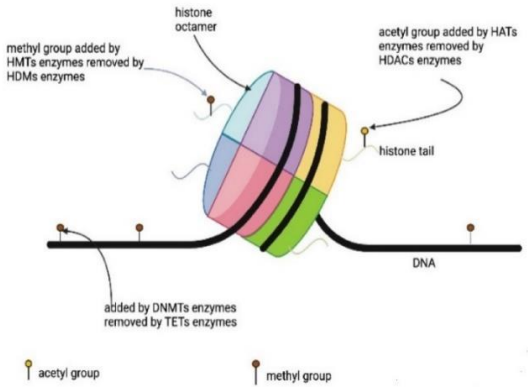

Figure 1: A representation of the basic structure of chromatin showing one of its repeated units, the nucleosome, and featuring common epigenomic modifications. HMTs= histone methyltransferases, HDMs= histone demethylases, HATs= histone acetyltransferases, HDACs= histone deacetylases, DNMTs= DNA-methyltransferases and TETs= ten eleven translocation enzymes.

In this review, we briefly outline the mechanisms involved in epigenomic modifications relevant to carcinogenesis and current therapeutic developments in the treatment of cancer through the targeting of the main players causing these modifications (Figure 2).

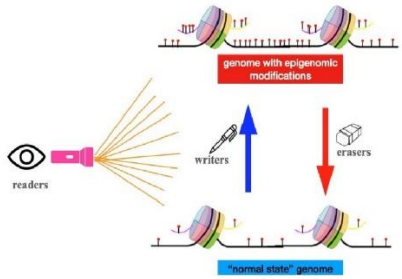

Figure 2: A visual illustration of the role of writers, erasers and readers in modifying the epigenome.

METHODS

Peer-reviewed articles, in the form of research studies and reviews published between January 2003 and January 2023, were examined using three search tools: PubMed, Google Scholar and ResearchGate. Publications before January 2003 were only considered in the survey if the initial reading of the collected articles suggested that they represent significant discovery and/or had a historical value. The key phrases and the keyword employed in the literature survey were epigenetics and cancer, epigenomics and cancer, cancer epigenome and epidrugs with the Boolean operator "and" requiring both terms on either side of it to be present in the article considered. For this narrative review, studies and reviews with inadequate data analysis or deemed insufficiently reflecting the aim of the present work were excluded.

Regulation of the epigenome in cancer and potential targets

Modifications to the epigenome can fall into three distinct classes: a) nucleic acids (DNA or RNA) modifications e.g., methylation, b) histone modifications e.g., methylation, acetylation, phosphorylation and ubiquitylation and c) the regulation by non-coding RNAs. These modifications can alter the chromatin structure by changing the covalent bonding between and within nucleosomes affecting the accessibility of DNA to transcription factors and other proteins that need to interact with the DNA [19]. Furthermore, these modifications can act as docking stations for specialized proteins capable of detecting the changes and acting upon them accordingly [15]. Modifications to the epigenome can either lead to an open chromatin configuration and enhanced gene expression or to a closed chromatin structure that inhibits gene expression depending on the type of alteration in question [2]. Together, these epigenome alterations ensure the desired regulation of gene expression to essentially maintain cellular identity [20]. Specialized proteins regulate nucleic acid and histone modification marks. Those proteins that add marks to the nucleotides of the DNA and specific amino acids of the histones are called "writers". Other proteins "erasers" act to remove the marks to achieve fine-tuning of gene expression and reversibility. Yet more specialized proteins can recognize and interpret the modified DNA or histones and these proteins are called "readers" (refer to Figs 3,4 and 5) [20,21]. Non-coding RNAs (ncRNAs) with genomic regulatory functions take up a large part of the human genome and are divided into two main categories: small non-coding RNAs of less than 200 nucleotides and long non-coding RNAs of more than 200 nucleotides [22,23]. The most studied small ncRNAs are micro-RNAs (miRNAs) which are highly conserved single-stranded molecules of around 20 nucleotides in length. The regulatory non-coding RNAs participate in the fine-tuning of gene expression through their post-transcriptional binding with their complimentary mRNA and more directly through DNA-methylation changes [23,24]. Non-coding RNAs have been the subject of intense investigations in recent years and will not be the focus of this simple review. In this overview, we will consider methylation of the DNA, methylation of the histones and acetylation of histones as they represent the three major epigenomic modifications.

DNA methylation

The most relevant epigenome modifications from a cancer point of view are DNA methylation. The methylation of DNA was first identified in 1983 and remains the most widely studied [25]. Alteration in the DNA methylation pattern is strongly linked to cancer progression and metastasis [5]. DNA methylation is a covalent modification that occurs on the 5-position of the pyrimidine ring of the cytosine base of the nucleotides and very often on the cytosine bases that are followed by guanines (what is commonly termed CpG islands or sites). The CG dinucleotides (the CpG islands) in mammalian cells are largely methylated whereas these dinucleotides are protected from methylation if they occur in the promoter regions of genes and remain mainly hypomethylated [26]. The CpG islands are not randomly distributed throughout the genome but exist as clusters primarily at the 5` regulatory regions (promoters) of genes. It is estimated that around half of all human genes contain CpG islands mainly in their promoters [27]. Methylation of the cytosine bases in these CpG islands causes transcriptional repression of their associated genes and can act as a silencing mechanism of tumour suppressor genes thus promoting carcinogenesis [28,29]. In contrast, the hypermethylation of the main body of the gene (as opposed to the promotor region) is a common feature in active genes compared with their flanking sequences [30]. Contrasting the methylated state of the promoter regions of the DNA, whether hypo- or hypermethylated, is simplified and illustrated in Figure 3.

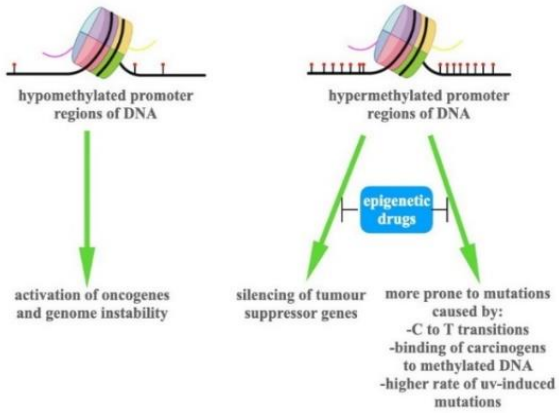

Figure 3: A schematic comparison between hypomethylated and hypermethylated epigenome concerning cancer and possible targets for epigenetic drugs. C= cytosine, and T= thymine.

To unravel the methylation status, the DNA is treated with sodium bisulphite which removes the amine group from unmethylated cytosines, converting them to uracil, leaving behind methylated cytosines unaffected and creating sequence differences which can be resolved by analysis [31].

DNA methylation writers

The methylation patterns are regulated by enzyme "writers" that add the methyl groups either *de novo* by a process in which methyl groups are added to cytosine at unmethylated DNA or through the preservation of existing formats during the copying of DNA strands. These enzymes belong to a family of proteins called DNA methyltransferases (DNMTs) which includes DNMT1, DNMT2, DNMT3A, DNMT3B and DNMT3L (Figure 4).

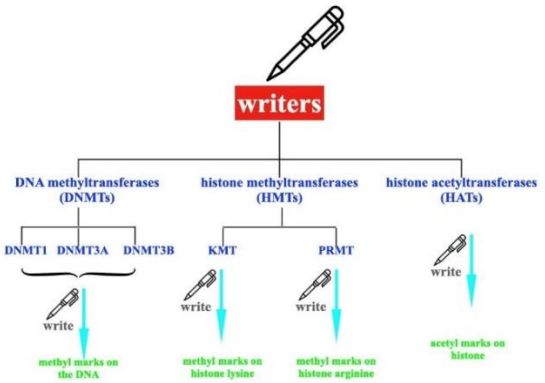

Figure 4: The main writers of epigenomic modifications. KMT= lysine methyltransferase, and PRMT=protein arginine methyltransferase.

The enzymes DNMT3A and DNMT3B are responsible for the *de novo* modifications while DNMT1 is responsible for the addition of methyl groups to maintain and preserve the *status quo* during DNA replication [15]. Despite DNMT2 sharing considerable sequence homology with other DNMTs, it does not appear to possess detectable cytosine-methylation properties. Instead, DNMT2 is the only methyltransferase that methylates the RNAs [13]. The Enzyme DNMT3L lacks the catalytic activity but surprisingly upon its association with DNMT3A and DNMT3B it increases their catalytic activity by as much as 15-fold and acts as a co-activator of DNMT3A and DNMT3B during the *de novo* methylation [32]. The hypermethylation of the promoter region, by DNMT enzymes, of the genes involved in carcinogenesis offers a good opportunity to target these enzymes in the development of cancer treatments. Inhibitors of DNMTs are mainly classified into three main groups: a) nucleoside analogues, b) synthetic non-nucleoside analogues and c) natural compounds. The nucleoside analogues, with their modified cytosine, can be incorporated into newly synthesized RNA/DNA chains and covalently bound to DNMTs thus inhibiting these enzymes from transferring methyl groups to the progeny cells [20]. The first two of these compounds to demonstrate inhibitory activity against DNMTs were 5-azacitidine (azacitidine) and 5-aza-2`-deoxycytidine (decitabine). Both drugs are FDA-approved for the treatment of myelodysplastic syndrome (a cancer of the bone marrow) acute myeloid leukaemia and chronic myelomonocytic leukaemia [20]. In May 2022 azacitidine was additionally approved for the treatment of juvenile myelocytic leukemia [33]. However, azacitidine and decitabine suffer from poor chemical and metabolic stability in addition to their relatively high toxicity and mutagenic risk [20]. A prodrug of decitabine called guadecitabine, a dinucleotide in the form of decitabine-p-deoxyguanosine, with improved stability, pharmacokinetic profile and longer half-life has now been developed and is currently in clinical trials for various cancers [34-37]. Owing to the mutagenic risk associated with the nucleoside analogues, product development is focusing on the non-nucleoside analogues as these do not get incorporated into the DNA. Instead, non-nucleoside analogues directly target the catalytic site of DNMTs as in the case of RG108, DC_517 and GSK3482364 which selectively target DNMT1 [38-40]. The selective inhibition of DNMTs can also be achieved using antisense oligonucleotides. One such molecule, MG98, was developed to inhibit the mRNA translation of DNMT1 resulting in the demethylation and reactivation of the tumour suppressor gene *CDKN2A* [41]. Hydralazine, a drug widely used to treat hypertension during pregnancy, is another example of the non-nucleoside analogues investigated for their possible benefits in the treatment of cancer. The link of hydralazine to epigenomic modifications is interesting and stems from its immunological reactions leading to the drug-induced lupus-like syndrome. This provided the clue to the DNA-demethylation effect of hydralazine as DNA-demethylation is quite often noticed in immunological disorders [42]. Compounds from natural sources such as epigallocatechin gallate (EGCG- a polyphenol in tea), curcumin (a chemical compound from plants) and genistein (a flavonoid found in legumes such as soya beans and fava beans) have all been able to block various DNMTs [43,44] and are the subjects of clinical trials for the treatment of various cancers [45,46].

DNA methylation erasers

Removing the methyl groups is achieved either actively through a group of enzyme “erasers” or passively through the non-engagement of DNMT1. The erasers that actively remove the methyl groups and reverse the chromatin modification belong to a family called ten-eleven translocation enzymes (or TET for short) (Figure 5).

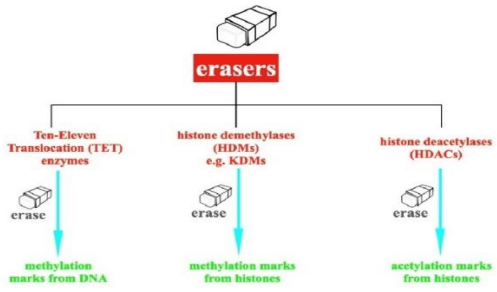

Figure 5: The main erasers of epigenomic modifications. KDMs =lysine demethylases.

The three prominent TET enzymes in this respect are TET1, TET2 and TET3. On the other hand, the passive removal of the methyl group is accomplished by default during DNA replication when DNMT1 does not participate in copying the previous pattern of methylation [6]. The TET enzymes can modify 5-methylcytosine (5-mC) by oxidation to 5-hydroxymethylcytosine (5-hmC) which is regarded as an intermediate product of the active demethylation process [47,48]. The

intermediate 5-hmC can go on to be further oxidized by the TET enzymes to 5-formylcytosine (5-fC) and 5-carboxycytosine (5-caC). The first study highlighting the role of the eraser enzymes, TETs, in carcinogenesis was the identification of TET1 as a partner of another enzyme called MLL (mixed lineage leukemia) in patients with acute myeloid leukemia [49]. The MLL protein was found to bind to the *TET1* promoter increasing its transcription and the level of the oxidation intermediate 5-hmC [50]. The expression levels of TET mRNAs and TET proteins were directly correlated with 5-hmC levels in various malignancies [48,51-53]. Moreover, low levels of TET mRNA and 5-hmC were indicative of poor survival [48,51]. Our literature searches, under the criteria employed, did not find studies aimed at manipulating the erasers of the DNA methylation status for cancer therapy. The link between the TET enzymes and cancer might provide a new exploratory pathway to find suitable treatments for cancer.

DNA methylation readers

As for the readers of the DNA-methylation status, three main families that have been identified and they are a) methyl CpG binding domain (MBD) family, b) methyl CpG binding Zn finger (ZnF) family and c) Set and Ring Associated (SRA) proteins (Figure 6).

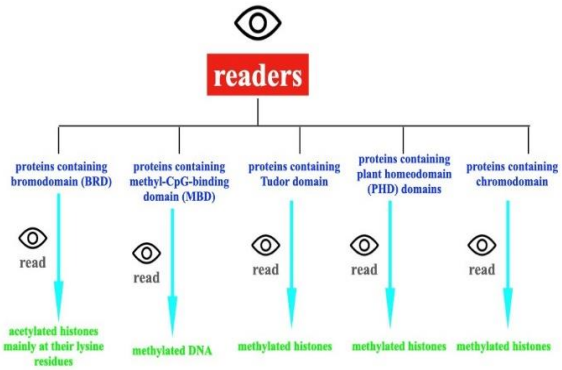

Figure 6: The main readers of epigenomic modifications.

The MBD family of enzymes, which consists of 11 enzymes so far, are the more intensively investigated and include MeCP2 the first one to be discovered [13,54]. While DNMT inhibitors can reverse hypermethylated promoters of tumour suppressor genes and release the break on their encoded proteins to stop the progression of cancer, they could also activate oncogenes [55]. For this reason, targeting the DNA-methylation readers might yield better outcomes in avoiding many of these oncogenic side effects. Prostate cancer cells treated with polyphenols from green tea reversed the hypermethylated state of a silenced tumour suppressor gene called GSTP1 (Glutathione-S-transferase Pi). The demethylation occurs through the downregulation of DNMT1, MeCP2 and several other members of the MBD family of readers [56]. Other studies revealed a reduction in the expression of DNMT1 and MeCP2 enzymes following treatments with resveratrol, curcumin, EGCG, genistein and withaferin [57]. The use of antisense oligonucleotides against MBD2, a member of the MBD family of readers, was also shown to be beneficial against lung and colorectal cancers *in vitro* and *in vivo* [58]. A few compounds that are either already approved for the treatment of cancer or have significant clinical value are shown in Table 1.

Histone methylation

The histone proteins, around which the DNA winds, can be methylated at their lysine (K) and arginine (R) residues. Histone methylation takes place mostly on H3, and to a lesser degree on H4, and occurs on the side-chain nitrogen atoms of lysine and arginine amino acids [59]. The methyl groups are added (written) by an S-adenosylmethionine (SAM)-dependent methyltransferases and the methylation status at these residues does not, by itself, alter the chromatin structure but rather acts as a docking site for other proteins [60,61].

Table 1: Compounds targeting the DNA methylation apparatus

Compound	Target	Indication	Reference
Azacitadine	*DNMT*	MDS, FDA-approved	107,108
Decitabine	*DNMT*	MDS, FDA-approved	107
Guadecitabine (SGI-110)	*DNMT*	MDS	109
RG108	Binds to DNMT1 enzyme active site	Colon and Esophageal cancer	38,110
MG98	Block mRNA translation of *DNMT1*	RCC	111
Hydralazine	Approved for hypertension but repurposed as a DNMT1 inhibitor	Various malignancies	112,113,114,115
Procaine	Approved for anesthesia but repurposed as a DNMT1 and DNMT3A inhibitor	Various malignancies	116,117,118
Green tea polyphenols	*DNMT1* and *MeCP2*	Prostate cancer	119
Antisense oligonucleotides	*MBD2*	Lung and colorectal cancers	58

The different histones and their different methylation states can elicit different transcriptional outcomes. Aberrant histone methylation has been frequently encountered in cancer making the methyltransferases and their erasers potential targets for the treatment of cancer [62,63].

Histone methylation writers

Lysine methylation is achieved by six different classes of lysine methyltransferases, KMT1-6 (where K represents the single letter designation of the amino acid lysine and MT stands for methyltransferase) (see Figure 4). Different classes of KMTs target different substrates with KMT1 methylating H3K9 (lysine position 9 on histone 3), KMT2 targeting H3K4, KMT3 methylating H3K36, KMT4 methylating H3K79, KMT5 methylating H4K20 and the substrate for KMT6 being H3K27. The functional importance of KMT6 and KMT4 in cancer attracted the most research interest in these two classes of lysine methyltransferases. The major enzyme of KMT6 class is EZH2 (enhancer of zeste homolog 2 also known as KMT6A) and to some extent EZH1 while DOT1L (disruptor of telomeric silencing 1-like also known as KMT4) is the archetypal representative of KMT4 class. The enzyme EZH2 mediates transcriptional repression, and its expression has been seen to progressively increase as cancer moves from benign to primary to metastatic malignancy with patients exhibiting higher expression of EZH2 showing worse survival rates [64]. The inhibition of EZH2 activity was thought to be an effective strategy to treat cancer. Several research groups and pharmaceutical companies have developed inhibitors against the catalytic activity of EZH2 to release the transcriptional repression this enzyme exerts on tumour suppressor genes. Treatment of cells with EZH2 inhibitors resulted in a global reduction in the transcriptionally recessive H3K27me2 (dimethylated lysine number 27 in histone 3) and H3K27me3 (trimethylated lysine number 27 in histone 3) within 72 hours [65-69]. Owing to the strong dependence of lymphoma on EZH2 activity, research on EZH2 inhibitors has focused largely on this disease although other cell populations have been found to exhibit a degree of sensitivity towards EZH2 inhibition [70-72]. The interest in DOT1L was based on its complex role in mixed-lineage leukaemia (MLL) which represents most infant leukaemia [73]. This cancer is caused by chromosomal translocations involving the *MLL* gene and several partner genes that can interact with DOT1L [74]. The recruitment of DOT1L to the fusion locus causes the methylation of the partner genes which is a mark of active transcription [75]. The resulting overexpression of these partner genes drives the carcinogenesis of MLL and puts DOT1L as a potential target for its treatment [76]. Compounds inhibiting EZH2 and DOT1L such as GSK343, UNC1999, EPZ005687, 5-ITC, EPZ004777 and SGC0946 have progressed into clinical testing [77]. Arginine methylation is achieved with the enzymes arginine methyltransferases which are capable of methylating not only the histones but other proteins as well. Methylated arginine is found in three different states: monomethylated arginine (MMA), asymmetrically dimethylated arginine (ADMA, when the same amine group is doubly methylated) and symmetrically dimethylated arginine (SDMA, when both amine groups are methylated) [78]. The arginine methyltransferases are grouped under three classes, PRMT I-III (protein arginine methyltransferases), based on specificity. All three classes produce MMA, however, class I PRMTs also produce asymmetric dimethylated arginine and class II PRMTs additionally yield symmetric dimethylated arginine (Figure 7).

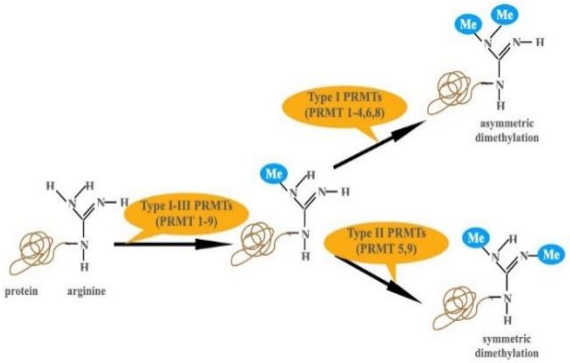

Figure 7: Different methylations induced by different protein arginine methyltransferases.me= methyl group, PRMT= protein arginine methyltransferase.

The only known member of class III PRMTs is PRMT7 which yields only monomethylated arginine [78]. The PRMTs methylate different arginine residues in different histones including H2AR3, H3R2, H3R8 and H4R3. Generally, the modification of arginine to ADMA is associated with active transcription while the generation of SDMA is linked to transcriptional repression [79,80]. The methylation of arginine and the levels of the different PRMTs have been associated with cancer [78]. The enzyme PRMT1 is the predominant member of class I and it is overexpressed in several cancers including leukaemia, lung and colorectal malignancies. It has been difficult to develop specific inhibitors for PRMT1 owing to the considerable homology between members of this class. A potent inhibitor called MS023 was developed that blocks the activity of most class I PRMTs and led to a global loss of ADMA and increases in MMA and SDMA [81]. This outcome attenuated the growth of cancer cell lines and suggested that class I PRMT inhibitors may hold therapeutic potential in the treatment of cancer. Class II PRMTs are the predominant type of these enzymes in mammalian cells and their knockout reduces the global SDMA levels [82]. The enzyme PRTMT5 is the most widely investigated member of class II PRTMs. The overexpression of PRMT5 was found sufficient to transform normal fibroblasts into

cancerous cells and high levels of this enzyme was encountered in many human tumours including lymphoma, glioma, breast cancer and lung cancer. The pharmaceutical companies GlaxoSmithKline and Epizyme have jointly developed several highly selective PRMT5 inhibitors including GSK3235025 (EPZ015666), GSK3203591 (EPZ015866) and GSK3326595 (EPZ015938) [83,84]. The latter compound, which is also called pemrametostat, was the first PRMT inhibitor to be tested in clinical trials in patients with lymphomas and solid tumours [85].

Histone methylation erasers

Lysine methylation is reversible and depending on the histone the consequence of removing the methyl group(s) might induce either a closed or open chromatin state which results in downregulation or an upregulation of gene expression respectively. The lysine demethylases are classified into two broad groups according to their catalytic mechanisms [86]. Group 1 of the lysine demethylases (KDMs) is the amine-oxidase enzymes KDM1A and KDM1B (also known as LSD1 and LSD2 for lysine demethylases 1 and 2). The enzyme KDM1A predominantly removes methyl groups from mono- and di-methyl lysine at H3K4 and induces gene repression in that process and can also demethylate non-histone proteins [87]. One study found that overexpression of KDM1A promotes cell proliferation in oral cancer illustrating the oncogenic-like nature of this enzyme [88]. The enzyme KDM1B has also been reported to catalyse the removal of methyl groups from H3K4 and mice exhibit embryonic lethality if the homolog of the gene responsible for this enzyme is knocked out highlighting the importance of this enzyme in normal biology [89]. Group 2 KDMs contains the Jumonji C domain (Jumonji means cruciform in Japanese) and includes KDM2-6. This group catalyses the removal of methyl groups from mono-, di- and trimethylated lysine residues at various sites. Several inhibitors have been developed to target the various KDMs in cancer and some were promising enough to be progressed to clinical testing. However, the complex role of KDMs in cancer is hampering their entry into clinical practice but they remain the focus of future investigations [86]. To erase the methyl marks from histone arginine, two demethylases have been identified: PAD4 (peptidyl arginine deaminase 4) and JMJD6 (Jumonji domain-containing protein 6). The demethylase PAD4 demethylates methylarginine to citrulline while JMJD6 directly converts methylarginine to arginine [90,91]. Further studies are needed to investigate the role of these two demethylases in cancer biology.

Histone methylation readers

Methylated lysine residues on the histone tails appear to be targeted by several divergent readers. These readers contain methyllysine-binding motifs such as PHD (plant homeodomain), BAH (bromo-adjacent homology), ADD (ATRX-DNMT3-DNMT3L where ATRX stands for alpha thalassemia mental retardation X-linked protein), CD (chromodomain, MBT (malignant brain tumour) and Zn-CW (Zinc finger named after its conserved Cytosine (C) and Tryptophan (W) residues) [92]. The review process employed in this work has not yielded significant articles where the readers of methylated Lysine residues on the histones were targeted for cancer therapeutics. Readers of methylated histone arginine marks have not been widely studied. A group of proteins containing the Tudor-domain appear to be one such family that interacts with sites having methylated arginine motifs [93]. Those readers, and others, require further investigations to elucidate their role in carcinogenesis. Some of the compounds that target histone methylation for a significant gain in cancer therapy are illustrated in Table 2.

Table 2: Compounds targeting the histones methylation apparatus

Compound	Target	Indications	Reference
Tazemetostat	EZH2	FDA-approved for follicular lymphoma and epithelioid sarcoma	120
Lirametostat	EZH2	B-cell lymphoma, prostate cancer (in clinical trials)	121
Valemetostat	EZH1 and EZH2	Approved in Japan for adult T cell leukaemia/lymphoma	122
Pinometostat	DOT1L	Acute myeloid leukaemia	123,124
Pemrametostat (GSK3326595)	PRMT5	Solid tumour and lymphomas	125

Histone acetylation

The acetylation of histones, particularly the lysine amino acid, can influence the compaction of the chromatin by neutralizing its positive (basic) charge thus weakening the hold between the negatively charged DNA molecule and the modified histone [94]. More recent studies suggested that this could be an oversimplification of the role of acetylation in the overall epigenomic regulation, particularly following the finding that histone acetylation may regulate cellular acidity [95,96]. Several tumours exhibit intracellular acidity, as measured by their pH, which correlates with a poor clinical outcome. Functionally, the acetylation event is mainly linked to active gene transcription and the acetylation of H4K16 has been linked to many cancers and was found to have a potential prognostic value [97,98]. Hyperacetylation can activate oncogenes and hypoacetylation, together with DNA methylation, can silence tumour suppressor genes [99]. As lysine is the main histone amino acid that is acetylated, we will focus primarily on this epigenetic modification.

Histone acetylation writers

The writers of the acetyl marks on lysine residues are termed lysine (K) acetyltransferases (KATs) or

more generally histone acetyltransferases (HATs) (see Figure 4). These enzymes can also acetylate lysine residues in non-histone proteins. In addition to their effect on the overall structure of the chromatin and enhancing the transcriptional activity, they can also act as docking sites for the readers of acetyl marks. Several studies showed that HATs can act as oncogenes as well as tumour suppressor genes depending on the context and illustrating the critical balance of acetylation that they must achieve [98,100,101]. Although HATs could be considered viable drug targets for the treatment of cancer, developing such drugs has been difficult and lagging behind those targeting other epigenomic modifiers.

Histone acetylation erasers

The erasers of the acetyl groups from histone lysine residues are called histone deacetylases (HDACs) (see Figure 6) and there are four main families of these enzymes: class I, class II, class III (sirtuins) and class IV [102]. Classes I, II and IV are Zn ions-dependent while class III is nicotinamide adenine dinucleotide-dependent. The overexpression of these enzymes is frequently observed in different cancers suggesting an association between the removal of the acetyl marks from histone lysine and this disease [103]. Class III HDACs (sirtuins) are believed to play an additional role in the cell response to a variety of stresses and are crucial for cell metabolism [104]. Two broad-spectrum HDAC inhibitors targeting both class I and II enzymes were FDA-approved for the treatment of cutaneous T cell lymphoma, vorinostat and romidepsin. However, that latter medicine, romidepsin, had to be withdrawn by its manufacturer in 2021 after phase III trial data showed insufficient efficacy.

Histone acetylation readers

The readers of the acetylation marks on the histone lysine residues are mainly proteins that contain the bromodomain motif (Figure 6). The value of targeting these readers for therapeutic benefit in cancer came to light upon using specific inhibitors of their function and the consequent reversal of the malignant phenotype [105,106]. Investigations are ongoing to further explore the possibility of targeting these readers in the treatment of cancer. Obtaining useful compounds for the treatment of cancer focused here on targeting HDACs and several molecules have been already approved for various malignancies in many countries (Table 3).

Epigenomic Profiling in the Clinic

The value of epigenomic profiling in the clinical setting is increasingly being recognized as a valuable tool in the fight against cancer. Tests to establish the methylation status of several genes, particularly their promoter regions, are currently licensed by several authorities worldwide for the management of cancer. Epigenomic profiling of cancer specimens can aid tumour classifications, cancer screening, selection of more effective treatments and predicting response from such therapies [6,13,99].

Table 3: Compounds targeting the histones acetylation apparatus

Compound	Target	Indications	Reference
Valproic acid	Class I HDACs	In clinical trials for various cancers	126
Vorinostat	Classes I, II and IV HDACs	Approved by USFDA for cutaneous T cell lymphoma	127
Belinostat	Classes I and II HDACs	Approved by USFDA for peripheral T cell lymphoma	128
Panobinostat	Classes I, II and IV HDACs	Approved by USFDA for multiple myeloma	129,130
Etinostat	Classes I and IV HDACs	In clinical trials for BC and NSCLC	131
Romidepsin	Class I HDACs	Approved by USFDA for cutaneous T cell lymphoma.	132

Selected examples of the use of epigenomic profiling in cancer therapy are given in Figure 8.

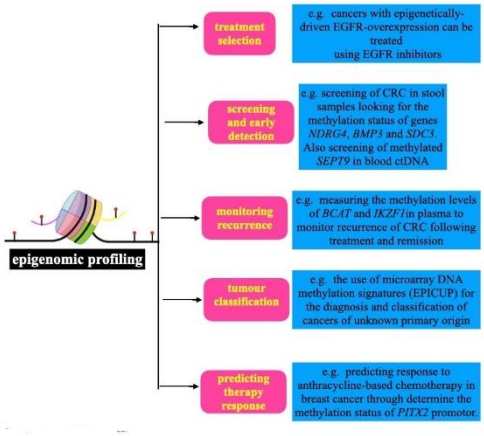

Figure 8: The importance of epigenomic profiling in the management of cancer. EGFR= epithelial growth factor receptor, CRC= colorectal cancer, *NDRG4*= N-myc downregulated 4 gene, *BMP3*= bone morphogenetic 3 gene, *SDC3*= syndecan 3 gene, *SEPT9*= septin 9 gene, ctDNA= circulating tumour DNA, *BCAT*= branched chain amino acid transaminase gene, *IKZF1*= ikaros zinc finger 1 gene, EPICUP= a method of "epigenomic profiling of cancer of unknown primary" and *PITX2*= pituitary homeobox 2 gene.

Conclusion

Modifications of the epigenome are important molecular mechanisms to control gene expression and enable crucial biological processes such as cell differentiation and embryogenesis to be executed. Strong and varied evidence has accumulated in recent years supporting the role of such epigenomic modifications in carcinogenesis [3,5,6]. Yet, despite the enormous progress in our understanding of the causes of cancer and the role the epigenome plays in this disease, most precision medicines target

genomic, rather than epigenomic, abnormalities. This narrative review illustrates the role of the main molecular players controlling the epigenome and how they could be targeted to obtain compounds useful in the overall management of cancer including its diagnosis, prognosis and treatment. A few of these compounds are already licensed by various regulatory global authorities and are on the market. A further and deeper understanding of the function the epigenome can have in the progression of cancer can only assist in the fight against this scourge which continues to claim many human lives every day.

Conflict of interests

The author declares no conflict of interests.

Source of fund

No specific fund received.

Data sharing statement

N/A

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