Pharmacology & Therapeutics 173 (2017) 118–134

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Associate editor: B. Teicher

Epigenetics in cancer: Fundamentals and Beyond

Subhankar Biswas, C. Mallikarjuna Rao *

Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal 576104, Karnataka, India

ARTICLE INFO

Available online 8 February 2017

Keywords:
Epigenetics
Cancer
Histones
DNA methylation
miRNA
Epigenetic drugs

ABSTRACT

Activation of oncogenes or the deactivation of tumor suppressor genes has long been established as the fundamental mechanism leading towards carcinogenesis. Although this age old axiom is vastly accurate, thorough study over the last 15 years has given us unprecedented information on the involvement of epigenetic in cancer. Various biochemical pathways that are essential towards tumorigenesis are regulated by the epigenetic phenomenons like remodeling of nucleosome by histone modifications, DNA methylation and miRNA mediated targeting of various genes. Moreover the presence of mutations in the genes controlling the epigenetic players has further strengthened the association of epigenetics in cancer. This merger has opened up newer avenues for targeted anti-cancer drug therapy with numerous pharmaceutical industries focusing on expanding their research and development pipeline with epigenetic drugs. The information provided here elaborates the elementary phenomena of the various epigenetic regulators and discusses their alteration associated with the development of cancer. We also highlight the recent developments in epigenetic drugs combining preclinical and clinical data to signify this evolving field in cancer research.

Contents

1. Introduction

Cancer is a group of disease that varies extensively with respect to its origin. As a result, the key hurdles associated with its treatment consist of inappropriate diagnosis leading to recurrences and drug resistance. In relation to this, one of the emerging therapeutic class of antineoplastic agent is aimed at targeting gene expression owing to the fact that irregularities in the expression pattern of a gene is a key feature in the development of cancer (Cavalieri, 1996). To accommodate the entire length of several meters of human DNA in the nucleus of a cell, DNA is coiled around histone proteins forming a complex called nucleosome, the basic unit of chromatin (Annunziato, 2008). In addition, post-translational modifications of histone tail alter the structure of chromatin leading to a change in gene expression which is an element of epigenetic regulation. With our improved understanding about cancer, it is now established that malignant growth is associated with both genetic and epigenetic abnormalities (Sadikovic, Al-Romaih, Squire, & Zielenska, 2008). In particular, epigenetic alterations occur early during neoplastic growth and finally develop into a malignant tumor. Although epigenetic modifications are inherited in somatic cells, yet these modifications are possibly reversible indicating that epigenetic alterations can be a promising therapeutic target to explore. Undoubtedly, the fast growing field of therapeutic epigenetic is being continually expanded by integrating

* Corresponding author.
E-mail address: mallik.rao@manipal.edu (C.M. Rao).

http://dx.doi.org/10.1016/j.pharmthera.2017.02.011
0163-7258/

laboratory results with clinical data suggesting us how epigenetic therapy can be best utilized for the benefit of patient. In this review we take a comprehensive look at the various epigenetic players, their involvement in the development of cancer and the drugs employed in altering those mechanisms.

2. Epigenetic phenomenon

The term Epigenetics was coined by C.H. Waddington in 1942 as "the causal interaction between genes and their products, which bring the phenotype into being". However with our increasing knowledge in molecular biology the definition has evolved as "the study of changes in gene function that are mitotically and/or meiotically heritable and that do not involve a change in the sequence of DNA" (Dupont, Armant, & Brenner, 2009). The manner in which chromatin structure is preserved and ordered is crucial in understanding the origin of epigenetic alteration. Certainly, each cell type in the body is genetically identical i.e. they share the same set of genes but needs to differentiate phenotypically into diverse type of cells and tissues to endure a normal functioning human body. This is controlled by highly synchronized regulatory mechanism which involves epigenetics. Epigenetic changes regulate gene expression by hindering the availability of transcription factors towards DNA. These modifications occur at different regions encircling the genome. The fundamental of epigenetic regulation of gene expression takes into account the manner in which DNA is wrapped around nucleosome and also considers the way in which each nucleosome are positioned throughout the genome. With our increased understanding about the biology of cancer attributed by the rapid advances in technology, it is now well established that cancer cell harbor global epigenetic alterations beside various genetic mutations representing a complex interplay between these players (Sadikovic et al., 2008). This phenomenon was evident from gene expression and DNA methylation studies providing the initial clues linking epigenetics with cancer. Emerging data are now strengthening our outlooks of the genome wide role of epigenetics. At present, the most studied epigenetic alterations associated with neoplastic phenotype are variation in DNA methylation, alteration in the structure of histone proteins and gene regulation by small noncoding microRNAs.

2.1. DNA methylation

Adenine, Thymine, Cytosine and Guanine are the key nitrogenous bases which are found in eukaryotic organisms. These bases usually comprise the majority of sequence found in eukaryotic DNA. Apart, from these four major bases, the existence of a fifth base i.e 5-methylcytosine, is one of the major covalent modification of DNA. In eukaryotes, DNA methylation is a common epigenetic alteration and these epigenetic marks are typical of heterochromatin. DNA methylation plays an important role in maintaining the stability of genome, genomic imprinting, inactivation of X-chromosome in females, regulation of transcription and also in the developmental process of an organism (Robertson & Jones, 2000). Methylated DNA is present primarily in repetitive genomic regions (including satellite DNA, like micro and mini-satellites), within centromeres and parasitic elements such as short interspersed transposable elements (SINEs) and long interspersed transposable elements (LINEs) where they function to silence genes and non-coding genomic regions. The 5^{th} carbon of cytosine residues are highly prone to methylation compared to other nitrogenous bases and consist of approximately 1% of the total nucleotides. Moreover, the majority of DNA methylation occurring on cytosine residue is present in the CpG dinucleotide distributed throughout the genome and is also densely found in regions known as CpG islands (Jones & Takai, 2001). These CpG islands overlap the promoter regions of approximately 60-70% of human gene. In normal cells, the promoter regions of genes, especially those preceded by CpG islands are usually unmethylated, allowing transcription factors and other associated proteins to interact with the gene and facilitate their expression. In contrast, the genomes of gametes and cells whose promoter regions are less enriched with CpG islands are frequently methylated during early development. However, we should bear in mind that these genes exhibit a distinct expression control during development and are always tissue specific.

The conversion of cytosine into 5-methyl cytosine (5mC) is carried out by the catalytic activity of a group of enzymes called DNA methyltransferases (DNMTs). These enzymes use S-adenosyl methionine (SAM) as a key methyl group donor which transfers methyl group to cellular elements like DNA, lipids and proteins. SAM is converted into S-adenosyl homocysteine (SAH) after the transfer of methyl group by DNMTs. There are two major categories of the DNMTs in mammalian cells, a maintenance methyltransferase and a *de novo* methyltransferase. The original DNA methylation pattern in a cell is greatly maintained by the catalytic activity of DNMT1, which prefers hemi-methylated DNA in place of non-methylated DNA as a substrate during replication, most likely with the support of UHRF1 (Ubiquitin like with PHD and ring finger domain 1) which also recognizes hemi-methylated sites, suggesting a role in maintaining the methylation patterns during cell division (Qin et al., 2015). In contrast, new DNA methylation pattern are established in the developmental phase of a cell utilizing DNMT3A and DNMT3B, which are expressed all over the cell cycle and shows equal preference for both hemi and unmethylated DNA making them *de novo* methyltransferase. Another enzyme, DNMT3L has been identified which is deficient in the conserved catalytic domain commonly associated with DNA methyltransferase. Although it is accepted that DNA methyltransferase are specific in their functions and non-overlapping, yet recent evidence suggests the overlapping role of *de novo* methyltransferases with maintenance methyltransferase (Walton, Francastel, & Velasco, 2011).

DNA methylation silence gene expression directly by impeding the binding of various transcription factors and indirectly by enrolling methyl-CpG binding domain (MBD) proteins. The MBD family contains five core proteins which include MBD1, MBD2, MBD3, MBD4 and the methyl cytosine binding protein 2 (MECP2). Apart from these, other MBD containing proteins are MBD5/6, SETDB1/2 and BAZ2A/B. The MBD protein employs histone modifying enzymes and chromatin remodeling complexes in methylated sites and facilitates transcriptional repression. Chromatin remodeling complex like NuRD binds with MBD2 protein and methylate DNA (Du, Luu, Stirzaker, & Clark, 2015). These mechanisms play a central role in establishing the critical role of DNA methylation in epigenetic gene regulation.

Although enzymes catalyzing DNA methylation has been well established, recent research has also identified mechanisms involved with the removal of methyl group. The discovery of ten-eleven translocation (TET) [which derives its name based on a recurrent chromosomal translocation t(10;11)(q22;q23)] and activation-induced cytidine deaminase (AID) family of enzymes has provided unprecedented information in our understanding of DNA demethylation (Scourzic, Mouly, & Bernard, 2015). DNA demethylation can be achieved by two processes involving passive and active demethylation. Passive demethylation occurs by the failure of maintenance DNMT enzyme to methylate DNA after replication. Whereas, active DNA demethylation utilizes TET and AID family of enzymes to hydroxylate, oxidize or deaminate 5mC. Three TET family members have been identified so far including TET1, TET2 and TET3 and each of them are involved in distinct cellular process. Hydroxylation of 5mC by TET proteins produces 5-hydroxy methylcytosine (5hmC) and its subsequent conversion into 5-formylcytosine (5-fC) and 5-carboxylcytosine (5caC) followed by deamination and entry into the subsequent base excision repair pathway (Fig. 1) (Zhao & Chen, 2013).

2.2. Histone modifications

The nucleosome core particle which is the basic element of chromatin wraps 147 base pair of DNA around an octamer of four core histone proteins in a 1.7 left handed super helical turn. The inherent positive

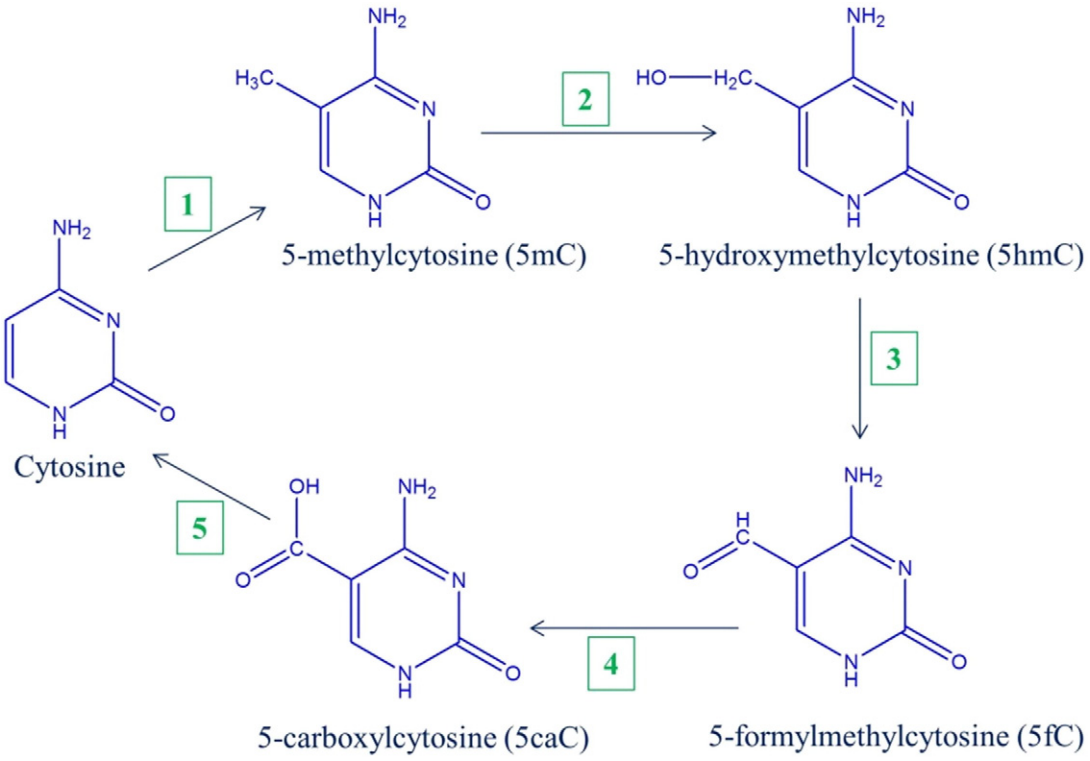

Fig. 1. Modification of cytosine residue. Cytosine residue present in DNA can be methylated at C5 position to form 5mC by the enzyme DNA methyltransferase (1). 5mC can be subsequently oxidized by TET1-3 enzymes (2) to form 5hmC. Over-activity of TET enzymes (3,4) can further oxidize 5hmC into 5fC and 5caC. The enzyme Thymine-DNA glycosylase (5) removes the carboxyl group from 5caC following which base excision repair pathway (5) converts it into unmodified cytosine.

charge of the basic histone proteins provides efficient binding with negatively charged DNA. The four core histone comprise of H2A, H2B, H3 and H4 which are present as H2A-H2B dimer and H3-H4 tetramer in association with a linker histone H1 which joins nucleosomes together. The sequence of amino acids comprising the histone proteins vary substantially among different species, however, the histone proteins are made up of a common structural domain called the histone fold. These folds comprises of a long central helix linked with two helix-strand-helix motifs at the opposite ends. The N-terminal tails of these proteins are highly flexible and are rich in lysine and arginine residues which can be extensively modified by a large number of cellular systems (Fig. 2) (Ramakrishnan, 1997).

A plethora of post-translational modifications on histone proteins are observed in the regions of transcriptionally active and inactive chromatin. The pioneering work carried out by Vincent Allfrey in the 1960s has given us valuable information about histones and their modifications (Allfrey, Faulkner, & Mirsky, 1964). Although, present research has provided a lot of information on chromatin structure we are still not assured whether all histone tail modifications directly regulate chromatin compaction. Because of the ubiquitous nature of chromatin, various DNA process such as repair, replication and recombination are also affected by these modifications. It is interesting to understand that although polymerase enzymes do not interact with histones directly, a modification in them alters the DNA wrapping style and influence gene expression.

Probably the most studied histone modifications include acetylation and methylation of lysine residues on the N-terminal tails of histone. Acetylation of lysine residue of histone tails is highly prevalent and their levels associate with transcriptionally active chromatin. Acetylation removes the net positive charge on the histone proteins by acetylating the ε-amino group of lysine residues using acetyltransferases (HATs) which utilizes acetyl-CoA as the acetyl group donor. These enzymes are generally categorized into two different types: Type A, which are found in the nucleus and Type B, which are found in the cytoplasm (Brownell & Allis, 1996). However, evidence has suggested various functions of HAT which are beyond these classifications (Ruiz-García et al., 1998). Nucleosomal histones within the nucleus are acetylated by Type A HAT, whereas housekeeping role are associated with Type B HAT where they are involved in acetylating newly synthesized histones present in the cytoplasm (Ruiz-Carrillo, Wangh, & Allfrey, 1975). The major families of histone acetyltransferases include GNAT family (Gcn5-related N-acetyltransferases), with a specificity towards H3K9, H3K14, H3K36 histones, MYST family with a specificity towards H4K5, H4K8, H4K12, H4K16, H3K14, H3K23 histones and CBP/p300 (cAMP response element binding protein) family with a specificity towards H2AK5, H3K9, H3K23, H3K56. Apart from these families the general transcription factor HATs include TFIID subunit TAF250 and the nuclear hormone related HATs like steroid receptor coactivator-1 (SRC1) and ACTR. HATs are associated with various protein complexes to perform their catalytic activity among which SAGA, TFTC, NuA4, Rtt109-Vps75 complexes are widely studied. The acetylated lysine on the histone tails are recognized by an evolutionary conserved motif called the bromodomain which are found in many transcriptional activators. Most of the nuclear HATs contain bromodomain as a catalytic component (Losson, 1997). The bromodomain motif contains 110 amino acids which are arranged in four left handed α helix connected with ZA and BC loops (Dhalluin et al., 1999). Very high degree of specificity for an acetylated target is obtained with the interaction of individual bromodomian with other structural elements within a protein. A classic example of this is the acetylated lysine on H4K12 which is found to interact selectively with the bromodomain protein Brd2 to initiate transcription. On the contrary, the bromodomain motif associated with TAF(II)250 and P/CAF bind to various acetylation sites on histone protein other than H3K14. For more information the readers are

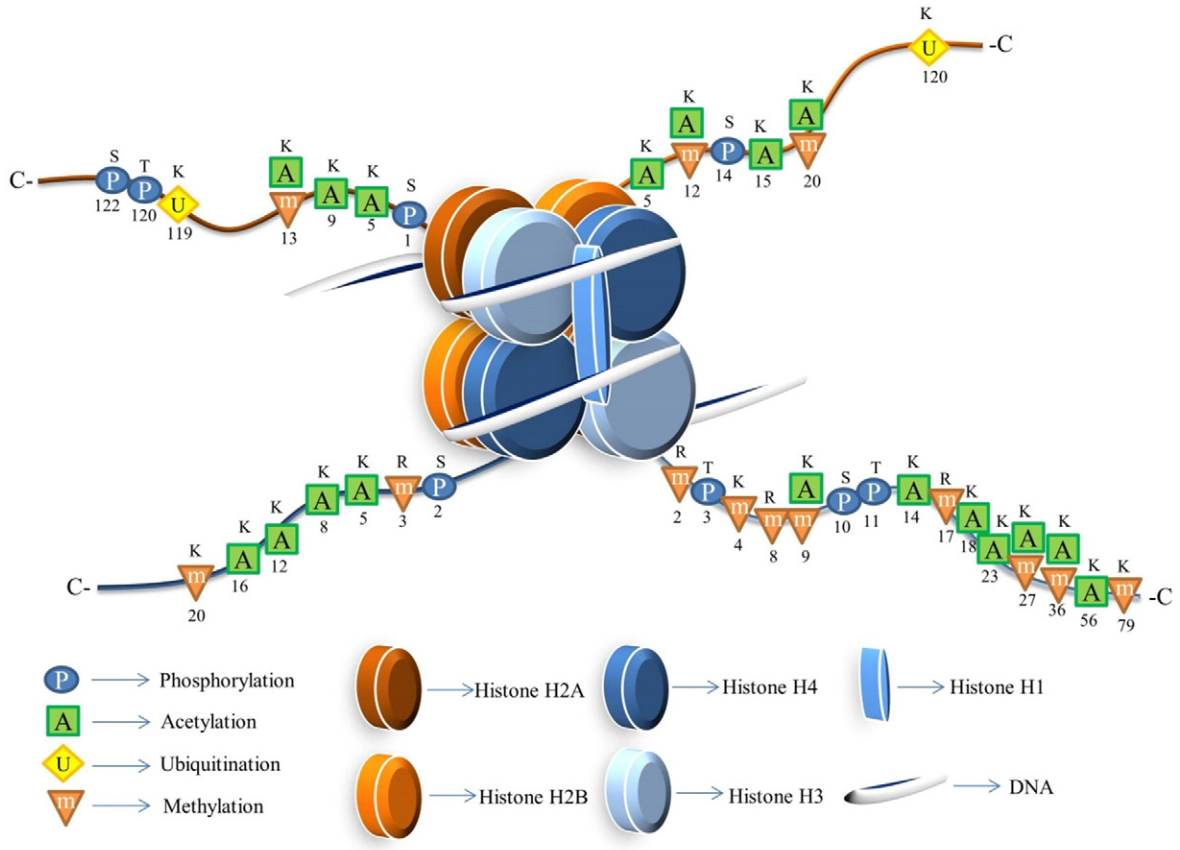

Fig. 2. Histone modifications. Nucleosomes are the basic unit of DNA packaging in eukaryotes. Each nucleosome core particle consists of an octamer of histone proteins including H2A, H2B, H3 and H4. It is assembled as a tetramer of two copies of H3 and H4 and a dimer of two H2A and H2B. The linker histone H1 keeps the DNA in place that is wrapped around the histone octamer. Histone N-terminal tail plays a crucial role in modulating nucleosome structure and function. Various modifications on the different residues of histone tail are being shown here where S, T, K and R represent Serine, Threonine, Lysine and Arginine respectively. Other possible modifications are also present which are not displayed.

suggested to go through the following excellent reviews on bromodomains proteins (Filippakopoulos & Knapp, 2014; Mujtaba, Zeng, & Zhou, 2007). Methylation of histone proteins is generally found on arginine and lysine residues, however unlike acetylation there is no alteration in the charge of the histone protein. Furthermore it should be kept in mind that this type of modification has a different level of intricacy. Three different forms of methylation have been observed on the lysine residues viz. mono-, di- and tri-methyl whereas arginine can be mono-methylated and symmetrically or asymmetrically di-methylated (Bedford & Clarke, 2009). A wide range of information is available because of the multiple methylation states associated with lysine and arginine. For example, H3K4me3 i.e. trimethylation of lysine 4 on histone H3 is abundant at active gene promoter, whereas H3K9me3 is associated with transcriptionally repressed gene promoters (Kouzarides, 2007; Liang et al., 2004). Histone methylation is catalyzed by three distinct families of enzymes namely, the SET-domain containing protein family, the non-SET domain protein family and the PRMT1 (protein arginine methyltransferases) family. Most of the enzymes with SET-domain methylate lysine present on H3 and H4. It is interesting to understand that although, SET 7/9 enzyme is only involved in monomethylating lysine 4 on H3 histone, a mutation in its amino acid can alter this specificity which is evident from the mutation of Tyr 3053 to Phe in SET 7/9. This kind of mutation transforms SET 7/9 into a dimethyltransferase. The SET and MYND domain containing protein 2 (SMYD2) is known to methylate H3K36 and H3K4 and acts as a transcriptional activator (Derissen, Beijnen, & Schellens, 2013). The histone methyltransferase EZH2 utilizes its SET domain to dimethylate and trimethylate lysine 27 on histone H3 (H3K27me2/3). Another histone methyltransferase SUV39H1 contain the SET domain protein which is involved in methylating H3K9 in the three different states. Evidence has also suggested that subset of Suv39h1 coexist in a megacomplex for performing its demethylating activities (Fritsch et al., 2010). Studies have identified a mammalian HATase G9a which plays a major role in methylating H3 lysine 9 (Constantinides, Jones, & Gevers, 1977). Moreover an important protein complex that is involved in methylating lysine-4 of histone H3 is the MLL1 protein complex which contains ASH2L, HCFC2/HCF1, WDR5 and RbBP5 as the core components (Avdic et al., 2011). Non SET domain containing protein also takes part in methylating histone proteins among which DOT1L is extensively studied. DOT1L is involved in methylating H3K79 and also in a number of vital processes including DNA damage response and cell cycle progression (McLean, Karemaker, & van Leeuwen, 2014). Although lysine methylation plays an important role in modifying the structure of chromatin, enzymes that methylate peptidyl arginine residues has been widely studied. Protein arginine methyltransferase (PRMTs) adds mono or dimethyl groups on the terminal guanidino nitrogen of the arginine residues. Recent advances have helped us in establishing the structure of PRMTs, categorizing it into various classes and stating its various functions (Yang & Bedford, 2013).

There is no doubt that apart from acetylation and methylation various other mechanisms prevail to modify histone tails and it is widely accepted that phosphorylation plays a major role in amending protein structures. The amino acids serine, threonine and tyrosine residues on the histone tails are prone to phosphorylation. It has been observed

that phosphorylation of serine 139 on histone variant H2AX plays a major role in generating the initial stages of DNA damage response (DDR). Although phosphorylation of threonine is a less common phenomenon, it contributes a major portion in epigenetic control of chromatin structure. Studies have demonstrated the phosphorylation of threonine 119 on H2A by nucleosomal histone kinase-1 which plays a major role in cell cycle progression (Aihara et al., 2004). Phosphorylation of H3S10, S28 and T11 is widely studied and it is associated with transcriptional activation. Studies have shown that H3S10 phosphorylation encourages acetylation of H3K14 (Lo et al., 2000). Phosphorylation of serine 1 of histone H4 is involved in the later stages of DDR and also in stabilizing new nucleosome structure by preventing their acetylation (Rossetto, Avvakumov, & Côté, 2012). The enzymes involved in phosphorylating the serine residues belong to the kinase family of enzymes among which Ribosomal S6 kinase (RSKs), Mitogen and stress activated protein kinase 1 and 2 (MSK1 and MSK2) and Aurora kinases are widely studied (Rossetto et al., 2012). They play a major role in phosphorylating various serine residues on histone H3.

Addition of ubiquitin and small ubiquitin-related modifier protein (SUMO) on specific lysine residues is another prominent histone post-translational modification. Ubiquitination of lysine 119 of Histone H2A and lysine 120 of H2B is one of the most important observations made in recent years (Jason, Moore, Lewis, Lindsey, & Ausió, 2002). It is seen that for dimethylation and trimethylation of H3K4 and H3K79 to occur ubiquitination of H2B is mandatory and therefore considered as a gene activating mark (Cole, Clifton-Bligh, & Marsh, 2015). On the contrary, ubiquitination of H2A is associated with transcriptional silencing with the involvement of two different E3 ubiquitin ligases, Ring1B and 2A-HUB (Spivakov & Fisher, 2007). Although ubiquitinaiton of a protein is generally directed towards proteasomal degradation the above role of ubiquitin on histone proteins suggests its epigenetic role. Almost analogous to ubiquitination on the basis of reaction mechanism and the class of enzymes used, sumoylation adds SUMO peptides to all the four core histone proteins. An important feature associated with sumoylation is its primary target which is lysine. Although lysines are the targets of various other modifications, sumoylation is usually related with repression of target genes (Nathan, Sterner, & Berger, 2003).

Histones are also modified by the vitamin Biotin which is catalyzed by the enzymes holocarboxylase synthetase (HCS) and biotinidase. Specifically, lysine residues are targeted by this system and are widely prevalent in repeat regions of the genome, contributing to genomic stability. Studies have identified that lysine on position 9, 13 and 129 on H2A; position 4, 9 and 18 on H3; position 8 and 12 on H4 are the sites of biotinylation (Camporeale, Shubert, Sarath, Cerny, & Zempleni, 2004; Sarath et al., 2004). Although studies are being conducted to explore more about this modification, evidence suggests a decrease in biotinylation of lysine residues with acetylation of adjacent lysines. Whereas, an enhancement in biotinylation of lysine residues are observed when the arginine residues are dimethylated (Martinez-Zamudio & Ha, 2012).

The consequence of histone ADP-ribosylation is not widely discussed however studies have established their role in regulating chromatin structure. In particular, with the identification of specific lysine residues on histone which acts as acceptor site for ADP-ribose suggests an equal importance with respect to other modifications. One of the best substrate for poly (ADP-ribose) polymerase is histone H1. Apart from that all core histones are also ADP ribosylated. The linker histone and the core histones are ADP ribosylated during various phases which include shortly after their synthesis in the cytoplasm or during their transport into the nucleus. Many reports suggest that alteration in chromatin structure is associated with the ADP-ribosylation of H1 histones. Recent evidence has also identified enzymes that reverse ADP-ribosylation belonging to two different classes which are ADP-ribosylhydrolases (ARHs) and PAR glycohydrolases (PARGs). Three ARHs (ARH1-3) and one PARG enzyme has been identified in humans. Although it is clear that the activities of these degrading enzymes determine the pattern of ADP-ribose modification we still lack a detailed understanding about the mechanism of these enzymes.

2.3. MicroRNAs (miRNAs)

Recent studies have identified a key player in epigenetic regulation and gene expression with the discovery of microRNAs. These are endogenous molecules consisting of small non-coding RNAs. miRNAs are approximately 16 to 22 nucleotide long RNA molecule that are transcribed by RNA polymerase II leading to the formation of primary miRNAs. These primary miRNAs are then acted upon by RNase III Drosha and DGCR8 (components of the microprocessor complex) inside the nucleus to form the precursor miRNAs. The precursor miRNAs generated in the nucleus are imperfect in their structure usually having a hairpin like organization, so they are exported out into the cytoplasm by forming a complex with exportin-5 and RAN-GTP for further processing. In the cytoplasm, RNase III Dicer processes the precursor miRNAs into a functional double stranded miRNAs. The mature miRNAs modulate gene expression by incorporating with a complex called RNA-induced silencing complex (RISC) whereas the other strand is likely to be degraded. In most cases the mature miRNA interacts with the 3′-UTR of the target mRNAs leading to their degradation and inhibition of translation (Fig. 3) (Garzon, Fabbri, Cimmino, Calin, & Croce, 2006).

The expression of miRNA is quite analogous to that of protein-coding genes as they are regulated by both genetic and epigenetic mechanisms. Recent research has mapped the presence of miRNA genes in the common breakpoint regions of oncogenes and tumor suppressor genes and fragile regions of the genome which are preferential site for deletion, translocation or amplification suggesting their involvement in driving the behavior of tumor growth. In addition studies have found a link between epigenetics and miRNA, for example various miRNAs are able to modulate the activity of epigenetic modifying enzymes associated with carcinogenesis (Guil & Esteller, 2009). miRNA serves as a part of the regulatory network which takes part in silencing gene expression by methylation and modifying the structure of chromatin. Although a number of miRNA has been identified till date, mir-127 was the first epigenetically regulated microRNA associated with cancer (Saito et al., 2006).

Indeed with the progress in the field of micro RNA it was possible to come across numerous microRNAs that are regulated by the epigenetic machinery viz. DNA methylation and histone modificaiton. One of the most common epigenetic alterations includes DNA methylation and it has been observed that numerous microRNA genes are hypermethylated resulting in miRNA silencing. Among them miR-9, mir-148, miR-124, miR-137, miR-34, miR-127, miR-512 are frequently reported to be silenced in various types of cancer. Apart from methylation, histone modifications have also been associated with miRNA expression. Studies have identified a link between histone modifications (especially H3K27 and H3K9) and miR-212 gene contributing towards the development of lung cancer (Incoronato et al., 2011).

On the other hand a different outlook of miRNA has also been established in controlling DNA methylation and histone modification i.e. they are capable of targeting genes that regulate the epigenetic pathway creating a highly organized feedback pathway (Denis, Ndlovu, & Fuks, 2011). An abnormal expression of these microRNAs, called epi-miRNAs has been associated with various diseases. Studies have identified numerous miRNA that control chromatin structure by altering histone deacetylase enzymes and polycomb group related genes (Sato, Tsuchiya, Meltzer, & Shimizu, 2011). For instance, miR-1 and miR-140 are involved in targeting HDAC4 isoenzyme whereas miR-449a binds to the HDAC1 and regulates their expression pattern (Chen et al., 2006; Noonan et al., 2009). Also noted is the fact that the expression of EZH2 a catalytic subunit of the polycomb repressive complex 2 (PRC2) is altered by the epi-miRNA miR-101(Varambally et al., 2008). Moreover, a family of miRNAs (miR-29) regulates the expression of maintenance DNA methyltransferases DNMT3a and DNMT3b. Studies

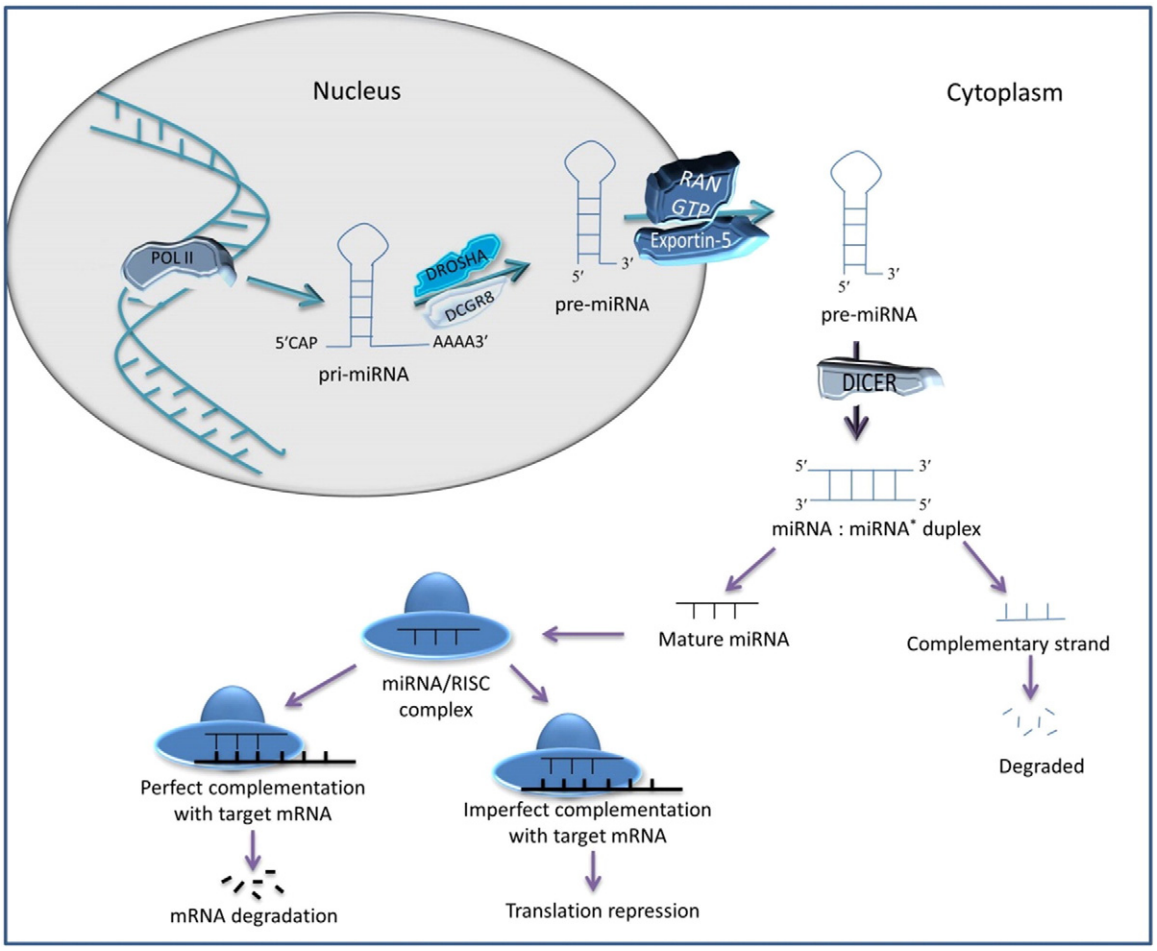

Fig. 3. miRNA biogenesis. Most miRNA are transcribed by the enzyme RNA polymerase II to form the initial transcript which is known as primary microRNA (pri-miRNA). The primary miRNA contains an imperfect structure with a long sequence extending from the 5′ and 3′ end. The pri-miRNA is processed by microprocessor complex containing DROSHA and DGCR8 to form the precursor miRNA (pre-miRNA). The pre-miRNA is exported into the cytoplasm by Exportin 5 in association with Ran-GTP. The pre-miRNA is further processed by DICER in the cytoplasm which removes the stem loop to generate a double stranded RNA of 20-25 nucleotide long. The double stranded RNA cleaves to form a mature miRNA which associates with RNA induced silencing complex (RISC). The mature miRNA guides RISC to recognize the target mRNA leading to mRNA degradation or translation repression.

have established that by repressing the activities of DNA methyltransferases, miR-29 suppresses tumorigenesis by altering the existing DNA methylation pattern in a cell (Morita et al., 2013). Taken together the role of epi-miRNA in epigenetic control of gene expression is enormous and a lot remains to be uncovered.

3. Epigenetic alterations in cancer

3.1. Aberrant DNA methylation in cancer

Alteration in DNA methylation pattern is very closely associated with the initiation and progression of cancer and it was also the first epigenetic alterations to be identified. Two seemingly opposing pattern of DNA methylation are observed in cancer cells which includes increased methylation of CpG islands and an overall decrease in global DNA methylation pattern (Feinberg & Vogelstein, 1983). Although the fundamental mechanisms leading to these alterations are widely investigated, it is fairly clear that minute changes occur early in the architecture of DNA leading to cancer initiation (Feinberg, Ohlsson, & Henikoff, 2006). For instance, a recent study investigated the prevalence of early DNA methylation changes associated with the development of breast cancer, where they confirmed frequent DNA methylation changes in promoters, far-upstream regions, introns, LINE-1 and satellite 2 DNA repeats (Rauscher et al., 2015).

Numerous reports have established the role of hypermethylated CpG islands in cancer development including the nature of genes involved (Esteller, 2002). Moreover, recent data have contributed to the fact that increased methylation are observed in promoter CpG islands of normally unmethylated genes specially the tumor suppressor genes in cancerous cells (Smiraglia et al., 2001). In this regard the gene CDKN2A, which is involved in cyclinD-Rb pathway for keeping the retinoblastoma protein in its active state is worth mentioning. This gene encodes a cyclin dependent kinase inhibitor $p16^{INK4A}$ which is critical in cell cycle progression. Promoter hypermethylation of $p16^{INK4A}$ leads to uncontrolled cell cycle progression which is commonly observed in almost all tumors (Herman et al., 1995). Furthermore, hypermethylation of $p16^{INK4A}$ gene in patients suffering from non-small cell lung cancer (NSCLC) and colorectal cancer (CRC) have a poor overall survival rate (Xing et al., 2013). Not only tumor suppressor gene, promoter hypermethylation is also connected with other genes that are strongly involved with tumorigenesis. The gene *p73*, which is closely related with *p53* is found to be hypermethylated in various cases of lymphomas (Pei et al., 2011). Similarly $p15^{INK4A}$, a gene related to $p16^{INK4A}$ is hypermethylated in numerous haematological malignancies (Quintás-Cardama et al., 2012). Moreover, silencing of the tissue inhibitor of metalloproteinase-3 (*TIMP-3*) gene, a negative regulator of angiogenesis leads to malignant growth. A recent study by Guan and colleagues (Guan, Zhang, Song, & Dai, 2013) suggested that hypermethylation of promoter region of *TIMP3* gene was associated with the development of gastric cancer and it has also been demonstrated by Lin H and colleague (Lin et al., 2012) that the levels of *TIMP-3* were significantly decreased in colorectal cancer tissue. Although DNA hypermethylation

can directly induce gene silencing, there are certain indirect ways by which hypermethylation can silence genes leading to tumorigenesis. These include silencing of DNA repair genes and various transcription factors. Alteration in DNA repair genes leads to the accumulation of DNA damage eventually promoting cancer development (Lahtz & Pfeifer, 2011). Hypermethylation occurring in DNA repair genes like *BRCA1* (Homologous Recombination Repair pathway), *MGMT* gene (Direct repair), *MLH1* and *MSH2* gene (Mismatch repair), ERCC1 gene (Non-homologous end joining) are some of the widely studied genes associated with cancer development. A recent study by Zhu and colleagues (Zhu et al., 2015) suggested that promoter hypermethylation of *BRCA1* gene decreases the overall survival of patients and can be used as a biomarker for triple negative breast cancer. Also, aberrant hypermethylation of *MLH1* promoter is highly common in endometrial carcinoma which is associated with microsatellite instability (Esteller, Levine, Baylin, Ellenson, & Herman, 1998). For more details readers are suggested to go through the excellent review by Esteller (2000). It has also been observed that silencing transcription factors such as *RUNX3, GATA-4, GATA-5* by hypermethylation leads to the inactivation of their downstream targets which are involved in various cellular process. RUNX3 belongs to the family of transcription factors that plays a vital role in TGF-β signalling pathway. Evidence suggests that in lung cancer cell lines and primary lung cancer specimen, RUNX3 is inactivated by aberrant DNA hypermethylation (Li et al., 2004). Similarly the GATA family of transcription factors are associated with gastrointestinal development, and it has been observed that in colorectal cancer promoter hypermethylation leading to transcriptional silencing of GATA-4 and GATA-5 are very frequent (Akiyama et al., 2003).

Global hypomethylation of DNA plays a key role in tumor formation and are found at various genomic locations including retrotransposons, CpG poor promoters, repeat sequence and numerous other sites (Rodriguez et al., 2006). The most common phenomenon associated with hypomethylation is the overexpression of proto-oncogenes and growth factors which are responsible for various hallmarks of cancer (Szyf, Pakneshan, & Rabbani, 2004). Hypomethylation of retrotransposable elements leads to their activation which leads to genomic instability. Moreover studies have demonstrated that in non-small cell lung cancer genomic instability due to retrotransposable element hypomethylation is very closely related (Daskalos et al., 2009). Similarly hypomethylation of repeat sequence is also associated with genomic instability and is highly prevalent between various cancer types (Ross, Rand, & Molloy, 2010). The most characterized feature of genomic instability is demonstrated in patients with immunodeficiency, centromeric region instability and facial anomalies (ICF) syndrome. This is a unique DNA methylation deficiency disease with a germ line mutation in DNMT3B enzyme leading to chromosomal anomalies and is observed in many cancers (Ehrlich, Jackson, & Weemaes, 2006). In addition, cancer associated hypomethylation also affects gene regions which can express aberrant protein products. For instance, the gene encoding urokinase type plasminogen activator (*PLAU*/*uPA*) is hypomethylated and leads to tumor progression in breast and prostate cancer (Ehrlich, 2009). The expression of *uPA* is strongly correlated with its hypomethylated status with studies reporting the overexpression of *uPA* and its receptor uPAR in malignant brain tumor (MacDonald, DeClerck, & Laug, 1998). They play a major role in the migration and invasion of gliomas. Similarly various growth factors are affected by hypomethylation. For example, Insulin-like growth factor 2 (IGF-2) has a monoallelic expression pattern in non-malignant cells, however loss of imprinting due to hypomethylation is observed in the second allele of malignant cells because of which biallelic expression of the growth factor takes place leading to uncontrolled tumor cell proliferation (Leick, Shoff, Wang, Congress, & Gallicano, 2012). Recent findings implicate that in certain cancer various genes harbour DNA hypomethylation in their gene body. For example the Alu repeat of *TGFB2* which is present in the intronic region of the gene is hypomethylated in some cancer cell line. Another hypomethylation is observed in the region overlapping a CpG island in the exon of *PRDM16* gene in cancer cell lines (Ehrlich & Lacey, 2013). Although the exact reasons bridging hypomethylation and cancer is still unclear, it is anticipated that deficiency of numerous enzymes might play a role. Also it should be taken into consideration that how specific genes are hypomethylated in cancer and others are spared is still unclear, providing unexplored areas for future research in this direction.

3.2. Alterations of Histone proteins in Cancer

Extensive findings have suggested that variation in the organization of histone PTMs is widely allied with cancer. These aberrations add a higher level of complexity in understanding tumorigenesis. Advancement in sequencing technology has facilitated the mapping of chromatin changes in the development of cancer. Moreover, it is also clear that alterations in histone proteins are found to be present globally across the genomic DNA and also at a specific locus of a gene (Bannister & Kouzarides, 2011).

The incorporation of acetyl group at a lysine residue of histone tail has the potential to alter the compaction state of chromatin and can also regulate the intracellular pH. Recent studies have identified that many tumors have low intracellular pH along with a reduction in the level of histone acetylation (McBrian et al., 2013). In addition, alteration in the global level of histone acetylation especially loss of lysine 16 acetylation on H4 histone (H4K16ac) and trimethylation of lysine 20 on H4 histone (H4K20me3) has been linked to a variety of cancer (Fraga et al., 2005). Acetyl groups are incorporated into the lysine tail of histone proteins by histone acetyltransferases. With the over-activity of these enzymes hyperacetylation occur leading to the activation of proto-oncogenes whereas tumor suppressor genes are silenced because of hypoacetylation. There exist a balance between histone acetylation and histone deacetylation which is very often altered in cancer cells. The HAT Gcn5 plays a pivotal role in regulating cell cycle, DNA damage, cell proliferation and regulating transcription suggesting that an anomalous activity in this enzyme can lead to cellular malfunction resulting in malignant growth. It was observed that Gcn5 along with a transcriptional adapter protein Ada3 plays a key role in breast cancer cell proliferation (Germaniuk-Kurowska et al., 2007). Moreover, a recent study demonstrated the role of Gcn5 in lung cancer where Chen and colleagues observed that Gcn5 potentiates non-small cell lung cancer growth with the involvement of E2F1 and various cell cycle regulatory proteins (Chen et al., 2013). A bulk of literature suggests the involvement of Tip60, MOZ, MORF in the development of cancer. These enzymes facilitate the activation of various proteins which in turn steers the cell towards malignant growth. Human Tip60 is associated with NuA4 complex and establishes connection with other subunit of the complex which seems to play a role in oncogenesis (Judes et al., 2015). The monocytic leukemia zinc finger protein (MOZ) is found to be fused with other transcription factors which are critical towards leukemogenesis (Timmermann, Lehrmann, Polesskaya, & Harel-Bellan, 2001). Especially the fusion of MOZ-TIF2 is associated with the gain of function of MOZ leading to AML in murine bone marrow (Deguchi et al., 2003). Mis-sense point mutation in the p300 gene was observed in colon and gastric adenocarcinoma and was associated with the loss of the wild-type allele (Iyer, Özdag, & Caldas, 2004). Moreover, in diffuse large B-cell lymphoma the HAT activity of p300/CBP are disabled due to point mutation and non-sense mutation (Haery, Lugo-Picó, Henry, Andrews, & Gilmore, 2014). Methylation of lysine residue on histone has been extensively studied for cancer formation. The Lysine methyltransferase SUV39H1 function to maintain genome stability and appears to play a tumor-suppressive role. However, in acute myeloid leukemia, tumor suppressor genes such as $p15^{INK4B}$ are silenced because of SUV39H1 mediated H3K9me (Lakshmikuttyamma, Scott, DeCoteau, & Geyer, 2010). EZH2, the enzymatic subunit of PRC2 is extensively linked with the metastasis of various cancers. Lysine 27 at histone H3

is methylated by EZH2, however it is not clear whether methylation of H3K27 drives the tumorigenic action of EZH2 (Kim & Roberts, 2016). Methylation of H3K79 is catalysed by DOT1L which causes transcription elongation and is associated with mixed lineage leukemia (MLL). McLean and colleagues suggested that MLL fusion proteins like AF9, AF10 recruit DOT1L to increase H3K79 methylation leading to the irregular expression of genes contributing to leukemia (McLean et al., 2014). H3K36 methylation is catalysed by SMYD2 and its levels are raised in various cancer including esophageal squamous cell carcinoma. In addition, a recent study identified the growth promoting role of SMYD2 in pancreatic cancer (Reynoird et al., 2016).

The activation of genes such as c-fos and c-jun has been linked with the phosphorylation of H3S10 (Nowak & Corces, 2004). It is noteworthy that these are proto-oncogenes and are involved in cell cycle progression, apoptosis and cellular proliferation linking histone phosphorylation with malignant transformation. The phosphorylation of H3S10 is mainly carried out by Aurora B kinase and it has been observed that in several cancers like glioblastoma, hepatocellular and colorectal cancer the levels of Aurora B are increased (Ota et al., 2002; Tanaka et al., 2008).

Impairment in DNA damage repair machinery leads to a higher risk of cancer development. The K63 linked polyubiquitination on H2A and H2AX is catalysed by the DNA damage response regulator RNF8 and RNF168 (Huen et al., 2007). Studies have suggested that loss of function of histone ubiquitination enzyme weakens DSB associated polyubiquitination of H2A and H2AX and increases ionizing radiation associated cell damage (Pandita et al., 2013). Monoubiquitinaiton of H2B at lysine 120 plays a crucial role in transcription, DNA damage response and it has been observed that in many cancers like lung, colorectal and breast levels of H2Bub1 are very low or absent (Cole et al., 2015). Moreover, USP22 an ubiquitin hydrolase is highly expressed in malignant cancer and it catalyses the removal of ubiquitin from monoubiquitinated histones H2A and H2B. The involvement of DSB towards genomic instability and cancer development is very well established and studies have suggested that a deficiency in the biotinylation of K12H4 is the initial signalling episode in response to DSB.

3.3. miRNA and cancer

An increasing amount of literature has suggested that miRNAs are epigenetically regulated and deregulation of miRNAs in cancer has been extensively studied. Most of the miRNAs are involved in regulating cell cycle progression, apoptosis, differentiation and other crucial process in the cell and alterations in them through epigenetic pathways are implicated in numerous cancer types (Kunej et al., 2011). Recent research has clearly documented the role of miRNAs in all the hallmarks of cancer. For example miR-15 and miR-16 was identified at the chromosome location 13q14.3 which is frequently deleted in chronic lymphocytic leukemia leading to an aberrant expression of anti-apoptotic genes (Calin et al., 2002). Although studies have identified the overexpression of miR-9 in brain (Nass et al., 2009), hypermethylation of miR-9 loci is evident in numerous tissue including colon, neck and lung carcinoma (Bandres et al., 2009; Kang et al., 2013; Liu, Chen, Yu, Xia, & Zhou, 2009). Moreover, the locus of miR-9-1 is heavily methylated both in invasive ductal carcinoma and the intra-ductal component of invasive ductal carcinoma of breast (Lehmann et al., 2008). In addition, recent study has indicated that CpG island methylation of miR-9 gene was considerably higher in gastric cancer tissue (Li et al., 2014). Furthermore, role of miR-9 in the metastasis of esophageal squamous cell carcinoma has been established via repressing E-cadherin (Song et al., 2014).

Members of miR-148/152 family consisting of miR-148a, miR-148b and miR-152 play a significant role in the development of cancer. Growing evidence has identified miR-148/152 family members as potential oncogenes and tumor suppressor genes. Studies have reported the up-regulation of miR-148a in the plasma of multiple myeloma patients leading to poor survival (Huang, Yu, Li, Liu, & Zhong, 2012). Moreover up-regulation of miR-148b was also observed in hepatocellular carcinoma (Yuan et al., 2012). On the other hand studies have indicated the anti-tumor effect of miR-148a especially in breast cancer where it was able to halt the proliferation and migration of breast cancer cells by targeting MMP-13 (Xue, Chen, Gu, Zhang, & Zhang, 2016). The expression of miR-148/152 family members is reduced due to methylation occurring at the CpG islands of miR-148/152 family member genes. Literature suggests that in gastric cancer overexpression of DNMT1 caused hypermethylation of miR-148a gene leading to its inactivation (Xia, Guo, Yan, & Deng, 2014). Moreover TGFβ signalling pathway pays a crucial role in carcinogenesis and is a target of miR-148 family members. Epigenetic inactivation of miR-148 family by DNA methylation leads to enhanced signalling of TGFβ leading to tumor growth and metastasis (Neuzillet et al., 2015).

miR-34a controls the production of various target proteins associated with cell cycle progression and apoptosis. miR-34a is inactivated by DNA methylation occurring in the CpG island next to its transcriptional start site which is a frequent observation in various malignancies (Lodygin et al., 2008). In addition, Kwon and colleagues demonstrated that expression of miR-34a is epigenetically silenced in human cholangiocarcinoma cells suggesting its tumor suppressive role (Kwon et al., 2016). Also in soft tissue sarcomas (STS), hypermethylation of miR-34b/c is very frequently observed in its late clinical stages (Xie et al., 2015).

Downregulation of miR-137 by CpG island methylation has been observed in several cancers (Balaguer et al., 2010; Deng et al., 2011; Zhao et al., 2012). Accumulating evidence has established that ectopic expression of miR-137 significantly lowered Cdc42 and Cdk6 levels leading to cell cycle arrest at G1 phase in lung cancer cells (Zhu et al., 2013). miR-124 is the most prevalent miRNA in the brain and an aberrant expression leads to central nervous system related malignancies. Diverse modes of miR-124 expression have been observed in numerous cancers including glioblastomas (Karsy, Arslan, & Moy, 2012). Recent report suggests that miR-124 acts as a tumor suppressor and might be useful in treating human glioblastoma by targeting STAT3 (Li et al., 2016). Moreover studies have identified that DNMT1 induction by Hepatitis C virus (HCV) led to the suppression of miR-124 in HCV related intrahepatic cholangiocarcinoma (Zeng et al., 2012). A greater frequency in the hypermethylation of miR-124-1 gene was observed in non-Hodgkin's lymphoma (Wong et al., 2011).

miR-200 is recognized as a cell's autonomous suppressor of epithelial to mesenchymal transition (EMT) and metastasis. Reports suggest that Zinc finger E-box binding homeobox transcription factor 1 (ZEB1) is involved in EMT and an overexpression of ZEB1 has been identified in numerous cancer (Liu, El-Naggar, Darling, Higashi, & Dean, 2008). Studies have identified that miR-200 overexpression inhibited ZEB1 mediated metastasis in colorectal cancer cells (Sun, Ding, Zhi, & Chen, 2015). Indeed it has been demonstrated that miR-200 silencing by CpG island hypermethylation causes the transition between EMT and vice versa leading to tumorigenesis (Davalos et al., 2012).

4. Epigenetic drugs for cancer

4.1. Drugs altering DNA methylation:

An amplified level of DNA methylation due to the over-activity of DNMTs, occurring in the CpG island of tumor suppressor genes leads to silencing of gene expression. Because of the reversibility of this type of modification, inhibition of DNMTs is considered an interesting therapeutic strategy as it may lead to the restoration of tumor suppressor gene activity. Advancement in the field of epigenetic research has led to the development of numerous DNMT inhibitors that are well categorized and tested in clinical trials. The DNMT inhibitors are divided into two broad classes: nucleoside and non-nucleoside analogues. The nucleoside analogues were initially designed as antimetabolites but only after 1977 their hypomethylating properties came to surface (Constantinides et al., 1977). Azacytidine (5-azacytidine) and

Decitabine (5-aza-2′-deoxycytidine) are the two oldest nucleoside analogue that gets intercalated with the DNA during S phase of the cell cycle where they are recognized by the DNMTs. These analogues then acts as a suicide inhibitor forming a covalent irreversible complex with the enzyme, ultimately leading to its proteasomal degradation. The toxic profile of the older nucleoside analogues has been addressed by the development of Zebularine and 5-fluoro-2′-dexoycytidine which are more effective nucleoside DNMT inhibitors. Even though Zebularine is used at a much higher dose compared with the suicide analogues to attain the same demethylation levels in the cell it is associated with less cytotoxicity at that concentration. Promising data from numerous clinical trials have led the approval of nucleoside analogues Azacitidine and Decitabine by FDA for the management of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) (Derissen et al., 2013). Although Zebularine was found to have better anti-cancer profile compared to Decitabine and Azacitidine its limited oral bioavailability and incompetent metabolism has hindered its clinical use (Holleran et al., 2005; Yang, Lay, Han, & Jones, 2010). Ongoing studies have led to the development of various new nucleoside inhibitors. NPEOC-DAC a 5 azacytidine derivative has been developed which gets converted into Decitabine by carboxylesterase 1 (Fahy, Jeltsch, & Arimondo, 2012). Although promising its use as a drug candidate has not been reported. RX-3117 is another nucleoside analogue with an ability to inhibit DNMT1 (Choi et al., 2012). Studies have also identified its anti-cancer effect *in vivo* (Yang et al., 2014). A nucleoside analogue that has currently undergone Phase I clinical trial for the treatment of MDS and AML is SGI-110 (Issa et al., 2015). It is a new hypomethylating agent that is derived from decitabine and is a promising candidate for MDS and AML. The investigational drug CP-4200, an elaidic acid ester of azacitidine works as a pro-drug and has demonstrated better therapeutic profile than azacitidine (Brueckner et al., 2010). A research team at the Southern Research Institute has investigated the potential of thiocytidine analogues as DNMT inhibitors. They have identified two analogues 4′-thio-2′-deoxycytidine and 5-aza-4′-thio-2′-deoxycytidine that can inhibit DNMT1 in both cancer cell lines and animal models of cancer (Thottassery et al., 2014).

Due to the cytotoxic property of the nucleoside analogues several non-nucleoside inhibitors of DNMTs are being developed with the idea that these compounds can bind at the catalytic site of the enzymes without directly incorporating into the DNA (Song, Han, & Bang, 2011). Studies conducted in 2009 reported a quinoline derivative SGI-1027 with an ability to inhibit DNA methyltransferase (Datta et al., 2009). It is a lipophilic quinoline derivative that can inhibit DNMT1, 3A and 3B and because of its basic property it binds weakly with the AT rich region of DNA. Recent studies have indicated it to be more selective towards human DNMT3A compared to human DNMT1 (Rilova et al., 2014). Although promising, very few reports are available establishing it as a clinical drug candidate. A quinone antibiotic nanaomycin A which was isolated from a strain of *Streptomyces* initially demonstrated to be a DNMT1 inhibitor from virtual screening (Kuck, Singh, Lyko, & Medina-Franco, 2010; Umezawa et al., 1975). However, biochemical analysis conducted using nanaomycin A suggested its selectivity towards DNMT3B enzyme and was able to reactivate silenced tumor suppressor genes in human cancer cells (Kuck, Caulfield, Lyko, & Medina-Franco, 2010). Further studies are vital to determine its potential in clinical trials. A phthalimido-L-tryptophan derivative RG108 was developed as a DNMT inhibitor through *in silico* drug design. Recent study demonstrated that RG108 induced the expression of E-cadherin in promyelocytic leukaemia cells given alone or in combination with HDAC inhibitors (Savickiene, Treigyte, Jazdauskaite, Borutinskaite, & Navakauskiene, 2012). Moreover studies have also indicated that RG108 was able to protect retinal pigment epithelial cells from oxidative stress by up-regulating methylated silenced genes involved in producing antioxidant enzymes (Tokarz, Kaarniranta, & Blasiak, 2016). Naturally occurring compounds, especially polyphenols have been widely studied for their DNMT inhibitory activity (Busch et al., 2015). The flavan-3-ol Epigallocatechin-3-gallate which is profuse in green tea inhibits DNMT1 enzyme directly (Yiannakopoulou, 2015). Studies have shown that flavonoids like Quercetin, Myricetin and Fisetin inhibited DNMT1 enzyme in a dose dependent manner (Subramaniam, Thombre, Dhar, & Anant, 2014). Moreover flavones and flavanones like apigenin and hespertin are also been proved to inhibit DNMT enzyme in human cancer cell lines (Fang, Chen, & Yang, 2007). Hydralazine which is indicated for the management of hypertension has been studied for its potential as a DNMT inhibitor. Graca and colleagues demonstrated that in prostate cancer cells hydralazine treatment lowered the production of DNMT1, DNMT3a and DNMT3b mRNA suggesting its potential in reducing the malignant growth through epigenetic alterations (Graça et al., 2014). Evidence also suggests that combining hydralazine with the HDAC inhibitor valproic acid would be a more effective treatment option in the epigenetic therapy of cancer (Dueñas-Gonzalez et al., 2014). The antiarrhythmic drug procainamide has been studied for its ability to alter the epigenentic machinery of a cell. Work carried out by Lee and colleagues suggested that procainamide was able to hinder the hemimethylase activity of DNMT1 without much alteration in the activity of DNMT3a and DNMT3b (Lee, Yegnasubramanian, Lin, & Nelson, 2005). Studies have reported that the expression of tumor suppressor genes like $p16^{INK4a}$, *RAR-β*, *GSTP1* was restored by procainamide treatment (Ren et al., 2011). The ester analogue of procainamide i.e. procaine also has the ability to hinder the levels of DNA methylation in a cell. Studies have suggested that it has a more affinity towards CpG rich sites and acting partially towards DNMT enzymes (Amatori, Bagaloni, Donati, & Fanelli, 2010). Treatment with procaine caused a reduction in 5-methylcytosine content in breast cancer cell line suggesting it as a promising DNA demethylating agent (Villar-Garea, Fraga, Espada, & Esteller, 2003). An antisense oligonucleotide designed to bind with the 3′ untranslated region of DNMT1 mRNA and hindering with its transcription is MG98. It is a second generation DNMT inhibitor specifically inhibiting DNMT1 without altering DNMT3 expression (Amato, 2007). Clinical study has been carried out with MG98 in combination with Interferon for the treatment of metastatic renal cell carcinoma and was proven to be safe at a particular dosage (Amato et al., 2012). However further studies needs to be carried out to market it as a drug candidate. Table 1 highlights the various DNMT inhibitors used in preclinical and clinical setup.

4.2. Drugs altering histone modification

Various posttranslational modifications of histone protein are carried out using numerous enzymes that are involved in the addition or deletion of several covalent attachments at specific histone residues. Alteration or deregulation in the functions of these enzymes is involved with malignant growth. Thus, the development of newer therapies directed towards these enzymes can be beneficial for the treatment of cancer. Currently histone deacetylase inhibitor is the only approved epigenetic therapy in clinics altering histone proteins. However, continuous efforts are being made for the development of specific inhibitors to target various other enzymes involved in the alteration of histone proteins. Fig. 4 summarizes the various epigenetic drug targets.

4.2.1. Histone methyltransferases inhibitor

Histone methyltransferases (HMT) are linked with gene regulatory complex and affect the pattern of gene expression. Histone lysine methyltransferases modify lysine residues generating various degrees of methylation. The overexpression of these enzymes is observed in numerous types of cancer suggesting them to be a potential therapeutic target (Wagner & Jung, 2012). The lysine methyltransferase DOT1L is a distinctive HMT that does not contain the SET domain and methylates H3K79 in the globular domain. DOT1L has an S-adenosyl methionine (AdoMet) binding domain and based on this EPZ004777 was developed by a team of researchers (Daigle et al., 2011). *In vitro* selectivity of EPZ004777 was observed against DOT1L enzyme in MLL cells and

Table 1
DNA methylation inhibitors (DNMTi).

DNA methylation inhibitors					
Category	Compound	Development stage	Tumor type	Developer/Marketer	References
Nucleoside analogues	Azacytidine	Approved	MDS	Celgene	Derissen et al. (2013)
	Decitabine	Approved	MDS	Otsuka, Janssen	Derissen et al. (2013)
	Zebularine	Preclinical	Solid tumors	NCI	Yang et al. (2010)
	RX-3117	Clinical	Pancreatic, Bladder	Rexahn Pharmaceuticals	Yang et al. (2014)
	SGI-110	Clinical	MDS, AML	Astex Pharmaceuticals	Issa et al. (2015)
	CP-4200	Preclinical	MDS	Clavis Pharma	Brueckner et al. (2010)
	SGI-1027	Preclinical	Solid tumors	SuperGen, Inc.	Rilova et al. (2014)
	Nanaomycin A	Preclinical	Lung, colon, leukemia	GCRC	Kuck, Caulfield, et al. (2010), Kuck, Singh, et al. (2010)
Non-nucleoside analogues	RG 108	Preclinical	leukemia	GCRC	Savickiene et al. (2012)
	Polyphenols	Preclinical	Solid tumor, leukemia	Naturally occurring	Busch et al. (2015)
	Hydralazine	Clinical	Solid tumor	Known molecule	Graça et al. (2014)
	MG98	Clinical	Renal	Methylgene Inc.	Amato et al. (2012)

Various experimental and clinical molecules serving as DNA methylation inhibitors are shown here. Apart from the compounds highlighted here numerous experimental compounds are being tested for their DNMT inhibitory properties. MDS-Myelodysplastic syndrome, NCI—National Cancer Institute, AML—Acute myeloid leukemia, GCRC—German Cancer Research Center

decreased H3K79 methylation. Moreover a new DOT1L inhibitor EPZ-5676 was found to inhibit H3K79 methylation leading to the reduction in MLL-fusion gene expression. In addition *in vivo* studies in xenograft model of MLL-translocated leukemia EPZ-5676 proved to be effective in reducing tumor growth without any significant toxicity (Daigle et al., 2013). A study conducted by Klaus and colleagues demonstrated that EPZ-5676 displayed synergistic antiproliferative effect in combination with cytarabine or daunorubicin in MLL-rearranged leukemia cells (Klaus et al., 2014). The core component of PRC2 includes EZH2 and it is associated with the methylation of H3K27 leading to repression of gene expression. Overexpression of this enzyme is found in numerous cancers including B-cell lymphoma, prostate and breast cancer (Volkel, Dupret, Le Bourhis, & Angrand, 2015; Zhou et al., 2015). Targeting the degradation of EZH2 utilizing S-adenosyl-L-homocysteine hydrolase inhibitor DZNep has been effective in inducing apoptosis and inhibiting metastasis in chondrosarcoma cells (Girard et al., 2014). Direct inhibitor

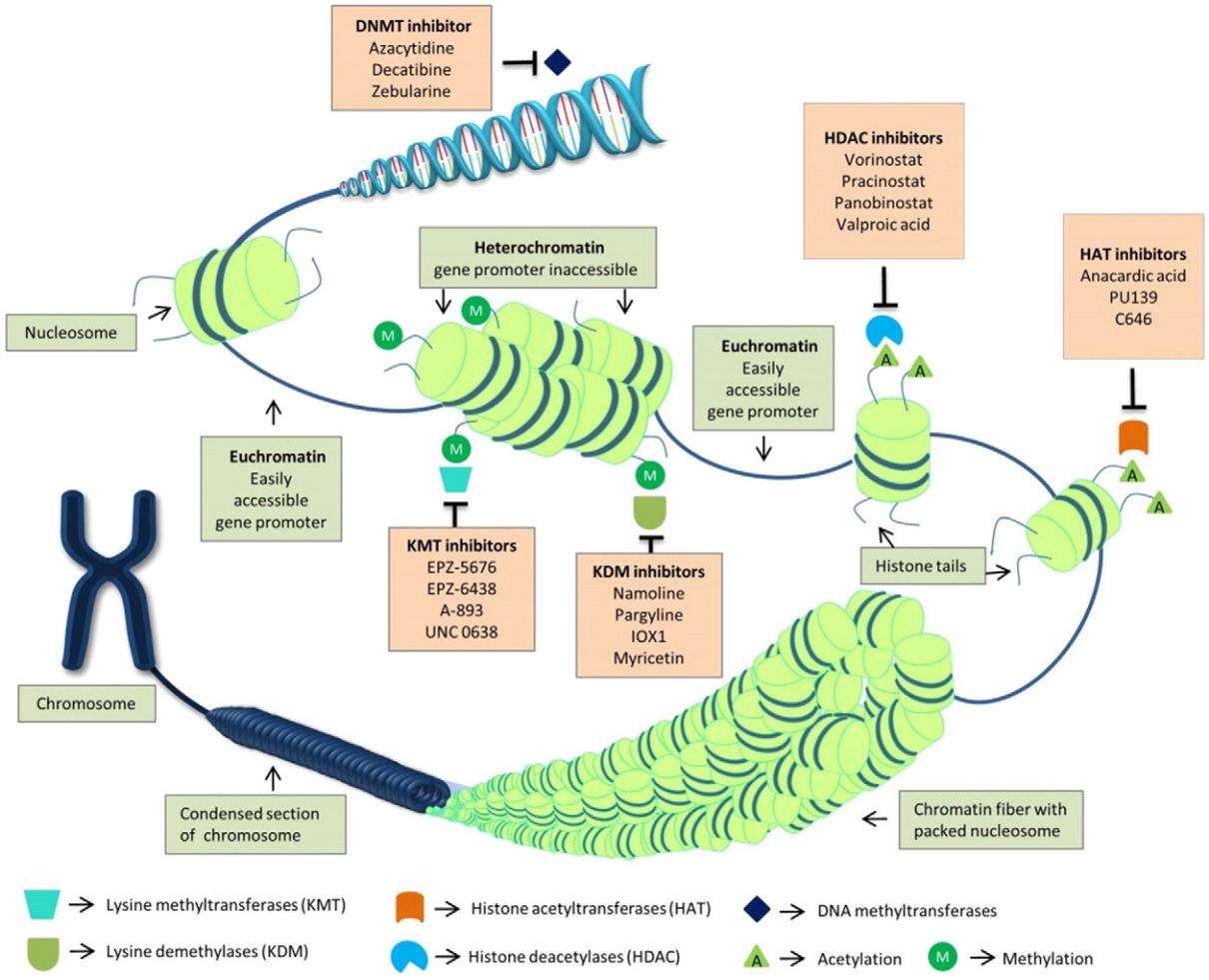

Fig. 4. Epigenetic targets. Modification of histone proteins on the nucleosome core is carried out by various enzymes including HAT, HDAC, KMT and KDMs. All these enzymes serve as epigenetic targets with numerous inhibitors already approved by the FDA and many undergoing clinical trials for the same. The enzyme DNA methyltransferases (DNMT) are also explored for the development of epigenetic drugs. Apart from the targets shown here other possible epigenetic targets for drug development are also available.

of EZH2, EPZ005687 has been developed by a team of researchers at Epizyme, Inc., with the ability to lower H3K27 methylation in lymphoma cells (Knutson et al., 2012). Another selective inhibitor of EZH2, EPZ-6438 was developed by Knutson and colleagues which was found to be superior to the previous molecule. This compound was effective in reducing tumor growth in EZH2 mutant non-Hodgkin lymphoma xenograft bearing mice model of cancer prompting further studies in patients (Knutson et al., 2014). The lysine methyltransferase, SMYD2 methylates lysine residues on Histone H2B, H3 and H4 apart from non-histone targets. A selective inhibitor of SMYD2, AZ505 was developed by a team of structural chemist at Astra Zeneca and reported the crystal structure of SMYD2 where AZ505 was bound at the peptide binding site of SMYD2 (Ferguson et al., 2011). However, few reports are available depicting further studies. Another selective inhibitor of SMYD2 is LLY-507 which is reported to inhibit the proliferation of several cancer cell lines including liver and breast cancer cell lines (Nguyen et al., 2015). These data warrants further *in vivo* studies to assess it potential in preclinical studies. A recent study was conducted by Sweis et al. where they analyzed the critical binding sites of AZ505 (Sweis et al., 2015). In modifying the structure they came up with A-893, a compound with increased potency for SMYD2. They predicted its increased activity due to the presence of hydroxyl group capable of forming hydrogen bond with lysine pocket. Preliminary *in vitro* data with the compound was encouraging portraying the development of new SMYD2 inhibitors. Methylation of H3K9 is catalyzed by the methyltransferase G9a. G9a is up-regulated in numerous cancer and a specific inhibitor of G9a would be beneficial. BIX-01294 was identified with the potential to selectively impair G9a activity after screening thousands of other compounds. *In vitro* studies demonstrated that the compound was able to lower H3K9 methylation levels in various cell lines (Kubicek et al., 2007). Another inhibitor of G9a UNC0638 was highly effective in *in vitro* setup (Vedadi et al., 2011). Recently a peptide-competitive inhibitor of G9a, A-366 was developed that was effective against leukemic cell lines (Pappano et al., 2015). Set 7/9 methylates H3K4 and is also associated in methylating the estrogen receptor. An X-ray crystal structure of an inhibitor bound to Set 7/9 domain has been reported. Recently cyproheptadine was identified as an inhibitor of Set 7/9 in breast cancer cell regulating estrogen-dependent gene expression (Takemoto et al., 2016). Table 2 shows various HMT inhibitors at different stage of development.

4.2.2. Histone demethylase inhibitor

Two classes of histone demethylase (KDMs) inhibitors have been identified: the amine oxidases like histone demethylases (Lysine-specific demethylases LSD1/2) belong to KDM1 subfamily and the iron and α-ketoglutarate dependent jumonji C (JmjC) domain containing demethylase belonging to larger KDM subfamilies (KDM2-8).

Phenelzine, Tranylcypromine and Pargyline were the initial compounds reported to inhibit LSD1. Prusevich and colleagues reported a novel phenelzine analogue capable of inhibiting LSD1 enzyme compared to LSD2 *in vitro*. Moreover they also observed a reduction in multiplication rate of prostate and lung carcinoma cells (Prusevich et al., 2014). Numerous reports suggests the role of tranylcypromine in inhibiting LSD1 and researches are continually modifying its structure to come up with new and better inhibitors of LSD1. Indeed, two new LSD1 inhibitors based on the structure of tranylcypromine has been developed and are currently undergoing clinical trials (Zheng et al., 2016). Recently the LSD1 inhibitor Pargyline has been demonstrated to inhibit the process of epithelial to mesenchymal transition in prostate cancer cell lines and delayed the progression of cancer from androgen dependent to independent state (Wang et al., 2015). Polyamine derivatives were also reported to inhibit LSD1 function with studies highlighting the role of polyamine analogues 2d or PG11144 in the treatment of breast cancer. They observed an increase in H3K4 methylation in MDA-MB-231 cell line with an alteration in the expression pattern of multiple genes (Zhu et al., 2012). In addition, derivatives of (bis)guanidines and (bis)biguanides exhibited increase in H3K4 marks by inhibiting LSD1 in lung cancer cell lines (Sharma et al., 2010). Namoline, a reversible inhibitor of LSD1, halts its demethylase activity leading to silencing of gene expression and impairment of androgen dependent proliferation in prostate cancer (Willmann et al., 2012). A recent study proved that inhibition of LSD1 with HCI-2509 reduced the levels of c-MYC in prostate cancer cell lines and has a potential in docetaxel-resistant prostate cancer (Gupta et al., 2016). However all the inhibitors described so far has only been evaluated in preclinical models and few reports are available describing clinical studies with those compounds.

With our increased understanding about the structure and function of JmjC domain it has been possible to design and develop novel inhibitors for this class of enzymes. N-oxalylglycine (NOG) which is closely related to α-ketoglutarate was initially found to inhibit prolyl hydroxylase is now known to inhibit JmjC KDMs (Hopkinson et al., 2013). Although promising very few reports are available pertaining to its clinical use. In the quest for identifying newer analogues directed towards JmjC KDMs, the hydroxamic acid derivative SAHA (vorinostat) demonstrated KDM4E inhibition (Rose et al., 2008). Screening of a series of 8-hydroxyquinoline derivative led to the identification of IOX1, which is a potent inhibitor of many KDM subtypes (Hopkinson et al., 2013). Similarly reports suggests that pyridine-2,4-dicarboxylic acid is an inhibitor of several JmjC KDMs and are more stable than NOG. Only cellular studies on this molecule have been carried out so far using it as a prodrug (Rose et al., 2008). In addition to all the inhibitors described so far a number of flavonoids have been shown to inhibit many subtypes of JmjC KDMs. Studies carried out on myricetin, epigallocatechin gallate and caffeic acid demonstrated their ability to inhibit few subtypes of KDMs in cell based analysis. For more comprehensive information about histone lysine demethylases inhibitors readers are suggested to go through the excellent review by Thinnes (Thinnes et al., 2014),

Table 2
Histone methyltransferase inhibitors.

Histone methyltransferase inhibitors					
Category	Compound	Development stage	Tumor type	Developer	References
DOT1L inhibitor	EPZ004777	Preclinical	Mixed lineage leukemia	Epizyme Inc.	Daigle et al. (2011)
	EPZ-5676	Clinical	Hematological malignancy		Daigle et al. (2013)
EZH2 inhibitor	DZNeP	Preclinical	Colon, Breast	NCI	Girard et al. (2014)
	EPZ005687	Preclinical	lymphoma	Epizyme Inc.	Knutson et al. (2012)
	EPZ-6438	Clinical	Lymphoma and Solid tumors		Knutson et al. (2014)
SMYD2 inhibitor	AZ505	Preclinical	Gastric cancer	AstraZeneca	Ferguson et al. (2011)
	LLY-507	Preclinical	Liver, Breast, Esophageal	Eli Lilly & Company	Nguyen et al. (2015)
	A-893	Preclinical	Lung	AbbVie Inc.	Sweis et al. (2015)
G9a inhibitor	BIX-01294	Preclinical	Neuroblastoma	Vienna Biocenter	Kubicek et al. (2007)
	UNC0638	Preclinical	Breast	University of North Carolina	Vedadi et al. (2011)
	A-336	Preclinical	Leukemia	AbbVie Inc.	Pappano et al. (2015)

Some preclinical and clinical compounds acting as histone methyltransferse inhibitors are being highlighted here. Numerous experimental compounds are also being evaluated for histone methyltransferase inhibitory potential which is not displayed here. NCI—National Cancer Institute.

Table 3
Histone demethylase inhibitor.

Histone demethylase inhibitors					
Category	Compound	Development stage	Tumor type	Developer	References
	Phenelzine analogue	Preclinical	Lung, Prostate	John Hopkins University	Prusevich et al. (2014)
	Tranylcypromine analogue	Clinical	Leukemia	Oryzon Genomics	Zheng et al. (2016)
	Pargyline	Preclinical	Prostate	Albert-Ludwigs-University	Wang et al. (2015)
LSD1 inhibitor	Polyamine analogues	Preclinical	Breast	John Hopkins University	Zhu et al. (2012)
	Bisguanidines	Preclinical	Lung	Wayne State & John Hopkins University	Sharma et al. (2010)
	Namoline	Preclinical	Prostate	Albert-Ludwigs-University	Willmann et al. (2012)
	HCI-2509	Preclinical	Prostate	Huntsman Cancer Institute	Gupta et al. (2016)
	8-hydroxy quinolines	Preclinical	Inhibition of target protein	University of Oxford	King et al. (2010)
JmjC domain inhibitor	Pyridine dicarboxylates	Preclinical	Inhibition of target protein	University of Oxford	Rose et al. (2008)
	Flavonoids	Preclinical	Inhibition of target protein	Naturally occurring	Thinnes et al. (2014)

Various experimental compounds inhibiting the two broad classes of histone demethylase enzymes are being highlighted here. The table only highlights few important histone demethylase inhibitors in various stages of development.

Hoffmann (Hoffmann et al., 2012) and McAllister (McAllister et al., 2016). Table 3 highlights some of the experimental histone demethylase inhibitors.

4.2.3. Histone acetyltransferase inhibitors

The involvement of histone acetyltransferases in transcriptional regulation make this groups of enzymes an important target. Numerous small molecule inhibitors have been described targeting a class of HAT. Among them isothiazolones consisting of N-alkyl and N-aryl substituents were proved to inhibit PCAF and p300 HATs causing a reduction in the proliferation of colon cancer cells (Stimson et al., 2005). In silico screening for a panel of compounds led to the identification of C646, which is a competitive inhibitor of p300. Recent study demonstrated that C646 was able to arrest cell cycle and induce apoptosis in AML1-ETO-positive acute myeloid leukemia (AML) cells (Gao et al., 2013). A naturally occurring chemical compound, Anacardic acid was proven to inhibit a member of the MYST family of HATs in cellular studies (Sun, Jiang, Chen, & Price, 2006). Based on this, numerous structural analogs of anacardic acid were being developed with studies reporting 6-alkylsalicylates as selective Tip60 inhibitors (Ghizzoni et al., 2012). All the above described experimental compounds have established their HAT inhibitory activity in cellular and biochemical studies, however very few reports are available with *in vivo* studies. Recently Gajer and colleagues revealed two pyridoisothiazolone derivatives PU139 and PU141 that were able to inhibit Gcn5, PCAF, CBP and p300 in cellular studies with PU141 displaying more selectivity towards CBP and p300. The efficacy of these compounds was also established in xenografts mice where they were able to reduce histone lysine acetylation (Gajer et al., 2015). Table 4 displays some preclinical HAT inhibitors.

4.2.4. Histone deacetylase inhibitor

The level of histone acetylation in a cell is preserved by the balance of HAT and HDAC activity. It is widely accepted that genome wide changes in histone acetylation is associated with cancer development and aberrant activity of HDAC is linked to oncogenic event. Hydroxamic acid derivative Vorinostat was the first HDAC inhibitor approved by the FDA. It is indicated for the treatment of cutaneous T cell lymphoma (CTCL). Other hydroxamic acid derivatives which are in various phases of development include Abexinostat, Pracinostat, Resminostat, Givinostat, Panobinostat. Abexinostat has demonstrated promising anticancer activity in preclinical study. Recently report from a phase 1 clinical trial with Abexinostat suggested that patients with follicular lymphoma were benefited with this drug (Morschhauser et al., 2015). Furthermore a phase I/II trial with Abexinostat showed that it was well tolerated and effective in follicular lymphoma (Evens et al., 2016). Pracinostat is another hydroxamic acid analogue with HDAC inhibitory potential. A phase II trial with pracinostat in patients with myelofibrosis displayed its tolerability with modest clinical activity (Quintás-Cardama et al., 2012). Recently FDA has approved Pracinostat with a designation of breakthrough therapy in combination with azacitidine for the treatment of AML. The HDAC inhibitor Resminostat (developed by 4SC) was assessed in patients with refractory or relapsed Hodgkin lymphoma and was found to possess admirable safety profile (Walewski et al., 2010). Moreover results from the SHELTER study showed that resminostat in combination with sorafenib can be useful for advanced hepatocellular carcinoma (Bitzer et al., 2016). Another novel HDAC inhibitor 4SC-202 was recently evaluated in a phase I trial in patients with advanced hematological malignancies (NCT01344707). Givinostat is another hydroxamate derivative with proven clinical activity in patients with Hodgkin's lymphoma and multiple myeloma. A phase II study was carried out with givinostat in combination with hydroxycarbamide (HC) to treat polycythemia vera. Results from the study proved that combined use of Givinostat and HC was clinically effective (Finazzi et al., 2013). The HDAC inhibitor Panobinostat was approved by FDA for the treatment of multiple myeloma. Farydak (Panobinostat) is to be taken in combination with bortezomib and dexamethasone for multiple myeloma. A phase I study in patients with advanced solid tumor has been carried out with an oral hydroxamate HDAC inhibitor Quisinostat with the sign of target modulation and antitumor activity (Venugopal et al., 2013). Recently a phase II trial was carried out with Quisinostat where it was found to be active in the treatment of patients with relapsed or refractory CTCL (Child et al., 2016). Clinical trial of a selective HDAC6 inhibitor Rocilinostat developed by Acetylon pharmaceuticals has suggested that rocilinostat alone or in combination with bortezomib and dexamethasone might be a treatment option for relapsed/refractory multiple myeloma (Raje et al., 2012). A dual acting HDAC and PI3K inhibitor CUDC-907 developed by Curis pharmaceuticals was found to be safe as a monotherapy in patients with relapsed or refractory lymphoma and multiple myeloma (Younes et al., 2016). Another small molecule

Table 4
Histone acetyltransferease inhibitors.

Histone acetyltransferase inhibitors					
Category	Compound	Development stage	Tumor type	Developer	References
p300 inhibitor	C646	Preclinical	Inhibition of target enzyme	John Hopkins University	Bowers et al. (2010)
Tip60 inhibitor	Anacardic acid	Preclinical	Breast	Naturally occurring	Sun et al. (2006)
	6-alkyl salicylates	Preclinical	Inhibition of target enzyme	University of Groningen	Ghizzoni et al. (2012)
Pyridoisothiazole derivative	PU139, PU141	Preclinical	Neuroblastoma	Albert-Ludwigs-Uiversity	Gajer et al. (2015)

The table highlights some of the experimental compounds developed as Histone acetyltransferase inhibitors. Other experimental compounds are also available which are not shown.

CUDC-101 which is a multi-target inhibitor of HDAC, EGFR and HER2 was recently evaluated in a phase I study in patients with head and neck squamous cell carcinoma (Galloway et al., 2015). An orally active anti-angiogenic drug Tasquinimod which is an allosteric modulator of HDAC4 has been evaluated clinically for the treatment of castration resistant prostate cancer (Sternberg et al., 2016). Another pan-HDAC inhibitor developed by Arno therapeutics (AR-42) which was evaluated clinically in hematologic malignancies is recently been evaluated in a phase I trial in patients with advanced solid tumors (NCT01129193). Benzamide derivatives are another class of HDAC inhibitors among which mocetinostat, entinostat and CI-994 (tacedinaline) has been evaluated clinically. Mocetinostat was found to be safe in patients with Myelodysplastic syndromes and displayed antileukemic activity. Moreover a phase II trial conducted in patients with chronic lymphocytic leukemia demonstrated its efficacy profile. Another benzamide derivative Entinostat has been evaluated for numerous cancer types in the clinics. In addition combination of entinostat with erlotinib for NSCLC patient was assessed and no significant change was observed in the outcome when compared with erlotinib alone (Witta et al., 2012). Tacedinaline has been evaluated in combination with gemcitabine in patients with advanced pancreatic cancer and non-small cell lung cancer (Richards et al., 2006) (NCT00005093). The short chain fatty acid denotes another class of HDAC inhibitors among which Valproic acid and phenylbutyrate are two well characterized compounds. Phase I clinical trial with valproic acid (VPA) was conducted in patients with refractory solid tumors or central nervous system tumors and was found to be well tolerated. Even for the treatment of neuroendocrine tumors VPA was found to be effective (Mohammed et al., 2011). Various cellular investigations have been carried out with phenylbutyrate with a recent report suggesting its ability to suppress the growth of glioblastoma cell line (Kusaczuk, Krętowski, Bartoszewicz, & Cechowska-Pasko, 2016). However few clinical reports are available suggesting its usage in the clinics. A plethora of natural compounds have been screened for their HDAC inhibitory potential. Among them Amamistatin, isolated from *Nocardia asteroids,* the natural cyclopeptide FR235222 isolated from the fermentation broth of *Acremonium sp*., chlamydocin from *Diheterospora chlamydosporia*, apicidin from *Fusarium* sp. are worth mentioning (Mottamal, Zheng, Huang, & Wang, 2015). For a more comprehensive information readers are suggested to refer the excellent reviews (Manal, Chandrasekar, Priya, & Nanjan, 2016; Mottamal et al., 2015). Table 5 highlights various approved HDAC inhibitors and some HDAC inhibitors which are evaluated clinically for various malignancies.

5. Concluding remarks

The widespread belief that cancer is a disease characterized by genetic irregularities has been challenged based on the advancement in genomic technologies which provided us with a bird's eye view about the molecular cause of cancer. Although defects in the blueprint of DNA remains as one of the obvious path leading towards malignant growth, the participation of epigenetic factors in oncogenesis cannot be refuted. The extensive alterations of the epigenome including modification of histone proteins and chromatin remodeling complex are some of the vital epigenetic changes associated with cancer. Indeed with the application of next generation sequencing technology researchers were able to identify that in human cancer, genes controlling the epigenome are highly mutated suggesting having consequence on multiple pathway relevant to cancer phenotype.

The abundance of genetic mutations occurring in the epigenetic regulatory complexes and proteins provide a number of fundamental targets in the field of epigenetic drug discovery. It is beyond the shadow of a doubt that these targets are some of the most widely studied research topic throughout the globe and has definitely incited its consideration in the R&D sector of the billion dollar pharmaceutical industry. Having said that, with an estimate of approximately 1.8 billion dollar (Paul et al., 2010) to bring a new chemical entity into the market the efficiency of the pharmaceutical R&D in choosing the target will play a crucial role. We should move beyond the traditional approach and delve into newer avenues to achieve maximum benefit in terms of R&D productivity. As most of these epigenetic targets are enzymes and with the success in developing effective inhibitors for erasing epigenetic marks (histone deacetylase enzymes), other targets such as readers of epigenetic marks and chromatin remodeling complex should be the contender of choice. Moreover, the presence of genetic mutations should not solely be the criteria to escort the drug development process, as we have seen that FDA has approved HDAC inhibitors which are used clinically without reports of any genetic alteration occurring in this enzyme. Molecular characterization of cancer through the use of high-throughput genotype/phenotype technologies and the identification of clinically relevant biomarkers should be a way forward in the drug discovery program.

In spite of the above avowals, there remains a lot to uncover before we can apply our current knowledge in a clinical setup. The burning issue of epigenetic drug discovery is that of selectivity. The approved HDAC inhibitors are non-selective towards the HDAC enzyme leading to various undesirable effects. Recent attention has been generated towards the development of isoform specific inhibitors with a belief that it will lead to superior therapeutic drugs with lower toxic profiles. However, looking at the failure in the drug development program the validity of this statement can only be accepted once we have selective isoform inhibitors in the clinics. Moreover, studies have suggested that hematopoietic malignancies are more vulnerable towards epigenetic therapies leaving a blind side about their effectiveness in solid tumor. The use of epigenetic therapies in combination with the conventional

Table 5
Histone deacetylase inhibitors

Histone deacetylase inhibitors Category	Compound	Development stage	Tumor type	Developer	References
Hydroxamic acid derivatives	Vorinostat	Approved	CTCL	Merck	Mann et al. (2007)
	Abexinostat	Clinical	Follicular lymphoma	Pharmacyclics	Evens et al. (2016)
	Pracinostat	Approved	AML	MEI Pharma	Quintás-Cardama et al. (2012)
	Resminostat	Clinical	HL, HCC	4SC	Bitzer et al. (2016)
	Givinostat	Clinical	HL, MM, PV, DMD	Italfarmaco S.P.A.	Finazzi et al. (2013)
	Panobinostat	Approved	Multiple myeloma	Novartis	Moore (2016)
Benzamide derivative	Mocetinostat	Clinical	MDS, CLL	Mirati Therapeutics	Boumber et al. (2011)
	Entinostat	Clinical	NSCLC	Syndax pharmaceuticals	Witta et al. (2012)
Cyclic Peptides	Romidepsin	Approved	CTCL, PTCL	Gloucester pharmaceuticals	VanderMolen et al. (2011)
Short chain fatty acids	Phenylbutyrate	Clinical	Glioblastoma, CRC	Known molecule	Kusaczuk et al. (2016)
	Valproic acid	Clinical	Solid tumors, CNS tumors	Known molecule	Mohammed et al. (2011)

Various clinical and preclinical compounds having histone deacetylase inhibitory property has been shown here. Numerous other experimental molecules are available which are not highlighted here. CTCL—Cutaneous T—Cell Lymphoma, AML—Acute Myeloid Leukemia, HL—Hodgkin lymphoma, HCC—Hepatocellular Carcinoma, MM—Multiple Myeloma, PV—Polycythemia Vera, DMD—Duchenne Muscular Dystrophy, MDS—Myelodysplastic syndrome, CLL—Chronic Lymphocytic Leukemia, NSCLC—Non-small cell lung carcinoma, PTCL—Peripheral T-cell lymphoma, CRC—Colorectal carcinoma

chemotherapy might be a valid strategy to look forward in treating solid tumors. These are few out of many other challenges facing epigenetic drug discovery program.

The road travelled so far in cancer epigenetics have provided unprecedented information but there is still a long and treacherous way ahead of us without a map leading towards success. We believe that the landscape of cancer will continue to evolve in the future and we hope that the field of cancer epigenetics will evolve with it and surprise us.

Conflict of interest

The authors declare that they have no conflict of interest to disclose.

Acknowledgements

Research in our lab is supported in part by the funds from All India Council for Technical Education through Research Promotion Scheme (AICTE-RPS), Modernization and Removal of Obsolescences (MODROB) scheme, Department of Biotechnology (DBT) Ministry of Science and Technology and the funds provided by the Department of Science and Technology — Science and Engineering Research Board (DST-SERB). SB is supported from Dr. T.M.A Pai fellowship provided by Manipal University. We sincerely apologize to the authors whose original work have substantially contributed to this field but were not cited in this review due to space constraints.

References

Aihara, H., Nakagawa, T., Yasui, K., Ohta, T., Hirose, S., Dhomae, N., ... Muramatsu, M. (2004). Nucleosomal histone kinase-1 phosphorylates H2A Thr 119 during mitosis in the early Drosophila embryo. *Genes & Development 18*, 877–888.

Akiyama, Y., Watkins, N., Suzuki, H., Jair, K. -W., van Engeland, M., Esteller, M., ... Herman, J. G. (2003). GATA-4 and GATA-5 transcription factor genes and potential downstream antitumor target genes are epigenetically silenced in colorectal and gastric cancer. *Molecular and Cellular Biology 23*, 8429–8439.

Allfrey, V., Faulkner, R., & Mirsky, A. (1964). Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *Proceedings of the National Academy of Sciences 51*, 786–794.

Amato, R. J. (2007). Inhibition of DNA methylation by antisense oligonucleotide MG98 as cancer therapy. *Clinical Genitourinary Cancer 5*, 422–426.

Amato, R. J., Stephenson, J., Hotte, S., Nemunaitis, J., Bélanger, K., Reid, G., & Martell, R. E. (2012). MG98, a second-generation DNMT1 inhibitor, in the treatment of advanced renal cell carcinoma. *Cancer Investigation 30*, 415–421.

Amatori, S., Bagaloni, I., Donati, B., & Fanelli, M. (2010). DNA demethylating antineoplastic strategies: A comparative point of view. *Genes & Cancer 1*, 197–209.

Annunziato, A. (2008). DNA packaging: Nucleosomes and chromatin. *Nature Education 1*, 26.

Avdic, V., Zhang, P., Lanouette, S., Groulx, A., Tremblay, V., Brunzelle, J., & Couture, J. -F. (2011). Structural and biochemical insights into MLL1 core complex assembly. *Structure 19*, 101–108.

Balaguer, F., Link, A., Lozano, J. J., Cuatrecasas, M., Nagasaka, T., Boland, C. R., & Goel, A. (2010). Epigenetic silencing of miR-137 is an early event in colorectal carcinogenesis. *Cancer Research 70*, 6609–6618.

Bandres, E., Agirre, X., Bitarte, N., Ramirez, N., Zarate, R., Roman-Gomez, J., ... Garcia-Foncillas, J. (2009). Epigenetic regulation of microRNA expression in colorectal cancer. *International Journal of Cancer 125*, 2737–2743.

Bannister, A. J., & Kouzarides, T. (2011). Regulation of chromatin by histone modifications. *Cell Research 21*, 381–395.

Bedford, M. T., & Clarke, S. G. (2009). Protein arginine methylation in mammals: Who, what, and why. *Molecular Cell 33*, 1–13.

Bitzer, M., Horger, M., Giannini, E. G., Ganten, T. M., Wörns, M. A., Siveke, J. T., ... Wege, H. (2016). Resminostat in combination with sorafenib as second-line therapy of advanced hepatocellular carcinoma—The SHELTER Study. *Journal of Hepatology*.

Boumber, Y., Younes, A., & Garcia-Manero, G. (2011). Mocetinostat (MGCD0103): a review of an isotype-specific histone deacetylase inhibitor. *Expert opinion on investigational drugs 20*, 823–829.

Bowers, E. M., Yan, G., Mukherjee, C., Orry, A., Wang, L., Holbert, M. A., et al. (2010). Virtual ligand screening of the p300/CBP histone acetyltransferase: identification of a selective small molecule inhibitor. *Chemistry & Biology 17*, 471–482.

Brownell, J. E., & Allis, C. D. (1996). Special HATs for special occasions: Linking histone acetylation to chromatin assembly and gene activation. *Current Opinion in Genetics & Development 6*, 176–184.

Brueckner, B., Rius, M., Markelova, M. R., Fichtner, I., Hals, P. -A., Sandvold, M. L., & Lyko, F. (2010). Delivery of 5-azacytidine to human cancer cells by elaidic acid esterification increases therapeutic drug efficacy. *Molecular Cancer Therapeutics 9*, 1256–1264.

Busch, C., Burkard, M., Leischner, C., Lauer, U. M., Frank, J., & Venturelli, S. (2015). Epigenetic activities of flavonoids in the prevention and treatment of cancer. *Clinical Epigenetics 7*, 1.

Calin, G. A., Dumitru, C. D., Shimizu, M., Bichi, R., Zupo, S., Noch, E., ... Rai, K. (2002). Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proceedings of the National Academy of Sciences 99*, 15524–15529.

Camporeale, G., Shubert, E. E., Sarath, G., Cerny, R., & Zempleni, J. (2004). K8 and K12 are biotinylated in human histone H4. *European Journal of Biochemistry 271*, 2257–2263.

Cavalieri, F. (1996). Drugs that target gene expression: An overview. *Critical Reviews in Eukaryotic Gene Expression 6*.

Chen, J. -F., Mandel, E. M., Thomson, J. M., Wu, Q., Callis, T. E., Hammond, S. M., ... Wang, D. -Z. (2006). The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nature Genetics 38*, 228–233.

Chen, L., Wei, T., Si, X., Wang, Q., Li, Y., Leng, Y., ... Zhu, S. (2013). Lysine acetyltransferase GCN5 potentiates the growth of non-small cell lung cancer via promotion of E2F1, cyclin D1, and cyclin E1 expression. *Journal of Biological Chemistry 288*, 14510–14521.

Child, F., Ortiz-Romero, P., Alvarez, R., Bagot, M., Stadler, R., Weichenthal, M., ... Cowan, R. (2016). Phase II multicentre trial of oral quisinostat, a histone deacetylase inhibitor, in patients with previously treated stage IB–IVA mycosis fungoides/Sézary syndrome. *British Journal of Dermatology*.

Choi, W. J., Chung, H. -J., Chandra, G., Alexander, V., Zhao, L. X., Lee, H. W., ... Kim, J. -H. (2012). Fluorocyclopentenyl-cytosine with broad spectrum and potent antitumor activity†. *Journal of Medicinal Chemistry 55*, 4521–4525.

Cole, A. J., Clifton-Bligh, R., & Marsh, D. J. (2015). Histone H2B monoubiquitination: Roles to play in human malignancy. *Endocrine-Related Cancer 22*, T19–T33.

Constantinides, P. G., Jones, P. A., & Gevers, W. (1977). *Functional striated muscle cells from non-myoblast precursors following 5-azacytidine treatment.*

Daigle, S. R., Olhava, E. J., Therkelsen, C. A., Basavapathruni, A., Jin, L., Boriack-Sjodin, P. A., ... Scott, M. P. (2013). Potent inhibition of DOT1L as treatment of MLL-fusion leukemia. *Blood 122*, 1017–1025.

Daigle, S. R., Olhava, E. J., Therkelsen, C. A., Majer, C. R., Sneeringer, C. J., Song, J., ... Xiao, Y. (2011). Selective killing of mixed lineage leukemia cells by a potent small-molecule DOT1L inhibitor. *Cancer Cell 20*, 53–65.

Daskalos, A., Nikolaidis, G., Xinarianos, G., Savvari, P., Cassidy, A., Zakopoulou, R., ... Liloglou, T. (2009). Hypomethylation of retrotransposable elements correlates with genomic instability in non-small cell lung cancer. *International Journal of Cancer 124*, 81–87.

Datta, J., Ghoshal, K., Denny, W. A., Gamage, S. A., Brooke, D. G., Phiasivongsa, P., ... Jacob, S. T. (2009). A new class of quinoline-based DNA hypomethylating agents reactivates tumor suppressor genes by blocking DNA methyltransferase 1 activity and inducing its degradation. *Cancer Research 69*, 4277–4285.

Davalos, V., Moutinho, C., Villanueva, A., Boque, R., Silva, P., Carneiro, F., & Esteller, M. (2012). Dynamic epigenetic regulation of the microRNA-200 family mediates epithelial and mesenchymal transitions in human tumorigenesis. *Oncogene 31*, 2062–2074.

Deguchi, K., Ayton, P. M., Carapeti, M., Kutok, J. L., Snyder, C. S., Williams, I. R., ... Gilliland, D. G. (2003). MOZ-TIF2-induced acute myeloid leukemia requires the MOZ nucleosome binding motif and TIF2-mediated recruitment of CBP. *Cancer Cell 3*, 259–271.

Deng, Y., Deng, H., Bi, F., Liu, J., Bemis, L. T., Norris, D., ... Zhang, Q. (2011). MicroRNA-137 targets carboxyl-terminal binding protein 1 in melanoma cell lines. *International Journal of Biological Sciences 7*, 133–137.

Denis, H., Ndlovu, M. N., & Fuks, F. (2011). Regulation of mammalian DNA methyltransferases: A route to new mechanisms. *EMBO Reports 12*, 647–656.

Derissen, E. J., Beijnen, J. H., & Schellens, J. H. (2013). Concise drug review: Azacitidine and decitabine. *The Oncologist 18*, 619–624.

Dhalluin, C., Carlson, J. E., Zeng, L., He, C., Aggarwal, A. K., & Zhou, M. -M. (1999). Structure and ligand of a histone acetyltransferase bromodomain. *Nature 399*, 491–496.

Du, Q., Luu, P. -L., Stirzaker, C., & Clark, S. J. (2015). *Methyl-CpG-binding domain proteins: Readers of the epigenome.*

Dueñas-Gonzalez, A., Coronel, J., Cetina, L., González-Fierro, A., Chavez-Blanco, A., & Taja-Chayeb, L. (2014). Hydralazine–valproate: A repositioned drug combination for the epigenetic therapy of cancer. *Expert Opinion on Drug Metabolism & Toxicology 10*, 1433–1444.

Dupont, C., Armant, D. R., & Brenner, C. A. (2009). Epigenetics: definition, mechanisms and clinical perspective. *Seminars in reproductive medicine vol. 27*. (pp. 351–357). © Thieme Medical Publishers.

Ehrlich, M. (2009). DNA hypomethylation in cancer cells. *Epigenomics 1*, 239–259.

Ehrlich, M., & Lacey, M. (2013). DNA hypomethylation and hemimethylation in cancer. *Epigenetic alterations in oncogenesis* (pp. 31–56). Springer.

Ehrlich, M., Jackson, K., & Weemaes, C. (2006). Immunodeficiency, centromeric region instability, facial anomalies syndrome (ICF). *Orphanet Journal of Rare Diseases 1*, 1.

Esteller, M. (2000). Epigenetic lesions causing genetic lesions in human cancer: Promoter hypermethylation of DNA repair genes. *European Journal of Cancer 36*, 2294–2300.

Esteller, M. (2002). CpG island hypermethylation and tumor suppressor genes: A booming present, a brighter future. *Oncogene 21*, 5427–5440.

Esteller, M., Levine, R., Baylin, S. B., Ellenson, L. H., & Herman, J. G. (1998). MLH1 promoter hypermethylation is associated with the microsatellite instability phenotype in sporadic endometrial carcinomas. *Oncogene 17*, 2413–2417.

Evens, A. M., Balasubramanian, S., Vose, J. M., Harb, W., Gordon, L. I., Langdon, R., ... Yue, J. (2016). A phase I/II multicenter, open-label study of the oral histone deacetylase inhibitor abexinostat in relapsed/refractory lymphoma. *Clinical Cancer Research 22*, 1059–1066.

Fahy, J., Jeltsch, A., & Arimondo, P. B. (2012). DNA methyltransferase inhibitors in cancer: A chemical and therapeutic patent overview and selected clinical studies. *Expert Opinion on Therapeutic Patents*.

Fang, M., Chen, D., & Yang, C. S. (2007). Dietary polyphenols may affect DNA methylation. *The Journal of Nutrition 137*, 223S–228S.
Feinberg, A. P., & Vogelstein, B. (1983). Hypomethylation distinguishes genes of some human cancers from their normal counterparts. *Nature 301*, 89–92.
Feinberg, A. P., Ohlsson, R., & Henikoff, S. (2006). The epigenetic progenitor origin of human cancer. *Nature Reviews Genetics 7*, 21–33.
Ferguson, A. D., Larsen, N. A., Howard, T., Pollard, H., Green, I., Grande, C., ... Wu, J. (2011). Structural basis of substrate methylation and inhibition of SMYD2. *Structure 19*, 1262–1273.
Filippakopoulos, P., & Knapp, S. (2014). Targeting bromodomains: Epigenetic readers of lysine acetylation. *Nature Reviews Drug Discovery 13*, 337–356.
Finazzi, G., Vannucchi, A. M., Martinelli, V., Ruggeri, M., Nobile, F., Specchia, G., ... Musolino, C. (2013). A phase II study of Givinostat in combination with hydroxycarbamide in patients with polycythaemia vera unresponsive to hydroxycarbamide monotherapy. *British Journal of Haematology 161*, 688–694.
Fraga, M. F., Ballestar, E., Villar-Garea, A., Boix-Chornet, M., Espada, J., Schotta, G., ... Petrie, K. (2005). Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. *Nature Genetics 37*, 391–400.
Fritsch, L., Robin, P., Mathieu, J. R., Souidi, M., Hinaux, H., Rougeulle, C., ... Ait-Si-Ali, S. (2010). A subset of the histone H3 lysine 9 methyltransferases Suv39h1, G9a, GLP, and SETDB1 participate in a multimeric complex. *Molecular Cell 37*, 46–56.
Gajer, J., Furdas, S., Gründer, A., Gothwal, M., Heinicke, U., Keller, K., ... Fichtner, I. (2015). Histone acetyltransferase inhibitors block neuroblastoma cell growth in vivo. *Oncogenesis 4*, e137.
Galloway, T. J., Wirth, L. J., Colevas, A. D., Gilbert, J., Bauman, J. E., Saba, N. F., ... Atoyan, R. (2015). A phase I study of CUDC-101, a multitarget inhibitor of HDACs, EGFR, and HER2, in combination with chemoradiation in patients with head and neck squamous cell carcinoma. *Clinical Cancer Research 21*, 1566–1573.
Gao, X. -n., Lin, J., Ning, Q. -y., Gao, L., Yao, Y. -s., Zhou, J. -h., ... Yu, L. (2013). A histone acetyltransferase p300 inhibitor C646 induces cell cycle arrest and apoptosis selectively in AML1-ETO-positive AML cells. *PloS One 8*, e55481.
Garzon, R., Fabbri, M., Cimmino, A., Calin, G. A., & Croce, C. M. (2006). MicroRNA expression and function in cancer. *Trends in Molecular Medicine 12*, 580–587.
Germaniuk-Kurowska, A., Nag, A., Zhao, X., Dimri, M., Band, H., & Band, V. (2007). Ada3 requirement for HAT recruitment to estrogen receptors and estrogen-dependent breast cancer cell proliferation. *Cancer Research 67*, 11789–11797.
Ghizzoni, M., Wu, J., Gao, T., Haisma, H. J., Dekker, F. J., & Zheng, Y. G. (2012). 6-alkylsalicylates are selective Tip60 inhibitors and target the acetyl-CoA binding site. *European Journal of Medicinal Chemistry 47*, 337–344.
Girard, N., Bazille, C., Lhuissier, E., Benateau, H., Llombart-Bosch, A., Boumediene, K., & Bauge, C. (2014). 3-Deazaneplanocin A (DZNep), an inhibitor of the histone methyltransferase EZH2, induces apoptosis and reduces cell migration in chondrosarcoma cells. *PloS One 9*, e98176.
Graça, I., Sousa, E. J., Costa-Pinheiro, P., Vieira, F. Q., Torres-Ferreira, J., Martins, M. G., ... Jerónimo, C. (2014). Anti-neoplastic properties of hydralazine in prostate cancer. *Oncotarget 5*, 5950–5964.
Guan, Z., Zhang, J., Song, S., & Dai, D. (2013). Promoter methylation and expression of TIMP3 gene in gastric cancer. *Diagnostic Pathology 8*, 1.
Guil, S., & Esteller, M. (2009). DNA methylomes, histone codes and miRNAs: Tying it all together. *The International Journal of Biochemistry & Cell Biology 41*, 87–95.
Gupta, S., Weston, A., Bearrs, J., Thode, T., Neiss, A., Soldi, R., & Sharma, S. (2016). Reversible lysine-specific demethylase 1 antagonist HCI-2509 inhibits growth and decreases c-MYC in castration-and docetaxel-resistant prostate cancer cells. *Prostate Cancer and Prostatic Diseases*.
Haery, L., Lugo-Picó, J. G., Henry, R. A., Andrews, A. J., & Gilmore, T. D. (2014). Histone acetyltransferase-deficient p300 mutants in diffuse large B cell lymphoma have altered transcriptional regulatory activities and are required for optimal cell growth. *Molecular Cancer 13*, 1.
Herman, J. G., Merlo, A., Mao, L., Lapidus, R. G., Issa, J. -P. J., Davidson, N. E., ... Baylin, S. B. (1995). Inactivation of the CDKN2/p16/MTS1 gene is frequently associated with aberrant DNA methylation in all common human cancers. *Cancer Research 55*, 4525–4530.
Hoffmann, I., Roatsch, M., Schmitt, M. L., Carlino, L., Pippel, M., Sippl, W., & Jung, M. (2012). The role of histone demethylases in cancer therapy. *Molecular Oncology 6*, 683–703.
Holleran, J. L., Parise, R. A., Joseph, E., Eiseman, J. L., Covey, J. M., Glaze, E. R., ... Egorin, M. J. (2005). Plasma pharmacokinetics, oral bioavailability, and interspecies scaling of the DNA methyltransferase inhibitor, zebularine. *Clinical Cancer Research 11*, 3862–3868.
Hopkinson, R. J., Tumber, A., Yapp, C., Chowdhury, R., Aik, W., Che, K. H., ... Chan, M. C. (2013). 5-Carboxy-8-hydroxyquinoline is a broad spectrum 2-oxoglutarate oxygenase inhibitor which causes iron translocation. *Chemical Science 4*, 3110–3117.
Huang, J. -j., Yu, J., Li, J. -y., Liu, Y. -t., & Zhong, R. -q. (2012). Circulating microRNA expression is associated with genetic subtype and survival of multiple myeloma. *Medical Oncology 29*, 2402–2408.
Huen, M. S., Grant, R., Manke, I., Minn, K., Yu, X., Yaffe, M. B., & Chen, J. (2007). RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. *Cell 131*, 901–914.
Incoronato, M., Urso, L., Portela, A., Laukkanen, M. O., Soini, Y., Quintavalle, C., ... Condorelli, G. (2011). Epigenetic regulation of miR-212 expression in lung cancer. *PloS One 6*, e27722.
Issa, J. -P. J., Roboz, G., Rizzieri, D., Jabbour, E., Stock, W., O'Connell, C., ... Walsh, K. (2015). Safety and tolerability of guadecitabine (SGI-110) in patients with myelodysplastic syndrome and acute myeloid leukaemia: A multicentre, randomised, dose-escalation phase 1 study. *The Lancet Oncology 16*, 1099–1110.
Iyer, N. G., Özdag, H., & Caldas, C. (2004). p300/CBP and cancer. *Oncogene 23*, 4225–4231.
Jason, L. J., Moore, S. C., Lewis, J. D., Lindsey, G., & Ausió, J. (2002). Histone ubiquitination: A tagging tail unfolds? *Bioessays 24*, 166–174.
Jones, P. A., & Takai, D. (2001). The role of DNA methylation in mammalian epigenetics. *Science 293*, 1068–1070.
Judes, G., Rifaï, K., Ngollo, M., Daures, M., Bignon, Y. -J., Penault-Llorca, F., & Bernard-Gallon, D. (2015). A bivalent role of TIP60 histone acetyl transferase in human cancer. *Epigenomics 7*, 1351–1363.
Kang, H. -W., Crawford, M., Fabbri, M., Nuovo, G., Garofalo, M., Nana-Sinkam, S. P., & Friedman, A. (2013). A mathematical model for microRNA in lung cancer. *PloS One 8*, e53663.
Karsy, M., Arslan, E., & Moy, F. (2012). Current progress on understanding microRNAs in glioblastoma multiforme. *Genes & Cancer 3*, 3–15.
Kim, K. H., & Roberts, C. W. M. (2016). Targeting EZH2 in cancer. *Nature Medicine 22*, 128–134.
King, O. N., Li, X. S., Sakurai, M., Kawamura, A., Rose, N. R., Ng, S. S., Quinn, A. M., Rai, G., Mott, B. T., & Beswick, P. (2010). Quantitative high-throughput screening identifies 8-hydroxyquinolines as cell-active histone demethylase inhibitors. *PloS one 5*, e15535.
Klaus, C. R., Iwanowicz, D., Johnston, D., Campbell, C. A., Smith, J. J., Moyer, M. P., ... Pollock, R. M. (2014). DOT1L inhibitor EPZ-5676 displays synergistic antiproliferative activity in combination with standard of care drugs and hypomethylating agents in MLL-rearranged leukemia cells. *Journal of Pharmacology and Experimental Therapeutics 350*, 646–656.
Knutson, S. K., Kawano, S., Minoshima, Y., Warholic, N. M., Huang, K. -C., Xiao, Y., ... Kumar, N. (2014). Selective inhibition of EZH2 by EPZ-6438 leads to potent antitumor activity in EZH2-mutant non-Hodgkin lymphoma. *Molecular Cancer Therapeutics 13*, 842–854.
Knutson, S. K., Wigle, T. J., Warholic, N. M., Sneeringer, C. J., Allain, C. J., Klaus, C. R., ... Song, J. (2012). A selective inhibitor of EZH2 blocks H3K27 methylation and kills mutant lymphoma cells. *Nature Chemical Biology 8*, 890–896.
Kouzarides, T. (2007). Chromatin modifications and their function. *Cell 128*, 693–705.
Kubicek, S., O'Sullivan, R. J., August, E. M., Hickey, E. R., Zhang, Q., Teodoro, M. L., ... Homon, C. A. (2007). Reversal of H3K9me2 by a small-molecule inhibitor for the G9a histone methyltransferase. *Molecular Cell 25*, 473–481.
Kuck, D., Caulfield, T., Lyko, F., & Medina-Franco, J. L. (2010a). Nanaomycin A selectively inhibits DNMT3B and reactivates silenced tumor suppressor genes in human cancer cells. *Molecular Cancer Therapeutics 9*, 3015–3023.
Kuck, D., Singh, N., Lyko, F., & Medina-Franco, J. L. (2010b). Novel and selective DNA methyltransferase inhibitors: Docking-based virtual screening and experimental evaluation. *Bioorganic & Medicinal Chemistry 18*, 822–829.
Kunej, T., Godnic, I., Ferdin, J., Horvat, S., Dovc, P., & Calin, G. A. (2011). Epigenetic regulation of microRNAs in cancer: An integrated review of literature. *Mutation Research, Fundamental and Molecular Mechanisms of Mutagenesis 717*, 77–84.
Kusaczuk, M., Krętowski, R., Bartoszewicz, M., & Cechowska-Pasko, M. (2016). Phenylbutyrate—a pan-HDAC inhibitor—suppresses proliferation of glioblastoma LN-229 cell line. *Tumor Biology 37*, 931–942.
Kwon, H., Song, K., Han, C., Zhang, J., Ungerleider, N., Yao, L., & Wu, T. (2016). Epigenetic silencing of microRNA-34a in human cholangiocarcinoma cells via DNA methylation and EZH2: Impact on regulation of Notch pathway. *The FASEB Journal 30*, 56.53.
Lahtz, C., & Pfeifer, G. P. (2011). Epigenetic changes of DNA repair genes in cancer. *Journal of Molecular Cell Biology 3*, 51–58.
Lakshmikuttyamma, A., Scott, S., DeCoteau, J., & Geyer, C. (2010). Reexpression of epigenetically silenced AML tumor suppressor genes by SUV39H1 inhibition. *Oncogene 29*, 576–588.
Lee, B. H., Yegnasubramanian, S., Lin, X., & Nelson, W. G. (2005). Procainamide is a specific inhibitor of DNA methyltransferase 1. *Journal of Biological Chemistry 280*, 40749–40756.
Lehmann, U., Hasemeier, B., Christgen, M., Müller, M., Römermann, D., Länger, F., & Kreipe, H. (2008). Epigenetic inactivation of microRNA gene hsa-mir-9-1 in human breast cancer. *The Journal of Pathology 214*, 17–24.
Leick, M. B., Shoff, C. J., Wang, E. C., Congress, J. L., & Gallicano, G. I. (2012). Loss of imprinting of IGF2 and the epigenetic progenitor model of cancer. *American Journal of Stem Cells 1*, 59–74.
Li, W., Huang, H., Su, J., Ji, X., Zhang, X., Zhang, Z., & Wang, H. (2016). miR-124 Acts as a Tumor Suppressor in Glioblastoma via the Inhibition of Signal Transducer and Activator of Transcription 3. *Molecular Neurobiology*, 1–7.
Li, Q. -L., Kim, H. -R., Kim, W. -J., Choi, J. -K., Lee, Y. H., Kim, H. -M., ... Ito, Y. (2004). Transcriptional silencing of the RUNX3 gene by CpG hypermethylation is associated with lung cancer. *Biochemical and Biophysical Research Communications 314*, 223–228.
Li, Y., Xu, Z., Li, B., Zhang, Z., Luo, H., Wang, Y., ... Wu, X. (2014). Epigenetic silencing of miRNA-9 is correlated with promoter-proximal CpG island hypermethylation in gastric cancer in vitro and in vivo. *International Journal of Oncology 45*, 2576–2586.
Liang, G., Lin, J. C., Wei, V., Yoo, C., Cheng, J. C., Nguyen, C. T., ... Gonzales, F. A. (2004). Distinct localization of histone H3 acetylation and H3-K4 methylation to the transcription start sites in the human genome. *Proceedings of the National Academy of Sciences of the United States of America 101*, 7357–7362.
Lin, H., Zhang, Y., Wang, H., Xu, D., Meng, X., Shao, Y., ... Wang, S. (2012). Tissue inhibitor of metalloproteinases-3 transfer suppresses malignant behaviors of colorectal cancer cells. *Cancer Gene Therapy 19*, 845–851.
Liu, X., Chen, Z., Yu, J., Xia, J., & Zhou, X. (2009). MicroRNA profiling and head and neck cancer. *Comparative and Functional Genomics 2009*.
Liu, Y., El-Naggar, S., Darling, D. S., Higashi, Y., & Dean, D. C. (2008). Zeb1 links epithelial-mesenchymal transition and cellular senescence. *Development 135*, 579–588.
Lo, W. -S., Trievel, R. C., Rojas, J. R., Duggan, L., Hsu, J. -Y., Allis, C. D., ... Berger, S. L. (2000). Phosphorylation of serine 10 in histone H3 is functionally linked in vitro and in vivo to Gcn5-mediated acetylation at lysine 14. *Molecular Cell 5*, 917–926.

Lodygin, D., Tarasov, V., Epanchintsev, A., Berking, C., Knyazeva, T., Körner, H., ... Hermeking, H. (2008). Inactivation of miR-34a by aberrant CpG methylation in multiple types of cancer. *Cell Cycle 7*, 2591–2600.

Losson, R. (1997). The bromodomain revisited. *Trends in Biochemical Sciences 22*(5), 151–153 (Waterborg, JH (2000). Steady-state levels of histone acetylation in).

MacDonald, T. J., DeClerck, Y. A., & Laug, W. E. (1998). Urokinase induces receptor mediated brain tumor cell migration and invasion. *Journal of Neuro-Oncology 40*, 215–226.

Manal, M., Chandrasekar, M., Priya, J. G., & Nanjan, M. (2016). Inhibitors of histone deacetylase as antitumor agents: A critical review. *Bioorganic Chemistry 67*, 18–42.

Mann, B. S., Johnson, J. R., Cohen, M. H., Justice, R., & Pazdur, R. (2007). FDA approval summary: vorinostat for treatment of advanced primary cutaneous T-cell lymphoma. *The oncologist 12*, 1247–1252.

Martinez-Zamudio, R., & Ha, H. C. (2012). Histone ADP-ribosylation facilitates gene transcription by directly remodeling nucleosomes. *Molecular and Cellular Biology 32*, 2490–2502.

McAllister, T. E., England, K. S., Hopkinson, R. J., Brennan, P. E., Kawamura, A., & Schofield, C. J. (2016). Recent progress in histone demethylase inhibitors. *Journal of Medicinal Chemistry 59*, 1308–1329.

McBrian, M. A., Behbahan, I. S., Ferrari, R., Su, T., Huang, T. -W., Li, K., ... Seligson, D. B. (2013). Histone acetylation regulates intracellular pH. *Molecular Cell 49*, 310–321.

McLean, C., Karemaker, I., & van Leeuwen, F. (2014). The emerging roles of DOT1L in leukemia and normal development. *Leukemia 28*, 2131–2138.

Mohammed, T. A., Holen, K. D., Jaskula-Sztul, R., Mulkerin, D., Lubner, S. J., Schelman, W. R. , ... LoConte, N. K. (2011). A pilot phase II study of valproic acid for treatment of low-grade neuroendocrine carcinoma. *The Oncologist 16*, 835–843.

Moore, D. (2016). Panobinostat (Farydak): a novel option for the treatment of relapsed or relapsed and refractory multiple myeloma. *Pharmacy and Therapeutics 41*, 296.

Morita, S., Horii, T., Kimura, M., Ochiya, T., Tajima, S., & Hatada, I. (2013). miR-29 represses the activities of DNA methyltransferases and DNA demethylases. *International Journal of Molecular Sciences 14*, 14647–14658.

Morschhauser, F., Terriou, L., Coiffier, B., Bachy, E., Varga, A., Kloos, I., ... Ribrag, V. (2015). Phase 1 study of the oral histone deacetylase inhibitor abexinostat in patients with Hodgkin lymphoma, non-Hodgkin lymphoma, or chronic lymphocytic leukaemia. *Investigational New Drugs 33*, 423–431.

Mottamal, M., Zheng, S., Huang, T. L., & Wang, G. (2015). Histone deacetylase inhibitors in clinical studies as templates for new anticancer agents. *Molecules 20*, 3898–3941.

Mujtaba, S., Zeng, L., & Zhou, M. (2007). Structure and acetyl-lysine recognition of the bromodomain. *Oncogene 26*, 5521–5527.

Nass, D., Rosenwald, S., Meiri, E., Gilad, S., Tabibian-Keissar, H., Schlosberg, A., ... Kharenko, O. (2009). MiR-92b and miR-9/9* are specifically expressed in brain primary tumors and can be used to differentiate primary from metastatic brain tumors. *Brain Pathology 19*, 375–383.

Nathan, D., Sterner, D. E., & Berger, S. L. (2003). Histone modifications: Now summoning sumoylation. *Proceedings of the National Academy of Sciences 100*, 13118–13120.

Neuzillet, C., Tijeras-Raballand, A., Cohen, R., Cros, J., Faivre, S., Raymond, E., & de Gramont, A. (2015). Targeting the TGFβ pathway for cancer therapy. *Pharmacology & Therapeutics 147*, 22–31.

Nguyen, H., Allali-Hassani, A., Antonysamy, S., Chang, S., Chen, L. H., Curtis, C., ... Li, F. (2015). LLY-507, a cell-active, potent, and selective inhibitor of protein-lysine methyltransferase SMYD2. *Journal of Biological Chemistry 290*, 13641–13653.

Noonan, E., Place, R., Pookot, D., Basak, S., Whitson, J., Hirata, H., ... Dahiya, R. (2009). miR-449a targets HDAC-1 and induces growth arrest in prostate cancer. *Oncogene 28*, 1714–1724.

Nowak, S. J., & Corces, V. G. (2004). Phosphorylation of histone H3: A balancing act between chromosome condensation and transcriptional activation. *Trends in Genetics 20*, 214–220.

Ota, T., Suto, S., Katayama, H., Han, Z. -B., Suzuki, F., Maeda, M., ... Tatsuka, M. (2002). Increased mitotic phosphorylation of histone H3 attributable to AIM-1/Aurora-B overexpression contributes to chromosome number instability. *Cancer Research 62*, 5168–5177.

Pandita, T. K., Kumar, R., Horikoshi, N., Singh, M., Gupta, A., Misra, H. S., ... Hunt, C. R. (2013). Chromatin modifications and the DNA damage response to ionizing radiation. *Frontiers in Oncology 2*, 214.

Pappano, W. N., Guo, J., He, Y., Ferguson, D., Jagadeeswaran, S., Osterling, D. J., ... Sweis, R. F. (2015). The histone methyltransferase inhibitor A-366 uncovers a role for G9a/GLP in the epigenetics of leukemia. *PloS One 10*, e0131716.

Paul, S. M., Mytelka, D. S., Dunwiddie, C. T., Persinger, C. C., Munos, B. H., Lindborg, S. R., & Schacht, A. L. (2010). How to improve R&D productivity: The pharmaceutical industry's grand challenge. *Nature Reviews Drug Discovery 9*, 203–214.

Pei, J. -H., Luo, S. -Q., Zhong, Y., Chen, J. -H., Xiao, H. -W., & Hu, W. -X. (2011). The association between non-Hodgkin lymphoma and methylation of p73. *Tumor Biology 32*, 1133–1138.

Prusevich, P., Kalin, J. H., Ming, S. A., Basso, M., Givens, J., Li, X., ... Hsiao, P. -Y. (2014). A selective phenelzine analogue inhibitor of histone demethylase LSD1. *ACS Chemical Biology 9*, 1284–1293.

Qin, W., Wolf, P., Liu, N., Link, S., Smets, M., La Mastra, F., ... Fellinger, K. (2015). DNA methylation requires a DNMT1 ubiquitin interacting motif (UIM) and histone ubiquitination. *Cell Research*.

Quintás-Cardama, A., Kantarjian, H., Estrov, Z., Borthakur, G., Cortes, J., & Verstovsek, S. (2012). Therapy with the histone deacetylase inhibitor pracinostat for patients with myelofibrosis. *Leukemia Research 36*, 1124–1127.

Raje, N., Hari, P. N., Vogl, D. T., Jagannath, S., Orlowski, R. Z., Supko, J. G., ... Lonial, S. (2012). Rocilinostat (ACY-1215), a selective HDAC6 inhibitor, alone and in combination with bortezomib in multiple myeloma: Preliminary results from the first-in-humans phase I/II study. *Blood 120*, 4061.

Ramakrishnan, V. (1997). Histone structure and the organization of the nucleosome. *Annual Review of Biophysics and Biomolecular Structure 26*, 83–112.

Rauscher, G. H., Kresovich, J. K., Poulin, M., Yan, L., Macias, V., Mahmoud, A. M., ... Tonetti, D. (2015). Exploring DNA methylation changes in promoter, intragenic, and intergenic regions as early and late events in breast cancer formation. *BMC Cancer 15*, 1.

Ren, J., Singh, B. N., Huang, Q., Li, Z., Gao, Y., Mishra, P., ... Jiang, S. -W. (2011). DNA hypermethylation as a chemotherapy target. *Cellular Signalling 23*, 1082–1093.

Reynoird, N., Mazur, P. K., Stellfeld, T., Flores, N. M., Lofgren, S. M., Carlson, S. M., ... Arrowsmith, C. H. (2016). Coordination of stress signals by the lysine methyltransferase SMYD2 promotes pancreatic cancer. *Genes & Development 30*, 772–785.

Richards, D., Boehm, K., Waterhouse, D., Wagener, D., Krishnamurthi, S., Rosemurgy, A., ... Clark, M. (2006). Gemcitabine plus CI-994 offers no advantage over gemcitabine alone in the treatment of patients with advanced pancreatic cancer: Results of a phase II randomized, double-blind, placebo-controlled, multicenter study. *Annals of Oncology 17*, 1096–1102.

Rilova, E., Erdmann, A., Gros, C., Masson, V., Aussagues, Y., Poughon-Cassabois, V., ... Novosad, N. (2014). Design, synthesis and biological evaluation of 4-amino-N-(4-aminophenyl) benzamide analogues of quinoline-based SGI-1027 as inhibitors of DNA methylation. *ChemMedChem 9*, 590–601.

Robertson, K. D., & Jones, P. A. (2000). DNA methylation: Past, present and future directions. *Carcinogenesis 21*, 461–467.

Rodriguez, J., Frigola, J., Vendrell, E., Risques, R. -A., Fraga, M. F., Morales, C., ... Ribas, M. (2006). Chromosomal instability correlates with genome-wide DNA demethylation in human primary colorectal cancers. *Cancer Research 66*, 8462–9468.

Rose, N. R., Ng, S. S., Mecinovic, J., Liénard, B. t. M., Bello, S. H., Sun, Z., ... Schofield, C. J. (2008). Inhibitor Scaffolds for 2-Oxoglutarate-Dependent Histone Lysine Demethylases ⊥. *Journal of Medicinal Chemistry 51*, 7053–7056.

Ross, J. P., Rand, K. N., & Molloy, P. L. (2010). *Hypomethylation of repeated DNA sequences in cancer*.

Rossetto, D., Avvakumov, N., & Côté, J. (2012). Histone phosphorylation: A chromatin modification involved in diverse nuclear events. *Epigenetics 7*, 1098–1108.

Ruiz-Carrillo, A., Wangh, L., & Allfrey, V. (1975). Processing of newly synthesized histone molecules. *Science 190*, 117–128.

Ruiz-García, A. B., Sendra, R., Galiana, M., Pamblanco, M., Pérez-Ortín, J. E., & Tordera, V. (1998). HAT1 and HAT2 proteins are components of a yeast nuclear histone acetyltransferase enzyme specific for free histone H4. *Journal of Biological Chemistry 273*, 12599–12605.

Sadikovic, B., Al-Romaih, K., Squire, J., & Zielenska, M. (2008). Cause and consequences of genetic and epigenetic alterations in human cancer. *Current Genomics 9*, 394–408.

Saito, Y., Liang, G., Egger, G., Friedman, J. M., Chuang, J. C., Coetzee, G. A., & Jones, P. A. (2006). Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. *Cancer Cell 9*, 435–443.

Sarath, G., Kobza, K., Rueckert, B., Camporeale, G., Zempleni, J., & Haas, E. (2004). Biotinylation of human histone H3 and interactions with biotinidase. *FASEB JOURNAL Vol. 18*. (pp. A103). 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998 USA: Federation Amer Soc Exp Biol.

Sato, F., Tsuchiya, S., Meltzer, S. J., & Shimizu, K. (2011). MicroRNAs and epigenetics. *FEBS Journal 278*, 1598–1609.

Savickiene, J., Treigyte, G., Jazdauskaite, A., Borutinskaite, V. V., & Navakauskiene, R. (2012). DNA methyltransferase inhibitor RG108 and histone deacetylase inhibitors cooperate to enhance NB4 cell differentiation and E-cadherin re-expression by chromatin remodelling. *Cell Biology International 36*, 1067–1078.

Scourzic, L., Mouly, E., & Bernard, O. A. (2015). TET proteins and the control of cytosine demethylation in cancer. *Genome Medicine 7*, 1.

Sharma, S. K., Wu, Y., Steinbergs, N., Crowley, M. L., Hanson, A. S., Casero, R. A., Jr., & Woster, P. M. (2010). (Bis) urea and (bis) thiourea inhibitors of lysine-specific demethylase 1 as epigenetic modulators. *Journal of Medicinal Chemistry 53*, 5197–5212.

Smiraglia, D. J., Rush, L. J., Frühwald, M. C., Dai, Z., Held, W. A., Costello, J. F., ... Wright, F. A. (2001). Excessive CpG island hypermethylation in cancer cell lines versus primary human malignancies. *Human Molecular Genetics 10*, 1413–1419.

Song, S. -H., Han, S. -W., & Bang, Y. -J. (2011). Epigenetic-based therapies in cancer. *Drugs 71*, 2391–2403.

Song, Y., Li, J., Zhu, Y., Dai, Y., Zeng, T., Liu, L., ... Zeng, M. (2014). MicroRNA-9 promotes tumor metastasis via repressing E-cadherin in esophageal squamous cell carcinoma. *Oncotarget*.

Spivakov, M., & Fisher, A. G. (2007). Epigenetic signatures of stem-cell identity. *Nature Reviews Genetics 8*, 263–271.

Sternberg, C., Armstrong, A., Pili, R., Ng, S., Huddart, R., Agarwal, N., ... Vogelzang, N. (2016). Randomized, double-blind, placebo-controlled phase III study of tasquinimod in men with metastatic castration-resistant prostate cancer. *Journal of Clinical Oncology 34*, 2636–2643.

Stimson, L., Rowlands, M. G., Newbatt, Y. M., Smith, N. F., Raynaud, F. I., Rogers, P., ... Bannister, A. (2005). Isothiazolones as inhibitors of PCAF and p300 histone acetyltransferase activity. *Molecular Cancer Therapeutics 4*, 1521–1532.

Subramaniam, D., Thombre, R., Dhar, A., & Anant, S. (2014). DNA methyltransferases: A novel target for prevention and therapy. *Frontiers in Oncology 4*, 80.

Sun, J., Ding, W., Zhi, J., & Chen, W. (2015). MiR-200 suppresses metastases of colorectal cancer through ZEB1. *Tumor Biology*, 1–7.

Sun, Y., Jiang, X., Chen, S., & Price, B. D. (2006). Inhibition of histone acetyltransferase activity by anacardic acid sensitizes tumor cells to ionizing radiation. *FEBS Letters 580*, 4353–4356.

Sweis, R. F., Wang, Z., Algire, M., Arrowsmith, C. H., Brown, P. J., Chiang, G. G., ... Li, F. (2015). Discovery of A-893, a new cell-active benzoxazinone inhibitor of lysine methyltransferase SMYD2. *ACS Medicinal Chemistry Letters 6*, 695–700.

Szyf, M., Pakneshan, P., & Rabbani, S. A. (2004). DNA methylation and breast cancer. *Biochemical Pharmacology 68*, 1187–1197.

Takemoto, Y., Ito, A., Niwa, H., Okamura, M., Fujiwara, T., Hirano, T., ... Ogawa, K. (2016). Identification of Cyproheptadine as an Inhibitor of SET Domain Containing Lysine Methyltransferase 7/9 (Set7/9) That Regulates Estrogen-Dependent Transcription. *Journal of Medicinal Chemistry 59*, 3650–3660.

Tanaka, S., Arii, S., Yasen, M., Mogushi, K., Su, N., Zhao, C., ... Miki, Y. (2008). Aurora kinase B is a predictive factor for the aggressive recurrence of hepatocellular carcinoma after curative hepatectomy. *British Journal of Surgery 95*, 611–619.

Thinnes, C. C., England, K. S., Kawamura, A., Chowdhury, R., Schofield, C. J., & Hopkinson, R. J. (2014). Targeting histone lysine demethylases—progress, challenges, and the future. *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms 1839*, 1416–1432.

Thottassery, J. V., Sambandam, V., Allan, P. W., Maddry, J. A., Maxuitenko, Y. Y., Tiwari, K., ... Parker, W. B. (2014). Novel DNA methyltransferase-1 (DNMT1) depleting anticancer nucleosides, 4′-thio-2′-deoxycytidine and 5-aza-4′-thio-2′-deoxycytidine. *Cancer Chemotherapy and Pharmacology 74*, 291–302.

Timmermann, S., Lehrmann, H., Polesskaya, A., & Harel-Bellan, A. (2001). Histone acetylation and disease. *Cellular and Molecular Life Sciences: CMLS 58*, 728–736.

Tokarz, P., Kaarniranta, K., & Blasiak, J. (2016). Inhibition of DNA methyltransferase or histone deacetylase protects retinal pigment epithelial cells from DNA damage induced by oxidative stress by the stimulation of antioxidant enzymes. *European Journal of Pharmacology 776*, 167–175.

Umezawa, H., Takeuchi, T., Iinuma, H., Ito, M., Ishizuka, M., Kurakata, Y., ... Obayashi, A. (1975). A new antibiotic, calvatic acid. *The Journal of Antibiotics 28*, 87–90.

VanderMolen, K. M., McCulloch, W., Pearce, C. J., & Oberlies, N. H. (2011). Romidepsin (Istodax, NSC 630176, FR901228, FK228, depsipeptide): a natural product recently approved for cutaneous T-cell lymphoma. *The Journal of Antibiotics 64*, 525–531.

Varambally, S., Cao, Q., Mani, R. -S., Shankar, S., Wang, X., Ateeq, B., ... Ramnarayanan, K. (2008). Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. *Science 322*, 1695–1699.

Vedadi, M., Barsyte-Lovejoy, D., Liu, F., Rival-Gervier, S., Allali-Hassani, A., Labrie, V., ... Siarheyeva, A. (2011). A chemical probe selectively inhibits G9a and GLP methyltransferase activity in cells. *Nature Chemical Biology 7*, 566–574.

Venugopal, B., Baird, R., Kristeleit, R. S., Plummer, R., Cowan, R., Stewart, A., ... Mcclue, S. (2013). A phase I study of quisinostat (JNJ-26481585), an oral hydroxamate histone deacetylase inhibitor with evidence of target modulation and antitumor activity, in patients with advanced solid tumors. *Clinical Cancer Research 19*, 4262–4272.

Villar-Garea, A., Fraga, M. F., Espada, J., & Esteller, M. (2003). Procaine is a DNA-demethylating agent with growth-inhibitory effects in human cancer cells. *Cancer Research 63*, 4984–4989.

Volkel, P., Dupret, B., Le Bourhis, X., & Angrand, P. -O. (2015). Diverse involvement of EZH2 in cancer epigenetics. *American Journal of Translational Research 7*, 175–193.

Wagner, T., & Jung, M. (2012). New lysine methyltransferase drug targets in cancer. *Nature Biotechnology 30*, 622–623.

Walewski, J., Paszkiewicz-Kozik, E., Borsaru, G., Moicean, A., Warszewska, A., Strobel, K., ... Henning, S. W. (2010). Resminostat in relapsed or refractory Hodgkin lymphoma: Initial results of the SAPHIRE phase II trial with a Novel Oral Histone Deacetylase (HDAC) Inhibitor. *Proceedings of the 52nd ASH Annual Meeting and Exposition, Orlando, FL, USA* (pp. 4–7).

Walton, E. L., Francastel, C., & Velasco, G. (2011). Maintenance of DNA methylation: Dnmt3b joins the dance. *Epigenetics 6*, 1373–1377.

Wang, M., Liu, X., Guo, J., Weng, X., Jiang, G., Wang, Z., & He, L. (2015). Inhibition of LSD1 by Pargyline inhibited process of EMT and delayed progression of prostate cancer in vivo. *Biochemical and Biophysical Research Communications 467*, 310–315.

Willmann, D., Lim, S., Wetzel, S., Metzger, E., Jandausch, A., Wilk, W., ... Janzer, A. (2012). Impairment of prostate cancer cell growth by a selective and reversible lysine-specific demethylase 1 inhibitor. *International Journal of Cancer 131*, 2704–2709.

Witta, S. E., Jotte, R. M., Konduri, K., Neubauer, M. A., Spira, A. I., Ruxer, R. L., ... Hirsch, F. R. (2012). Randomized phase II trial of erlotinib with and without entinostat in patients with advanced non-small-cell lung cancer who progressed on prior chemotherapy. *Journal of Clinical Oncology 30*, 2248–2255.

Wong, K. Y., So, C. C., Loong, F., Chung, L. P., Lam, W. W. L., Liang, R., ... Chim, C. S. (2011). Epigenetic inactivation of the miR-124-1 in haematological malignancies. *PloS One 6*, e19027.

Xia, J., Guo, X., Yan, J., & Deng, K. (2014). The role of miR-148a in gastric cancer. *Journal of Cancer Research and Clinical Oncology 140*, 1451–1456.

Xie, Y., Zong, P., Wang, W., Liu, D., Li, B., Wang, Y., ... Cui, X. (2015). Hypermethylation of potential tumor suppressor miR-34b/c is correlated with late clinical stage in patients with soft tissue sarcomas. *Experimental and Molecular Pathology 98*, 446–454.

Xing, X. -B., Cai, W. -B., Luo, L., Liu, L. -S., Shi, H. -J., & Chen, M. -H. (2013). The prognostic value of p16 hypermethylation in cancer: A meta-analysis. *PloS One 8*, e66587.

Xue, J., Chen, Z., Gu, X., Zhang, Y., & Zhang, W. (2016). MicroRNA-148a inhibits migration of breast cancer cells by targeting MMP-13. *Tumor Biology 37*, 1581–1590.

Yang, Y., & Bedford, M. T. (2013). Protein arginine methyltransferases and cancer. *Nature Reviews. Cancer 13*, 37–50.

Yang, X., Lay, F., Han, H., & Jones, P. A. (2010). Targeting DNA methylation for epigenetic therapy. *Trends in Pharmacological Sciences 31*, 536–546.

Yang, M. Y., Lee, Y. B., Ahn, C. -H., Kaye, J., Fine, T., Kashi, R., ... Kim, D. J. (2014). A Novel Cytidine Analog, RX-3117, Shows Potent Efficacy in Xenograft Models, even in Tumors that Are Resistant to Gemcitabine. *Anticancer Research 34*, 6951–6959.

Yiannakopoulou, E. C. (2015). Targeting DNA methylation with green tea catechins. *Pharmacology 95*, 111–116.

Younes, A., Berdeja, J. G., Patel, M. R., Flinn, I., Gerecitano, J. F., Neelapu, S. S., ... Clancy, M. S. (2016). Safety, tolerability, and preliminary activity of CUDC-907, a first-in-class, oral, dual inhibitor of HDAC and PI3K, in patients with relapsed or refractory lymphoma or multiple myeloma: An open-label, dose-escalation, phase 1 trial. *The Lancet Oncology 17*, 622–631.

Yuan, K., Lian, Z., Sun, B., Clayton, M. M., Ng, I. O., & Feitelson, M. A. (2012). Role of miR-148a in hepatitis B associated hepatocellular carcinoma. *PloS One 7*, e35331.

Zeng, B., Li, Z., Chen, R., Guo, N., Zhou, J., Zhou, Q., ... Zheng, L. (2012). Epigenetic regulation of miR-124 by Hepatitis C Virus core protein promotes migration and invasion of intrahepatic cholangiocarcinoma cells by targeting SMYD3. *FEBS Letters 586*, 3271–3278.

Zhao, H., & Chen, T. (2013). Tet family of 5-methylcytosine dioxygenases in mammalian development. *Journal of Human Genetics 58*, 421–427.

Zhao, Y., Li, Y., Lou, G., Zhao, L., Xu, Z., Zhang, Y., & He, F. (2012). MiR-137 targets estrogen-related receptor alpha and impairs the proliferative and migratory capacity of breast cancer cells. *PloS One 7*, e39102.

Zheng, Y. C., Yu, B., Jiang, G. Z., Feng, X. J., He, P. X., Chu, X. Y., ... Liu, H. M. (2016). Irreversible LSD1 inhibitors: Application of tranylcypromine and its derivatives in cancer treatment. *Current Topics in Medicinal Chemistry 16*, 2179–2188.

Zhou, Z., Gao, J., Popovic, R., Wolniak, K., Parimi, V., Winter, J. N., ... Chen, Y. -H. (2015). Strong expression of EZH2 and accumulation of trimethylated H3K27 in diffuse large B-cell lymphoma independent of cell of origin and EZH2 codon 641 mutation. *Leukemia & Lymphoma 56*, 2895–2901.

Zhu, Q., Huang, Y., Marton, L. J., Woster, P. M., Davidson, N. E., & Casero, R. A., Jr. (2012). Polyamine analogs modulate gene expression by inhibiting lysine-specific demethylase 1 (LSD1) and altering chromatin structure in human breast cancer cells. *Amino Acids 42*, 887–898.

Zhu, X., Li, Y., Shen, H., Li, H., Long, L., Hui, L., & Xu, W. (2013). miR-137 inhibits the proliferation of lung cancer cells by targeting Cdc42 and Cdk6. *FEBS Letters 587*, 73–81.

Zhu, X., Shan, L., Wang, F., Wang, J., Shen, G., Liu, X., ... Yang, H. (2015). Hypermethylation of BRCA1 gene: Implication for prognostic biomarker and therapeutic target in sporadic primary triple-negative breast cancer. *Breast Cancer Research and Treatment 150*, 479–486.