SciMedCentral

Journal of Cancer Biology & Research

Mini Review

Epigenetics and Cancer Therapy

Srikanth Kaithoju*

University of Aberdeen, Department of Medicine and Therapeutics, UK

Abstract

Epigenetic approach in treating different diseases has gained importance as the inhibition of the enzymes involved in epigenetic modifications can reverse the disease condition. The major epigenetic markers include DNA methylation, histone acetylation and methylation that alter chromatin structure resulting in changes in cellular functions. The DNA methylation and histone modifications in cancer cells are thought to be important in tumorigenesis and the epigenome modifying enzymes are considered as epigenetic targets in cancer drug discovery. This review will discuss about different epigenetic dysregulations in tumors as well as epigenetic targets for drug discovery and development of novel cancer agents. This article also highlights the recent success in identifying new epigenetic targets and cancer epigenetic drug discovery.

***Corresponding author**

Mr. Srikanth Kaithoju, 388 Abbeydale road, Sheffield, UK, Tel: 447867490848; E-mail: kaitojusrikanth@gmail.com

Submitted: 01 December 2014

Accepted: 01 December 2014

Published: 03 December 2014

Keywords

- Epigenetics
- Epigenetic therapy
- Epigenetic cancer therapy
- Dna Methylation
- Histone modifications

ABBREVIATIONS

DNA: Deoxyribonucleic acid, RNA: Ribonucleic acid, ATP: Adenosine triphosphate, DNMTS: DNA methyl transferase, HDAC: Histone deacetylases, HMT: Histone methyl transferase, HAT: Histone acetyl transferases, HDM: Histone demetylases, SAM: S-adenosyl methionine, LINEs: Long interspersed nuclear elements, CDKI: Cyclin-dependent kinase inhibitor, MAGE: Melanoma – associated antigen, AML: Acute myeloid leukemia, TSG: umour Suppressor Genes, HCC: Hepatocellular carcinoma, PRC2: Polycomb repressive complex 2, EZH2: Enhancer of zeste homolog 2, DOT1L: DOT1-like histone methyltranferase, MLL: Mixed lineage leukaemia, MRTs: Malignant rhabdoid tumours, ATRTs: Atypical teratoid rhabdoid tumours, BRD: Bromodomain family, BCP: Bromodomain containing proteins, BET: odomaiBromn and Extra terminal, NMC: NUT midline carcinoma, NUT: Nuclear protein testis.

INTRODUCTION

Epigenetics is defined as "the study of mitotically and / or meiotically heritable changes in gene function that cannot be explained by change in DNA sequence". The expression of mammalian genes is regulated by different mechanisms such as change in DNA sequence, ATP dependent chromatin remodelling, DNA methylation, Histone modifications, and non-coding RNA molecules regulating transcription, post-transcriptional control and translational control [1]. In this article we focus on DNA methylation and Histone modifications which are associated with different types of cancers.

DNA methylation

DNA methylation plays a critical role in the control of embryonic development, transcription, X chromosome inactivation, and genomic imprinting [2]. In mammals, DNA methylation takes place at the 5th position carbon of the pyrimidine cytosine residues within CpG dinucleotide which is carried out by DNMTS (DNA methyl transferase) and a methyl donar SAM (S-adenosyl methionine). Methylation is mainly carried out by 3 types of DNMTS: DNMT1, DNMT3a and DNMT3b which require methyl donar SAM to add methyl group to cytosine residues [3]. In normal cells, the methylation of CpG dinucleotides maintains chromosomal stability and protects from abberent transcription of repetitive elements like LINEs (long interspersed nuclear elements) and Alu repeats which may result in insertional mutagenesis. In normal genome, 70 – 80% of all CpG dinucleotides are methylated and the un-methylated CpG islands are actively transcribed. Hyper-methylation of promoter CpG islands is found to silence the tumour suppressor genes and CpG sites outside the island are hypo-methylated in cancer cells [4]. Aberrant methylation is associated with cancer as it alters the normal gene regulations and these alterations are of three types: hypermethylation, hypomethylation and loss of imprinting. This kind of aberrant methylation occurs mainly in the promoter CpG island regions which are un-methylated in the normal condition. DNA hypermethylation has been associated with transcriptional repression of tumour suppressive genes which results in tumour genesis [5]. For example, p16^{INK4} gene acts as a cyclin-dependent kinase inhibitor (CDKI) during the cell cycle and it is silenced by hyper-methylation of CpG islands on the promoter of the gene. This gene has been found silenced in different types of cancers [6]. Tumour suppressor genes such as Rb in retinoblastoma, MLH1 in colon cancer, and BRCA1 undergo tumour specific silencing through hyper-methylation. DNA hypo-methylation increases genomic instability, reactivation of transposable elements, and loss of imprinting. DNA hypo-methylation may activate growth-promoting genes such as R-Ras and MAPSIN in gastric cancer, MAGE (melanoma – associated antigen) in melanoma, and S-100 in colon cancer. DNA hypo-methylation results in abberent activation of genes and non-coding regions that may contribute in tumour genesis [7].

Histone modifications

Histones are abundant proteins in eukaryotes which are considered a hub for gene regulation. The DNA is wrapped around histone octamer of two copies of each of the core histones (H3, H2B, H2A and H3). Post-transcriptional modifications of histones regulate the chromatin accessability and recruit specific binding proteins. Post-transcriptional modifications of histones such as methylation, phosphorylation and acetylation were found to be associated with tumour genesis [8]. Histone acetylation facilitates the transcriptional machinery by neutralizing the histone charges and weakens the histone – DNA interaction. Histone acetylation along with other modifications (methylation, phosphorylation and ubiquination) to form histone codes which dictate gene transcription and various biological outcomes [9]. Loss of acetylated H4K16 and H4K20me3 is commonly found in human cancers. Decrease in histone acetylation is involved in tumorogenesis, invasion and metastasis in gastro-intestinal tumours [10]. Histone methyl transferase (HMT) such as PRDM5 is down regulated in breast cancer, colorectal cancer, liver and ovarian cancer. Down regulation of SMYD4 a HMT is associated with medulloblastoma and breast cancer. HMTs such as NSD3, EHMT1, SMYD2 and PRDM12 are either deleted or amplified in different types of cancers [11]. Histone methyltransferases (HMT), histone acetyl transferases (HAT) and histone deacetylases (HDAC) are directly or as a part of oncofusion proteins have been implicated in pathology of leukaemia. For example, the HATs such as MOF, MOZ or p300 and HMT MLL are found in translocations in acute myeloid leukemia (AML) [12].

CURRENT EPIGENETIC APPROACHES

DNMT

DNA methyl transferases are enzymes that are responsible to maintain CpG methylation which regulate the expression of various genes. Hypermethylation of CpG is a common feature of carcinogenesis which leads to silencing of Tumour Suppressor Genes (TSG) such as $P14^{ARF}$, $P15^{INK4Bb}$, $P16^{INK4a}$, CDH1 or EXT1 [13]. Each type of cancer has a typical hyper-methylome such as $P15^{INK4b}$ in leukaemia and $P16^{INK4a}$, $P14^{ARF}$, RARβ2, RASSF1A, MGMT, TIMP3, in colon cancer and together with GSTPi in prostate cancer. Azacitidine and Decitabine are two DNMT inhibitors which are FDA approved for the treatment of Myelodysplasic syndrome [14] (Table 1).

HDAC

HDAC enzymes act as transcriptional repressors through histone deacetylation which causes DNA condensation. HDAC act through removal of acetyl groups and allowing the interaction between negatively charged DNA and positively charged histones which results in hetero-chromatin and silencing of genes. HDAC is responsible in modulating the function of proteins involved in regulatory process including proliferation and angiogenesis [15]. Over expression HDAC2 and HDAC3 in colon cancers and HDAC6 has been reported in cutaneous T-cell lymphoma [16]. Vorinostat is a HDAC inhibitor which has been approved by FDA for treating different haematological malignancies such as Hodgkin lymphoma, diffuse large B-cell lymphoma, and cutaneous T-cell lymphoma. Romidepsin has been approved by FDA for treating patients with cutaneous T-cell lymphoma [17] (Table 1).

EZH2

EZH2 is a catalytic subunit of polycomb repressive complex 2 (PRC2) which catalyses the mono-, di-and tri-methylation of H3K27. Hyper-trimethylation of H3K27 has been associated with different human cancers and elevated levels are often seen at

Table 1: Different Classes of epigenetic inhibitors in clinical development.

CLASS	Enzyme	Condition	Investigational stage
DNA methyltransferase Inhibitors			
Azacitidine	DNMT	Myelodysplasic Syndrome	FDA approved(14)
Decitabine	DNMT	Myelodysplasic Syndrome	FDA approved(14)
SGI-110	DNMT	Advanced Hepatocellular Carcinoma (HCC)	Phase II (ClinicalTrials.gov Identifier: NCT01752933)
Histone deacetylase Inhibitors			
Vorinostat	HDAC	Hodgkin lymphoma, Large B-cell	FDA approved(17)
Romidepsin	HDAC	lymphoma & Cutaneous T-cell lymphoma	FDA approved(17)
Resminostat	HDAC	Cutaneous T-cell lymphoma Relapsed or refractory Hodgkins lymphoma	Phase II (ClinicalTrials.gov Identifier: NCT01037478)
Histone methyltransferase Inhibitors			
E-7438	EZH2	B-cell lymphomas or Advanced Solid tumors	Phase I/II (ClinicalTrials.gov Identifier: NCT01897571)
EPZ-5676	DOT1L	Leukaemias involving translocation of MLL genes	Phase I (ClinicalTrials.gov Identifier: NCT02141828)
Bromo domain inhibitors			
GSK525762	BET	NUT midline Carcinoma	Phase I (ClinicalTrials.gov Identifier: NCT01587703)
OTX015	BET	Acute leukemia	Phase I (ClinicalTrials.gov Identifier: NCT01713582)
RVX208	BET	Stable Coronary artery disease	Phase II (ClinicalTrials.gov Identifier: NCT01058018)

tumour suppressive gene loci in variety of cancers. Progression of different cancers like prostate, breast, kidney and lung cancer have been associated with dysregulation of EZH2. The loss of demethylase activity at H3K27 also results in hyper-methylation in an equivalent manner to elevation of PRC2 methyl transferase which would cause transcriptional repression of tumour suppressor genes. EZH2 inhibition has been a new approach for the treatment of different cancers [18-21].

Recent studies have shown that EZH2 inhibition results in selective killing of lymphoma cells with EZH2 mutations which suggests that EZH2 acts as a driver of proliferation in the mutation bearing cells [22-23]. SWI/SNF complexes are considered as tumour suppressors, and specific inactivating mutations in SWI/SNF units are found in human cancers. Inactivated SMARCB1 subunit (SNF5, INI1 or BAF47) mutations are found in nearly all malignant rhabdoid tumours (MRTs), and atypical teratoid rhabdoid tumours (ATRTs) which don't have any effective therapy. SMARCB1 –deficient tumours show elevated expression of EZH2 and co-deletion of EZH2 suppressed tumorigenesis completely which suggests the antagonistic interaction between PRC2 and SWI/SNF [24]. E-7438 is an EZH2 inhibitor that is being studied in Phase1/2 clinical trials in subjects with B-cell lymphomas or advanced solid tumors (ClinicalTrials.gov Identifier: NCT01897571).

DOT1L

DOT1L is a histone methyl transferase that catalyzes the specific methylation of histone H3 at lysine 79 (H3K79) and this leads to transcriptional activation. In MLL (Mixed lineage leukemia) the recruitment of DOT1L leads to enhanced expression of different leukemogenic genes including HOXA9 and MEIS1. Chromosomal translocation at MLL gene can result in fusion between MLL and number of proteins result in translocation complexes that recruit DOT1L at aberrant loci which leads to activation of different leukemogenic genes. Recent studies have shown that DOT1L inhibition caused a selective killing of mixed lineage leukaemia (MLL) [25-26]. Deregulation of MLL accounts for 70% of ALL (acute lymphoblastic leukaemia) in infants and 5 to 10% of AML (acute myeloid leukaemia) in adults which shows the requirement of an effective therapy [27].

In-vitro studies have displayed the anti-proliferative effect of DOT1L inhibitor EPZ-5676 and its synergistic effect in combination with hypomethylating agents in MLL-rearranged leukaemia cells [28]. In-vivo study of EPZ-5676 in a rat xenograft model of MLL- rearranged leukaemia showed that EPZ-5676 a potent DOT1L inhibitor that causes tumour regression [29]. EPZ-5676 is currently being studied in phase I clinical trials in patients with leukemia's involving translocation of MLL gene (ClinicalTrials.gov Identifier: NCT02141828).

BET

Bromodomain family (BRDs) has been studied most intensively as it is recognized as a key component of variety of physiological process that involves abnormal expression that leads to tumour genesis. Bromodomain containing proteins (BCP) have been found to play a vital role in cell cycle, apoptosis, metastasis, and proliferation which proves that they are the most ideal targets for developing treatment. BET (Bromodomain and Extra terminal) subgroup of BRDs, this includes BRD2, BRD3, BRD4 and BRDT. BRD3 or BRD4 has been associated with NUT midline carcinoma (NMC) where chromosomal translocation results in in-frame fusion of BRD4 and nuclear protein testis (NUT). NMC has been an aggressive cancer with no effective treatment which makes it more vital to study BET inhibitors [30-31]. Over expression of BRD4 in NMC, c-MYC in multiple myeloma and acute myeloid leukaemia has been associated with tumour genesis. BET inhibitors in pre-clinical studies have shown therapeutic potential through down regulating BRD4 and MYC expression. But, exact mechanism of action of these inhibitors is still not fully understood [32-34]. (Table 1) shows the epigenetic drugs that are in different phases of clinical trials.

LIMITATIONS

The major limitation of epigenetic therapies is the lack of specificity. Drugs targeting DNMTs or DNA methylation may cause global demethylation which is considered cytotoxic [13]. The major limitation of HDAC inhibitors is their lack of specificity especially the lack of isoform selectivity and lack of known targets. Identifying genomic targets of HDAC complexes in relevant tissue and resolving the interaction of HDACs with other proteins is important for better specificity [35]. Epigenetic therapies targeting HDACs and HATs may cause unintended consequences as they are a part of macromolecular protein complexes. The inhibitors of HMTs and HDMs mainly target cofactors and cofactor binding sites which are involved in multiple processes can cause non-specific inhibition of these processes [36]. Studying these processes would be beneficial for better targeting of the drugs.

CONCLUSION

The epigenetic modifications are important in activating and silencing of different genes maintaining cellular functions. The dysregulation of these modifications are observed in different diseases and these modifications are reversible. Dysregulation of these modifications and modifying enzymes have been studied in detailed in different types of cancers. Epigenetic enzymes have been successful targets in discovery and development of novel cancer drugs. New epigenetic inhibitors such as EZH2, DOT1L, and BET inhibitors are currently in clinical trials and this shows the success of this approach. The limitation for the epigenetic inhibitors has been their specificity and better understanding of epigenetic mechanisms, histone codes and macromolecular protein complexes will help in targeting specific epigenetic modifications.

REFERENCES

1. Roloff TC, Nuber UA. Chromatin, epigenetics and stem cells. Eur J Cell Biol. 2005; 84: 123-135.
2. Hirst M, Marra MA. Epigenetics and human disease. Int J Biochem Cell Biol. 2009; 41: 136-146.
3. Vaiopoulos AG, Athanasoula KCh, Papavassiliou AG. Epigenetic modifications in colorectal cancer: molecular insights and therapeutic challenges. Biochim Biophys Acta. 2014. 1842; 971-980.
4. Kristensen LS, Nielsen HM, Hansen LL. Epigenetics and cancer treatment. Eur J Pharmacol. 2009. 625; 131-142.
5. Sandoval J, Esteller M. Cancer epigenomics: beyond genomics. Curr

Opin Genet Dev. 2012; 22: 50-55.

6. Kawasaki H, Abe H. Epigenetics in cancer and inflammation. Pers Med Universe. 2012; 1: 7–12.

7. Shikhar Sharma, Theresa K. Kelly,and Peter A. Jones. Epigenetics in cancer. Carcinogenesis. 2010; 31: 27-36.

8. Waldmann T, Schneider R. Targeting histone modifications--epigenetics in cancer. Curr Opin Cell Biol . Ltd; 2013 Apr [cited 2014 Sep 4];25(2):184–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23347561

9. Herceg Z. Epigenetic information in chromatin and cancer. Eur J Cancer. 2009; 45:442–444.

10. 10. Ropero S, Esteller M. The role of histone deacetylases (HDACs) in human cancer. Mol Oncol. 2007; 1: 19–25.

11. Albert M, Helin K. Histone methyltransferases in cancer. Semin Cell Dev Biol. 2010; 21:209–220.

12. Hassler MR, Egger G. Epigenomics of cancer - emerging new concepts. Biochimie. 2012; 94: 2219–2230.

13. Yang X, Lay F, Han H, Jones P a. Targeting DNA methylation for epigenetic therapy. Trends Pharmacol Sci. 2010; 31: 536–546.

14. Gros C, Fahy J, Halby L, Dufau I, Erdmann A, Gregoire J-M, et al. DNA methylation inhibitors in cancer: recent and future approaches. Biochimie. 2012; 94: 2280–2296.

15. Perego P, Zuco V, Gatti L, Zunino F. Sensitization of tumor cells by targeting histone deacetylases. Biochem Pharmacol. 2012; 83: 987–994.

16. Marks P a. Histone deacetylase inhibitors: a chemical genetics approach to understanding cellular functions. Biochim Biophys Acta. 2010; 1799: 717–725.

17. Mercurio C, Minucci S, Pelicci PG. Histone deacetylases and epigenetic therapies of hematological malignancies. Pharmacol Res. 2010; 62: 18–34.

18. Dhanak D, Jackson P. Development and classes of epigenetic drugs for cancer. Biochem Biophys Res Commun. 2014.

19. You JS, Jones P a. Cancer genetics and epigenetics: two sides of the same coin? Cancer Cell. 2012; 22: 9–20.

20. Copeland R a. Protein methyltransferase inhibitors as personalized cancer therapeutics. Drug Discov Today Ther Strateg. 2012; 9: 83–90.

21. Campbell RM, Tummino PJ. Cancer epigenetics drug discovery and development: the challenge of hitting the mark. J Clin Invest. 2014; 124: 64-69.

22. Copeland R a. Molecular pathways: protein methyltransferases in cancer. Clin Cancer Res . 2013; 19: 6344–6350.

23. Knutson SK, Kawano S, Minoshima Y, Warholic NM, Huang K-C, Xiao Y, et al. Selective inhibition of EZH2 by EPZ-6438 leads to potent antitumor activity in EZH2-mutant non-Hodgkin lymphoma. Mol Cancer Ther. 2014; 13: 842–54.

24. Knutson SK, Warholic NM, Wigle TJ, Klaus CR, Allain CJ, Raimondi A, Porter Scott M . Durable tumor regression in genetically altered malignant rhabdoid tumors by inhibition of methyltransferase EZH2. Proc Natl Acad Sci U S A. 2013; 110: 7922-7927.

25. Yu W, Smil D, Li F, Tempel W, Fedorov O, Nguyen KT, et al. Bromo-deaza-SAH: a potent and selective DOT1L inhibitor. Bioorg Med Chem. 2013; 21: 1787–1794.

26. Daigle SR, Olhava EJ, Therkelsen CA, Majer CR, Sneeringer CJ, Song J, et al. Selective killing of mixed lineage leukemia cells by a potent small-molecule DOT1L inhibitor. Cancer Cell. 2011; 20: 53-65.

27. Cao F, Townsend EC, Karatas H, Xu J, Li L, Lee S, et al. Targeting MLL1 H3K4 methyltransferase activity in mixed-lineage leukemia. Mol Cell. 2014; 53: 247–261.

28. Klaus CR, Iwanowicz D, Johnston D, Campbell C a, Smith JJ, Moyer MP, et al. DOT1L Inhibitor EPZ-5676 Displays Synergistic Antiproliferative Activity in Combination with Standard of Care Drugs and Hypomethylating Agents in MLL-Rearranged Leukemia Cells. J Pharmacol Exp Ther. 2014; 350: 646–656.

29. Daigle SR, Olhava EJ, Therkelsen CA, Basavapathruni A, Jin L, Boriack-Sjodin PA, Allain CJ . Potent inhibition of DOT1L as treatment of MLL-fusion leukemia. Blood. 2013; 122: 1017-1025.

30. Prinjha RK, Witherington J, Lee K. Place your BETs: the therapeutic potential of bromodomains. Trends Pharmacol Sci. 2012; 33: 146–153.

31. Chung C, Tough DF. Bromodomains: a new target class for small molecule drug discovery. Drug Discov Today Ther Strateg. 2012; 9: 111–20.

32. Mair B, Kubicek S, Nijman SMB. Exploiting epigenetic vulnerabilities for cancer therapeutics. Trends Pharmacol Sci. 2014; 35: 136–145.

33. Wyce A, Degenhardt Y, Bai Y, Le B, Korenchuk S, Crouthame MC, McHugh CF . Inhibition of BET bromodomain proteins as a therapeutic approach in prostate cancer. Oncotarget. 2013; 4: 2419-2429.

34. Ott CJ, Kopp N, Bird L, Paranal RM, Qi J, Bowman T, Rodig SJ. BET bromodomain inhibition targets both c-Myc and IL7R in high-risk acute lymphoblastic leukemia. Blood. 2012; 120: 2843-2852.

35. Delcuve GP, Khan DH, Davie JR . Roles of histone deacetylases in epigenetic regulation: emerging paradigms from studies with inhibitors. Clin Epigenetics. 2012; 4: 5.

36. Bojang P, Ramos KS. The promise and failures of epigenetic therapies for cancer treatment. Cancer Treat Rev. 2014; 40: 153–169.

Cite this article

Kaithoju S (2014) Epigenetics and Cancer Therapy. J Cancer Biol Res 2(3): 1052.