Invited Article

Epigenetics - A Paradigm shift in Cancer Management

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Kurinji Pandiyan completed her doctorate in Human Genetics and Molecular Biology from the Johns Hopkins School of Medicine, Baltimore, MD, USA. Her thesis research was conducted at Johns Hopkins University and at the University of Southern California, under the mentorship of Stephen Baylin, MD, and Peter Jones, PhD, DSc., distinguished professors in the field of cancer epigenetics. Kurinji's thesis work resulted in the development of a novel methodology to coordinately characterize nucleosome positioning and DNA methylation and is currently widely adopted by the field. Her graduate work has been published in several high-impact, peer-reviewed journals.

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Introduction

The importance of epigenetics was recognized decades ago by C.H. Waddington, when he coined the term and described it as "the causal interactions between genes and their products, which bring the phenotype into being" in 1942[1]. Although the definition of epigenetics has evolved over the decades that followed, the concept posited by Waddington as to the importance of epigenetics in controlling gene expression has withstood the test of time.

Currently, epigenetics is regarded as the study of mitotically and meiotically heritable changes in gene expression that are not caused by changes in the underlying DNA sequences[2]. A majority of these heritable modifications are established during embryogenesis and faithfully maintained through the divisions of somatic cells, allowing for the adoption of distinct cell identities despite identical genetic information. In our current understanding, epigenetic mechanisms of gene expression control include DNA methylation changes at CpG (cytosine followed by guanosine) sites, packaging of DNA into nucleosomes, octamers of histone proteins, and their positioning, modification of histone tails on the nucleosomes and the expression of small and large non-coding regulatory RNA species (Table 1)[3-6]. In concert, these define the epigenetic landscape of a cell, impact gene expression and hence cell state definition.

Epigenetic modification	Function
DNA methylation	Represses gene expression when present at gene promoters
Histone modifications	Can repress or activate gene expression depending on the mark
Histone variants	Some variants de-stabilize the nucleosome structure and activate gene expression
Nucleosome positioning	Represses gene expression when present at gene promoters
Regulatory RNAs	Generally repress of gene transcripts

Table 1: Components of the epigenetic machinery

All of these epigenetic mechanisms of control have been shown to go awry in cancer. Although it was originally believed that genetic changes are the primary causal events in tumorigenesis, it has now been established that some of the epigenetic aberrations seen in cancers can drive malignant potential[7,8]. These aberrations have been identified both at the individual gene level and on a genome-wide scale[9-11]. The prevalence of global epigenetic aberrations reinforces the concept that epigenetic disruption is truly a hallmark of cancer[12].

Since the identification of the first aberrantly hypermethylated gene promoters, numerous papers have observed these epigenetic changes in a multitude of genes that code for proteins with tumor suppressive function, such as cell cycle checkpoint proteins[13], DNA damage repair proteins[14] and adhesion proteins[15]. It is now widely accepted that promoter DNA hypermethylation results in turning off gene expression and locking genes in a repressed state. Global hypomethylation in tumors has also been implicated in destabilizing the genome and activating proto-oncogenes[16-18]. More recently, overexpression of the repressive polycomb group of proteins that administers the repressive trimethylation mark on the 27th lysine of histone H3 has been implicated in tumorigenesis[19]. Alterations in the expression state of other components of the epigenetic machinery, such as chromatin remodelers, histone methyltransferases, demethylases, and deacetylases have all been observed in tumors[8]. This indicates that the faithful inheritance of epigenetic processes is critical to the maintenance of the non-tumorigenic state.

There is excitement revolving around the identification of epigenetic changes in fuelling carcinogenesis since these changes are potentially reversible by pharmacological intervention. Even though epigenetic states are heritable, they are dynamic and do not affect the primary DNA sequence in most situations, which makes them excellent targets for drug development. The vast quantities of data generated by recent genome-wide studies have established a picture of the

cancer epigenome, supporting the development of epigenetic therapies, the focus of which is currently on reverting aberrant gene silencing events. The last several decades has seen the emergence of numerous DNA methyltransferase inhibitors (DNMTi), histone deacetylases inhibitors (HDACi) and inhibitors antagonizing histone modifying enzymes. These drugs have been tested in both preclinical and clinical studies with encouraging results, predominantly in hematological malignancies [12,20-22].

Epigenetic Aberrations in Cancer

For decades it was believed that cancer was a disease of genetic mutations and that mutations and translocations were exclusively the causal events behind tumorigenesis[7]. Several studies have established that epigenetic silencing of tumor suppressor genes can cause and contribute to tumorigenesis (Figure 1)[9,23]. Epigenetic gene silencing has been shown to serve as a "second hit", resulting in the loss of function of genes that have one allele inactivated by mutations (eg. CDH1). There are also some genes that are rarely mutated and only silenced by DNA methylation (eg. SFRP1)[8,9]. More recently, it was found that genetic and epigenetic aberrations in cancer are not independent events and are, in fact, two sides of the same coin[8]. This notion arose from the unexpected finding that numerous components of the epigenetic machinery, such as DNA/histone methyltransferase enzymes and chromatin remodelers, harbor genetic disruption in cancers[8]. Abnormal expression of these genes or the presence of misfolded isoforms of the proteins results in the disruption of the epigenome and could trigger epigenetic alterations that lead to carcinogenesis[18]. Hence, a study of the aberrant cancer epigenome (Figure 1) is critical in order to understand the pathogenesis of cancer and the best ways to target it therapeutically. The following section describes the aberrant DNA methylation changes that have been observed in cancers.

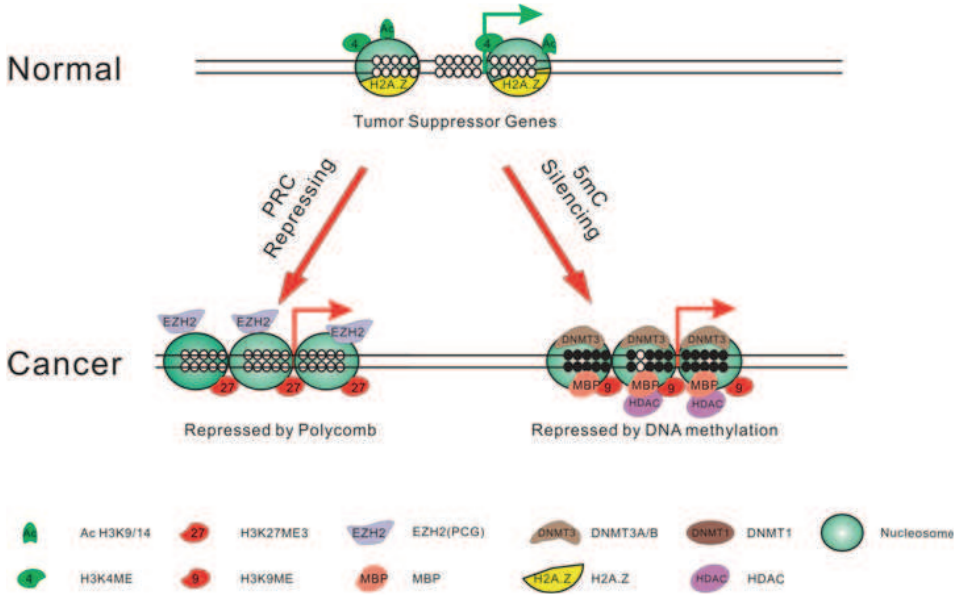

Figure 1: Aberrant epigenetic repression in cancer. *The above schematic depicts epigenetic repression in cancers that results from polycomb repression or from DNA methylation induced silencing. Red arrows on the genes represent transcription start sites (TSS); open circles represent unmethylatedCpG sites and filled circles represent methylated CpGdinucleotides. Tumor suppressor genes that are in an active configuration in normal cells are unmethylated, have nucleosomes with histone variants (e.g. H2A.Z) as well as histones with active marks (H3K4me3 and a nucleosome-depleted region (NDR) at the TSS, permissive for transcription. These tumor suppressor genes have been found as silenced by the polycomb H3K27me3 mark (applied by EZH2), independent of DNA methylation, or by DNA methylation (applied by DNMTs). Compounding of repression can occur when the methylated CpG sites recruit methyl-binding proteins (MBP). In turn, these recruit HDACs, that further repress chromatin by removing histone acetylation, as well as histone methyltransferases, that lock in methylation by applying trimethylation to H3K9. Nucleosome compaction is seen in both contexts of gene repression.*

The figure has been adapted from a recent paper12.

Aberrant DNA methylation in cancer

Disruption of DNA methylation patterns has been observed genome-wide in cancers. Key features of this disruption include global hypomethylation and promoter - specific hypermethylation[11,18,24]. Hypomethylation of repetitive elements such as those seen in regions of retrotransposon insertion can result in genomic instability and potential chromosomal translocations and breakage[16,24]. This is an understudied area and deserves more attention in the future. In contrast, focal hypermethylation at CpG island genes has garnered substantial interest. Studies have established that several tumor suppressor genes, encoding cell cycle regulators, DNA damage response genes, pro-differentiation factors as well as tumor suppressive microRNAs and other non-coding RNAs are found to be abnormally silenced by promoter DNA methylation[25-28]. A few examples of these well-studied tumor suppressor genes that have been found to harbor DNA hypermethylation in cancers include RB13, CDKN2A, MLH1, and BRCA1[11,29,30]. These genes are crucial to the maintenance of normal cellular physiology and their silencing could trigger tumorigenesis[31]. When differentiation inducing transcription factors are methylated, as seen frequently

for GATA4 and GATA5 in colon and gastric tumors, appropriate lineage specification is prevented[32]. A return of a stem cell signature in cancers has been frequently observed and could be a key driver of tumorigenesis[33].

Although it has been firmly established that DNA methylation patterns are disrupted in cancer, the field is a long way from understanding the mechanisms by which certain regions are targeted for hyper- or hypo-methylation. An initial hypothesis was that an aberrant increase in the levels of DNMTs could cause hypermethylation of genes[34]. This model does not explain the global hypomethylation that occurs in conjunction with the hypermethylation. Alternatively, recent studies have shown that certain genes have a predisposition for DNA hypermethylation due to the repressive H3K27me3 marks that they harbor in the embryonic state (along with the active H3K4me3 in the bivalent state) and in the adult stem cell state, a process that is potentially co-regulated by the polycomb group and DNMTs[35-38].The process by which a promoter that is repressed by the polycomb mark attains de novo DNA methylation has been termed "epigenetic switching", wherein the state of reduced epigenetic plasticity is locked into a state of irreversible silencing on application of DNA methylation[18,22,37]. It is unclear as to whether the DNA methylation machinery directly interacts with the polycomb machinery to induce silencing. There has been some evidence for this concept in recent studies that have uncovered interactions of EZH2[39] and CBX7 40 with DNMTs in cancer. It has also been demonstrated that the DNMTs themselves can achieve gene silencing without the application of DNA methylation, perhaps by acting as a scaffold for other repressive proteins[41].

Epigenetic therapy

The excitement surrounding the identification of epigenetic driver events in tumorigenesis is due to the relatively reversible nature of epigenetic aberrations. This is in contrast to genetic mutations that cannot be altered at the sequence level but, rather, can be compensated for by the use of inhibitors and protein substitutes to compensate for mutated proteins, depending on the consequences of the defect[42]. Since epigenetic changes are potentially erasable at the source, the research community has been triggered to develop a number of therapeutic measures to reverse the abnormalities. DNA methylation inhibitors have been the most widely studied and are currently the first line therapy for myelodysplastic syndrome (MDS). Drugs targeting other components of the epigenetic machinery are in development and have the potential to greatly impact cancer management[2].

DNA methyltransferase inhibitors

Without knowledge of the drugs' DNA demethylation potential, scientists at the Institute of Organic Chemistry and Biochemistry in Prague first synthesized 5-Azacytidine (AZA) and 5-aza-2□deoxycytidine (DAC), the rogue cytidine analogs that can replace cytidine in DNA. They were thought to be traditional chemotherapeutic agents and did in fact prove to induce cytotoxicity at high doses. The efficacy of these drugs, especially in liquid tumors such as acute myelogenous leukemia[43], was identified shortly thereafter. The demethylating effects of these drugs finally surfaced a few decades later when they were found to induce muscle differentiation in mouse embryonic cells[44,45]. It is now well established that the mechanism of action of these drugs involves incorporation into DNA following which DNA methyltransferases are covalently bound to these analogs and targeted for proteasomal degradation[46,47]. The loss of the DNMTs results in heritable global DNA demethylation and re-expression of genes that were aberrantly silenced by DNA methylation[12].

After years of clinical trials, the two DNMT inhibitors, AZA and DAC, have been approved by the Food and Drug Administration (FDA) for the treatment of myeloid malignancies[12]. Due to their high toxicity, these drugs were nearly abandoned years ago. Dose de-escalation has been key to the re-introduction of these drugs in the clinic and there remains continued interest in the ability of these drugs to induce sustained reprogramming of cancer cells[48]. The most effective demethylation induced by these drugs is at lower doses, since cell division and DNA synthesis is critical to their action[49]. AZA is also capable of directly incorporating into RNA, which has been shown to result in the disruption of cellular processes and, hence, inhibition of protein synthesis[50,51]. The schematic in Figure 2 depicts the action of DNA methylation in conjunction with other epigenetic therapies that have not been discussed in this review. Table 2 highlights select clinical studies that have shaped the understanding of these therapeutics.

Epigenetic Drug	Function	Disease targeted
Azacitidine	DNA methylation inhibitor	MDS/AML (2002-10)
Decitabine	DNA methylation inhibitor	MDS (2006-09), Refractory solid tumors (2009)
Azacitidine + Entinostat	DNA methylation inhibitor + histone deacetylase inhibitor	MDS/AML (2009)
Decitabine + Valproic acid	DNA methylation inhibitor + histone deacetylase inhibitor	Advanced leukemias (2007)

Table 2: Select clinical studies using epigenetic drugs

Numerous clinical trials are underway, attempting to expand the therapeutic reach of these drugs to solid tumors[2], wherein DNA hypermethylation is also observed. Despite encouraging results in myeloid malignancies, the results of treating solid tumors have been disappointing. A major cause of this is the inability of the drug to effectively reach the target tumor site, due to instability in aqueous solution. The drugs get readily hydrolyzed and become easily deaminated by cytidinedeaminase[52-55].

To circumvent this stability problem, other cytidine analogues with longer half-lives and improved aqueous stabilities have been developed. Zebularine is one such drug engineered to lack an amino group in the 4th position of the pyrimidine ring, which has rendered it less chemically labile and cytotoxic. The drug has proven effective in reactivating methylated tumor suppressor genes in breast cancers[56] and in vivo in MIN mice studies[57]. Another more stable version of AZA and DAC is their prodrug form, such as the analog S110. This is a dinucleotide with a 5-azacytosine ring that is more resistant to deamination. S110 is effective in re-expressing genes such as p16 in mouse models[58] and is the current focus of clinical trials in leukemias, under the umbrella of the Stand up to Cancer program.

A serious concern with the use of drugs that incorporate into DNA is potential mutagenic and DNA damage events that could result from the incorporation. Hence, another focus of the drug development community is to engineer non-nucleoside DNMT inhibitors, which are capable of targeting DNMTs for degradation without incorporating into DNA[18,59]. The few such inhibitors that have been developed to date, RG108 and MG98, have shown some promise in reactivating genes 12 by blocking the active site of DNMT1[60,61] but have limited potency in demethylation and, hence, have not been actively pursued for clinical applications[62].

Another concern in the use of these inhibitors is their lack of specificity in targeting the demethylation at tumor suppressor genes and the global hypomethylation that is induced as a result of usage. While it is possible that targeting of demethylation using zinc finger nucleases coupled with DNMT inhibitors is a future focus of drug development, the non-specificity of DNA methylation inhibition could, in fact, be a strength of the treatment since cancer is a multi-faceted disease with numerous epigenetic aberrations[2]. Finally, although demethylation is non-specific and genome-wide, the predilection for rebounding of methylation could be specific to some regions. Hence, regions with sustained demethylation coupled with chromatin opening may, in fact, be a lot less random than anticipated, and potentially be the drivers of therapeutic response post-therapy.

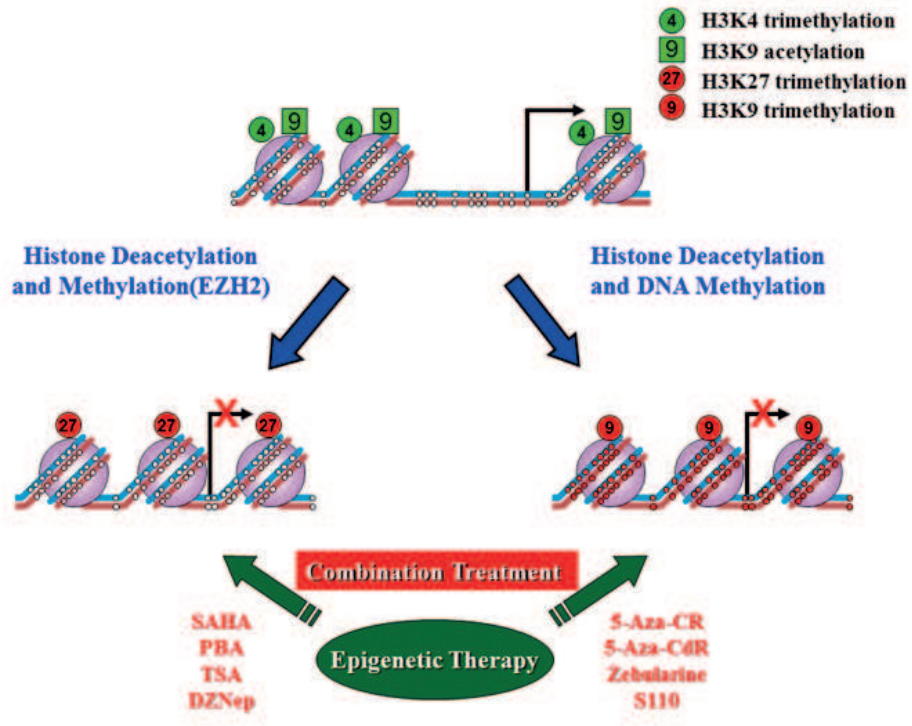

Figure 2: Epigenetic therapies can reverse aberrant epigenetic modifications in cancer. *Genes that are expressed in normal cells, such as tumor suppressor genes, have an open chromatin structure, consisting of an unmethylated promoter, active histone marks (marked in green) and a nucleosome-free region immediately upstream of the transcription start site. During tumorigenesis, genes can be silenced through one of the two silencing mechanisms: polycomb repressive complex (PRC) reprogramming and de novo DNA methylation. PRC mediated silencing can be reversed upon treatment with EZH2 inhibitors, such as DZnep. The de novo methylation mediated silencing can be reversed upon treatment with DNA methylation transferase inhibitors, such as 5-Aza-CdR, 5-Aza-CR, Zebularine, and S110. The therapeutic value of above reagents may be enhanced when combining with HDAC inhibitors, such as SAHA, PBA and TSA. Open and closed circles represent unmethylated and methylated CpG sites, respectively.*

Adapted from a recent paper 12

Conclusions

Epigenetic processes significantly impact and contribute to embryonic development, the maintenance of normal biological processes as well as in the initiation and progression of numerous diseases including cancer. Despite the outpouring of literature, we have but scratched the surface of this vast and important field of study. With rapidly advancing technologies, our ability to study epigenetic processes and changes has been better than ever before. This will allow us to dissect the intricacies of the epigenetic landscape and the tight control that is needed for maintenance of normal processes.

It is becoming increasingly important to obtain a holistic view of the field and to study different facets of the epigenome together, and not in isolation. In order to address this concern and to make the combined study of DNA methylation and chromatin accessibility readily available to clinical researchers and basic scientists alike we have developed a novel method, named AcceSsslble,to study DNA methylation and nucleosome positioning in a coordinate manner[63].

Epigenetic aberrations are prevalent yet potentially reversible with pharmacological interventions. Although, several epigenetic therapies have been developed and have shown some clinical promise, there are many unanswered questions that need to be addressed before epigenetic therapies in development can be translated from the bench to the bedside. As the paradigm has shifted in the usage of epigenetic modulators, from high cytotoxic doses to low reprogramming doses, the metrics for determining clinical trial efficacy and the regulatory barriers for drug approval also need to evolve. Future basic science, clinical and regulatory studies will, hopefully, address this burgeoning field of epigenetic therapy and allow for tailoring to meet individual patient needs, as an important component of personalized medicine.

References

1) Waddington, C. H. The epigenotype. 1942. Int J Epidemiol41, 10-13, doi:10.1093/ije/dyr184 (2012).

2) Azad, N., Zahnow, C. A., Rudin, C. M. & Baylin, S. B. The future of epigenetic therapy in solid tumours-lessons from the past. Nat Rev Clin Oncol, doi:10.1038/nrclinonc.2013.42 (2013).

3) Bernstein, B. E., Meissner, A. & Lander, E. S. The mammalian epigenome. Cell128, 669-681, doi:10.1016/j.cell.2007.01.033 (2007).

4) Kouzarides, T. Chromatin modifications and their function. Cell128, 693-705, doi:S0092-8674(07)00184-5 [pii] 10.1016/j.cell.2007.02.005 (2007).

5) Suzuki, M. M. & Bird, A. DNA methylation landscapes: provocative insights from epigenomics. Nat Rev Genet9, 465-476, doi:nrg2341 [pii] 10.1038/nrg2341 (2008).

6) Hwang, H. W. & Mendell, J. T. MicroRNAs in cell proliferation, cell death, and tumorigenesis. Br J Cancer94, 776-780, doi:10.1038/sj.bjc.6603023 (2006).

7) Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. Cell144, 646-674, doi:S0092-8674(11)00127-9 [pii] 10.1016/j.cell.2011.02.013 (2011).

8) You, J. S. & Jones, P. A. Cancer genetics and epigenetics: two sides of the same coin? Cancer Cell22, 9-20, doi:S1535-6108(12)00257-7 [pii] 10.1016/j.ccr.2012.06.008 (2012).

9) Baylin, S. B. & Jones, P. A. A decade of exploring the cancer epigenome - biological and translational implications. Nat Rev Cancer11, 726-734, doi:10.1038/nrc3130 (2011).

10) Egger, G., Liang, G., Aparicio, A. & Jones, P. A. Epigenetics in human disease and prospects for epigenetic therapy. Nature429, 457-463, doi:10.1038/nature02625 nature02625 [pii] (2004).

11) Jones, P. A. DNA methylation and cancer. Oncogene21, 5358-5360, doi:10.1038/sj.onc.1205597 (2002).

12) Yang, X., Lay, F., Han, H. & Jones, P. A. Targeting DNA methylation for epigenetic therapy. Trends Pharmacol Sci31, 536-546, doi:S0165-6147(10)00142-2 [pii] 10.1016/j.tips.2010.08.001 (2010).

13) Greger, V., Passarge, E., Hopping, W., Messmer, E. & Horsthemke, B. Epigenetic changes may contribute to the formation and spontaneous regression of retinoblastoma. Hum Genet83, 155-158 (1989).

14) Herman, J. G. et al. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. Proc Natl Acad Sci U S A95, 6870-6875 (1998).

15) Galm, O., Herman, J. G. & Baylin, S. B. The fundamental role of epigenetics in hematopoietic malignancies. Blood Rev20, 1-13, doi:10.1016/j.blre.2005.01.006 (2006).

16) Eden, A., Gaudet, F., Waghmare, A. & Jaenisch, R. Chromosomal instability and tumors promoted by DNA hypomethylation. Science300, 455, doi:10.1126/science.1083557 300/5618/455 [pii] (2003).

17) Howard, G., Eiges, R., Gaudet, F., Jaenisch, R. & Eden, A. Activation and transposition of endogenous retroviral elements in hypomethylation induced tumors in mice. Oncogene27, 404-408, doi:10.1038/sj.onc.1210631 (2008).

18) Sharma, S., Kelly, T. K. & Jones, P. A. Epigenetics in cancer. Carcinogenesis31, 27-36, doi:bgp220 [pii] 10.1093/carcin/bgp220 (2010).

19) Valk-Lingbeek, M. E., Bruggeman, S. W. & van Lohuizen, M. Stem cells and cancer; the polycomb connection. Cell118, 409-418, doi:10.1016/j.cell.2004.08.005 S0092867404007457 [pii] (2004).

20) Chung, Y. M. et al. FOXO3 signalling links ATM to the p53 apoptotic pathway following DNA damage. Nat Commun3, 1000, doi:ncomms2008 [pii] 10.1038/ncomms2008 (2012).

21) Juergens, R. A. et al. Combination epigenetic therapy has efficacy in patients with refractory advanced non-small cell lung cancer. Cancer Discov1, 598-607, doi:2159-8290.CD-11-0214 [pii] 10.1158/2159-8290.CD-11-0214 (2011).

22) Kelly, T. K., De Carvalho, D. D. & Jones, P. A. Epigenetic modifications as therapeutic targets. Nat Biotechnol28, 1069-1078, doi:nbt.1678 [pii] 10.1038/nbt.1678 (2010).

23) Sandoval, J. & Esteller, M. Cancer epigenomics: beyond genomics. Curr Opin Genet Dev22, 50-55, doi:S0959-437X(12)00019-6 [pii] 10.1016/j.gde.2012.02.008 (2012).

24) Sharma, S., Kelly, T. K. & Jones, P. A. Epigenetics in cancer. Carcinogenesis31, 27-36, doi:bgp220 [pii] 10.1093/carcin/bgp220 (2009).

25) Esteller, M. Cancer epigenomics: DNA methylomes and histone-modification maps. Nat Rev Genet8, 286-298, doi:nrg2005 [pii] 10.1038/nrg2005 (2007).

26) Lujambio, A. et al. A microRNA DNA methylation signature for human cancer metastasis. Proc Natl Acad Sci U S A105, 13556-13561, doi:0803055105 [pii] 10.1073/pnas.0803055105 (2008).

27) Toyota, M. et al. Epigenetic silencing of microRNA-34b/c and B-cell translocation gene 4 is associated with CpG island methylation in colorectal cancer. Cancer research68, 4123-4132, doi:68/11/4123 [pii] 10.1158/0008-5472. CAN-08-0325 (2008).

28) Saito, Y. et al. Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. Cancer Cell9, 435-443 (2006).

29) Jones, P. A. The DNA methylation paradox. Trends Genet15, 34-37, doi:S0168-9525(98) 01636-9 [pii] (1999).

30) Baylin, S. B. DNA methylation and gene silencing in cancer. Nat Clin Pract Oncol2 Suppl 1, S4-11, doi:ncponc0354 [pii] 10.1038/ncponc0354 (2005).

31) Sherr, C. J. Principles of tumor suppression. Cell116, 235-246, doi:S0092867403010754 [pii] (2004).

32) Akiyama, Y. et al. GATA-4 and GATA-5 transcription factor genes and potential downstream antitumor target genes are epigenetically silenced in colorectal and gastric cancer. Mol Cell Biol23, 8429-8439 (2003).

33) Ben-Porath, I. et al. An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. Nature genetics40, 499-507 (2008).

34) Kautiainen, T. L. & Jones, P. A. DNA methyltransferase levels in tumorigenic and nontumorigenic cells in culture. J Biol Chem261, 1594-1598 (1986).

35) Ohm, J. E. et al. A stem cell-like chromatin pattern may predispose tumor suppressor genes to DNA hypermethylation and heritable silencing. Nature genetics39, 237-242, doi:ng1972 [pii] 10.1038/ng1972 (2007).

36) Schlesinger, Y. et al. Polycomb-mediated methylation on Lys27 of histone H3 pre-marks genes for de novo methylation in cancer. Nat Genet39, 232-236, doi:10.1038/ng1950 (2007).

37) Gal-Yam, E. N. et al. Frequent switching of Polycomb repressive marks and DNA hypermethylation in the PC3 prostate cancer cell line. Proc Natl Acad Sci U S A105, 12979-12984, doi:0806437105 [pii] 10.1073/pnas. 0806437105 (2008).

38) Easwaran, H. et al. A DNA hypermethylation module for the stem/progenitor cell signature of cancer. Genome research22, 837-849, doi:10.1101/gr.131169.111 (2012).

39) Vire, E. et al. The Polycomb group protein EZH2 directly controls DNA methylation. Nature439, 871-874, doi:nature04431 [pii] 10.1038/ nature04431 (2006).

40) Mohammad, H. P. et al. Polycomb CBX7 promotes initiation of heritable repression of genes frequently silenced with cancer-specific DNA hypermethylation. Cancer research69, 6322-6330, doi:0008-5472.CAN-09-0065 [pii] 10.1158/0008-5472.CAN-09-0065 (2009).

41) Clements, E. G. et al. DNMT1 modulates gene expression without its catalytic activity partially through its interactions with histone-modifying enzymes. Nucleic Acids Res40, 4334-4346, doi:10.1093/nar/gks031 (2012).

42) Heyn, H. & Esteller, M. DNA methylation profiling in the clinic: applications and challenges. Nature reviews. Genetics13, 679-692, doi:10.1038/nrg3270 (2012).

43) Cihak, A. Biological effects of 5-azacytidine in eukaryotes. Oncology30, 405-422 (1974).

44) Constantinides, P. G., Jones, P. A. & Gevers, W. Functional striated muscle cells from

non-myoblast precursors following 5-azacytidine treatment. Nature267, 364-366 (1977).

45) Jones, P. A. & Taylor, S. M. Cellular differentiation, cytidine analogs and DNA methylation. Cell20, 85-93, doi:0092-8674 (80)90237-8 [pii] (1980).

46) Taylor, S. M. & Jones, P. A. Mechanism of action of eukaryotic DNA methyltransferase. Use of 5-azacytosine-containing DNA. J Mol Biol162, 679-692 (1982).

47) Christman, J. K., Mendelsohn, N., Herzog, D. & Schneiderman, N. Effect of 5-azacytidine on differentiation and DNA methylation in human promyelocytic leukemia cells (HL-60). Cancer Res43, 763-769 (1983).

48) Tsai, H. C. et al. Transient low doses of DNA-demethylating agents exert durable antitumor effects on hematological and epithelial tumor cells. Cancer Cell21, 430-446, doi:S1535-6108(12)00041-4 [pii] 10.1016/j.ccr.2011.12.029 (2012).

49) Qin, T., Jelinek, J., Si, J., Shu, J. & Issa, J. P. Mechanisms of resistance to 5-aza-2'-deoxycytidine in human cancer cell lines. Blood113, 659-667, doi:blood-2008-02-140038 [pii] 10.1182/blood-2008-02-140038 (2009).

50) Li, L. H., Olin, E. J., Buskirk, H. H. & Reineke, L. M. Cytotoxicity and mode of action of 5-azacytidine on L1210 leukemia. Cancer research30, 2760-2769 (1970).

51) Stresemann, C. & Lyko, F. Modes of action of the DNA methyltransferase inhibitors azacytidine and decitabine. International journal of cancer. Journal international du cancer123, 8-13, doi:10.1002/ijc.23607 (2008).

52) Issa, J. P. & Kantarjian, H. M. Targeting DNA methylation. Clin Cancer Res15, 3938-3946, doi:1078-0432.CCR-08-2783 [pii] 10.1158/1078-0432.CCR-08-2783 (2009).

53) Jackson-Grusby, L., Laird, P. W., Magge, S. N., Moeller, B. J. & Jaenisch, R. Mutagenicity of 5-aza-2'-deoxycytidine is mediated by the mammalian DNA methyltransferase. Proc Natl Acad Sci U S A94, 4681-4685 (1997).

54) Pohlmann, P. et al. Phase II trial of cisplatin plus decitabine, a new DNA hypomethylating agent, in patients with advanced squamous cell carcinoma of the cervix. Am J Clin Oncol25, 496-501 (2002).

55) Samlowski, W. E. et al. Evaluation of a 7-day continuous intravenous infusion of decitabine: inhibition of promoter-specific and global genomic DNA methylation. J Clin Oncol23, 3897-3905, doi:JCO.2005.06.118 [pii] 10.1200/JCO.2005.06.118 (2005).

56) Billam, M., Sobolewski, M. D. & Davidson, N. E. Effects of a novel DNA methyltransferase inhibitor zebularine on human breast cancer cells. Breast Cancer Res Treat120, 581-592, doi:10.1007/s10549-009-0420-3 (2010).

57) Yoo, C. B. et al. Long-term epigenetic therapy with oral zebularine has minimal side effects and prevents intestinal tumors in mice. Cancer Prev Res (Phila)1, 233-240, doi:1940-6207. CAPR-07-0008 [pii] 10.1158/1940-6207. CAPR-07-0008 (2008).

58) Chuang, J. C. et al. S110, a 5-Aza-2'-deoxycytidine-containing dinucleotide, is an effective DNA methylation inhibitor in vivo and can reduce tumor growth. Mol Cancer Ther9, 1443-1450, doi:1535-7163.MCT-09-1048 [pii] 10.1158/1535-7163.MCT-09-1048 (2010).

59) Cortez, C. C. & Jones, P. A. Chromatin, cancer and drug therapies. Mutat Res647, 44-51, doi:S0027-5107(08)00136-X [pii] 10.1016/j.mrfmmm.2008.07.006 (2008).

60) Amato, R. J. Inhibition of DNA methylation by antisense oligonucleotide MG98 as cancer therapy. Clin Genitourin Cancer5, 422-426, doi:S1558-7673(11)70119-7 [pii] 10.3816/CGC.2007.n.029 (2007).

61) Datta, J. et al. A new class of quinoline-based DNA hypomethylating agents reactivates tumor suppressor genes by blocking DNA methyltransferase 1 activity and inducing its degradation. Cancer research69, 4277-4285, doi:0008-5472.CAN-08-3669 [pii] 10.1158/0008-5472.CAN-08-3669 (2009).

62) Chuang, J. C. et al. Comparison of biological effects of non-nucleoside DNA methylation inhibitors versus 5-aza-2'-deoxycytidine. Mol Cancer Ther4, 1515-1520, doi:4/10/1515 [pii] 10.1158/1535-7163.MCT-05-0172 (2005).

63) Pandiyan, K. et al. Functional DNA demethylation is accompanied by chromatin accessibility. Nucleic Acids Res41, 3973-3985, doi:10.1093/nar/gkt077 (2013).