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■ RESEARCH ARTICLE

A Comprehensive Overview of Epigenetics

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ABSTRACT: This review article focuses on the field of epigenetics. Since its inception in the 20th century, there have been several major developments which induced the change in what the word "epigenetics" actually meant. In this review, the discoveries made so far are summarised and the potential applications of these new discoveries are discussed. Some things to keep in mind are that the field of epigenetics is relatively young compared to many other fields of research, so there are bound to be areas in which the understanding of the topic is lacking. Despite this, and partly due to recent advances in technology, several epigenetic therapeutics, some of which have already been approved for use, have been developed.

KEYWORDS: Cellular and Molecular Biology; Molecular Biology; Epigenetics; Epigenetic Mechanisms; Review; Bioinformatics.

■ Introduction

Epigenetics is a relatively new field. Up until the 1950s, the word was used to define the biological processes starting from the fertilization of the zygote to the mature organism.[1] Conrad Waddington who introduced the term 'epigenetics' in the early 1940s defined it as "the branch of biology which studies the casual interaction between genes and their products, which bring the phenotype into being." This definition is now out of date due to various new discoveries. The discovery of DNA as the main carrier of genetic information prompted the creation of a new definition, which separates epigenetics from genetics. Currently, it is defined as "the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence."[2] The redefining process has also helped to deepen our understanding of the subject.[1]

During the early 20th century, evidence for the presence of epigenetic modifications slowly accumulated from different experiments. One such experiment was carried out by Rollin D. Hotchkiss where the separation of purines, pyrimidines, and nucleosides lead to one of the earliest descriptions of covalent modifications on DNA nucleotides.[3] Further information accumulated when studies of the processes showed how phenotypic variations could be passed down by dividing cells.[1] Recently, there has been an explosion of knowledge associated with epigenetics, thanks to advancements in both technology and the rapid accumulation of knowledge in the past decade. Since the discovery of chemical agents (e.g., nucleoside analogue 5-azacytidine (5-aza-CR), that could reverse DNA methylation in late 1970's by Jones and Taylor[4]), therapeutics that involve epigenetic alterations have been studied extensively with many of them in clinical trials. The increase in epigenetic research has led scientists to see how cancer is caused not only by mutations in genes, but also by epigenetic alterations.[5] This has given humanity a new direction to work on and may, in the near future, give rise to many previously unseen possibilities of treatment in regard to cancer. In this review, the fields of epigenetics and the mechanisms of epigenetic modalities and how they are linked to health and disease are explored.

How is epigenetics different from traditional genetics?:

The name of epigenetics can be split up into two parts, "epi-" and "genetics". The "epi-" part of the word is a Greek prefix that denotes the meaning "above". Together, the word "epigenetics" denotes the meaning of "above" genetics, something added on top of genes. Epigenetics is different from traditional genetics in two respects. Firstly is that it involves changes that do not directly change the DNA base sequence. Secondly, these changes are reversible. Epigenetics does not involve any direct alterations of the DNA sequence itself. As a result, although all cells in the body will contain the same genetic information; genetic expressions mediated by epigenetic alterations give rise to the magnitude of diverse applications of somatic cells (Figure 1).

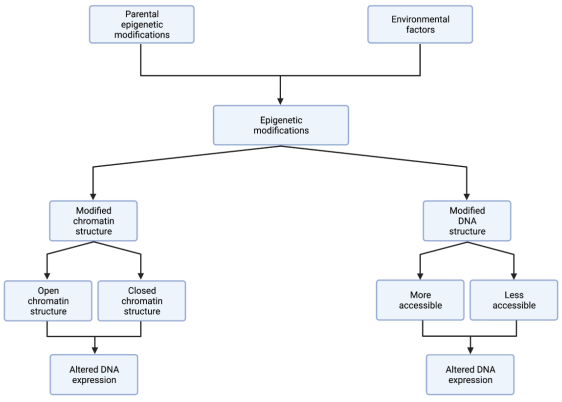

Figure 1: A flowchart showing how epigenetic alterations may cause changes to the expression of DNA sequences. Created with BioRender.com

Types of epigenetic modifications :

There are three major types of epigenetic alterations that can be carried out to either silence or express a gene. The most studied type to date is DNA methylation where methyl groups are added directly onto the DNA base sequence. Other

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modifications can be added to the histone proteins or to RNA molecules.

DNA methylation:

DNA methylation is the addition of a methyl group to the DNA. This includes the addition of methyl groups at the C5 and N4 positions on cytosine, N6 positions on adenine and the O^6 position on guanine. Currently, research around DNA methylation is focused specifically on the methylation of the C5 position on cytosine bases.[6,8]

This methylation forms 5-methylcytosine (5mC) and the process is regulated by the family of DNA methyltransferase enzymes.[6,9] DNA methylation is important for its role as a gene suppressor. In the human genome, DNA methylation can occur at any cytosine site available, but a study shows that more than 98% of DNA methylation occurs on the CpG dinucleotide.[9] Furthermore, ~ 70% of cytosine in CpG dinucleotides in the human genome are methylated.[10] CpG islands are a chain of nucleotides where there is a higher occurrence of CpGs. These islands are often 1000 base pairs long, about 70% of all gene promoters within the human body reside in CpG islands. As a result, the methylation of CpG islands results in stable silencing of promoter activity (Figure 2). The regulation of CpG islands carried out by methylation is particularly important for the establishment of genomic imprinting, in which the paternal or the maternal allele of a gene is expressed. Furthermore, methylation of CpG islands also regulates genetic expression during development and differentiation of cells.[6]

DNA methylation is also important for the silencing of transposable and viral elements, where it stops them from being able to jump between different parts of the human genome. Roughly 45% of the human genome is made up of transposable elements and ~ 8% is made up of viral elements. Transposable viral elements can cause mutations while moving around the genome, the mutation can be silent, but it could also be detrimental. The probability of having a malignant mutation is very high as over 50% of the entire human genome is made up of these "jumping genes". The mutations caused by the L1 transposon can be used to demonstrate this point. A study carried out by Kazazian et al in 1988 showed that the insertion of L1 into Factor VIII caused hemophilia;[11] in 1992, Miki *et al* showed that L1 transposons are present in a mutated form of the *APC* gene which causes colon cancer.[12,13]

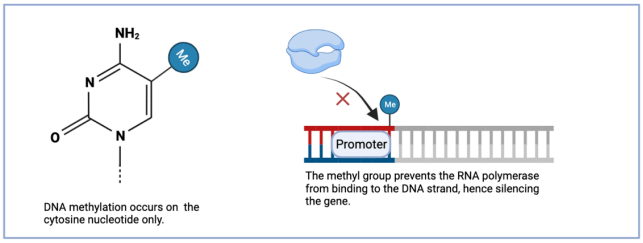

Figure 2: A schematic illustration shows the site of DNA methylation on the cytosine molecule and the general method of action. Created with BioRender.com.

Regulation of DNA methylation:

DNA methylation is regulated through DNA methyltransferases (DNMTs) that catalyse DNA methylation. These enzymes consist of a family of five DNMTs: DNMT1, 2, 3A, 3B, and 3L. Of note, the enzyme DNMT3L does not appear to have any inherent enzymic activities, instead, it acts by binding to DNMT3A2 to increase its catalytic speed by up to 20-fold.[10,14,15] The DNMT1 enzyme primarily works to maintain current existing methylations (i.e. transferring existing methylation patterns onto newly synthesised, hemimethylated DNA) and DNMT3A and DNMT3B preferentially methylates unmethylated DNA, acting as "*de novo*" enzymes. Instead of methylating DNA, DNMT2 specifically methylates "cytosine-38 in the anticodon loop of aspartic acid transfer RNA." according to Jin B. *et al*. Despite the separation of the functions of DNMTs, there is also clear evidence of functional overlapping between *de novo* DNA methylation and DNA methylation maintenance.[14]

Histone modification:

The study of histone modifications was pioneered by Vincent Alfred in the 1960's when he discovered that histones were modified at post-translational levels.[18] There has been an ever increasing amount of research dedicated to the study of histone modifications since the 2000's,[16] resulting in a large number of known different histone post-translational modifications (PTMs), including but not limited to histone acetylation, histone phosphorylation and histone methylation (Figure 3). Histone octamers are made up of eight core histone molecules. From each core histone molecules, protrude histone tails, which are either highly basic or acidic depending on their terminal group and play an important role in nucleosome interaction and gene expression. Most modifications occur on these histone tails and these modifications can affect the chromatin in a variety of different ways. It can change the shape of nucleosomes; furthermore, it may also affect transcription, replication, repair, and recombination.

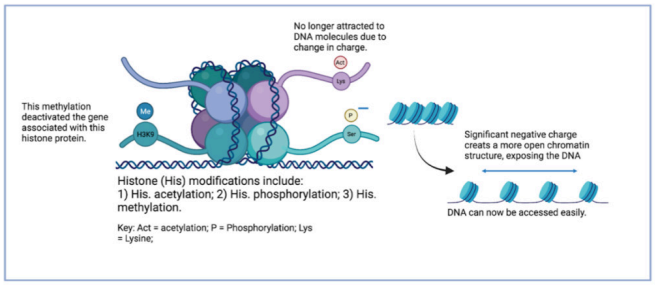

Figure 3: Cartoon depicts three main types of histone modifications: histone acetylation, histone phosphorylation, and histone methylation. Created with BioRender.com.

Histone acetylation:

Histone acetylation is the process through which an acetyl group is attached to a lysine residue on the histone tails. This modification controls the expression of genes. Generally, acetylation of histone N-terminal tails leads to the activation of the gene, on the contrary, de-acetylation leads to repression of gene expression. There are cases however, where this might not be true, a 2003 paper suggests that histone acetylation may recruit SUMO-conjugating enzymes which will on the contrary, repress the expression of genes.[17] The acetylation of histone tails is regulated by two different enzymes. Histone acetyltransferases (HATs) and histone deacetylases (HDATs). HATs attach acetyl groups to target ε-amino group of a target

lysine with the aid of acetyl CoA, this action neutralises the positive charge of lysine and as a result will cause disruptions to the electrostatic interaction between DNA and histones.[18]

There are two types of HATs, types A and B. Type-B is predominantly cytoplasmic; hence their main role is to acetylate newly synthesised histones. Type-B more specifically acetylates the K5 and K12 sites on the H4 histone and certain sites on H3 as well, such as at Lys 9, 14, 18, and 23. These acetylations are important in terms of the deposition of these histones in place, after which the sites are deacetylated. Type-A HATs are a more diverse group of HATs compared to Type-Bs. The type-A HATs can be further separated into three subcategories depending on the amino acid sequence-homology and conformational structure. The three types are GNAT, MYST and CBP/p300 families. These three enzymes can catalyse a wide range of acetylations on multiple locations on histone N-terminal tails. Other than the acetylation of histone tails, these enzymes also play a role in the acetylation of the globular histone cores themselves. The HATs are often associated with in large protein complexes, similar to other histone-modifying enzymes. The way in which the enzymes are associated with other proteins plays an important role in determining the action of the enzyme. For example, purified scGCN5 acetylases free histones whereas scGCN5 present in SAGA complexes can effectively acetylate nucleosomal histones.[18]

To deacetylate histones, HDAC enzymes are recruited. There are 4 different classes of HDAC, class I through to class IV. The deacetylation of the lysine amino acid restores its positive charge and hence stabilises the positive histone structure. This makes it harder for DNA transcription to happen hence causing an effect opposing DNA acetylation. HDAC enzymes generally have a relatively low substrate specificity; a single enzyme can deacetylate multiple sites within histones. Determination of which HDAC deacetylates which site is still under research as HDACs are often present with other HDACs in multiple distinct complexes. There is one recently discovered histone modification that is very similar to histone acetylation - lysine crotonylation. This modification has competing sites with histone acetylation. Lysine crotonylation has a similar effect on histones in that it also neutralise the positive charge of the ε-amino group of the lysine side chain, but there is increasing evidence that it is functionally distinctive from histone acetylation.[19,20]

Histone methylation:

Unlike acetylation and phosphorylation, histone methylation does not change the charge of the histone proteins. Histone methylation occurs on lysine and arginine. The lysine may be mono-, di- or tri-methylated, whereas arginines may be mono-, symmetrically, or asymmetrically di-methylated. Histone lysine-methyltransferase (HKMT) are the enzymes which carry out histone lysine methylation. They catalyse the reaction which transfers a methyl group from a S-adenosylmethionine (SAM) group to a lysine's ε-amino group. The HKMTs tend to be very specific enzymes, both in terms of the sites which they target but also the degree to which they methylate the histones.[18]

The degree to which histones are methylated is determined by a key residue within the active site. This residue limits the size to which the substrate can "grow" when methylated, hence preventing the methylation of the substrate past a certain point. There are several positions where once methylated, can lead to activation of the genes or deactivation of genes. H3K9, H3K27, and H4K20 are examples of inactivation markers once methylated. H3K4 and H3K36 are considered to be activation markers due to their position on the histone protein.[18,21] Protein arginine methyltransferases (PRMT) are a family of enzymes that methylates arginine in histone proteins. There are two classes of them, type-1 and type-2; they both transfer a methyl group from the universal donor SAM to the ω-guanidino group.[18]

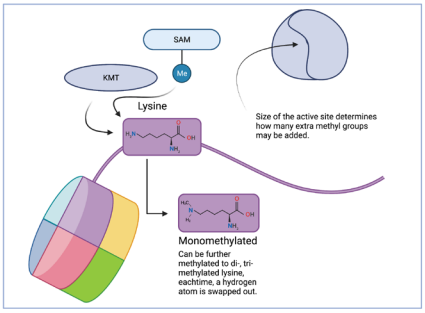

Figure 4: Schematic illustrating the mechanism through which a methyl group is added. The KMT transfers a methyl group to the lysine residue from the universal methyl group donor SAM. Created with BioRender.com.

Like DNA methylation, arginine methylation plays important roles in DNA damage repair pathways. For example, PRMTs methylates transcription factor Krüppel-like factor 4 (KLF4). KLF4 is important in its role in regulating diverse cellular processes such as cell growth, proliferation and differentiation. In DNA damage response pathways, KLF4 is important in its role as part of the DNA repair mechanism.[22]

Histone phosphorylation:

Histone phosphorylation takes place on multiple amino acids including serines, theronines, and tyrosines; like acetylation, histone phosphorylation mostly affects the N-terminal histone tails. This modification is carried out by kinases that add phosphate groups. Phosphatases remove phosphate groups. All identified kinases remove phosphate groups from ATP to add to the hydroxyl group of the target amino-acid side chain; this action adds significant negative charge to the histone hence causing disruptions within the structure. The significant negative charge results in a more open structure of the chromatin, this allows for actions such as DNA damage repair, DNA transcription, and also chromatin remodeling.[19]

Other histone modifications:

Deamination is another histone modification. This reaction involves the conversion of an arginine or a mono-methyl arginine (but not poly-methyl arginine) to citrulline. This reaction is catalysed by the peptidyl deiminase PADI4, which converts arginine to citrulline. The result of this reaction is the neutralisation of this amino acid location as citrulline is neutral whereas arginine is positively charged.[23]

Another modification is the addition of single β-N-acetylglucosamine (O-GlcNAc) sugar residues to their serine and

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development of cells, though there remains large gaps in our understanding of m⁶A and other modifications.[31]

Modifications on the tRNA molecule can be split into modifications outside the anticodon loop and those inside the loop, but both types carry out the role of quality checkpoints. Modifications outside the tRNA anticodon loop have a more specific role of maintaining tRNA stability or modulate tRNA folding. Modifications inside the tRNA anticodon loop have the role of tuning decoding capacity and to also control decoding accuracy.[31] An example of a tRNA modification is the deamination of the A_{37} position on the anticodon loop, this modification has been found to be important in terms of cell viability.[32]

The modification of RNA molecules is carried out by three groups of enzymes, "writers", "erasers" and "readers". The "writers" catalyse the reaction to modify specific sites; an example of this is the METTL3 enzyme which is an m⁶A-methyltransferase. This enzyme often complexes with the METTL14 enzyme which is another m⁶A-methyltransferase. The FTO enzyme was the first m6A "eraser" identified, this enzyme removes the methyl group through successive oxidation and produces two intermediates in the process. These two intermediates are stable for a few hours before they are converted back to adenosine. The "readers" recognises and binds to the modified groups and the binding of these proteins results in the triggering of one or multiple cellular pathways. One such example is the YTHDF2 protein that recognises m⁶A-modified RNA. The binding of YTHDF2 results in the m⁶A-containing mRNA to be localised to an mRNA decay site to allow for mRNA degradation.[31,33]

Further regulation of epigenetic alterations:

Epigenetic alterations are constantly changing; hence, it is important to understand the factors affecting these alterations. As previously discussed, epigenetic alterations are affected by metabolic actions, namely enzymes and response pathways. However, there are also other factors. Here two different types, non-coding RNA molecules and environmental factors, will be discussed.

Role of RNA molecules in epigenetic regulation:

There are several types of RNA molecules that can regulate epigenetic expressions, one of them being the long non-coding RNA. Long non-coding RNAs are not translated into proteins. This group can be roughly divided up into housekeeping non-coding RNAs and regulatory non-coding RNAs. The regulator non-coding RNAs can be further divided up into three groups according to their lengths shown in Figure 8.[31]

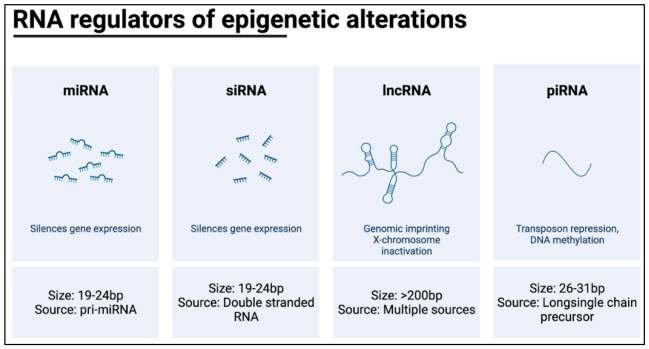

Figure 7: : Diagram shows the different types of non-coding RNAs and their regulatory roles in epigenetics. Adapted from Jian *et al.*[34] Created with BioRender.com.

siRNA can lead to transcriptional gene silencing through DNA methylation and histone modification. One example of this is the methylation of the CpG dinucleotide through its silencing of the *ezh2* gene. From Figure 8, we can see that the size of siRNA and miRNA are the same, so they are differentiated through two other differences.[25] The siRNA is thought to have originated from viral infections, hence exogenous, whereas the miRNA is mainly endogenous, stemming from the biological gene. The second difference is that miRNA consists mainly of incomplete double stranded hairpin-shaped double stranded RNA, on the other hand, siRNA is the product of a fully complementary, long double-stranded RNA.[34]

Currently, there is no evidence that miRNAs directly participate in epigenetic modifications, but it has been found that changes in miRNA expression can indirectly cause changes to epigenetics through their regulation of histone modifications. In addition to this, miRNAs can also induce overexpression of tumour suppressor genes, hence acting as tumour suppressors. It has been found that piRNAs have the ability to promote euchromatic histone modifications in Drosophila melanogaster. Furthermore, studies suggest a relationship between piRNA and chromatin regulation.[34]

Long non-coding RNAs (lncRNA) have two different roles. They can participate both in the process of X chromosome inactivation and in the process of genomic imprinting.[34] Non-coding RNAs have also been shown to bind directly to DNA molecules themselves, becoming the epigenetic modification. These RNAs have been shown to recruit proteins to bind to DNA promoters and operators, acting as targets for these proteins and enzymes to bind to. For example, RNA have been shown to bring with them the histone modifying complex Polycomb complex PRC2, which is involved in chromatin compaction.[35]

Epigenetic changes over a lifetime:

Some of the epigenetic changes are programmed into our DNA. These changes are intrinsic epigenetic modifications. Intrinsic factors are very important in its role of regulating epigenetic changes over time. The fact that epigenomes of monozygotic twins are more similar than dizygotic twins shows clearly the importance of intrinsic factors. Another example is the programmed epigenetic mechanisms during female puberty, recent studies showing that at least in part, the neuroendocrine pubertal components are mediated by epigenetic mechanisms.[25]

Many studies have shown that environmental pressures during development in both prenatal and childhood can affect epigenetic developmental programming. Exposure to toxic compounds and nutritional status are two very important environmental factors, these effects have given rise to the term "developmental origin of health and disease", which is an approach of medical research where the focus is on the effects of prenatal and perinatal on the development of diseases during adulthood. During embryonic development, the two main environmental influences are the lifestyle of the mother-hence exposure, and the phenotype of the mother, such as the size of

the uterus and placenta. This period is especially important for epigenetics as these changes can be inherited and amplified in future cells.[25]

In the adult epigenome, the influence of external factors highly depends on the type of tissue involved. For example, it is obvious that UV light will affect skin tissue more than the lungs. Furthermore, just like in embryos, if a stem cell is affected, the cells differentiated from it may cause serious problems. The most important thing is that if the germline was affected, reproductive disorders might result and these epigenetic alterations may be passed down.[25]

Chemical and environmental stressors:

Epigenetic marks can be affected by exposure to metals, air pollution, organic pollutants, benzene, and electromagnetic radiation. These chemicals have an amplified effect when an embryo is exposed to them. This leads to an increase in risk of developing diseases in the F1, F2, and F3 generations. Environmental exposure to a variety of metals such as mercury, lead, arsenic, and nickel can have severe impacts on the health of the individual. Many recent studies have shown that exposure to metal can have a major role in epigenetic changes leading to disease phenotypes. These metals can be found in most polluted industrial wastes, sometimes they can also be found naturally in high quantities, such as arsenic which is present in rocks, soil, water, insecticides, and many other places.[25]

As an example, analysis has found that DNA methylation changes after exposure to arsenic. Arsenic exposure is capable of inducing H3Kme3 and H3K9ac enrichment and H3K27me3 decrease due to its effect on histone-modifying enzymes. Overexposure to arsenic has also been found to correlate with an increased probability of developing cancer where there is a DNA hypermethylation of the *p16* and *RASSF1A* gene. Lead is another common heavy metal that it is used in building construction, batteries, fishing weights among other consumer goods. Exposure to lead has been found to induce changes in methylation in genes involved in neurogenetic pathways, which could lead to a neurodevelopmental deficit in children. In females, exposure to lead has been found to correlate with COL1A2 promoter hypomethylation.[25]

Furthermore, exposure to these metals has been found to affect not only DNA methylation and histone modifications, but also miRNA profiles. There is an inverse proportionality between the amount of metals exposed to and the amount of miRNA present. Air pollutants can cause illness and one factor contributing to this is its ability to alter the epigenome. High exposure to particulate matter (PM) in steel workers have been shown to induce hypomethylation in the nitric oxide synthase promoter region. Furthermore, black carbon, which is prevalent in vehicle exhaust, has also been found to cause aberrant global DNA methylation patterns. There is also evidence that air pollution is linked to the regulation of miRNAs; both the upregulation and downregulation of several miRNA caused by diesel exhaust particle (DEP) exposure have been associated with human airway diseases. Exposure to another well-known pollutant, asbestos, have also been found to cause DNA methylation of many tumour suppressor genes such as *APC, CCND2, CDKN2A, CDKN2B* and many more.[25]

There is also risk related to the exposure to endocrine disruptors, namely different plastics. It has been shown that the exposure to bisphenol A (BPA) causes global hypomethylation; moreover, it has been shown to disrupt the DNA methylation patterns of many imprinted genes. Electromagnetic radiation has been found to change the epigenetics of cells. Upon chronic ultraviolet (UV) light exposure, the H3 and H4 histone proteins have been found to have hypermethylation and hypomethylation in mouse models. Studies which show the inactivation of the cell apoptotic genes and also the inactivation of the mitotic control genes and aberrant methylation patterns in genes such as Cip1/p21 and p16 INK4a upon chronic exposure to UV light further support the notion that important epigenetic changes are being mediated by the chronic exposure to UV electromagnetic radiation.[24]

Diet and Lifestyle:

The intake of vitamins that cannot be synthesised by the human body could be a very important factor in the determination of epigenetics, especially for the intake of folate and other methyl donor groups. During the prenatal period, the intake of vitamin B6 is important as it is the cofactor in the synthesis of the methyl donor group 5-methyltetrahydrofolate, which is in turn the methyl donor for SAM, a methyl donor group responsible for maintaining methylation levels. Another important vitamin is the vitamin B9 which is used in the synthesis of tetrahydrofolate that will become 5-methyltetrahydrofolate. A lack of these essential vitamins might lead to hypomethylation and contribute to cancer, which has been demonstrated in animal models. This is true not only during the prenatal period, but also during adulthood. Folate deficiencies have been linked to epigenetic statuses in humans; methylation changes in colon cancer and hyperhomocysteinemia have been related to folate deficiencies. Another type of dietary habit to effect epigenetic alterations is caloric restriction (CR). It has been shown to reduce oxidative stress and a change in the regulation of metabolic pathways, it has also been shown to attenuate age related epigenetic changes.[25]

Lifestyle choices such as smoking and drinking have been shown to cause significant changes in epigenetics. Maternal tobacco smoke exposure (MTSE) has been shown to cause global DNA hypomethylation and an increase in DNA promoter-specific methylation in newborns. In adults, tobacco smoke has been shown to cause an increase in promoter gene-specific DNA methylation, such as the p16 gene. A more in-depth research project has revealed that tobacco smoke can cause changes in DNA methylation of many CpG sites. These sites are related to "the development and function of cellular, cardiovascular, detoxification, haematological, immune, tumorigenic, and reproductive systems." Tobacco smoke also affects histone modifications and the expression of miRNAs. Alcohol consumption inhibits methionine synthase, which means that long term consumption of alcohol may lead to a decreased level of SAM. Alcohol consumption will also affect the regulation of methylation patterns and miRNA regulation. In addition to these, ethanol may lead to immune system dysfunction as ethanol increases histone acetylation.[25]

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Vaping is a recent addition to the recreational drugs, because of this, there have not been conclusive research conducted on the effects of vaping on global epigenetic patterns. In a recent investigation using 45 human peripheral blood samples including exclusive vapers, smokers, and nonusers, significantly reduced LINE-1 repeats and global hydroxymethylation were observed in smokers and vapers compared to nonusers. Furthermore, out of the 24 miRNAs affected in smokers and 17 miRNAs affected in vapers, there were 9 overlapping miRNAs identified, suggesting the presence of similarities between the effects of smoking and vaping. There are further animal models showing how vaping alters epigenetics such as DNA methylation. However, the effects of these epigenetic alterations have not been fully explored.[35]

Stress in early life has been reported to induce aberrant DNA methylation for many genes such as the glucocorticoid receptor gene. Stress has also been shown to produce changes in histone modifications, such as increased levels of H3K4me3 and reduction of H3K9me3 levels in the dentate gyrus. The benefit of physical exercise on epigenetics is not very well known, but epigenetic changes have been observed in germ cells, skeletal muscle, and the brain following a period of exercise.[25]

Lastly, epigenetic changes can be induced by pharmaceutical drugs. The drug used to prevent pregnancy disorders, diethylstilbestrol (DES), is responsible for the increased risk in breast cancer, vaginal, and cervical adenocarcinoma. It is suggested that the reason behind this is the drug's ability to cause epigenetic change. In mice uteruses, synthetic estrogen has been observed to decrease DNMT expression and alterations in DNA methylation. DES has also been observed to upregulate and downregulate multiple miRNAs. There are many other drugs that have an effect on the epigenetics of cells within the human body, such as pyrazinamide, a classic antituberculosis drug.[25]

Epigenetic therapeutics

Epigenetic therapeutics in cancer:

There is currently a wide range of epigenetic drugs undergoing clinical trials and seven agents have been approved by the FDA for use. Among them are 5-azacytidine and 5-Aza-2-deoxycytidine which are both methylation inhibitors; FK-228, SAHA, PXD101, and LBH589 which are all HDAC inhibitors (HDACi); and tazemetostat which is an EZH2 inhibitor.[36]

Methylation inhibiting drugs:

Within methylation inhibiting drugs, there are three different categories or ways of inhibiting the addition of methyl groups by DNMTs. They are nucleoside-like compounds, antisense oligonucleotides and competitive inhibitors.

Nucleoside-like compounds:

Nucleoside-like compounds are analogues of nucleosides. An example of this is 5-azacytidine, a drug first synthesised in 1964 by Piskala and Sorm; its cytotoxic properties were tested and reviewed in the 1970's.[37]

The drug itself is a cytidine analogue, with a nitrogen atom in place of the carbon 5. This analogue is incorporated into the DNA sequence during DNA replication. DNMT1 recognizes the need to add a methyl group to this newly synthesised sequence, but when the DNMT1 binds to 5-azacytidine, it forms an irreversible DNMT1-aza linkage. This triggers the degradation of DNMT1 that in turn leads to the widespread reduction in methylation. This drug however is toxic, relatively unstable and cannot be taken orally.[38] But there is an analogue to 5-azacytidine, dihydro-5-azacytidine (DHAC) which is relatively less toxic. There is a non-FDA approved drug, zebularine, that is also a DNMT inhibitor. It has shown promising results on mouse models. This drug can be taken orally and is quite stable in both acidic and aquatic environments. However, its clinical application is hindered by its bioavailability.[36]

Antisense oligonucleotides:

Antisense oligonucleotides (ASO) are small pieces of DNA or RNA molecules that can bind to specific RNA molecules which block them from working.[39] An example is the drug MG98, which binds to the 3' untranslated DNMT1 gene.[38] This drug has entered phase I and II clinical trials. However, some of the most recent published articles seem to be from 2008 and 2009, suggesting a decrease in research-oriented use of this drug.[40,41]

Some of the application of ASO drugs include diabetes, hyperlipidaemias, cardiovascular diseases, neurodegenerative diseases, and cancer. A recent paper published in 2018 suggests that ASO drugs may be used for supressing the growth of advanced prostate cancer.[42]

Competitive Inhibitors:

Competitive inhibitors target DNMT1's active sites specifically, preventing the enzyme from being able to carry out methylation. An example of this type of drug is RG108. When used *in vitro* on human cancer cell lines, it has been demonstrated to cause significant demethylation and the re-expression of p16 tumour suppressor genes. As a result, slower cancer cell growth has been observed. The nature of these types of drugs - inhibitors, means that enzyme trapping is not involved (as is the case of nucleoside-like compounds) and hence there will be lower drug toxicity.[38]

Protein methyltransferase inhibitors:

Protein methyltransferase (PMT) inhibitors are another class of inhibitors that prevents methylation from being carried out by PMTs. An example of this type of drug is BIX-01294, the first selective inhibitor of this type and an inhibitor of the G9a histone methyltransferase. This drug is highly potent, but the trade-off is that it is toxic in high concentrations.[16,38]

Bromodomain Inhibitors:

Bromodomains are structural motifs or features associated with chromatin-modifying protein; currently, they are the only protein structure discovered to recognise acetylated lysine residues. As the recognition of this site is often a prerequisite for further chromatin modifications, epigenetic therapeutics may target these sites to control and mediate epigenetic alterations.[38]

Bromodomains are present on bromodomain and extra-terminal (BET) proteins. Many bromodomain inhibitors currently under clinical trials and undergoing laboratory testing specifically target this family of proteins.[36,38] An example of this is the first-generation synthetic inhibitor-JQ1, it potently inhibits BRD2, BRD3, BRD4, and the testis-specific protein BRDT in mammals. However, this drug has shown

various undesirable side effects such as heightened anxiety and impaired long-term memories in mouse models.[36]

HAT Inhibitors:

Most of the HAT inhibitors are not very selective and target multiple classes of protein. Although, there has been the discovery of one inhibitor, C646, that is both potent and selective which makes it a very good candidate for cancer treatments. This compound can reliably bind to the predicated druggable pocket of p300 and acts as a cofactor competitor. As discussed previously, HATs are often present in larger protein complexes, so further information regarding these complexes may be needed for further discoveries of HAT inhibitors.[38]

HDAC inhibitors:

HDAC inhibitors (HDACi) reduces histone deacetylation, and this leads to two results; either the direct induction of cell death or the sensitization of cancer cells to other drugs. An example of this type of drug is Vorinostat, however, this drug induces multiple side effects such as diarrhoea, fatigue, nausea, and anorexia due to the fact that it has multiple targets.[38]

There have been studies that show increased drug cell specificity and reduced toxic side effects when a combination of epigenetic drugs are being used.[36,38]An example of this interaction is when vincristine and Vorinostat are being used together. When administered together, these two drugs demonstrated synergic antitumour effects.[36] Furthermore, studies have also shown that HDACi can sensitise breast and ovarian cancer cell lines to a variety of cytotoxic drugs such as calpeptin, TRAIL, and telomere homologue oligonucleotides. There are other combinations where HDACi is paired with the previously discussed aza methylation inhibitors. An example of this is the interaction of aza and entinostat, studies show that this cocktail is tolerated by a majority of the patients who showed a decrease in methylation in at least two hypermethylated promoters.[38]

Uses in diseases other than cancer:

A reduction in memory loss and neuroprotective effects after administration of HDACi have been demonstrated in many studies. When HDACi have been administered to mice, there is evidence that memory deficits can be reversed. Although further investigation is needed, this technique has the potential to become the cure for neurodegenerative diseases such as Alzheimer disease. Investigations have also shown that the administration of valproic acid after cerebral ischaemia significantly reduces infarct size and neurological deficit scores. This gives rise to the possibility of HDACi being used to prevent permanent brain damage following strokes.[38]

Perspectives:

The field of epigenetics has seen an explosion of knowledge since the 2000s, evidenced by the increased amount of literature containing the keyword "epigenetics".[43] This led to deeper understanding of epigenetics and its concepts, allowing for a better understanding of many biological processes. Catalysed by the recent realization of the role abnormal epigenetic modifications play in cancer, there has been the development of many experimental drugs targeting currently untreatable diseases. These discoveries allow for the development of more specific therapeutics targeting these abnormal modifications, aiming to either return these abnormalities to the norm or further alter the epigenetic modifications to make cancer cells more susceptible to other drugs.[5] However, the current available epigenetic therapeutics do not offer astonishing results and, at the same time, cause side effects for the patient, so further research must be carried out to discover more effective compounds. With the development of these improved compounds, better patient welfare may be achieved. However, despite the current seemingly immense wealth of knowledge surrounding epigenetics, what is currently known is only the tip of the iceberg. Most of the epigenetic alterations currently present may be mapped out, but at the same time, for many of alterations, the role which they play within cells is still a mystery. Many of the mechanisms through which they act are also still hypotheses rather than having solid experimental proofs such as the mechanism of ADP-ribosylation. Furthermore, there seems to be a distinctive lack of research focused on the effects of our diet in recent years despite its inherent importance being part of our daily life. Hence, there is still a monumental amount of work to do in order to uncover the complete facade of epigenetics.

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References

1. Frasendeld, G. A Brief History of Epigenetics. *Cold Spring Harb Perspect Biol*. [Online] 2014, 6(1), a018200. National Centre for Biotechnology Information (NCBI) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3941222/ (accessed May 15, 2021)
2. Dupont, C.; Armant, D.R.; Brenner, C.A. Epigenetic: Definition, Mechanisms and Clinical Perspective. *Semin Reprod Med*. [Online] 2009, 27(5), 351-357. NCBI. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2791696/ (accessed May 15, 2021)
3. Hotchkiss, R. D. The Quantitative Separation of Purines, Pyrimidines, and Nucleosides by Paper Chromatography. J *Biol Chem*. [Online] 1948, 175(1), 315-32. Pub Med. https://pubmed.ncbi.nlm.nih.gov/18873306/ (accessed Sep 10, 2021)
4. Jones, P.A.; Taylor, S.M. Cellular Differentiation, Cytidine Analogues and DNA Methylation. *Cell*. [Online] 1980, 20(1), 85-93. https://pubmed.ncbi.nlm.nih.gov/6156004/ (accessed Sep 10, 2021)
5. Ahuja, N.; Sharma, A.R.; Baylin, S.B. Epigenetic Therapeutics: A New Weapon in the War Against Cancer. *Annu Rev Med*. [Online] 2016, 67, 73-89. NCBI. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4937439/ (accessed May 17, 2021)
6. Moore, L.D.; Le, T.; Fan, G.; DNA methylation and its Basic Function. *Neuropsychopharmacology*. [Online] 2013, 38(1), 23-38. Nature. https://www.nature.com/articles/npp2012112 (accessed May 17, 2021)
7. Low, D.A.; Weyand, N.J.; Mahan, M.J. Roles of DNA Adenine Methylation in Regulating Bacterial Gene Expression and Virulence. *Infect Immun*. [Online] 2001, 69(12), 7197-7204. NCBI. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC98802/ (accessed Sep 15, 2021)
8. Warren. J.J; Forsberg .L.J.; Beese, L.S. The Structural Basis for the Mutagenicity of O^6-methyl-guanine Lesions. *PNAS*. [Online] 2006, 103(52), 19701-19706. PNAS. https://www.pnas.org/content/103/52/19701 (accessed Sep 15, 2021)

DOI: 10.36838/v4i2.15

9. Jin, B.; Li, Y.; Robertson, K.D. DNA methylation: Superior or Subordinate in the Epigenetic Hierarchy? *Genes Cancer*. [Online] 2011, 2(6), 607-617. NCBI. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3174260/ (accessed May 17, 2021)
10. Mortusewicz, O.; Schermelleh, L.; Walter, J.; Cardoso, M.C.; Leonhardt, H. Recruitment of DNA Methyltransferase I to DNA Repair Sites. *Proc Acad Sci U S A*. [Online] 2005, 102(25) 8905-8909. NCBI. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1157029/(accessed May 21, 2021)
11. Kazazian, H.H.; Moran, J.V. The Impact of L1 Retrotransposons on the Human Genome. *Nat Genet*. [Online] 1998, 19, 19-24. Scitable. https://www.nature.com/scitable/content/The-impact-of-L1-retrotransposons-on-the-29852/# (accessed Sep 10, 2021)
12. Pray, L. Transposons: The Jumping Genes. *Nature Education*. [Online] 2008,1(1). Scitable. https://www.nature.com/scitable/topicpage/transposons-the-jumping-genes-518/(accessed May 17, 2021)
13. Miki, Y.; Nishisho, I.; Horii, A.; Miyoshi, Y.; Utsunomiya, J.; Kinzler, K.W.; Vogelstein, B.; Nakamura, Y. Disruption of the APC Gene by a Retrotransposal Insertion of L1 Sequence in a Colon Cancer. *Cancer Res*. [Online] 1992, 52(3), 643-5. Pub Med. https://pubmed.ncbi.nlm.nih.gov/1310068/ (accessed Sep 10, 2021)
14. Jin, B.; Robertson, K.D. DNA Methyltransferases, DNA Damage Repair, and Cancer. *Adv Exp Med Biol*. [Online] 2013, 754, 3-29. NCBI. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3707278/ (accessed May 19, 2021)
15. Kareta, M.S.; Botello, Z.M.; Ennis, J.J.; Chou, C.; Chédin, F.; \ Reconstitution and Mechanism of Simulation of de novo Methylation by Human DNMT3L. *J. Biol Chem*. [Online] 2006, 281(36), 25893-25902. Journal of Biological Chemistry (JBC) https://www.jbc.org/article/S0021-9258(19)35117-8/fulltext (accessed May 19, 2021)
16. Ciechomska, I.A.; Przanowski, P.; Jackl, J.; Wojtas, B.; Kaminska, B. BIX01294, an inhibitor of histone methyltransferase, induces autophagy-dependent differentiation of glioma stem-like cells. *Sci Rep.* [Online] 2016, 6, 38723. NCBI. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5146656/ (accessed Jul 30, 2021)
17) Nathan, D.; Sterner, D.E.; Berger, S.L. Histone Modifications: Now Summoning Sumoylation. *PNAS*. [Online] 2003, 100(23), 13118-13120. PNAS. https://www.pnas.org/content/100/23/13118.full (accessed Sep 15, 2021)
18. Bannister, A.; Kouzarides, T. Regulation of Chromatin by Histone Modifications. *Cell Res*. [Online] 2011, 21, 381-395. Nature. https://www.nature.com/articles/cr201122 (accessed May 24, 2021)
19. Ntorla, A.; Burgoyne, J.R. The Regulation and Function of Histone Crotonylation. *Frontiers in Cell and Developmental Biology.* [Online] 2021, 9, 729. https://www.frontiersin.org/articles/10.3389/fcell.2021.624914/full (accessed May 30, 2021)
20. Wan, J.; Liu, H.; Chu, J.; Zhang, H. Functions and Mechanisms of Lysine Crotonylation. *J Cell Mol Med*. [Online] 2019, 23(11), 7163-7169. Wiley Online Library. https://onlinelibrary.wiley.com/doi/full/10.1111/jcmm.14650 (accessed May 25, 2021)
21. Singh, D.; Nishi, K.; Khambata, K.; Balasinor, N.H. Introduction to Epigenetics: Basic Concepts and Advancements in the Field. *Epi and Rep Health*. [Online] 2020, 21, 25-44. SD. https://www.sciencedirect.com/science/article/pii/B9780128197530020018?via%3Dihub (accessed May 28, 2021)
22. Ghaleb, A.M.; Yang, V.W. Krüppel-like factor 4 (KLF4): What we currently know. *Gene*. [Online] 2017, 611, 27-37. NCBI. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5391259/
23. Lorton, B.M.; Schechter, D. Cellular consequences of Arginine Methylation. *Cell Mol Life Sci*. [Online] 2019, 76(15), 2933-2956. Springer Link. https://link.springer.com/article/10.1007/s00018-019-03140-2 (accessed May 29, 2021)
24. Zhang, S.; Roche, K.; Nasheuer, H.P.; Lowndes NF. Modification of histones by sugar β-N-acetylglucosamine (GlcNAc) occurs on multiple residues, including histone H3 serine 10, and is cell cycle-regulated. *J Biol Chem*. 2011, 286(43), 37483-37495. NCBI. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3199494/ (accessed Jul 28, 2021)
25. Toraño, E.G.; García, M.G.; Fernández-Morera, J.L.; Niño-García, P.; Fernández, A.F. The Impact of External Factors on the Epigenome: In Utero and Over Lifetime. *Biomed Res Int*. [Online] 2016, 2016, Article ID 2568635. Hindawi. https://www.hindawi.com/journals/bmri/2016/2568635/ (accessed Jun 3, 2021)
26. Zhou, P.; Wu, E.; Alam, H.B.; Li, Y. Histone cleavage as a mechanism for epigenetic regulation: current insights and perspectives. *Curr Mol Med*. [Online] 2014, 14(9), 1164-1172. NCBI. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4824947/ (accessed Jul 26, 2021)
27. Gajendra, K.A.; Swagatika, S.; Kumawat, M.; Kumawat, R.; Tomar, R.S. Modifying Chromatin by Histone Tail Clipping. *JMB*. [Online] 2018, 430(18), 3051-3067. SD. https://www.sciencedirect.com/science/article/abs/pii/S0022283618302948?via%3Dihub (accessed May 30, 2021)
28. Yun, M.; Wu, J.; Workman, J.L.; Li, B. Readers of Histone Modifications. *Cell Res*. [Online] 2011, 21(4), 564–578. NCBI. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3131977/ (accessed Jul 27, 2021)
29. MODOMICS: a Database of RNA Modification Pathways. http://genesilico.pl/modomics/ (accessed Sep 16, 2021).
30. Meng, S.; Zhou, H.; Feng, Z.; Xu, Z.; Tang, Y.; Wu, M. Epigenetics in Neurodevelopment: Emerging Role of Circular RNA. *Frontiers in Cellular Neuroscience*. [Online] 2019, 13, 327. NCBI. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6658887/ (accessed May 20, 2021)
31. Jiang, X.; Liu, B.; Nie, Z.; Duan, L.; Xiong, Q.; Jin, Z.; Yang, C.; Chen, Y. The Role of m6A Modification in the Biological Functions and Diseases. *Sig Transduct Target Ther*. [Online] 2021, 6, 74. Nature. https://www.nature.com/articles/s41392-020-00450-x#Sec13 (accessed Jul 28, 2021)
32. Swinehart, W.E.; Jackman, J.E. Diversity in mechanism and function of tRNA methyltransferases. *RNA Bio*. [Online] 2015, 12(4), 398-411. https://www.tandfonline.com/doi/pdf/10.1080/15476286.2015.1008358 (accessed Jul 31, 2021)
33. Boo, S.H.; Kim, Y.K. The Emerging Role of RNA Modifications in the Regulation of mRNA Stability. *Exp Mol Med*. [Online] 2020, 52, 400–408. NCBI. https://www.nature.com/articles/s12276-020-0407-z#citeas (accessed Aug 15, 2021)
34. Jian, W.W.; Kai, H.; Chao, Y.; Chun, S.K. Non-coding RNAs as Regulators in Epigenetics (Review). *Oncology Reports.* [Online] 2016, 37, 3-9. Spandidos Publications. https://www.spandidos-publications.com/or/37/1/3 (accessed May 31, 2021)
35. Margueron, R.; Reinberg, D. The Polycomb complex PRC2 and its mark in life. Nature. [Online] 2011, 469, 343–349. *Nature*. https://www.nature.com/articles/nature09784#citeas (accessed Jun 1, 2021)
36. Xie, Z.; Rahman, I.; Goniewicz, M.L; Li, D. Perspectives on Epigenetics Alterations Associated with Smoking and Vaping. Function. [Online] 2021, 2(3), zqab022. Oxford Academic https://academic.oup.com/function/article/2/3/zqab022/6247762?login=true (accessed Sep 15, 2021)
37. Nepali, K.; Liou, J.P. Recent Developments in Epigenetic Cancer Therapeutics: Clinical Advancement and Emerging Trends. J Biomed Sci. [Online] 2021, 28, 27. Journal of Biomedical Sciences. https://jbiomedsci.biomedcentral.com/articles/10.1186/s12929-0

21-00721-x#citeas (accessed Jul 29, 2021)
38.Christman, J. 5-Azacytidine and 5-aza-2'-deoxycytodine as Inhibitors of DNA Methylation: Mechanistic Studies and Their Implications for Cancer Therapy. Oncogene. [Online] 2002, 21, 5483-5495. *Oncogene*. https://www.nature.com articles/1205699#citeas (accessed Sep 11, 2021)
39.Heerboth, S.; Lapinska, K.; Snyder, N.; Leary, M.; Rollinson, S.; Sarkar, S. Use of Epigenetic Drugs in Disease: An Overview. *Genet Epigenet*. [Online] 2014, 6, 9-19. NCBI. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4251063/ (accessed Jul 30, 2021)
40.National Cancer Institute Dictionary. https://www.cancer.gov/publications/dictionaries/cancer-terms/def/antisense-oligonucleotide?redirect=true (accessed Jul 30, 2021)
41.ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/study/NCT00003890 (accessed Jul 29, 2021) MG98 in Treating Patients with Advanced Solid Tumors.
42.Winquist, E.; Knox, J.; Ayoub, J.; Wood, L.; Wainman, N.; Reid, G.K.; Pearce, L.; Shah, A.; Eisenhauer, E. Phase II Trial of DNA Methyltransferase 1 Inhibition with the Antisense Oligonucleotide MG98 in Patients with Metastatic Renal Carcinoma: a National Cancer Institute of Canada Clinical Trials Group Investigational New Drug Study. *Invest New Drugs*. [Online] 2006, 24(2), 159-67. PubMed. https://pubmed.ncbi.nlm.nih.gov/16502349/ (accessed Sep 16, 2021)
43.Xiao, L.; Tien, J.C.; Vo, J.; Tan, M.; Parolia, A.; Zhang, Y.; Wang, L.; Qiao, Y.; Shukla, S.; Wang, X.; Zheng, H.; Su, F.; Jing, X.; Luo, E.; Delekta, A.; Juckette, K.M.; Alice, X.; Cao, X.; Alva, A.S.; Kim, Y.; MacLeod, A.; Chinnaiyan, A. M. Epigenetic Reprogramming with Antisense Oligonucleotides Enhances the Effectiveness of Androgen Receptor Inhibition in Castration-Resistant Prostate Cancer. *Cancer Res*. [Online] 2018, 78(20), 5731-5740. NCBI. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6191320/ (accessed Sep 16, 2021)
44)Google Ngram Viewer. https://books.google.com/ngrams/graph?year_start=1800&year_end=2019&corpus=26&smoothing=7&case_insensitive=on&content=epigenetics&direct_

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