Jonathan Lee
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Epigenetic Therapy: The Use of Epigenetics to Treat Cancer and Diseases

Epigenetics is the study of genetic control through changes other than the actual DNA sequence. It is the study of how genes are turned on and off, how cells interpret genes, and ultimately, how protein production is effected. Epigenetics are the factors that effect phenotype without changing the actual DNA sequence, which is what gets translated and transcribed.[4] Epigenetics is a very important field because it is more prevalent that it seems. The development of cells greatly relies on epigenetic mechanisms. All cells of an organism have the same DNA sequence, but are able to differentiate into many different types of cells through epigenetic methods. By promoting, suppressing, or silencing certain genes, each cell has the ability to differentiate from a stem cell into a different type of cell. In addition to cell differentiation, epigenetics is seen in other natural processes such as X chromosome silencing in females. In addition, because of epigenetic influences, some twins have the same exact genetic sequence but show different phenotypes. Epigenetic factors plays a large role in natural processes but also are linked to various types of diseases and cancers. By learning and exploiting these epigenetic mechanisms, scientists are discovering ways to treat diseases with epigenetic therapy.[5]

Before the links between epigenetics and disease are discussed, it is important to understand the three main systems of epigenetics. These systems help give a better understanding of how they are mechanically related to diseases and cancer. The three main systems of epigenetics are DNA methylation, histone modification, and RNA linked silencing.[5]

DNA methylation is an enzymatic chemical process that adds a methyl group to the DNA strand. These enzymes are known as DNA methyltransferases (DNMTs) and they add methyl

groups to cysteine bases in the DNA sequence. The cysteine is usually found adjacent to a guanine base nucleotide. The methyl group that is transferred to the cysteine base in the DNA strand comes from a compound called S-Adenosyl methionine (SAM), which is produced and consumed in the liver in the body. Adding methyl groups to the DNA strand changes the structure of the strand and therefore effects how the sequence interacts with the mechanism of replication within the cell's nucleus. A DNA strand has regions with specific functions such as the promoter region. Methylation of a region like this could effect its interaction with repressor proteins, promoter proteins, or the RNA polymerase enzyme.

In addition to influencing replication mechanisms, methylation is also used for genetic imprinting. After a methylated gene is replicated, the newly generated strand can be methylated to imitate the methylation pattern of the original gene. Due to this ability, methylation patterns are used as signals that are epigenetically inherited. Imprinting is the use of methylation on DNA sequences to silence a gene in a parent of origin specific manner.[8] Areas of genes have regions of alternative CG nucleotides known as CpG islands. The methylation of these islands is used to mark and imprint the gene. When an allele is passed on to offspring from the parents, depending on the sex of the offspring, certain maternal and paternal genes have to either be imprinted or expressed. For example, insulin growth factor 2 (IGF2) is a maternally imprinting gene, meaning the copy of the gene from the mother is silenced and the copy of the gene from the father is the only one expressed.[10] Since DNA can be methylated or unmethylated it is a dynamic process. Methylation leads to gene silencing because certain proteins recognize the 5-methyl cytosine nucleotides in the DNA and compact the shape of the gene. Compacting the DNA results in eliminated transcription and silenced gene expression.[14,15]

The other main system of epigenetics is called histone modification. DNA is wrapped around proteins called histones to create chromatin. The proteins that make up histones act like the spool that DNA can wind up around. Enzymes can modify base nucleotides on the "tails" of histone proteins. Similar to the methylation of cysteine nucleotides in DNA methylation, lysine nucleotides are methylated on the histone tail. In addition to methylation, nucleotides can be acetylated and phosphorylated. Modification of histone tails of H3 and H4 are known to play a large role in various cellular processes. Depending on the modification, the chromatin may open up to the euchromatin form and allow transcription of the DNA strand. On the other hand, the modification causes the chromatin to become condensed and compact, not allowing for any DNA transcription. Methylation of H3 K9 is an example that results in the inactivation of the X chromosome in females. On the contrary, H3 K4 methylation is a gene marker that promotes expression.[5]

The third system of epigenetics is RNA-associated silencing. This is better known as RNA interference, where small-interfering RNAs (siRNAs), are used to silence genes. These siRNAs are only 21-25 nucleotides long and are designed specifically to align as the complementary strand to the mRNA. After attaching, the siRNA then silences the gene by cleaving the target mRNA, which then gets degraded by the cell. In plants there is a mechanism called RNA-dependent DNA methylation (RdDM). This mechanism use DCL family proteins to split dsRNA into siRNA that are identical to the target gene promoter region. These siRNAs are 24-26 nucleotides long and are able to promote methylation of the promoter region of the target gene, causing the silencing of the that gene. These siRNAs induce other methyltransferases such as MET1 or CMT3 to methylate the promoter region. A similar mechanism is seen to occur in

humans and mammals, however the mechanism in which the siRNAs are transported into the nucleus to access the chromatin and histones are not yet fully understood.[13]

Through these three known mechanisms, it is clear that epigenetic systems are important for normal cellular functions, yet could also play major roles in attributing to diseases. Because of the large influence epigenetics has on DNA transcription, small changes can have large influences through an organism. A silencing of a vital gene such as p53 or overexpression of a moderated gene can lead to diseases and cancers. ATR-X syndrome, Fragile X syndrome, ICF syndrome, Prader-Willi syndrome, BWS, Rett syndrome, alpha-Thalasseamia, Leukaemia, Rubinstein-Taybi syndrome, Coffin-Lowry syndrome, and various cancers are examples of diseases that are known to be linked to epigenetics. This paper will further investigate the links to these diseases and see where epigenetic research can be used for possible treatment or drug therapy.[5]

There are a couple of factors that play a role in the link between epigenetics and cancer. As mentioned, there are CpG islands near the promoter regions of genes. Certain genes should be unmethylated in these areas, however they are found to be hyper-methylated in cancer cells. Therefore the methylated CpG islands are silencing the genes that are supposed to be expressed. These genes usually encode for tumor suppressing proteins such as p16, a cyclin-dependent kinase inhibitor. Without these tumor-suppressing genes, cells lose the ability to stop the cell cycle or induce apoptosis of the cell, resulting in tumor overgrowth.[5]

By definition, it is known that epigenetics does not involve the actual modification or change of the DNA sequence. However it does not mean that an epigenetic change cannot influence other factors to result in a change in DNA sequence. For example, the methylation of GpC islands near promoter regions of DNA repair genes such as BRCA1 and MLH1 can

eliminate gene repairing ability.[12] MLH1's biological GO functions show that it is a DNA mismatch repair protein.[3] BRCA1's biological GO functions also show that it is a DNA repair protein and also has multiple functions as a methylation and acetylation regulator.[2] By silencing these genes, there is not enough of each respective protein to monitor and repair DNA throughout transcription. The epigenetics influence on these regulator and repair genes results in the increased number of allowed mistakes in DNA, which ultimately leads to cancerous cells and tumor growth.

In addition to inducing mutations that cause cancerous cells, methylation can lead to the creation of microsatellites. These DNA sequences are tandem repeats of 2 nucleotide bases, C and G, and are found widely throughout the genome, acting as the "fingerprint" region of the DNA. If DNA repair genes are silenced, spontaneous mutations in the microsatellites are not repaired and can lead to instable microsatellites. These instable microsatellites have been shown to be linked to colon, gastric, endometrium, ovarian, hepatobiliary tract, urinary tract, brain, and skin cancer.[11]

The other main types of diseases that are linked to epigenetic influences are mental retardation diseases. Fragile X syndrome is an epigenetically linked mental retardation diseases that is inheritable through the X chromosome. Normal individuals have the nucleotides CGG repeated about 5-40 times in the FMR1 gene. However, affected individuals have this CGG triplet repeated more than 200 times. The increased CGG codons in the FMR1 gene leads to hyper-methylation of the CpG islands and causes silencing of the gene. The FMR1 gene plays a critically role in transcribing FMRP, which regulates other proteins essential for synapse development. The lack of FMRP and nerve cell connections leads to the mental retardation phenotype.[7]

The wide influences of epigenetic changes are seen through understanding of their system mechanisms. Given this knowledge there have been advancements made to exploit these systems and create drug therapies that combat these diseases and cancers. As we know DNA methylation is a dynamic process and can therefore be revered. Technically, if a disease is caused by methylation of CpG islands, sending enzymes to demethylate them could be a viable strategy. Two popular types of drugs currently being used are 5-azacytidine and 5-aza-2'deoxycytidine. These are drugs act as cytosine nucleotides that incorporate themselves into DNA as it is being replicated. They are identical to cytosine except that they block the methylating action of DNMT proteins. If these drugs block methylation they are able to stop the silencing of the gene. Both these drugs are FDA approved for myelodysplastic syndrome (MDS). Another way to combat silencing of genes epigenetically is called histone deacetylase inhibitors (HDAC). When histones are deacetylated, the chromosome condenses, halting transcription and silencing the gene. These HDAC inhibitors combat this by inhibiting deacetylase enzymes, leaving chromatin open and available for transcription. Common examples of HDAC inhibitors are phenylbutyric acid, SAHA, depsipeptide, valproic acid, and panobinostat.[1]

Even though epigenetic drug therapy makes logical sense through theory, there have been many problems with it. The entire body relies on methylation patterns on all of the genetic material for normal functions. The study of the complete set of epigenetic patterns of a cell's genetic make up is known as epigenomics. Due to the body's wide reliance on methylation patterns, introducing a drug to change methylation patterns are found to effect methylation at more than just the target gene or cancerous cells. The lack of targeting is one of the biggest drawbacks of epigenetic therapy today. Recent technology is being used to help solve this issue. For example, restriction landmark genomic scanning is being used to analyze methylation

patterns in thousands of CpG islands. By studying associating methylation patterns on a large scale for certain types of cancer, global methylation cancer profiles can be made. By understanding global cancer epigenomics, epigenetic therapy can be designed to be more effective.[16] Delivery of the drugs to the correct cells is also a challenge. The reason why are 5-azacytidine and 5-aza-2'deoxycytidine are successful may be due to the fact that MDS is a blood disease and therefore can be treated more easily. Researchers had a difficult time using 5-azacytidine and 5-aza-2'deoxycytidine to cure mice with diseases and cancer in areas other than the blood. More research is currently being done to find ways to make these drug therapies more specific and efficient. By combining epigenetic drug therapies with existing treatments, such as chemotherapy, researchers to hope to make the effects of the therapy longer lasting and less toxic.[9]

Even with all the research going into epigenetics and epigenomics, there is a large knowledge gap in how and why methylation patterns are generated. Studies show that there is a correlation between samples from cancer patients and hyper-methylation in certain CpG islands, however that does not tell why or what is causing the hyper-methylation to cause the cancer in the first place. Because hyper-methylation of CpG islands is found in specific subsets of genes and not all genes, it is thought that these errors in methylation are not random. A paper by Estécio and Issa has hypothesized a few factors that they believe play a role in methylation alterations. One factor is identified as gene microenvironments. After analyzing microenvironments, a researcher named Feltus identified 7 short DNA motifs that are associated with frequently methylated genes. These DNA motifs and other proteins are chromatin signatures that seem to denote which CpG islands are supposed to be resistant to or likely for methylation. This research is promising as it was once assumed that methylation occurred

randomly and proceeded through a growth advantage selection to determine the final epigenomics pattern.[6]

The second factor that researchers have found to play a role in methylation is called transcriptional programs. It was shown that the actual DNA sequence might play a role in the methylation pattern observed DNA methylation in cancer is tissue-specific. By studying PcG proteins in embryonic stem cells, researchers found a link that genes which were targeted by PcG proteins were found to be later be methylated when in a cancer state. This finding further pushes the notion that the methylation is not random and is influenced by other factors.[6]

The final factor is cellular and host factors. It is thought that with aging, the increased number of cell replications results in greater chances of sequence mutations, which would result in higher rates of cancer. In addition, with older age, the individual is exposed to higher levels of carcinogens and other environmental influences over time. The key concept is that DNA replication requires the replication of the methylation pattern on the new strand. With old age and increased exposure to environmental carcinogens, it is possible that errors in the methylation pattern replication are also leading to increased chances of diseases and cancer. Infections and carcinogens such as *H. pylori* and tobacco smoke are also related to higher rates of cancer. Infections and tobacco smoke cause inflammation in the damaged areas and require more rapid cell replication to repair or replace the damaged cells. Inflammation induced rapid cell replications produces the same effect that is seen in older cells in older aged individuals. These areas of research are important and necessary to understand what causes alterations and errors in epigenomics patterns.[6]

The field of epigenetic therapy is a very exciting one as a lot of research is being done to potentially use this information to cure cancers and diseases. The drugs that are currently in

clinical trial and on the market are a good start, yet the topics of specificity, consequential toxicity, and inefficiency are holding the therapies back. More research is necessary to understand why methylation occurs in the specific patterns that it does and how epigenomics can be incorporated more to profile cancer patterns. These pattern profiles are promising due to their ability to quickly diagnose or even predict the onset of a cancer. By understanding more about where and why the methylation errors occur and how epigenomics changes with age and carcinogens, researchers can produce better and more efficient therapies. This field and research is promising because of its potential use in drug therapy. By learning more about factors that influence epigenomes, this research can hopefully be expanded to learning about how to improve our epigenomic health to prevent cancer in addition to curing it.

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