Epigenetic Dysregulation in Cancer Stem Cells

Sadhvi Reddy

Abstract:

Epigenetic dysregulation, itself, plays a role in gene expression, yet it does not change the DNA sequence. This type of dysregulation refers to abnormality within the structure of DNA and its proteins. Such modifications change gene expression, DNA accessibility, and chromatin structure, without changing the DNA sequence. Epigenetic change responses can play a significant role in rapid cancer growth. Modifications can be inherited directly from the parent or can be re-established through DNA replication– cancer-inducing ones would lead to the malignancy of normal stem cells into cancer stem cells, promoting the tumor. Epigenetic modifications (PTM), allow the DNA to be modified in certain cells. These codes play a significant role in gene expression. This paper analyzes the lethal relationship between cancer stem cells (CSC) and epigenetic dysregulation. To determine such factors, the review will analyze multiple, published, research works that include various clinical trials and studies to correlate significant discoveries.

Key Words: Epigenetic Dysregulation, Cancer Stem Cells, Post-Translational Modifications, Tumor, DNA

Introduction:

Cancer stem cells are an integral part of cancer. Regular stem cells offer raw material that is later specialized, whereas a CSC is a material from which cancer and tumors can be further instigated; they can cause cancer relapses as well. CSCs have properties of self-renewal, and differentiation, and can develop tumors. CSCs are present in various tumors and cancers.CSCs have great malleability; they are easily changed through various radiotherapeutics and aging tumor cells. Senescence (cell decline due to age) causes tumors to shrink by regulating an anti-tumor environment where the tumor's growth is then subsided and immune cells are attracted. Cancer cells indicate a tumor whereas a CSC maintains that tumor and has self-renewal and multipotency capabilities. Various epigenetic markers can contribute to the expression of a certain biomarker or silence certain other genes on CSCs or cancer cells. Epigenetic functions are partially responsible for the initiation and maintenance of CSCs. Epigenetic codes being nonfunctional may also contribute to the worsening nature of CSCs. This review will look at the link between, epigenetic dysregulation and CSCs, the various epigenetic codes and their impact on cancer induction, the underlying causes of epigenetic dysregulation in CSCs, what epigenetic dysregulation leads to within CSCs, and will explore options to prevent the spread of malignancy through epigenetics while reviewing the efficacy of current methods.

The Correlation Between Cancer Stem Cells and Epigenetic Dysregulation:

The recognition of cancer stem cells occurred over a century ago by scientists Virchow and Cohnheim. It was speculated that CSCs come from the remnants of regular stem cells and that they could activate embryonic tissue parts. Virchow emphasized how the proliferative qualities CSCs have given the ability to grow functional, resistive, tumors. being found in various cancers, are neoplastic; in fact, CSCs are known to begin the initiation of various tumors because of

metastasis and their continuous regenerative abilities. The modification of epigenetic codes has contributed to the development of cancer due to gene expressive changes. Additionally, epigenetic dysregulation properties can result in the transformation of stem cells to CSCs. The plasticity of epigenes allows CSCs to constantly change concerning their environments while enhancing their growth.

Epigenetic Codes in Cancer:

Epigenetics, which may be heritable, does not alter DNA sequence; somatic cells within the same individual have the same DNA sequence, but epigenetics controls which genes are expressed or silenced. There are three main types of epigenetic codes: *(1) DNA Methylation, (2) Histone Modifications, and (3) ncRNA (non-coding RNA).*

(1) *DNA Methylation*: Chemical modification of DNA molecules through DNA methyltransferases (DNMT). This involves adding a methyl group to DNA. When added, the methyl group usually decreases protein expression in the area it's placed (and vice-versa with demethylation). Silencing genes through methylation mainly occurs in CpG islands. CpG islands are areas with highly phosphate-linked Cytosine and Guanine pairs. If dysregulated, DNMT can further disease and malignancy. This alteration can lead to tumor cells inactivating the expression of certain genes– leading to malignancy. A recent study, *DNA methylation-associated dysregulation of transfer RNA expression in human cancer* (Tortella 22), concluded that there was a link between tRNA (RNA that plays a role in protein synthesis) and dysregulation of DNA methylation. It was found that tumor cells had defects in tRNA, causing cell proliferation and tumorigenesis; the study attributed DNMT to these defects. If methylated promoters are silenced, DNMT will follow patterns of gene expression and open a void of disease development. Concluding that the observation of DNMT can act as a biomarker for early tumor diagnosis. Now, various types of DNMT contribute to the methylation process as a whole:

> *DNMT3a, DNMT3b:* "De novo methylation"; methylation of cytosines to a 5-methylcytosine. Establish a DNA methylation pattern within a cell. Contributes to signaling which genes should be turned off in different cells.
>
> *DNMT1:* An enzyme that catalyzes methyl group transfer. It copies existing DNA methylation patterns into daughter strands during DNA replication. The parent cell is methylated whereas the daughter cell is unmethylated (hemi-methylated DNA which signals to the cell that replication isn't quite done yet). DNMT1 comes back through replication and methylation of adenine residues within the origin to signal that replication is finished.

(2) Histone modifications: stones are a protein that helps pack chromatin structure in a cell, thus, it's a post-translational modification (PTM). Due to the PTM nature, histone modifiers can't involve themselves in silencing or expressing certain genes. Therefore, they use various chemical groups to facilitate a wrapped or unwrapped state of a gene; this changes chromatin structure, thus affecting gene expression. This PTM changes histone tails (i.e. H2A, H2B, H3, and H4) that are catalyzed by "writers" (introduce chemical modifications on DNA and

histones) and “erasers” (remove chemical tags). Alternated histone modifications can activate oncogenes. For example, histone mutations within the SWI/SNF complex (involved in nucleosome positioning and a tumor suppressor) are present in ~25% of all cancers; histone mutations in H3K27M, H3K36M, and H4G34V/R/W/L are also common in pediatric cancer. There are currently nine forms of histone modifications (chemical groups) that have been discovered. A few of them include (a) acetylation/deacetylation, (b) methylation/demethylation, and (c) Phosphorylation/Dephosphorylation.

(a) Acetylation/Deacetylation: Acetylation occurs when acetyl groups are integrated into histones on the lysine residues of tails from histones, pushing the chromatin out and making the DNA susceptible to RNA polymerase; vice-versa for deacetylation. Naturally, this alters gene expression as the chromatin structure is changed. Acetylation is known to promote transcription, which is a system usually catalyzed through histone acetyltransferase (HAT) or histone deacetylase (HDAC). The induction of these dangerously transcripted genes leads to wrongdoings in the cell cycle, proliferation, and apoptosis. However, overexpression of HDAC has been found to drive malignancy and oncogenes (ex. p300 which is essential in cell growth and division).

(b) Methylation/Demethylation: Methyltransferases (KMT) add methyl groups to lysine in the histone, whereas histone lysine demethylases (KDMs) remove such groups– modifying the histone. This process can be complex, as methylation labels effector proteins. For example, one histone marker is H3K4me3 which communicates with transcription factor proteins (involved with gene promoters) where the nucleosome complex is blocked by H3K4me311,13. However, the invariably binding of H3K9me3 to structural amino acid components leads to gene repression. KMT and KDMs, similar to other epigenetic codes, affect gene expression. The mutation of histone-modifying enzymes has led to the dysregulation of methylation, correlating this epigenetic code to cancer cells. For example, the irregularity of H3K27me3 caused by histone methyltransferases leads to cancerous gene expression,

(c) Phosphorylation/Dephosphorylation: Phosphorylation is the addition of a phosphate group, whereas dephosphorylation is vice versa. Phosphorylation, specifically, alters DNA structure because it adds a negative charge to histone tails which changes the factors of gene expression involved in the cell cycle and proliferation. Dysregulation of this function can develop tumors; for example, the deletion of a catalytic subunit, spurred by irregularity, would inhibit the epithelial cell from undergoing biochemical changes by the magnification of phosphorylation.

(3) Non-Coding RNA: Modulates epigenetics by controlling post-transcriptional regulation of gene expression; the majority of eukaryotic genome transcription is implemented through ncRNA. NcRNA is regularly used for regulation and responds to environmental stimuli. Additionally, it has been postulated that this epigenetic code regulates the development of CSCs by failing to act as a tumor suppressor or an oncogene. This can partially be attributed to the fact that lncRNAs (long- ncRNA) have been expressed in various cancers including, but not limited to prostate, breast, brain, renal, and colorectal cancer.

Cancer can be caused or prevented by the expression or silencing of certain genes; consequently, various tumor suppressors stop malignancy. However, a mutation in such suppressors can result in the initiation of cancer. Regardless of the provided treatment aimed to battle these mutations or other factors of cancer induction, many cancer stem cells may remain harmful because their cancerous agents are posited through epigenetics.

However, due to the pliability of CSCs, post-chemotherapy may result in the plasticity quality of CSCs increasing which promotes the immortality of the stemness– leading to tumor relapse and the spread of cancer. When they are being transformed, CSCs may over-express oncogene, a mutated carcin-instigating gene, and tumor suppressors are inactivated. Additionally, other expressed biomarkers (carcinoembryonic antigen (CAE), cytokeratin 19 (YFRA 21-1) (58), and alpha-fetoprotein (AFP)) play a role in provoking the tumor.

The Beginnings of Epigenetic Dysregulation in Cancer Stem Cells:

Epigenetics is a normal part of the cell, the issue arises as epigenetic codes are dysregulated. These dysregulations can be caused by drugs, environmental factors, and more. Environmental factors that regulate epigenetic mutations can include unhealthy lifestyles, food, and/or exposure to toxicity.

Increased selenium or polyphenols, which are antioxidants in fruits and vegetables, can dysregulate DNA methylation, acting as a carcinogen. Excess amounts of curcumin, found in cosmetics, food spices, and food coloring, although an antioxidant, can cause mutated epigenetic codes in cells. Specifically, the toxicity of curcumin alters the PTM of histones. Sulforaphane, in broccoli, and/or butyric acid, in cheeses, can inhibit histone deacetylase. In addition, eating lots of fat leads to the hypermethylation of DNA promoters, reducing the effectiveness of tumor suppressor genes. In addition, it was confirmed that the common carcinogen, smoking, affects the methylation of tumor suppressor genes in *A Systematic Review of Smoking-Related Epigenetic Alterations* (Kaur 19). The tumor induction itself can occur in differentiated cells or during the transformation of resident stem cells. This transformation to a cancer cell may occur during tissue regeneration and can be catalyzed through infections, toxicities, the environment, or more. This transformation into cancer stem cells leads to the overexpression of oncogenes and inactivation of tumor suppressors, contributing to the proliferative quality of the cells. Through this, differentiated cells may reverse their differentiability and receive their true cancer stem cell nature. In *SMAD4 suppresses WNT-driven dedifferentiation and oncogenesis in the differentiated gut epithelium* (Res 18), mice were studied to decide that differentiated intestinal epithelial cells have the potential to become CSCs. Additionally, adult differentiated cells can also promote tumor creation in the liver.

Additionally, many medications or therapies alter gene expression. For example, diethylstilbestrol, a non-steroidal estrogen used to prevent miscarriages, can induce breast cancer risk alongside vaginal cancer and other reproductive irregularities. Diethylstilbestrol alters DNMT, and consequently, DNA Methylation.
A toxic environment full of pollutants also contributes to cancer induction. Pollutants such as chromium, cadmium, nickel, mercury, and arsenic are hypothesized to alter epigenetic codes. Mercury, itself, is known to be highly toxic; exposure to the chemical via intake or through the

environment, can generate epigenetic alterations. In a study concluded by Deborah Antwih, it was concluded that mercury-exposed animals had overactivity of proteolysis matrix metalloproteinase 9 (MMP9), which was caused by the demethylation of the regular MMP9 region.

The dysregulation of epigenetics leads to changed gene expression, resulting in cancer. The causes mentioned above are a few of the many various reasons for such tumor induction. Identifying why epigenetic dysregulation occurs heavily contributes to recognizing effective treatment processes.

The Effects of Epigenetic Dysregulation in Cancer Cells and CSCs:

Accordingly, dysregulation within the epigenetic process leads to the transfiguration of stem cells and regular cells. The cancerous effects of self-renewal, differentiation blockade, therapy resistance, heterogeneity/plasticity, and metastasis promotion all occur.

The induction of cancer spurs the necessity to maintain self-renewal of CSCs. Being one of the most common cancer causes, epigenetic mutations call for the constant reorganization of the epigenome. Cancer, itself, means unregulated cell growth. So, epigenetic dysregulation would lead to the unchecked nature of cellular division; specifically, this could include the lack of checkpoints, mutated stages of nuclear division, or mutations of other prominent self-renewal factors. Abnormal epigenetic mechanisms can affect cell normalcy by interjecting cell-intrinsic and cell-extrinsic differences, establishing a toxic environment.

Consequently, CSCs can lead to therapy resistance. The mutation of epigenetic codes can lead to the silencing of tumor-suppressing genes, allowing CSCs to potentially evade chemotherapy, radiation, and other treatment effects. CSCs express multidrug resistance (MDR); investigations regarding MDR isolated specific proteins that contribute to this nature: P-glycoprotein (P-go), multidrug resistance-associated protein (MRP1), lung resistance protein (LRP), and breast cancer resistance protein (BCRP).

Solvency: Effectivity and Prevention of Epigenetic-Based Interventions in Cancer:

By analyzing the causes of CSC development and their effects, it becomes easier to detect markers that may contribute to malignancy, preventing cancer induction in the first place. The presence of epigenetic dysregulation influences the induction of cancer. By recognizing how to prevent the induction of epigenetic dysregulations or identifying the efficacies of various treatments, it becomes easier to terminate cancer.The most basic effort that can be made to prevent cancer is for one to reduce their risk of acquiring cancer by being mindful of unhealthy intakes. Eating increased selenium, polyphenols, curcumin, sulforaphane, or butyric acid would influence the mutation of epigenetic mechanisms. Intaking toxic substances such as nicotine or other harmful substances will result in increased risk.

In terms of treatment against gene mutations, the presence of histone modifications in the SWI/SNF complex is abundant. The identification of the loss of function within this complex has only been recently discovered. A normal functioning SWI/SNF complex uses ATP energy in

interjecting nucleosomes and thus remodeling the DNA. Mutation in one SWI/SNF subunit increases its dependency on other SWI/SNF complexes. For example, the SMARCA4-mutant leads to dependence on (non-mutated) SMARCA2. The loss of function in one gene can only partially be compensated by another and makes the paralogue increasingly vulnerable. An inhibitor of both SMARCA4 and SMARCA2 has been identified to inhibit cell growth (Papillon 18). As this is a recent discovery, clinical trials and research about the issue are still being conducted. A recurrence in many of the trials is the attempt to inhibit the activity of certain genes whilst acting as a tumor suppressor. By studying immunotherapy, inhibitors, and DNA mediation, these trials have all been leading to the augmentation and diversification of clinical trials.

In addition, the aberrancy of histone modification has been attempted to be solved via medication. However, the majority of epigenetic drugs remain in the clinical phase, reiterating the importance of clarifying the abnormal pattern of epigenetics in cancer. An understanding of the post-translational modifications is crucial to elucidating the apparatus of tumorigenesis and then developing treatment plans. The disruption of histone modifier enzymes, such as HDAC, is a consequence of the disruption within the histone modification procedure itself. In PTM histone acetylation/deacetylation, HDAC is known to thrust malignancy and wrongful expression of CBP/p300. CBP/p300 is a gene that is shown to be affiliated with resistant malignant cells when overexpressed; whereas, if deleted or lost, the lack thereof leads to tumorigenesis. However, the methodical presence of p300 and CBP are tumor suppressors in blood disorders. This suggests integrating a process that would allow for the regulation of these two genes if they are a present factor in one's cancer.
Furthermore, a potential for targeting mutated ncRNA transcripts would be to use smaller RNAs such as eRNAs or PARs. Specifically, eRNAS are produced through enhancer regions and play a role in transcriptional gene activation. There would be a potential therapeutic outlook if minute RNAs prevented or treated circumstances caused by epigenetic changes.

There are a multitude of studies and methods being used in attempts to prevent the malignancy of epigenetic mutations. Below, a few techniques are presented alongside a brief description of their relationship to epigenetics in cancer and an explanation of their estimated functionality.

Current Method	***Description***	***Effectivity***
Histone Deacetylase Inhibitors (HDACI)	HDACI's changes affect chromatin structure, thus changing gene expression. This inhibitor is taken, in the form of a drug, to inhibit HDAC. HDAC1 is infamous for catalyzing deacetylation of H3K27 and STAT1 (gene promoters), weakening the cells' immune response, promoting malignant stem cells. HDACI may influence cell behavior (ex. promoting apoptosis, gene differentiation, downregulation of benefactory genes,	HDACIs are deemed limited in their capabilities because they cannot target solid tumors. The pleiotropic nature of HDACIs results in the possibility that HDACI may be more effective if combined with other target agents, so that unaffected structures may be targeted alongside HDACI-affected regions. In the US, as of 2021, there are 4 FDA approved

Current Method	*Description*	*Effectivity*
	DNA repair proteins, and interference of various complexes). However, studies proved that HDACIs selectively target varied cells, thus enabling the approval of certain treatments (ex. hematologic malignancies). However, the process of using HDACIs is extremely relative as their abilities are not transferable to other malignancies and tumors.	HDACIs for cancer treatment: Istodax, Beleodap, Vorinostat, and Panobinostat. However, Istodax's (aka Romidepsin) usage is declining in Canada due to its ineffectiveness. The effectiveness of HDACIs may be retained as various studies continue to advocate for the mixture of HDACIs alongside other drugs and treatment options.
Tazemetostat (EZH2)	Tazemetostat, a relatively new form of an interventional agent in cancer, aims to target mutated SWI/SNF complexes (caused by dysregulated histone modifications). Tazemetostat is often taken orally to block a specific enzyme function, inhibiting EZH2 (which produces histone methyltransferase). This kills or slows cancer in individuals whose cancer is caused by such mutations. Additionally, tazemetostat inhibits EZH2 surplus mutations: Y646X and A687V. Such inhibition prevents proliferation caused by EZH2 issues. This treatment form continues to be researched, but most cases are still near the phase 2 stage.	In the ongoing clinical trials, Tazemetostat has been shown in a positive light. One of the many newly forming epigenetic regulators, Taezemetosat is shown to successfully inhibit EZH2 in many processes. In the near future, a combination of Tazemetostat and therapy foretells synergistic results.
Stem Cell Transplant	Rather than attacking cancerous cells, stem cell transplant, in addition to other treatments, allows the body to continue to produce healthy blood cells, preventing cancerous growth. This transplant involves the injection of non-cancerous stem cells that usually come from the bone marrow, or, if stored, the umbilical cord. It suppresses the negative effects of certain treatments, deflecting the consequences of various treatments,	Stem cell transplants seem to have very direct drawbacks. Although they pose as beneficiaries by donating to the cancerous bloodstream, they fail to change the degree of cancer the patient is undergoing. However, in certain cancers, such as leukemia, the stem cell transplant can make a lasting impact. Due to graft-versus-leukemia, the

Current Method	*Description*	*Effectivity*
	such as intensive chemotherapy, instead of directly fighting the cancer. This transplant is not shown to replace CSCs or to terminate the cancer. There are various types of stem cell transplant: *Autologous*– the transplanted stem cells are received through the affected individual's own body; risk of cancer or cancer stem cells being reinjected. *Allogeneic*– the transplanted stem cells are received from a donor with similar matching capabilities; risk of the activated immune system, thus destroying the transplants.	relapse rate of the cancer decreases, and the WBC donation attacks cancerous cells. Despite stem cell transplants targeting the effects of cancer treatment rather than cancer in most cases, the effectiveness of stem cell transplants is extremely selective.

Figure 1 A review of multiple treatments regarding stem cells and epigenetic dysregulation.

Conclusion:

Epigenetic dysregulation, cancer, and cancer stem cells are all intertwined. As the medical field continues to persevere in research targeting epigenetic mutations, the knowledge gained by viewing cancer on a cellular scale allows researchers to identify various contributors to the disease– a weighted factor including epigenetics; as clinical trials continue, it's important to delve further into the combination efficacies of multiple treatments; for example, exploring the potency of Tazemetostat in addition to chemotherapy or other treatment methods. This review was able to highlight the epigenetics within CSCs, the true meaning of epigenetic dysregulation, and identify epigenetic mutation origins and their implications on an individual. The review was concluded by examining popular remedy methods. Additionally, this review confirms the evident link between cancer stem cells and epigenetics and the substances around them.

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