Epigenetic Therapy for Cancer Stem Cells*
A New Arrow in the Quiver

Akshatha E. Nagarkatte and Prathibha Ranganathan

Substantial evidence has shown that tumors can emerge from a distinct, small population of cells known as cancer stem cells (CSCs), which have a vital role in the initiation, maintenance, metastasis, drug resistance, and relapse of cancer. Hence, it is critical to identify drugs that selectively target and eliminate CSCs to induce a long-lasting therapeutic response and better patient outcomes. Modulations in epigenetic regulation contribute to cancer progression as it is crucial for both stem cell biology and chemoresistance. Polycomb group (PcG) and trithorax group (TrxG) proteins are identified as the key modulators of cellular memory that direct whether a stem cell will self-renew or differentiate. The dynamic interaction of these two groups of proteins with opposing effects on gene expression has opened up new avenues for understanding their role in tumorigenesis. Therefore, it is essential to elucidate the underlying mechanisms of aberrant epigenetic modifications, without which designing drugs becomes implausible. The existing cancer treatments like radiotherapy and chemotherapy have major limitations owing to treatment failure and recurrence of cancer. However, the application of epigenetic therapy has shown promising therapeutic results in clinical trials with its ability to reverse the aberrant epigenetic modifications that result in cancer and chemotherapy resistance. Future research aimed at developing drugs that are target specific is necessary to prevent off-target effects. To overcome the limitations of the current epidrugs, novel approaches like CRISPR/Cas9-based epigenetic editing are emerging as new hopes for targeted therapy.

Akshatha E. Nagarkatte has completed her MSc in Human Disease Genetics from the Centre for Human Genetics. Her areas of interest include cancer biology, epigenetics, and cytogenetics. She aspires to pursue PhD in the field of cancer biology.

Prathibha Ranganathan heads the Cancer Lab at the Centre for Human Genetics. She is interested in understanding the molecular basis of chemoresistance in cancer. Her group works on elucidating the signaling, epigenetic, and gene expression changes during the development of resistance in various models of cancer.

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in cancer. This article gives an overview of the till-date understanding of the role of epigenetics in cancer stem cell biology and recent developments in epigenetic therapy.

Introduction

Cancer is among the deadliest diseases and is one of the leading causes of death globally, constituting about 10 million deaths in 2020 (World Health Organization). Having said this, the striking question is how do cancers originate? Many models/theories have been proposed to explain this. The most widely accepted models for the origin of cancer are the stochastic model and the cancer stem cell (CSCs) model (Figure 1) [1].

According to the stochastic model, also known as the clonal evolution model, a cell could become tumorigenic by gaining a significant number of alterations, either aberrant genetic or epigenetic changes. The aberrations in the tumor cell would add growth advantage to compete and out-proliferate other cells [1]. On the other hand, the CSCs model or the hierarchy model suggests the presence of a hierarchical organization of cells in the tumor, with CSCs at the top. CSCs are distinct from the other cells in the tumor, mainly due to their extensive ability to self-renew and differentiate into other cell types [2]. Therefore, as per this model, only certain sub-populations of cancer stem cells have the ability to drive cancer progression. If the CSCs model holds, then CSCs would be the most vital cells in supporting tumorigenesis, emphasizing that identifying specific (intrinsic) characteristics of CSCs is crucial for managing the disease. Having this understanding, targeting CSCs specifically, could be the best strategy to eliminate the tumor without having to battle the whole tumor.

Substantial research has been carried out to identify the mechanisms that confer CSCs the ability to initiate and maintain the tumor. A multitude of evidence has indicated that genetic and epigenetic changes in CSCs cause significant resistance to chemotherapy and radiation by enhancing self-renewal, differentiation and metastasis [3]. Various genetic defects like translocations, muta-
tions, deletions and amplifications can result in the transformation of a normal cell into a cancerous cell.

**Epigenetic Regulation of Gene Expression**

The discovery that cancer can be driven above and beyond the level of our DNA marks a new era with the association of epigenetic modifications in cancer initiation and progression. The term epigenetics refers to the heritable changes in gene expression that occur without causing an alteration in the DNA sequence. Different epigenetic processes include methylation, acetylation, phosphorylation, ubiquitylation, and sumoylation that occur either on DNA or histones as post-translational modifications. These modifications mainly modify chromatin structure and accessibility of DNA to the transcriptional machinery, hence altering gene expression patterns by modulating shift from the condensed inactive heterochromatin to relaxed active euchromatin or the other way around. For instance, methylation or addition of methyl groups on the DNA or histones, results in chromatin compaction, leading to transcriptional repression. In contrast, acetylation marks help in opening up the chromatin, resulting in transcriptional activation. Therefore, epigenetic mechanisms are crucial for the adult organism’s proper development and differentiation of diverse cell

**Figure 1.** Schematic representation of the stochastic model and cancer stem cell (CSC) model to explain the origin of cancer. (a) Stochastic model: Every cell in the tumor has a low but equal ability to proliferate limitless and form a tumor. (b) CSC model: Only distinct cells within the tumor population have the potential to self-renew and the ability to initiate tumor growth and reproduce the hierarchy of cell types that comprise the tumor.

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Figure 2. Epigenetic regulation of gene expression. Epigenetic modification like acetylation facilitates the opening up of the chromatin and enables the binding of RNA polymerase II, leading to transcriptional activation. On the other hand, methylation results in chromatin condensation, thereby preventing the binding of RNA polymerase II and resulting in transcriptional repression.

Knowing the importance of epigenetics in gene regulation, one can imagine how aberrant epigenetic modifications in DNA methylation, histone modifications, RNA methylation, and noncoding RNAs can reprogram normal stem cells to CSCs and help in their maintenance and survival.

Knowing the importance of epigenetics in gene regulation, one can imagine how aberrant epigenetic modifications in DNA methylation, histone modifications, RNA methylation, and noncoding RNAs can reprogram normal stem cells to CSCs and help in their maintenance and survival. Mainly due to their nature, epigenetic modifications are reversible unlike genetic alterations, and they are proving to be promising targets in the treatment of cancer. Consequently, drugs that target the components of these epigenetic pathways would aid in the eradication of both CSCs and the overall tumor population. The article focuses on understanding (1) how CSCs play a vital role in tumor initiation and progression, (2) the role of epigenetics in CSCs formation, and (3) how epigenetic therapy can be used to target CSCs by reversing the aberrant epigenetic modifications and their limitations.

Cancer Stem Cells

CSCs are a subpopulation of cells in cancer with ‘stem-like’ properties. Like normal stem cells, they have the ability to differenti-
ate and generate different cell types that encompass the whole tumor. What is more interesting is these cells can differentiate and repopulate the cell types found in the original tumor. Therefore, CSCs have potent tumor-initiating capacity. Due to the above-mentioned properties, they form heterogeneous cancer cells. The heterogeneity poses a critical problem in treatment because it ensures the survival of cancer cells in difficult to live conditions, making them resistant to treatment. Since CSCs are not eliminated by most conventional treatment strategies, they eventually metastasize, ultimately leading to tumor relapse after many years of primary treatment. Hence, self-renewability, metastasis, and drug resistance are the three main properties of CSCs that contribute to the challenges in cancer treatment [1, 2].

**How Are CSCs Identified?**

CSCs were first identified as a critical tumor-initiating subset of cells in acute myeloid leukemia (AML) by Bonnet and Dick in 1997 [4]. The cell surface markers, such as the receptors and antigens, were used as the most effective approach in identifying CSCs in various cancers. Nevertheless, tumors were later shown to originate from cells that lacked these markers. Thus, the current gold standard to functionally identify CSCs is to assess their ability to generate a phenotypic mimic of the original tumor in an orthotopic transplantation model. In this approach, the CSCs are seeded into anatomic locations or tissues from where the original tumor is derived and checked if they can regenerate the tumor owing to their tumor-initiating capacity. Conversely, in mice models, it is shown that non-CSCs failed to regenerate the tumor.

**How Do CSCs Originate?**

Multiple explanations describe the origin of CSCs. Firstly, somatic cells acquiring mutations can give rise to CSCs. Second, adult stem cells with dysregulated signaling pathways result in the formation of CSCs. Another hypothesis is that CSCs originate from progenitor cells (progenitor cells differ from stem cells in
being targeted to differentiate to a specific type of cell). It is also believed that CSCs can originate from differentiated and mature cells undergoing de-differentiation to attain stem cell-like properties. In addition, the epithelial-mesenchymal transition (EMT) is a critical developmental program activated during cancer invasion and spread. EMT is a process wherein epithelial cells lose their differentiation and polarity and become mesenchymal. Following the loss of intracellular adhesion protein E-cadherin, initiation of invasion of surrounding tissues and metastasis occur due to the inverse relationship between cell migration and E-cadherin levels. Studies have indicated cancer cells undergo transient induction of EMT, acquire mesenchymal characteristics and express stem-cell markers, ultimately contributing to the formation of CSCs [3]. Above all, it is hypothesized that genetic and epigenetic alterations in differentiation genes, tumor suppressor genes, or signalling pathways associated with pluripotency have resulted in non-stem cancer cells’ ability to self-renew and attain stemness [3] (Figure 3).

**Why CSCs Pose a Challenge in Therapy?**

Investigations on CSCs have shown that they can exit the cell cycle and enter a state of quiescence (G0 phase). As most chemotherapeutic drugs like cisplatin, oxaliplatin, and doxorubicin target cells that actively divide and synthesize DNA, resistance of CSCs might be attributed to their quiescent state and lack of DNA synthesis. CSCs also have a higher level of a DNA-repair enzyme than non-CSCs, which helps to repair the damage caused by the chemotherapeutic drugs and protects DNA from radiation-induced damage. Therefore, several drugs that inhibit DNA repair proteins, e.g., RAD51, poly (ADP)-ribose polymerase (PARP) involved in homologous recombination, are being investigated to target CSCs [5]. Besides, they escape apoptosis by mutating and inactivating pro-apoptotic genes like p53 and overexpressing anti-apoptotic proteins like AKT and BCL2. Another interesting aspect of the resistance mechanism is the overexpression of multidrug resistance (MDR) proteins, such as ATP binding cassette
Figure 3. Models proposed to explain the origin of CSCs—progenitor cells and normal stem cells on acquiring mutations activate self-renewal genes; differentiated cells and somatic cells upon de-differentiation and cancer cells on induction of EMT can result in the formation of CSCs.

(ABC) transporters. These transporters contribute to chemoresistance by promoting efflux of anti-cancer drugs from the cancer cells [3]. In addition, CSCs have the ability to metastasize, which means the ability of the cancer cells to break off from the site of original tumor and spread to a distant site in the body. In order to achieve this, cancer cells undergo EMT. The environment around a tumor cell is called the tumor microenvironment (TME) and consists of blood vessels, fibroblasts, immune cells, signalling molecules and extracellular matrix. Epigenetic alterations and signals from the TME enhance EMT and stemness in the cells by affecting signalling pathways. As a result, drugs that inhibit EMT are considered to be an effective strategy to eradicate CSC [6] (Figure 4).
Figure 4. Properties of CSCs that confer stemness and drug resistance: Quiescence, enhanced activity of repair enzymes, expression of drug efflux transporters, activation of EMT, expression of anti-apoptotic proteins, vascular niche, hypoxia, and altered stemness signaling pathways are some of the properties of CSCs that confer them chemoresistance.

What is the Role of Niche in CSCs?

CSCs are found in a unique part of the tumor microenvironment, called the ‘niche’, which plays a vital role in keeping the CSCs plastic, protect them from exposure to damaging agents, induces EMT, and enhances their ability to invade and metastasize. As the tumor cells divide rapidly, it results in a state of reduced oxygen in the tumor microenvironment. Hypoxia, in turn, contributes to treatment resistance of CSCs by inducing cell cycle arrest, reducing cell proliferation, conferring protection to the cells from external stress, and inhibiting apoptosis and senescence. Thus, hypoxia has been established to be a critical component of the niche that increases the CSC population and preserves stemness. In response to hypoxia, hypoxia-inducible transcription factors (HIFs) are induced, and these further promote angiogenesis, the formation of new blood vessels for tumor tissues to obtain oxygen and nutrients. As a result, CSCs, rely on the cells and factors found in a niche to sustain their function and population. Therefore, CSCs and the niche work synergistically to
create a favorable environment for CSC maintenance and tumor progression [6].

**What Are the Pathways Associated With Self-renewal?**

Wingless (Wnt)/β-catenin, Notch, and Sonic Hedgehog (Shh) signalling are the most critical signalling pathways associated with self-renewal properties. Any alteration in these pathways, either by genetic, epigenetic, or other changes, result in the formation of CSC. Thereby, targeting the self-renewal pathways could be a potential strategy to eliminate CSCs [7].

Although there is a lot of knowledge regarding the characteristics of CSCs that can assist us in efficiently targeting them, there are numerous challenges to overcome before achieving the full potential. The first step is to identify a strategy to target only CSCs in order to prevent harming normal tissue stem cells, thereby minimizing side effects. Second, as CSCs may not be eliminated, combinational therapy for successful tumor elimination should be investigated. As a result, targeting CSCs specifically will be a promising future therapeutic.

**Role of Epigenetics in CSCs**

Epigenetic modifications involving DNA methylation, histone modifications, and RNA methylation play a vital role in the programming stem cell differentiation [3] (*Figure 5*). Thus, aberrant epigenetic alterations can induce the transformation of normal stem cells to the CSC phenotype.

Stem cells have an exceptional ability to either self-renew or differentiate into specified groups of cells. The activation or inactivation of specific genomic regions via alteration of chromatin conformation allows stem cells to achieve the multipotency required to maintain tissue homeostasis. So, the question that arises is—how does epigenetics regulates stem cell fate? Epigenetics, in its simplest form, offers a mechanism for cellular memory, which allows the cell to maintain its identity throughout life. The cel-
**Figure 5.** Different epigenetic modifications involved in programming stem cell fate. Three main epigenetic mechanisms that contribute to gene regulation are histone modifications, DNA methylation, and noncoding RNAs (ncRNA).

**Figure 6.** Role of PcG and TrxG proteins. The trithorax group of proteins (TrxG) adds acetylation marks and helps in transcriptional activation, while the polycomb group of proteins (PRC1 and PRC2) aids in transferring methylation marks to the histones and result in transcriptional repression.

Lobar memory is regulated by two components namely, the polycomb group (PcG) and the trithorax group (TrxG) protein families [8]. The PcG–TrxG proteins are not only evolutionarily conserved, but an imbalance in this system in mammals has been associated with impaired stem cell renewal and an increased risk of cancer (Figure 6).
Polycomb group (PcG) genes, a highly conserved gene family, were first identified for their role in Drosophila body plan segmentation. PcG genes encode a family of epigenetic silencers, which are structured in two multiprotein complexes—polycomb repressive complex 1 and 2 (PRC1 and PRC2). The different components of PRC1 majorly play a role in chromatin compaction, and PRC2 contains a variety of histone methyltransferase, like EZH2, which add methylation marks to histones and results in the silencing of gene expression.

The trithorax group of proteins (TrxG) is another family of proteins involved in the cellular memory system. They were discovered during genetic screens for mutations that rescued segmentation defects in PcG-mutated flies, suggesting that this group of proteins has a role opposite to that of PcG proteins. TrxG proteins function by counteracting the repressive effects mediated by PcG proteins, i.e., mainly, these proteins are involved in adding acetylation marks to the chromatin and leading to transcriptional activation.

By realizing the opposing roles of the polycomb and trithorax group of proteins, one can appreciate how an imbalance in these epigenetic regulators can alter the normal stem cell fate and result in the formation of CSCs [8]. Therefore, a study in this field is extremely useful to develop anti-cancer drugs to correct the disruptions in the PcG–TrxG cellular memory system.

**Epigenetics and Chemoresistance in CSCs**

Although many cancers are initially sensitive to chemotherapy, over time, they develop resistance, and this property is known as chemoresistance. Among the various mechanisms contribute to chemoresistance, epigenetic modifications are one of the important factors that confer the characteristic of drug resistance in CSCs. During cancer progression, DNA methylation and histone post-translational modifications regulate complex gene networks, contributing to tumor growth, metastasis, and drug resistance. Thus, several studies, both in solid tumors and leukemias,
have now recognized the importance of epigenetic modifications in predicting patient prognosis and response to chemotherapy.

Here are a few examples demonstrating the effect of aberrant epigenetic modifications in chemoresistance. EMT is a crucial event that allows tumor cells to metastasize to other organs and is represented by the loss of the membrane protein E-cadherin, which is involved in cell-cell adhesion. E-cadherin loss can occur either as a result of a mutation in its encoding gene CDH1 or due to the silencing of E-cadherin expression by methylation of the E-cadherin promoter resulting in transcriptional silencing [3]. Therefore, cells with both CSC traits and an EMT-like phenotype are considered to be more resistant to chemotherapeutic treatments, implying that the acquisition of CSC and EMT traits by epigenetic modifications traits most likely influence tumor cell’s response to therapy. In addition, increased drug resistance in CSCs is often due to enhanced expression of drug efflux transporters, such as the ATP-binding cassette family of proteins [3]. These drug transporters use ATP to transport drugs out of cells against a concentration gradient. Studies have shown permissive (active) histone modifications result in enhanced expression of drug efflux transporters contributing to chemoresistance.

Thus, it becomes essential to elucidate the epigenetic mechanisms that contribute to the chemoresistance property of CSCs, without which developing drugs to combat chemoresistance would be impossible. As a result, epigenetic therapy targeted specifically at CSCs, either alone or in combination with chemotherapy, becomes beneficial in the treatment of drug-resistant tumors to produce a long-lasting response and prevent tumor relapse (Figure 7).

**Epigenetic Therapy in CSCs**

Conventional therapies against cancer, including chemotherapy and radiotherapy, have several limitations owing to treatment failure and cancer recurrence. These limitations include the toxicity of these drugs and the relapse of cancer due to the emergence of
chemoresistant tumors. The latter majorly results from the escape of CSCs from therapeutic agents. Thus, it becomes vital to discover drugs that specifically target and eliminate CSCs. There are multiple ways by which CSCs can be targeted. Some of the methods include targeting the surface biomarkers, key signaling pathways involved in CSCs self-renewal and differentiation, tumor microenvironment that sustains CSC properties, and drug-efflux pumps involved in chemoresistance and inducing apoptosis and differentiation in CSCs.

Amidst these therapies, epigenetic therapy has gained significant attention in recent years. Since epigenetic alterations are reversible, active research is ongoing to explore novel drug targets to destroy CSCs. As epigenetic mechanisms play a significant role in modifying stem cell characteristics in cancer cells, targeting components of these epigenetic pathways would aid in the eradication of both CSCs and the overall tumor population. Indeed, many of these treatment approaches aim to induce CSC differentiation, make the CSCs susceptible and sensitize them to chemotherapy, with the ultimate goal of curtailing tumor recurrence and enhancing patient survival.

Epidrugs are classified based on the enzyme that they target. The US Food and Drug Administration (FDA) has approved two classes of epigenetic drugs—DNA methylation inhibitors (DNMTi) and histone deacetylase inhibitors (HDACi). DNMTi removes methyl

**Figure 7.** Chemoresistance in CSCs. Conventional therapies like chemotherapy and radiotherapy are not specific and fail to eradicate CSC. The CSCs which remain unharmed during the therapy with their ability of self-renewal contribute to the tumor relapse. Therefore, therapies that specifically target CSCs would be beneficial and promote the regression of tumor.

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Figure 8. General mechanism of epigenetic drugs like DNMTi and HDACi. As the tumor suppressor (TS) genes are often silenced in cancer, DNMTi inhibits DNA methyltransferase (DNMT) and helps reactivate silenced TS genes. Histone deacetylases (HDACs) remove acetylation marks rendering the gene inactive, thus HDACi inhibits HDAC contributing to the reactivation of TS genes. Similarly, oncogenes are activated in cancer. Histone acetyltransferases (HATs) add acetylation marks and result in the activation of oncogenes. Thus, HATi inhibits HATs and helps in the inactivation of the oncogenes.

groups on the DNA and results in gene expression, while HDACi removes acetylation marks from the histone and silence the gene. Depending on the type of epigenetic modification involved, DNMTi or HDACi are used to revert to the normal epigenetic modification pattern (Figure 8).

DNMT Inhibitors (DNMTi)

5-azacitidine and 5-aza-2'-deoxycytidine (decitabine) are the two DNMTi approved by FDA and used to treat patients with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) [3]. They are cytosine analogs that disrupt the activity of DNMTs by interfering in DNA structure and covalently binding to enzymes. DNMT inhibition results in the loss of methylation mark, which ultimately inhibits DNA methylation. Thus, DNMTi can be used to reactivate aberrantly silenced tumor suppressor genes and restore their expression and functional activity. Studies have shown that DNMTi reduces the proliferation and tumorigenic capabilities of CSCs by upregulating the differentiation genes and downregulating the genes involved in self-renewal. Furthermore, DNMTi has also been shown to reprogram CSCs and sensitize them to chemotherapeutic drugs. For instance, in the case of ovarian cancer, low doses of SGI-110, a newer DNMT inhibitor, re-sensitized these cells to platinum (chemotherapeutic drug) [9].
Histone Deacetylase Inhibitors (HDACi)

HDACs remove acetylation marks from histone tails to generate a condensed chromatin state. Aberrant gene silencing in tumors is frequently associated with abnormal histone deacetylation. The HDAC inhibitors are of two types—pan HDAC inhibitors (target all HDACs) and class-specific inhibitors (target class-specific HDACs). Four HDACi, have been approved by the US FDA. Vorinostat (SAHA), belinostat, romidepsin, and panobinostat are the FDA-approved HDACi [3]. Similar to DNMTi, HDACi induces the differentiation of CSCs and restricts their proliferative ability. HDACi is also known to reverse the EMT, and studies have proved the effectiveness of HDAC inhibitors in decreasing CSC invasiveness and tumor metastasis [3].

Combinatorial Therapy

While epigenetic inhibitors have been evaluated preclinically and clinically as single agents, subsequent research has indicated that they are more effective when used in combination with other therapies. DNMT and HDAC inhibitors are a typical combination used in a variety of epigenetic therapy. Pathania et al. demonstrated that the combination of azacitidine (DNMT inhibitor) with butyrate (HDAC inhibitor) greatly reduced the CSC population in breast cancer [3]. Also, in phase I/II clinical study, a combination of azacitidine with the HDAC inhibitor entinostat at low dosages showed persistent and favorable responses in patients with treatment-resistant non-small cell lung cancer (NSCLC) [3]. In recent years, a growing number of studies have described the use of epigenetic drugs, in combination with traditional chemotherapeutics, to re-sensitize resistant CSCs to drugs or prime cancer cells for subsequent therapies [10]. Epigenetic therapy has also been used, in combination with immunotherapy, indicating that using epigenetic drugs along with immunotherapy can enhance the reversal of immunological tolerance in cancer cells, including CSCs [10].
EZH2 Targeted Therapy

In addition to the above-mentioned epdrugs, enhancer of zeste homolog 2 (EZH2) inhibitors, tazemetostat is FDA approved for the treatment of relapsed or refractory follicular lymphoma [11]. EZH2, a subunit of PRC2, catalyzes trimethylation of Lys-27 residue in histone 3 (H3K27me3). Alteration of methylation induced by EZH2 is evident in multiple cancers, including solid tumors and hematological malignancies. Additionally, overexpression of EZH2 is associated with poor prognosis, signifying its role in tumorigenesis. Thus, considering the role of EZH2 in cancer progression, metastasis, drug resistance, and maintenance of CSCs’ self-renewal properties, it has become the hotspot of research, making it a potential target for cancer therapy. Several other EZH2 inhibitors are investigated, and are in pre-clinical trials or phase 1/2 clinical trials. A combinatorial therapy, involving EZH2 inhibitor, tazemetostat, and PD-1 blockade, pembrolizumab, to treat advanced non-small cell lung cancer is in phase 1/2 clinical trials (NCT05467748). Therefore, EZH2 targeted therapy is emerging as a novel approach for cancer treatment.

Case Report Signifying the Application of Epigenetic Therapy

In order to understand the practical application of epigenetic therapy in cancer, a study was conducted by a research group at Johns Hopkins Kimmel Cancer Centre [12]. In this study, 45 late-stage non-small-cell lung cancer patients were subjected to a combination of epigenetic drugs, azacytidine (DNMTi) and entinostat (HDACi). Each patient received azacytidine for nine days and entinostat for two days a month. The team performed a follow-up on patients who had received epigenetic therapy, and the results surprised them. Since most of the patients who received the therapy were in late-stage lung cancer that had metastasized to different organs and had stopped responding to conventional chemotherapy, the team assumed the patients would have passed
away. In contrast, the results were not what they expected, instead, many of the lung cancer patient were still alive and had an extended life expectancy. In a few patients, there was a reversal in gene methylation of the key genes involved in cancer, and their life expectancy was increased compared to the remaining untreated patients. In two patients, dramatic tumor shrinkage was observed. There was a complete response in one patient, where the tumor proliferation stopped, and the patient survived longer. In another patient where the lung tumor had metastasized to the liver, upon treatment with epigenetic therapy, the metastases were cleared, and there was a significant reduction in the original lung tumor. Therefore, these results were a breakthrough and were virtually unheard of earlier, which excited the team. The promising results of epigenetic therapy in cancer treatment hence paved the way for further investigations.

These findings, however, instill a question of how epigenetic therapy helps in the treatment of cancer. Researchers at John Hopkins have suggested that epigenetic therapy does not aim to kill cancer cells directly like chemotherapy rather, they reprogram the gene expression pattern of the cancer cells so that cancer cells lose their potential for uncontrolled growth (Stephen Baylin, M. D). Higher doses of the drug killed cancer cells, but at lower doses, the cancer cells were reprogrammed to behave like normal cells. Thus, this study stands out as it demonstrates the effectiveness of epigenetic therapy in cancer treatment [12].

Limitations of Epigenetic Therapy and the Need for Epigenetic Biomarkers

This complex cancer heterogeneity influences the therapy responsiveness and can explain the reason for the failure of some current cancer therapies since they are designed to treat all patients with a standard treatment that does not consider the unique profile of each patient (Figure 9).

Despite the promising results of epigenetic therapies, majority of drugs lack target specificity [13]. For instance, DNMTi tar-

Complex cancer heterogeneity influences the therapy responsiveness and can explain the reason for the failure of some current cancer therapies since they are designed to treat all patients with a standard treatment that does not consider the unique profile of each patient.
Figure 9. Limitations of epigenetic therapy. Epigenetic drugs have a few limitations, including off-target effects and drug toxicity, low specificity, poor solubility, low bioavailability, poor pharmacokinetic effects, low stability, poor permeability, and pleiotropic effect.

Getting DNA methylation are not locus-specific and thus can result in large-scale gene expression changes in the genome. They might not only induce re-expression of improperly silenced genes in cancer but can result in adverse effects by transcriptional activation of oncogenes that promote tumorigenesis. Such unintended outcomes of these drugs restrict them from being used for prolonged periods. Thus, the major limitations of the drugs are low specificity and pleiotropic effects, as these drugs are not selective in inhibiting different isoforms of DNMTs and HDACs. Other limitations of drugs are poor solubility, permeability, and poor pharmacokinetic effects with low stability and bioavailability. Due to these reasons, some drugs have failed the clinical trials and are withdrawn owing to their off-target effects and drug toxicity, hence limiting their clinical application. Therefore, the existing challenge is to develop new anti-cancer drugs conjugated with cancer-specific biomarkers, with enhanced specificity and stability as well as a site-specific delivery system capable of optimizing treatment efficacy while reducing toxicity.

Nevertheless, ‘omics’ technologies are expanding our understanding of cancer genetics and epigenetics, enabling the identification of patient-specific biomarkers and the delineation and classification of distinct cancer subtypes, laying the foundation for personalized-targeted therapy. Validation of epigenetic biomarkers will aid in the diagnosis, drug response prediction, and even-
tually, the identification of patients, who are responding to the therapy. Newer therapies that specifically target epigenetic writers and readers are emerging with the potential to be used in personalized cancer medicine [10].

Conclusion and Future Prospects

Cancer chemoresistance and recurrence are two of the most pressing issues in conventional cancer treatment. CSCs are cells with the ability to self-renew, differentiate, metastasize and exhibit treatment resistance. Given the role of CSCs in therapeutic resistance, understanding the characteristics of CSCs among other tumor cells, as well as the factors influencing their formation, will aid in the development of targeted cancer drugs. Epigenetic modifications play a crucial role in the biology of CSCs. Polycomb and trithorax groups of proteins are key participants of the cellular memory system. An imbalance in this system is associated with the formation of CSCs. Abnormal PcG–TrxG expression can be used in the diagnosis of cancer as well as to predict the prognosis of cancer. Also, drugs aimed at restoring the balance of this system would eliminate CSCs and overcome chemotherapy resistance. Hence, future research that sheds light on the processes through which PcG–TrxG-mediated pathways converge to regulate cancer could provide new insights. Epigenetic inhibitors have shown assuring results in clinical trials in many studies. However, before administering epigenetic therapy to treat cancer, it becomes essential to understand certain key questions like the optimal dose of drugs for single and combined therapies and the sequence of delivery of drugs in combined therapies.

Overall epigenetic therapy may be an appealing way to target CSCs if three conditions are met:

- Identification of CSC specific targets
- Appropriate pharmacokinetic/pharmacodynamic profiling
- Combination with conventional chemotherapy

Future challenges should include increasing specificity and effi-
ciency in targeting CSCs in order to avoid the toxicity of normal tissue stem cells, determining the optimal doses required for single and combined and the sequence of delivery of drugs in combined therapies, and developing new drug delivery and retention strategies within CSCs. Recent research has shown nano-drug-mediated drug delivery to be an effective approach in the targeted delivery of epigenetic drugs allowing enhanced stability and retention of epiprides in cancer treatment [14]. In addition, new technologies like CRISPR-Cas9-based techniques have been investigated in targeted epigenome editing [10]. The fundamental idea that underlies CRISPR/Cas9-mediated epigenome editing, involves combining the Cas9 protein with a transcription repressor or activator domain, known as an epigenetic effector (epieffector). Firstly, the Cas9 is deactivated (dCas9), hence lacks nuclease action, and serves as a DNA-binding domain. Experimental trials have been performed by fusing the dCas9 protein to the catalytic core of human acetyltransferase p300, wherein the fusion protein catalyses histone H3 lysine 27 acetylation (H3K27ac) at a target site resulting in transcriptional activation [15]. Therefore, such methods of epigenetic targeting via CRISPR are in trials and appear to be a promising approach in the future for targeted epigenetic therapy. Overall, a detailed study on epigenetics and CSCs would open new avenues to understand and develop drugs aimed at eradicating CSCs and hence treating cancer.

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Suggested Reading


Address for Correspondence
Akshatha E. Nagarkatte
Centre for Human Genetics
Biotech Park
Electronics City Phase 1
Bengaluru 560 100
Email: akshatha99@gmail.com
Prathibha Ranganathan
Centre for Human Genetics
Biotech Park
Electronics City Phase 1
Bengaluru 560 100
Email: pranganathan@chg.res.in