Combining Histone Modification Therapy and Immune Checkpoint Inhibitors for Tumor Treatment

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Abstract. Histone modifications play a crucial role in chromatin packaging and gene regulation during cell development. Differentiated alterations in histone modifications have the potential to adjust gene expression patterns, affecting tumorigenesis and cancerogenesis. Immune checkpoint inhibitors are effective treatments for attacking tumors and cancer cells; they are able to block the binding of protein receptors, allowing for the destruction of malignant cells through T-cells or NK cells. By combining the two therapies, tumor growth is prevented, and T-cells can more effectively target the malignant cells, resulting in lower doses of immune checkpoint inhibitors and lower risks of autoimmune diseases. This review discusses existing histone modifications and their roles in solid tumor development to identify therapeutic drugs that can reorganize gene expression patterns. Furthermore, this review summarizes existing research on immune checkpoint inhibitors and how a combination of both techniques can lead to more effective treatments. Future research may focus on other epigenetic mechanisms that can be paired with immune checkpoint inhibitors to remove tumors. Studies may also research the effects of pairing histone modifications with other immunotherapies for cancer treatment.

Keywords: Histone modifications, Immune checkpoint inhibitors, Cancer.

1. Introduction

Cancer is a complex disease that involves the abnormal growth and uncontrolled spread of cells. Currently, cancer has the potential to become the leading cause of death globally, with indications that it is still rapidly growing. As of 2020, cancer accounted for nearly 10 million deaths [1]. The current rate of cancer development every year is approximately 450 in every 100,000 men and women, and the current rate of death from cancer every year is about 150 in every 100,000 men and women [2]. Although there is an estimated increase of 6 million cancer survivors for 2030, the number of recorded rates of cancer incidences has been slowly increasing [3]. Therefore, it is crucial that more research is applied to how scientists can effectively regulate mechanisms of gene expression for the development of better therapies to fight cancer.

Epigenetic modifications are changes in how the body reads and transcribes gene sequences. Unlike normal genetic changes, epigenetic modifications are reversible and do not change the specific nucleotide sequence [4]. Because of its ability to modify the expression of certain gene sequences, it has been identified to have a significant impact on carcinogenesis and tumorigenesis. Common modifications include DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs. Through these modifications, researchers have been able to discover many epigenetic drugs-with several that have gained approval from the FDA. However, there are still many drugs that are undergoing trials and testing for approval, suggesting that further research is necessary to determine the exact mechanisms of these drugs, to facilitate their clinical applications.

Thus, this paper aims to elucidate the current roles of histone modifications-including histone acetylation, methylation, phosphorylation, and ubiquitination-and immune checkpoint inhibitors of CTLA-4, PD-1, and PDL-1 in treating tumorigenesis. Furthermore, FDA-approved drugs targeting these histone biomarkers and immune checkpoints are discussed.

2. Histone Modifications

Histone modification is a type of epigenetic mechanism that contributes to certain activation or repression of gene sequences through covalent post-translational modifications, where chromatin structure is altered, and modifiers of histones are recruited [5]. Histones are proteins that serve as a base for DNA strands to wrap around. Histones are positively charged, while DNA is negatively charged due to its phosphate groups, allowing there to be a strong electrostatic attraction. For every packaged DNA, there are eight core histones that it wraps around. These core histones consist of the basic 4-H2A, H2B, H3, and H4, which are duplicated to form a total of eight. Histone modifications occur when certain chemical groups attach to the N-terminus tails of these core histones (Fig. 1). There are three protein groups that contribute to the addition or removal of histone the chemical groups. The proteins are classified as writers (add marks), erasers (remove marks), and readers (recruits and recognizes marks) [6]. The chemical groups commonly modulated include acetyl groups, methyl groups, phosphate groups, and ubiquitin moieties, which make up modifications known as histone acetylation, methylation, phosphorylation, and ubiquitination (Fig. 1) [7].

The first of these modifications to be discovered were acetylation and methylation, discovered in 1964 by Allfrey et al. [8]. Allfrey set the precedent for future research and the discoveries of histone phosphorylation, ubiquitination, sumoylation, and many more modifications. All of these changes play critical roles in the regulation of gene expression, which is crucial in cancerogenesis and tumorigenesis.

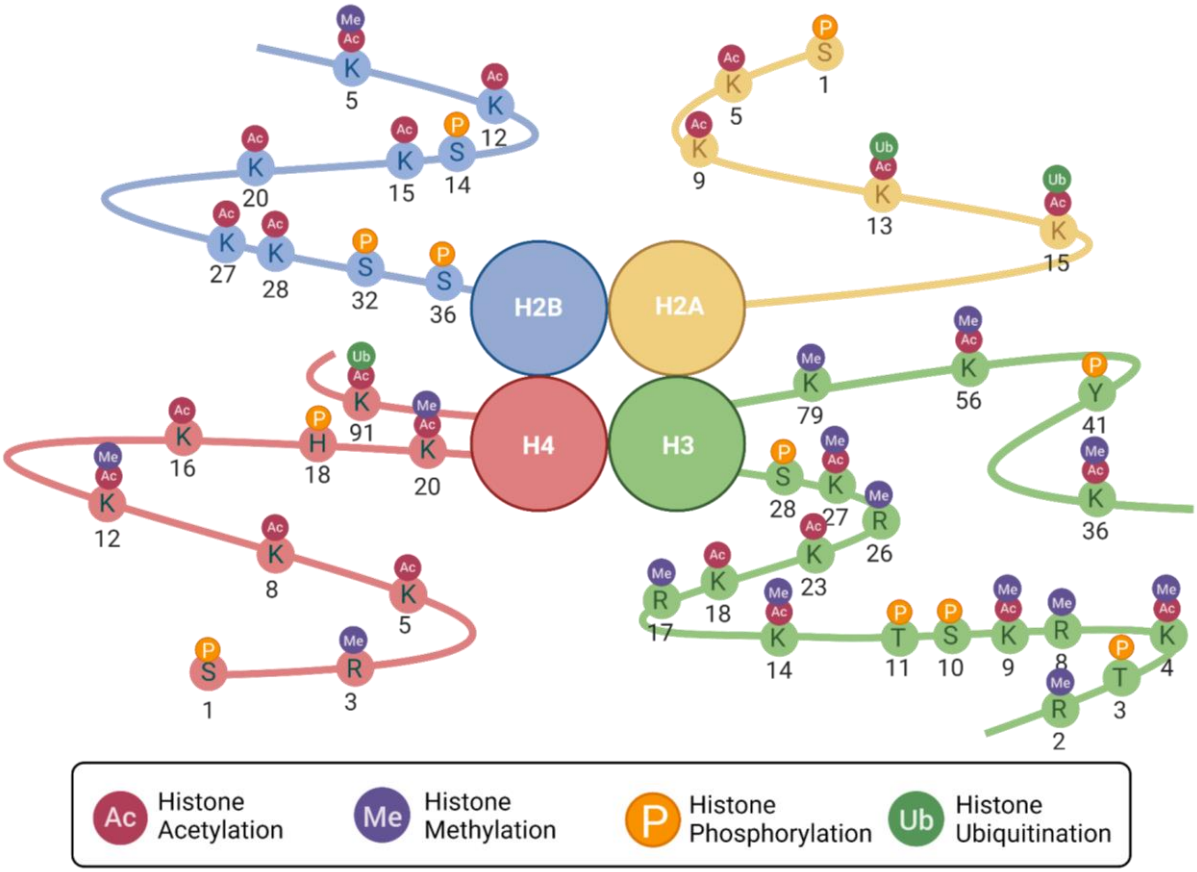


Figure 1. Overview of histone modifications involved in cancerogenesis. Various modulations could occur along the N-terminus tails of the four core histones H2A, H2B, H3, and H4. Modification marks consist of Ac (histone acetylation), Me (histone methylation), P (histone phosphorylation), and Ub (histone ubiquitination). Along the tails, certain amino acids can be modified more than once from different chemical groups, including H3K27ac/me, H3Kac/me, H4K91ac/ub, and H2AK13ac/ub. The most common modifications are acetylation and methylation, which usually occur on lysine sites. Figure credit: original. Made in BioRender.com.

2.1. Histone Acetylation

Histone acetylation occurs on lysine residues of the N-terminus tails of histones. The lysine group, NH2, which is positively charged, helps to neutralize the strong attraction to the negatively charged DNA backbone [8]. As a result, the histones and DNA are packaged together more loosely, leading to increased efficiency in gene expression. The process of adding acetyl groups (writer) is managed by histone acetylase enzymes, and the process of removing acetyl groups (eraser) is regulated by histone deacetylases (HDACs) [9].

Numerous studies have shown that changes in histone acetylation can lead to cancer development. Enhanced activity of HDACs has been demonstrated to increase the chances of tumor development as oncogenes are overly expressed. Possible treatments for this have been identified through specific HDAC inhibitors (HDACi)-which function by promoting cell-cycle arrest and apoptosis [10]. Currently, there are four FDA-approved HDAC inhibitors: vorinostat, romidepsin, belinostat, and panobinostat (Table 1 and Fig. 2) [11].

Table 1. Overview of the four FDA-approved HDAC inhibitors [11, 12]

HDACi Type	FDA Approved Indication	Classification	Clinical Trials
Vorinostat (SAHA)	Cutaneous T-Cell Lymphoma	Hydroxamate	Multiple myeloma Mesothelioma Neuroblastoma Glioblastoma Breast cancer Lymphoma Sarcoma
Romidepsin (FK-228)	Cutaneous T-Cell Lymphoma Peripheral T-Cell Lymphoma	Cyclicpeptide	Ovarian cancer Hepatocarcinoma Acute myeloid leukemia
Belinostat (PXD-101)	Multiple melanoma	Hydroxamate	Multiple myeloma Metastatic melanoma Prostate cancer Soft tissue sarcoma Acute myeloid leukemia
Panobinostat (LBH589)	Cutaneous T-Cell Lymphoma	Hydroxamate	Cervical cancer Ovarian cancer Breast cancer

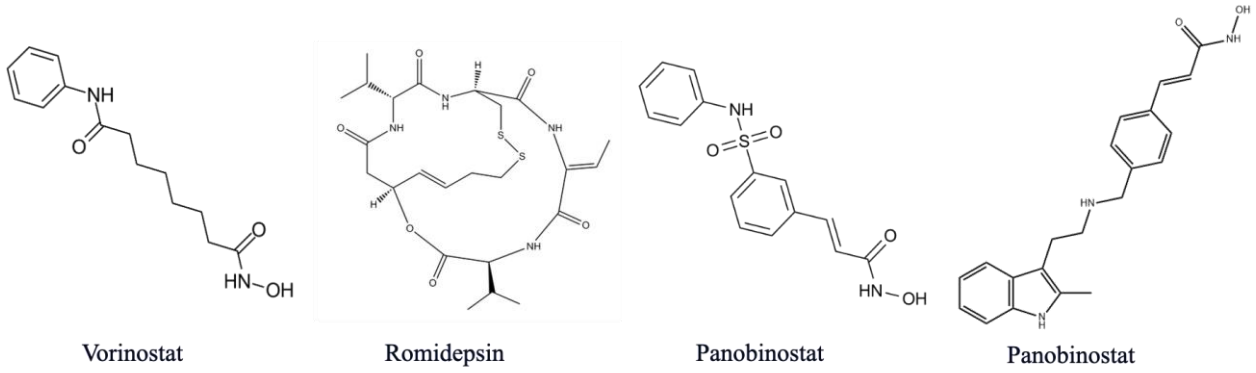


Figure 2. Chemical structure of the four FDA-approved HDAC inhibitors.

2.2. Histone Methylation

Histone methylation has been discovered to be a more complex, yet more durable and stable posttranslational modification than histone acetylation. Similarly to histone acetylation, histone

methylation occurs on lysine residues of the amino termini of core histones. However, histone methylation can take place both at a lysine (K) residue or at an arginine (R) residue. When methylation takes place on lysine, it can either mono-methylate, di-methylate, or tri-methylate the amino acid. On the other hand, when methylation involves an arginine, it can only mono-methylate or di-methylate it. The abundance and variety of these modifications serve to create an incredibly diverse array of methylation patterns, leading to the ability of histone methylations to regulate gene expression. Thus, histone methylation is a very context-dependent epigenetic mark [13].

Research has shown that changes in methylation patterns have had a significant impact on tumor development. In particular, the histone mark H3K27me3 is one of the commonly known disordered modifications that cause aberrant gene expression and cancer. Changes in this mark are usually associated with mutations in the gene encoding enhancer of zeste homologue 2 (EZH2), which functions as a histone methyltransferase. Despite the current understanding of mutated histone methyltransferases, a more comprehensive elucidation of the abnormal patterns in methylation is necessary to develop new therapies or treatments.

2.3. Histone Phosphorylation

Histone phosphorylation occurs on the N-terminus tails of core histones. The amino acids that are involved with phosphorylation often include serine, threonine, and tyrosine residues. Contrary to histone acetylation, histone phosphorylation confers a negative charge to the core histone, neutralizing the positive charge on it and increasing gene expression [14].

Similar to histone acetylation and histone methylation, differentiated histone phosphorylation patterns can also lead to tumor development. One well-known modification, H3S10P, is related to increased transcription levels. Other histone marks, including H4S1 and H3.3 have been shown to also mediate gene expression levels. Although more research is still needed in this field, histone phosphorylation has shown a significant impact on tumorigenesis and has indicated the potential to provide new insights into novel therapies [11].

2.4. Histone Ubiquitination

Histone ubiquitination involves the addition of a ubiquitin moiety, consisting of a 76-amino acid polypeptide, to specific lysine residues [15]. Ubiquitination of lysine can either be mono-ubiquitinated or poly-ubiquitinated-with the number of moieties contributing to different functions [7].

Research has identified that histone ubiquitination is closely tied to histone methylation. For instance, in order for methylation of H3K4 and H3K79 to take place, ubiquitination along the core histone H2B must first occur. This suggests that ubiquitination of histone residues may also play a role in tumorigenesis [7].

3. Immune Checkpoint Inhibitors

Immune checkpoints are a crucial part of our body's immune systems. Their role is to prevent immune responses from becoming overactive, preventing the destruction of healthy and functional cells in the body. Immune checkpoints normally function by acting like a switch to start an immune response [16]. When proteins on the receptors of specific anti-cancer immune cells bind to partner receptors on other cells, the immune checkpoints release "off" signals that prevent immune cells from destroying the partner cell. Cancer takes advantage of this mechanism to perform immunosuppression. Tumor cells will bind their receptors to immune cells, keeping them from killing the tumor cells in the body [17]. In 1995, Jim Allison et al. labeled the immune checkpoint molecule cytotoxic T-lymphocyte associated protein 4 (CTLA-4) as the first potential future anti-cancer therapy target, and in March of 2011, the FDA approved CTLA-4 for the treatment of advanced melanoma [18]. Since

then, many more immune checkpoint molecules have been discovered-with currently a total of seven treatments approved by the FDA (Table 2) [19].

Immune checkpoint inhibitors (ICIs) are a type of immunotherapy that has revolutionized the treatment of cancers and tumors. ICIs- which consist mainly of antibodies- work by blocking the receptors from binding with each other, preventing the "off" signal from being sent, and allowing immune cells to destroy harmful cells [12].

Table 2. FDA-approved immune checkpoint inhibitors [20]

Immune Checkpoint Inhibitor Type	Mechanism	FDA Approved Indications
Ipilimumab	Inhibits CTLA-4	Colorectal cancer Hepatocellular carcinoma Non-small cell lung cancer Renal cell carcinoma
Cemiplimab	Inhibits PD-1	Basal cell carcinoma Cutaneous squamous cell carcinoma Non-small cell lung cancer
Nivolumab	Inhibits PD-1	Colorectal cancer Squamous cell carcinoma Hepatocellular carcinoma Hodgkin lymphoma Head and neck squamous cell carcinoma Non-small cell lung cancer Renal cell carcinoma
Pembrolizumab	Inhibits PD-1	Breast cancer Colorectal cancer Cutaneous squamous cell carcinoma Endometrial carcinoma Gastric carcinoma Hepatocellular carcinoma Hodgkin lymphoma Merkel cell carcinoma Non-small cell lung cancer
Atezolizumab	Inhibits PD-L1	Breast cancer Hepatocellular carcinoma Non-small cell lung cancer Small cell lung cancer Urothelial carcinoma
Avelumab	Inhibits PD-L1	Merkel cell carcinoma Renal cell carcinoma Urothelial carcinoma
Durvalumab	Inhibits PD-L1	Non-small cell lung cancer Small cell lung cancer Urothelial carcinoma

3.1. Immune Checkpoint Inhibitors and T-cells

T lymphocytes (T-cells) are immune cells that are highly effective at killing malignant cells. They produce and release cytokines that attack cancerous cells to regulate immune responses [21]. Cancer has the ability to inactivate these killer cells when it binds to the receptors of T-cells through immune checkpoint molecules. Two commonly researched immune checkpoint molecules that engage with T-cells are CTLA-4 and PD-1, which act as negative regulators of T-cell function. As previously mentioned, CTLA-4 was the first to be discovered as an immune checkpoint molecule and has proven

to be associated with immunosuppression in cancer. The treatment drug that blocks CTLA-4 is ipilimumab, also known as Yervoy [22]. Another highly researched example is the interaction between the receptor PD-L1 on tumors and PD-1 on T-cells. When these two proteins bind together, the T-cell receives the "off" signal and does not destroy the harmful tumor cell [17]. Researchers have discovered multiple treatments for this checkpoint molecule including atezolizumab, avelumab, and durvalumab [22].

3.2. Side Effects of Immune Checkpoint Inhibitors

Although ICIs have been proven to have high efficacy towards cancer treatment, they also impose a risk of autoimmune diseases. This occurs when normal T-cells misread what is harmful in an individual's body and proceed to attack healthy organs and cells that are vital to survival. Autoimmune attacks can take place anywhere throughout the body. For example, it has been shown to be a significant contributor to pneumonitis, colitis, skin rashes, nerve or brain inflammation, liver inflammation, and more. Although there is currently an effective treatment for autoimmune diseases by using steroids, autoimmune diseases have also proven to be long-lasting and deadly. Thus, to minimize the chance of these life-threatening autoimmune attacks, research has turned to combining ICIs with other cancer therapies [16].

One such combinatorial method is with histone modification therapy and ICIs. HDAC inhibitors and other tumor suppressing histone modifying drugs have demonstrated significance in preventing tumor growth. ICIs have shown high efficacy in getting rid of tumors with low proliferation rates. Therefore, a combination of these two therapies allows T-cells to attack non-growing tumor cells without high doses of ICIs, resulting in lower chances of autoimmune diseases.

4. Conclusion

Histone modifications, such as methylation, acetylation, phosphorylation, and ubiquitination, play a significant role in the modulation of gene expression. Through drug targeting on specific histone biomarkers, the rate of transcription can be adjusted and mediated, allowing for malignant cell growth to be effectively regulated. Immune checkpoint inhibitors, which function by binding to receptors on the surface of cells, enable T-cells to properly attack malicious groups of cells through the release of cytokines. By combining histone modifications and ICIs, treatment for tumorigenesis and cancerogenesis can become more efficient as T-cells are able to destroy tumors without combatting continued growth. Future clinical studies on combinatorial methods with epigenetics and ICIs will further elucidate the extent to which these therapies can be used to treat cancer.

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