Epigenetic Approaches to Boost the Efficacy of CAR T-Cell Therapy in Treating Carcinomas

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Abstract

Cancer is one of the most common and deadly diseases known to humans. The application of immunotherapies to treat solid tumors (carcinomas) has been a topic of discussion and research. A common limitation seen throughout this development is T-cell exhaustion, which this paper aims to explain, and discuss how this problem can be neutralized using Epigenetic Modifications. Therefore, this paper explores how epigenetic modifications can be leveraged to overcome T-cell exhaustion and enhance the efficacy of CAR T-cell therapy in treating solid tumors. CAR T-cell therapy has already been developed and applied to patients, however it is still a relatively new immunotherapy and has some limitations, especially for the treatment of solid tumors. Overcoming T-cell exhaustion is important because it revives the CAR T-cell therapy from a state of exhaustion, to an active state, allowing to accurately identify and eradicate cancerous cells. There have been significant research studies that have used histone modifications, DNA methylation, demethylation, and acetylation to decrease T-cell exhaustion and improve T-cell treatment. This paper amplifies the importance of leveraging epigenetics to increase the efficiency of T-cell therapy for the treatment of cancers.

Introduction

CAR T-Cell therapy is a highly regarded cancer immunotherapy that serves as a treatment for hematological malignancies (Sterner and Sterner). Immunotherapies are a form of therapy that leverages a patient's immune system to help attack and deter the development of the patient's cancer (Esfahani et al.) Hematological malignancies are blood cancers, also known as B-cell cancers. The FDA has approved CAR T-cell therapy to treat multiple B-cell cancers, such as follicular lymphoma, diffuse large B-cell lymphoma, high-grade B-cell lymphoma, and primary mediastinal lymphoma. This therapy consists of 4 key components. Each component has a unique and important function. Together, all 4 components communicate and interact together through complex yet efficient processes to produce a successful CAR T-Cell therapy treatment. The 4 main components include the Extracellular target Antigen-Binding domain, hinge region, transmembrane domain, and multiple intracellular signaling domain. The extracellular target Antigen-Binding domain is a component of the CAR protein, which is responsible for identifying the specific target antigens to attack (G. Zhang et al.). The hinge region connects the Extracellular Target Antigen-Binding Domain with the transmembrane domain (Hudecek et al.; Jensen and Riddell). The hinge region allows for flexibility and overcoming physical barriers. The transmembrane domain enables the receptor to be securely attached to the T-cell's membrane. By ensuring stability by anchoring the CAR protein to the T cell membrane. The intracellular domain's function is to transmit signals through the T-cell once the CAR protein binds to its target antigen (Gross et al.). The signals are meant to initiate T cell activation. The process of how CAR T-cells work can be explained in a few steps. Essentially, CAR T-cell therapy is where the patient's T-cells are leveraged to attack and kill cancer tumor cells (*CAR T Cells*). As the CAR protein identifies specific antigens on the tumor cells and binds to them. Once this has been achieved, the T-cell can swiftly eliminate the tumor cells. To summarize, CAR T-cell therapy is an immunotherapy developed to treat hematological malignancies. The therapy consists of a CAR protein which is made up of 4 key components, and the patient's own T-cell. The CAR protein targets specific antigens on the tumor cells, and the T-cell kills the targeted cells. This therapy has great potential in treating solid tumors with its simple yet powerful structure, high levels of efficacy and accuracy, and the ability to modify this structure.

With all this success, it is important to note that this therapy has faced many issues, but also shown great potential in the treatment of solid tumors such as; glioblastomas, sarcomas, neuroblastomas, adenocarcinoma, melanoma, and retinoblastomas (Nazha et al.; Castelletti et al.; C. K. Tang et al.; Arteaga). A large issue is the patient's tumor microenvironment (TME), which is the surrounding environment that the tumor thrives in (Daei Sorkhabi et al.; Anderson and Simon). The tumor microenvironment consists of many barriers and obstacles which are poorly vascularized, foster hypoxic conditions, and negatively control and restrict the T-cell infiltration (Kakarla and Gottschalk; Robbins et al.) Hypoxia is when there is a lower quantity of oxygen in your blood tissue. Vascularity represents the veins which bring blood to the tissues. Blood contains oxygen, however the TME is poorly vascularized. This increases hypoxia and consequently makes the TME a very difficult place to survive in. Other limitations for the treatment of CAR T-cells include decreased T-cell proliferation, T-cell exhaustion, and antigen heterogeneity (Rodriguez-Garcia et al.). Decreased proliferation is when fewer T-cells are being multiplied, which is an event that occurs when a T-cell recognizes a target antigen. Antigen heterogeneity is where there is a greater variation in the tumor's antigens, making it difficult for the CAR T-cells to eliminate the cancer. Additionally, there are a variety of components in the vast TME that prevent the CAR T-cell therapy from eliminating the cancerous cells. Overall, the TME is a very harsh environment that fosters hypoxia, and contains components which decrease T-cell proliferation, increase T-cell exhaustion, and contribute to antigen heterogeneity, which makes it difficult for the CAR T-cell therapy to treat solid tumors.

T-cell exhaustion is where the CAR T-cell is exposed to the target antigen for a long time under the harsh conditions of the tumor microenvironment (B. Zhang et al.). The tumor microenvironment contains myeloid-derived suppressor cells (MDSCs) (Yang et al.). MDSCs are cells in the TME with immunosuppressive properties. This means these cells combat the CAR T-cells. Additionally, these cells can produce reactive oxygen species (ROS), which are toxic to CAR T-cells. Inhibitory cytokines are molecules that are created from the immunosuppressive cells in the TME (Milone et al.; X.-Y. Tang et al.; Ramos et al.). These molecules decrease and prevent the CAR T-cell proliferation, which can reduce the efficiency and power of the treatment. Proliferation is a process where the CAR T-cells multiply when the target antigen is located. With a decreased quantity of CAR T-cells, less damage is done to the tumor. The inhibitory cytokine also impairs the CAR protein's function in identifying the target antigens. This allows the cancerous cells to evade detection. Inhibitory signaling pathways are essentially pathways within the TME that suppress the T-cell function. A way researchers have found to combat this issue is epigenetic modifications (Saitakis). A core principle of epigenetic modifications is histone methylation. Histone methylation is where certain methyl groups are added to the histone H3 in specific locations. Histones are proteins that give support to the chromosome by wrapping around it. DNA methylation is where methyl groups are added to DNA molecules. This limits how easily the gene can be read and expressed, but as a result, silences the gene. The epigenetic modification creates a genetic disruption that prevents the gene from becoming silenced.

To summarize, CAR T-cell therapy consists of the CAR protein, which helps identify antigens on cancer cells, and the T-cells, which attack these cells. However, an increasingly imminent threat to this therapy occurs from a condition called T-cell Exhaustion, which inhibits the T-cell from being as effective and accurate as before. Furthermore, scientists and researchers have found a strategy to prevent this by implementing epigenetic modifications on the CAR T-cell, which change the histone methylation order and components in certain areas. Therefore, this paper will examine how epigenetic modifications can be utilized in CAR T-cell therapy to combat solid tumors.

Understanding T-Cell Exhaustion

T-cell exhaustion is a condition that makes the T-cell ineffective in the tumor microenvironment and its ability to neutralize tumors (Blank et al.). This exhaustion primarily arises from prolonged activation in the tumor microenvironment in order to neutralize threats, especially persistent antigens. Persistent antigens force the T-cell to first activate, then proliferate, and finally produce cytokines, molecules. Proliferation is the term for a process where T-cells multiply at a rapid rate to respond to an antigen, infection, or tumor microenvironment component that is becoming an obstacle and preventing the T-cell from completing its task. The cytokine molecules are signaling molecules that are produced by T-cells. Their role is to communicate with other T-cells to aid the T-cell that produced the cytokine molecules. This results in a more effective form of neutralizing the antigen, obstructing component, or infection.

Overall, there are a variety of factors and reasons that cause T-cell exhaustion in tumors. The general reasoning is that this condition occurs when the T-cell has been activated, proliferating and producing cytokine molecules at high rates (Brownlee). An environment that is heavily associated with CAR T-cell exhaustion is the TME (Zhu X, et al.). This can include components outside the tumor but right around it, such as blood vessels, surrounding cells, or molecules. Tumors have a reputation that precedes them. These tissues are known to foster harsh and hostile conditions (Z. Zhang et al.). Chronic antigen exposure is one of the tumor microenvironment's large contributions to T-cell exhaustion (Zhao et al.). Anytime the immune system identifies an antigen, it is interpreted as a threat to the body. Consequently, those cells are attacked. In parallel, the CAR protein helps the T-cell identify the antigens so the T-cell can promptly attack the cancer cells. However, as the T-cell suffer from over stimulation from antigens, they suffer from a term called desensitization (Wherry and Kurachi; Odorizzi and Wherry; C. Wang et al.). This means that over time, the T-cells will just become constantly activated. However, the drawback is that each time it responds, it becomes less efficient since it has been activated for so long and is now suffering exhaustion.

A more profound aspect of T-cell exhaustion and how this condition is identified can be found through analyzing the T-cell's cellular and molecular characteristics. (Catakovic et al.; Wherry; Blank et al.). A T-cell that is not exhausted can produce high levels of cytokine molecules, such as interferon-gamma (IFN-y) and interleukin-2 (IL-2) (Wherry). High cytokine levels are necessary for successful immune responses, which are delivered effectively and accurately. On the other hand, an exhausted T-cell will be unable to produce high levels of cytokine molecules. In fact, their production of cytokine molecules highly contrasts a non-exhausted T-cells production rate, with extremely low cytokine molecules being produced.

Furthermore, an exhausted T-cell cannot proliferate as effectively and as much when exposed to resistant antigens such as plasmacytoid dendritic cells (PMC). These cells play a role in immune responses, and the tumor exploits this mechanism by expressing high levels of these two components and linking them to T-cells. Consequently, the T-cells shut down, and this can lead to exhaustion as they can no longer activate, proliferate, and produce cytokine molecules at the same levels they were able to do before. Yet, these inhibitors have shown profound success in treating specific cancers, like melanoma and non-small cell lung cancer.

Drastic changes in behavior shown through the cellular and molecular differences between non-exhausted and exhausted T-cells help signal the inhibitory receptors, such as PD-1 and CTLA-4. These receptors are located on the surface of the Th0 Cells. Their task is to control the T-cell activity and can be viewed as an “off switch”. This is important because when these receptors are signaled, they reduce or entirely stop the T-cell’s activity, since it is not in a state of exhaustion. On a more molecular aspect of T-cell exhaustion, the main characteristics used to identify this condition occur in the signaling pathways. A present stimulation of antigens can lead to a constant expression of inhibitory receptors. This then changes the intracellular signaling. For example, T-cells can experience changes to their metabolic pathways, which would inevitably reduce their energy production. This matters because metabolism affects how much energy the CAR T-cell has and is another contributor to exhaustion. Consequently, other factors of the T-cells would become non-optimal in both function, accuracy, and effectiveness. Components in the TME, such as immunosuppressive cytokines and regulatory cells can increase the likelihood of T-cell exhaustion, as they foster an environment that constantly suppresses T-cell activity. This means that the T-cells would have to rely on proliferation and the production of cytokine molecules to survive and combat the tumor, and its immunosuppressive activity.

However, there are new approaches to combating T-cell exhaustion. Researchers and scientists are developing strategies and components which can ameliorate the symptoms of T-cell exhaustion and prematurely prevent the condition. A beneficial and practical approach is using immune checkpoint inhibitors (Budimir et al.). These drugs prevent the interaction between the programmed death cell protein 1(PD-1) and the T-cell. These drugs also present the interaction between the programmed death-ligand 1(PD-L1). This will overcome T0 Cell exhaustion since T-cell exhaustion occurs from overstimulation, and by reducing interactions which creates stimulation, the likelihood of exhaustion reduces significantly. While there are many approaches to preventing T-cell exhaustion, epigenetic modifications bring promising results with its ability to modify the genes state of being turned on and off to help save energy, and reactivate the T-cell. Additionally, the mechanism must prevent the state of exhaustion from ever happening again, since it is caused by overstimulation.

Epigenetic mechanisms in T-cell regulation

Since CAR T-Cells can attain a state of T-cell exhaustion to constant T-cell stimulation, and combating of components in the TME and solid tumor cancer cells, epigenetic modifications can be applied to help prevent this state (Dutta et al.). Essentially, epigenetic modifications help turn on and off genes. Epigenetic modifications utilize specific enzymes and proteins to control DNA methylation patterns in T-cells. Leveraging this tool can help efficiently control and use the energy and functions of CAR T-cells. These methods can also affect the differentiation, exhaustion, function, and activation of the T-cells (Tu et al.). Modifying the degree to which genes can be turned off, known as methylation, or on, which is methylation, can be accomplished through methods such as histone modification, and DNA methylation and methylation. where a methyl group is added to the DNA sequence. Methylation is when a methyl group is added to the DNA sequence, and a gene is turned off. Demethylation is when a methyl group is removed, which would reverse the change made by the methyl group. To elaborate, genes are the core mechanisms of our body and contain instructions which our body utilizes for everything. In this scenario, genes contain the instructions and code how the T-cells respond to stimulation and functions. However, through doing so, this means that even when performing functions that utilize only specific genes, all the genes are usually turned on. This puts extreme stress on the CAR T-cell because multiple functions are on even if they're not being used, which takes away more power. It is as if you have multiple devices to listen to music in your room and when listening to one device, all the other devices are also playing music which you do not intend to listen to. Even if you are focusing on the music from one device, the other devices are also playing music, which is wasting electricity. However, if you turn the other devices off, and keep the device you are listening to music on only, then the electricity consumption and usage would be much more efficient as well as the quality of listening. Now, there are multiple ways that the turning off and on of genes can be done. However, first, understanding what Histones are, is important for learning more about the methods that can be used to turn on and off genes. Histones are proteins which tightly wrap around the DNA, which helps give structural support while also controlling gene expression. This is important because Epigenetic Modifications strive to regulate gene expression, and Histones play a key role in this aspect. Examples of proteins that modify histones are Menin, Dot1 l, and HDac3 (Scheer et al.; Onodera et al.; Schuettengruber et al.). For example, Dot1 l can prompt differentiation through methylating the H3K79 gene. Now, differentiation is the turning on and off of genes to produce specific proteins. The difference in genes turned on and off produces different proteins, hence the name differentiation. Dot 1 does this by utilizing S-adenosylmethionine. S-adenosylmethionine is a cofactor. Cofactors refer to a molecule that is not considered a protein, which is vital for an enzyme to function. Cofactors can help connect the enzyme to aid it in executing its function. Co factors are the components which provide the methyl group and methyl tags to the enzyme which is trying to methylate a gene. So as S-adenosylmethionine provides the protein Dot 1 with the methyl group, Dot 1 can now methylate the gene H3K79. Even though most cells perform epigenetic modifications, this mechanism of modifying DNA expression could be applied to CAR T-Cell exhaustion. This is because controlling methylation and demethylation of genes in the CAR T-cell can provide more efficiency, since components are functions that are only used when necessary and not always turned on. Through these methylation patterns and histone modifications, the activation of the T-cell gets controlled and altered since the cytokine productions vary.

While methylation turns off genes, sometimes this contributes to the exhaustion (Zebley et al.). By adding a methyl group, specific genes are consequently silenced. However, this means that the genes which did not receive a methyl group gain the responsibility of making sure the CAR T-cell has proper and efficient functioning capabilities. Sometimes, this can create a lot of pressure because specific genes are forced to function when they would normally be silent. However, when the cell becomes over-stimulated, it commits a great energetic cost, which can consequently cause the cell to shut down and enter a state of exhaustion. By demethylating certain genes, the genes which were suppressed and originally turned off will be turned on to reverse the state of exhaustion. By creating an epigenetic function which allows for the fine-tuning of genes, cells can be modified such that efficiency can be improved and exhaustion can be avoided. Much research has shown insight that a transistor state can be seen during the process of T-cell exhaustion. This state displays the on and off genes. An example of this is, can be seen with the CXCR1 expression. This expression can often be associated with T-cell exhaustion, since it becomes demethylated to prevent the state of exhaustion. Furthermore, silent effector and memory genes which are pertinent include TCF7, LEF1, and CX3CR1, BATF. 5-Azacytidine is a drug used to inhibit the methylation and create demethylation. Along with this are histone modifications such as histone deacetylase. Overall, this example illustrates that using epigenetic modifications and applications such as silent effector and memory genes allow for the fine tuning of genes in the therapy, increasing efficiency and preventing exhaustion.

Since the Chromatin landscape is essentially how the DNA is packed around histones, understanding this component is crucial (Ford et al.). This would help researchers in figuring out the genes necessary to be turned on, how to navigate through this landscape, and how to strategically use the epigenetic modifications under these factors. By remodeling the chromatin landscape, it is essentially allowing histone modifications, drugs, and proteins to enter and inhibit the methylation, allow for demethylation, and turn on and off genes. Histones tightly wrap around DNA, giving both structural support wise and regulating gene expression. However, to modify a histone, one must gain access to it. This is because the histone coils around the DNA and to change the regulated gene expression, access must be gained appropriately to achieve this. The histone modifications only work if the access to the histone is gained, and this occurs through changing the landscape of the chromatin, which sheds light on the histone arrangement (*Histone H3 Phosphorylation, Immediate-Early Gene Expression, and the Nucleosomal Response: A Historical Perspective 1This Article Is Part of Special Issue Entitled Asilomar Chromatin and Has Undergone the Journal's Usual Peer Review Process.*; Sawicka and Seiser; Rossetto et al.; Swygert and Peterson; Bannister and Kouzarides). The chromatin is composed of the genes and DNA in the cell. The therapy's genes can be turned on and off by gaining access to DNA through histone modification. This process represents the change in the chromatin landscape (Petty and Pillus). The HDAC inhibitors, spoken about before, inhibit the DNA methylation by performing acetylation which therefore transcribes the turned off genes to reactivate the T-cell (Harb et al.; Fierz and Muir; Angiolilli et al.; Wapenaar and Dekker; Ceccacci and Minucci). Acetylation is adding acetyl groups to DNA to turn on a gene. However, this time, the DNA is displayed to components such as transcription factors which allow the Gene to be turned on. In the process, the chromatin landscape becomes looser and electrically changed.

Furthermore, the use of HDACis treatment has increased the expression of memory genes, while in parallel, decreasing the expression of exhausted genes. This is done through epigenetic modifications, where genes are turned on and off through acetylation, methylation, and demethylation. These processes significantly change the chromatin landscape. Now, the memory genes which are turned on remember the characteristics, details, and antigens that are being targeted. The HDAC inhibitors only heighten these senses in the therapy by turning these types of genes on. The memory genes are effective since the stored information can be used since the exhausted genes cannot properly function.

While turning on and off the genes has been spoken about, the ability to edit the genes itself has now become possible, thanks to scientific breakthroughs and countless years of research done by researchers and scientists.. By editing the genes, one can alter the state of them, whether they are turned on and off. This is another way of using epigenetic modifications. Examples of this include DNA methylation and histone modifications. Now CRISPR is a gene editing sequence concept where DNA is cut at specific sentences based on palindromic sequences (Ito et al.). This can be extremely useful because genes can be easily silenced to see which transitions can cause the exhaustion. This process can be characterized by the term

CRISPR Screening. Now, this can be useful because sometimes, being able to silence the right genes can be complicated due to navigation of the chromatin landscape and getting access to histones. Even so, once DNA methylation is completed, demethylation occurs, which can be time-consuming and difficult. Furthermore, this process can also be expensive. CRISPR is an effective and accurate technology that can complete this objective in one fell swoop.

Conclusion and Future Directions

This secondary research paper discusses the uses of epigenetic modifications on CAR T-cell therapy to prevent and alleviate T-cell exhaustion to treat solid tumors and increase efficacy. To summarize, CAR T-cell therapy can enter a state of exhaustion when it is constantly stimulated in the tumor microenvironment for long periods. Epigenetic modifications are used to save energy and control what functions are used to prevent exhaustion. This is done by turning genes on and off through many methods. Methods include using histone modifications to manipulate the chromatin landscape by adding acetyl groups and adding and removing methyl groups. A significant challenge in the treatment of solid tumors is the role of tumor heterogeneity. This occurs when target antigens used to identify cancer cells in solid tumors vary in tumor types (primary and metastatic), and even between patients. This makes it difficult for the therapy to identify cancer cells, since they can differ at large scales.

Furthermore, tumor-associated antigens from solid tumors can pose a threat to patients using the therapy. These antigens are expressed at low levels in healthy tissue but high levels in the solid tumor. However, this means that while the treatment may attack the solid tumor, through the process it may also attack the healthy tissue. Due to structural barriers, correctly entering the tumor site and ability to attack cancer cells pose a significant issue. For example, the tumor stroma is a term that refers to a thick set of tissue that prevents the CAR T-cells from successfully infiltrating the tumor.

Another example can be seen from the deviating vasculature. This makes it difficult for the CAR T-cell therapy to infiltrate the tumor from the bloodstream compared to blood cancers, which are literally in the blood. This makes it easily accessible for therapies. The final limitations that will be discussed are that roots from the inhospitable environment of the TME are tumor-associated macrophages, and myeloid-derived suppressor cells (MSDCs). Macrophages are a core component of the immune system with many functions and benefits, including killing harmful microorganisms, stimulating other immune system cell actions, as well as removing dead cells (National Library of Medicine) Additionally, TAAs are a vital component in the TME because they provide a variety of functions and benefits in the context of tumor progression (Yin et al.). TAAs release cytokines which are responsible for fostering tumor growth and spread (O'Shea et al.; Clevealand). However, studies have shown that specific types of cytokines are responsible for helping increase tumor growth and progression (Zamarron and Chen). Now MSDCs are immature myeloid cells which suppress immune responses and promote tumor growth (Y. Wang et al.). These cells grow during pathological conditions like cancer (Gabrilovich and Nagaraj). While this treatment option is essential for helping those with cancer the cost of getting these treatments is immense. Clinical translations with CAR T-cell issues include USD 70,000 (Cliff et al.). The highest costs of the CAR T-cell therapies can reach up to 400,000 USD and sometimes 1 million USD. Along with that, applying this therapy on solid tumors is also difficult on a cellular, and molecular level, and as recapped before, due to the TME, T-cell exhaustion, tumor infiltration, tumor heterogeneity, and other potential limitations. Furthermore, finding people to test this therapy on safely can be difficult. This is due to a lengthy and complex process fueled with regulations and restrictions from the government, and completion of specific stages of preclinical and clinical trials.

Utilizing epigenetic modifications in CAR T-cell therapy for combating T-cell exhaustion in treating solid tumors has proven to have massive potential in the cancer sector. However, the race hasn't ended since science is all about learning and innovation. One future emerging technology in this sector of cancer is single-cell sequencing. This is where one's cells can be analyzed at a much deeper scale (DNA, RNA, etc) (Huang et al.). Another interesting and emerging topic is performing epigenetic modifications on cancer cells to prevent their functions, which can lead to tumor growth and suppression of immune responses. Forms of this include DNA methyltransferase and DNA deacetylase (Rocha et al.). Epigenetic modifications is one of the many new technologies in this growing field of medicine and science. Now more than ever, the amount of research and people involved in research, finding new treatment, and improving patient life, is only increasing. All in all, it is vital to understand that T-cell exhaustion, a significant barrier in treating solid tumors, can be solved by epigenetic modifications. Epigenetic modifications reverse and prevent T-cell exhaustion, reviving the CAR T-cell therapy and effectively treating the patient's solid tumors.

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