Cancer Stem Cell as Target of CAR-T Cell Therapy

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Abstract. Chimeric antigen receptor-T cell (CAR-T) therapy has been studied intensively these years and is considered a promising cancer treatment. So far, Food and Drug Administration has approved 2 CAR-T cell therapy for patients with refractory leukemia and the result is positive. However, CAR-T cell therapy is still facing several challenges, including antigen escape, which will diminish the efficacy of treatment and lead to relapse. This review investigates the potential of cancer stem cell (CSC), a small group of cancer cells that contribute to tumorigenesis, metastasis, therapy resistance and relapse, as the target of CAR-T cell therapy, focusing on representative CSC surface markers: CD123, CD133 and CD44. Evidence indicates that CAR-T cell therapy directed by CSC surface markers is effective and feasible. Therefore, CSC targeted CAR-T cell therapy is a prospective treatment for cancer.

1 Introduction

Chimeric antigen receptor-T cell (CAR-T) therapy is a promising cancer treatment. CAR-T cells are genetically engineered T cells consisting 3 parts: an extracellular antibody-like surface domain, a transmembrane domain and an intracellular signaling domain 1. The extracellular part responsible for antigen recognition is made of single-chain variable fragment(scFv) targeting specific tumor-associated antigen (TAA)2. CD19, a highly and frequently expressed surface marker on B-cell leukemias and lymphomas, is one of the most common antigens targeted by CAR-T1. The transmembrane domain derived from CD3-ζ, CD4, CD8, or CD28 molecules connects the extra- and intracellular domains3. For the currently approved CAR-T cell therapy, the intracellular signaling domain containing a costimulatory domain (CD28 or 4-1BB) and the CD3ζ chain activates the cell1. The CAR-T cell therapy is highly customized, collecting the T cells from the patient, genetically modifying them into CAR-T cells and injecting CAR-T cells back to the patient4. As a "living drug", the CAR-T cells would multiply in the patient's body, recognize and kill the cancer cells that they recognize5.

CAR-T cell therapy has moved far beyond an experimental theory in the laboratory and launched successfully as a revolutionary treatment into clinical use. Up to date, FDA has approved two CAR-T cell therapy: Kymriah for patients up to 25 with B-cell precursor acute lymphoblastic leukemia (ALL) and adult patients with relapsed or refractory (r/r) large B-cell lymphoma and Yescarta for adult patients with relapsed or refractory large B-cell lymphoma67. Both treatments demonstrate auspicious result: Kymriah has an overall response rate (ORR) of 50% and Yescarta has an ORR of 72%67. This novel therapy certainly brings hope and health to many patients who have suffered for years. Because of its success, the field studying CAR-T cell therapy is blooming and the public are looking forward to its performance in treating other types of cancer.

Despite its current promising outlook, CAR-T cell therapy still has various problems that need to be overcome as it advances into treating solid tumors. One of the major challenges is the relapse caused by antigen escape. In an ideal situation the TAA used for CAR-T cell therapy are expected to be expressed by all the cancer cells, but unfortunately, as scientists make further progress in the field, it is discovered that with the persistent presence of CAR-T, the expression rate of the TAA decrease and the ratio of tumor cells without the TAA increase8. One of the current approaches is identifying other potential target, for instance the dual targeting of CD19 and CD123 shows promising outcome9. CD123 is a cancer stem cells surface marker and the current success raises cancer stem cells, a subpopulation of tumor cells, as potential targets for CAR-T therapy because of their stem cell like characteristics and their contribute to tumorigenesis and relapse10. In this review, we discuss cancer stem cells as potential targets for CAR-T cell therapy and summarize the result of current experiments on CAR-T directed by CD123, CD133 and CD44, all of which are expressed across various types of cancer as indicated by Table 1.

Table1. Prevalence of CSC markers CD123, CD133 and CD44

Surface marker	prevalence
CD123	hematologic malignancies
CD133	hepatocellular carcinoma, pancreatic cancer, brain tumor, breast cancer

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CD44	leukemia, hepatocellular carcinoma, lung cancer, ovary cancer

2 Cancer Stem Cells (CSCs) Characteristics

As the name suggests, CSCs demonstrate stem cell characteristics: self-renewal and differenciation11. They also contribute to cancer metastasis and show resistance to chemotherapy and radiation therapy11. Self-renewal allows the preservation of CSCs through mitosis and differentiation allows CSCs to generate bulk of tumor cells and enlarge the tumor12. Metastasis is caused by circulating tumor cells: tumor cells that are shed from the original tumor into the circulatory system, can seed at a different site and develop into a new tumor13. Some of the circulating tumor cells express cancer stem cell surface markers and are highly tumorigenic14. With their multi-layers self-defense lines and the ability to rapidly repair DNA, CSCs are considerably more resistant to chemo- and radio- therapy compared with normal tumor cell and would relapse1516. These characteristics indicate that CSCs play a significant role in the development of tumor, the resistance to clinical treatment and the relapse of cancer. They also imply that CSCs are useful targets for cancer therapy because once CSCs are removed, the severity of tumor would decrease remarkably.

3 CD123 as Target

Previous studies have shown that CD123, also known as interleukin-3 receptor alpha chain, has been overly expressed in hematologic malignancies, including acute myeloid leukemia (AML) and B-cell acute lymphoblastic leukemia and the presence of CD123 is always associated with poor prognosis. Previous works conducted by Dr MUÑOZ from Hospital de la Santa Creu i Sant Pau, Spain, analyzed CD123 expression in patients and 43 out of 45 patients with AML and all 13 patients with B-cell ALL were positive for CD12317. The expression was homogenously on blast tumor cell with moderate intensity17. Another experiment on AML indicates that CD123 is highly co-expressed with CD34 and that CD123 plays a significant role in the initiation and maintenance of leukemia population18. Patient with higher rate of CD123 have a significantly lower number of remission and higher number of relapses compared with patient with lower rate of CD12319. Because of its wide presence and negative impact on patient with AML, CD123 is a potent target for CAR-T cell therapy and is becoming a new focus for research.

In the past decade, scientists have targeted CD123 with CAR-T cell therapy, either by itself or with another TAA. The results suggest that CD123 CAR-T is able to target and eliminate primary AML cells in vitro and in vivo and the survival rate of mice with AML treated by CD123 CAR-T is significantly higher than those treated with phosphate-buffered saline (PBS) or CD19R T cells20. Another group took a different approach and designed and anti-CD123 CAR cytokine‐induced killer (CIK) cells, a specific type of T cells with natural killer cell like phenotype21. The ability for anti-CD123 CAR CIK to target and kill AML cells was also good and this approach appeared to have lower side effect as anti-CD123 CAR CIK showed limited activity towards normal cells with low CD123 expression21. A research conducted in 2014 on CD33 CAR and CD123 CAR also confirmed that CD123 CAR was effective in killing AML blasts and expressed less damage to normal hematopoietic stem/progenitor cells compared with CD33 CAR22.

CD123 has also been used together with another surface marker, CD19. Researchers have tried dual CD123 and CD19 targeting T cell to prevent the relapse caused by antigen loss after the treatment of CD19 CAR T9. The research noticed that B cell ALL cells expressing CD123 were not eliminated by CD19 CAR T and were responsible for the antigen loss relapse9. Mice model with B-ALL has shown that while those received only CD19 CAR T expressed progression of CD19-negative tumor cells, the ones that received CD19 CAR T and CD123 CAR T showed no sign of disease9. All the success that have achieved in CD123 CAR T imply that CD123, either by itself or as a supplement for another TAA, is a worthy target for further investigation.

4 CD133 as Target

Scientists have studied CD133 as a cancer stem cell marker extensively in the past decades and learned that CD133 is prevailing in various types of cancer, including hepatocellular carcinoma23, pancreatic cancer24, brain tumor25 and breast cancer26. CD133 contributes to tumor initiation25, metastasis, and drug resistance26 and high level expression of CD133 indicates poor prognosis27. Previous study demonstrates that CD133 is tumorigenic: 1000 CD133+ medulloblastoma cells injected to mice model lead to development of tumor and differentiation in tumor cells while 105 CD133- cells did not form tumor25. In a clinical analysis of patients with breast cancer, CD133 is found to be positively associated with tumor size and lymph node metastasis28. CD133+ CTC from breast cancer were enriched after chemotherapy while CD133- CTC decreased dramatically, indicating that CD133 contributes to chemoresistance29. Previous analysis of 723 patients with pancreatic cancer showed that patients with overly expressed CD133 had significantly lower survival rate compared with those who has low CD133 expression27. In summary, the prevalence of CD133 across different types of cancer and its contribution to tumorigenesis, metastasis, chemotherapy resistance and poor prognosis make CD133 a significant target for cancer therapy.

CD133 directed CAR-T cell therapy demonstrated its practicability and effectiveness in the lab and in a phase I clinical trial3031. Dr Parvez Vora and his team constructed 3 different approaches targeting orthotopic xenograft glioblastoma cells, anti-CD133 immunoglobulin G, CD133 dual antigen T cell engager, and CAR-T CD133, and compared their anti-tumor

ability31. The result indicates that CAR-T CD133 had the strongest power in inhibiting the development of cancer and the treatment showed no acute toxicity against normal cells expressing CD13331. The phase I clinical trial enrolled 14 patients with hepatocellular carcinoma, 7 patients with pancreatic carcinomas, and 2 patients with colorectal carcinomas and their cancer were in advanced stage, reoccurred after lesion and showed metastasis30. All patients received an initial four-week treatment cycle and could continue the treatment if it was beneficial to their condition30. The actual treatment ranged from 9 to 63 weeks for different patients and outcome was desirable: 3 patients achieved partial remission, 14 patients had a stable disease period for 9 weeks to 15.7 months and 3 patients had continuous response to the treatment30. Because of its success in the clinical trial, CAR-T CD133 demonstrates future potential in treating advanced metastasis malignancies and in treating solid tumor with CAR-T.

5 CD44 as Target

CD44 is widely expressed across different types of cancers, including leukemia32, hepatocellular carcinoma33, lung cancer34, and ovary cancer35. Similar to the previous CSC surface markers, CD44 plays critical roles in drug resistance36. In leukemia, enhanced CD44 in mouse and human T-ALL cell lead to enhanced chemoresistance while CD44 knockout lead to chemosensitivity36. The high expression of CD44 indicates poor prognosis hepatocellular carcinoma and the correlation is confirmed through patient-derived xenograft model37. CD44v6, a variation of CD44, when expressed in high level, is associated with short survival time for patient with epithelial ovarian cancer because CD44v6 drives tumor metastasis and drug resistance38. Because of these characteristics, CD44 presents itself as a good candid for CAR-T cell therapy.

Researchers have tested CD44 directed CAR-T against different types of cancer and the outcome is positive. Experiment conducted by Monica Casucci in 2013 indicates that CD44v6 directed CAR-T could effectively abolish CD44v6+ acute myeloid leukemia and multiple myeloma cells, which are associated with tumor initiation and metastasis39. The toxicity caused by CAR-T therapy was a concern for the experiment but the researcher proposed a solution: CD44v6 directed CAR-T co-expressing a suicide gene, which would not compromise the efficacy of the treatment but able to have a better control over the side effect39. Recent research using mice model with lung and ovary adenocarcinoma suggests that CD44 directed CAR-T can specifically and efficiently eliminate CD44+ tumor cells than thereby inhibit the development of tumor and increase overall survival rate of the mice40. Another research took a slightly different approach and used minicircle DNA-mediated CD44 directed CAR-T against hepatocellular carcinoma33. The result is consistent with the previous ones that CD44 directed CAR-T suppressed hepatocellular carcinoma development in vitro and in vivo while the addition of minicircle DNA enables the therapy to avoid side effect caused by virus33. The effectiveness and specificity of CD44 directed CAR-T therapy reflected through the previous experiment reveal the potential of CD44 as a target for CAR-T therapy across the treatment of various types of cancer.

6 SUMMARY

This review presents the potential of using CSC as the target of CAR-T cell therapy and discusses CSC surface markers CD123, CD133 and CD44 directed CAR-T in detail. The CSC contributes to tumorigenic, metastasis, chemoresistance, and relapse and is associated with poor prognosis. When CSCs are eliminated, the development of tumor is inhibited. CD123 is overly expressed in hematologic malignancies while CD133 and CD44 are expressed across various types of cancer. Experiments and trails of CAR-T cell targeting CD123, CD133 and CD44 indicate the specificity and effectiveness of the therapy: cancer cells expressing the targeted CSC surface marker decrease dramatically and the tumors are inhibited. Admittedly, CAR-T cell therapy is still facing multiple challenges and one of the concerns for CSC directed CAR-T is the toxicity41. Scientists have investigated the problem and proposed a possible solution. Previous research tested CD133 directed CAR-T cells with CRISPR/Cas-9 mediated programed cell death protein 1 and the result showed decreased cytotoxicity and enhanced anti-tumor ability42. In summary, these evidences suggest that CSCs directed CAR-T cell therapy might be an effective, practical and safe treatment with limited aversive effect.

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