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Challenges and Feasible Solutions of CART Therapy in Solid Tumors

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Abstract:

CAR-T cell therapy has emerged as a significant advancement in cancer immunotherapy, particularly in treating hematologic malignancies with notable success. Yet, its efficacy in addressing complex solid tumors remains constrained. A principal challenge in the deployment of chimeric antigen receptor T-cell therapy for solid tumors lies in the limited availability of tumor-specific target antigens. Presently, the field of CAR-T cell research is marked by the limited availability of antigens that are genuinely specific to tumors. Even if tumor-specific antigens with high specificity can be identified, the heterogeneity of their expression in tumor tissues often further complicates the treatment. This review aims to delve into the issue of tumor-specific antigens and, based on current research progress, propose strategies for improving the search for new tumor antigens and CAR structural design to provide reasonable theoretical support and practical guidance for the safe and effective targeting of solid tumors by CART cells. By continuously optimizing the treatment strategies of CAR-T cell therapy, we aim to offer solid tumor patients more precise and efficient immunotherapy.

Keywords: solid tumors, antigen recognition, tumor specificity, CAR-T cells

1. Introduction

In previous decades, the field of cancer treatment has undergone a profound revolution, shifting from traditional nonspecific therapies to more precise and efficient antibody-based immunotherapies. Among the various branches of immunotherapy, CAR-T-cell therapy has garnered widespread attention due to its highly specific tumor recognition and killing capabilities. While CAR-T cell therapy has shown significant success in treating hematologic malignancies[1], developing effective CAR-T therapies for solid tumors remains challenging. Overcoming obstacles such as T-cell migration and tumor infiltration, along with the immunosuppressive tumor microenvironment, to improve the effectiveness of CAR-T cell treatments targeting solid tumors[2]. Identifying the optimal target surface antigen is a pressing issue in addressing the challenges posed by solid tumors. Clinical trial reports on CAR-T cell treatments targeting solid tumors reveal that most therapies remain in the preliminary "targeting" phase, with precise tumor targeting yet to be fully realized. A META-analysis combining 42 hematologic and 18 solid tumor clinical trials involving 913 subjects in 2019 revealed a significant difference in efficacy between hematologic and solid tumor patients receiving CD19 CAR-T therapy. While 54.4% of hematologic patients experienced complete symptom relief, this proportion dropped significantly to 4.1% in solid tumor patients, highlighting the differential effects of CD19 CAR-T therapy between hematologic and solid tumors[3]. Presently, CAR-T cell treatments confront several challenges in addressing solid tumors, accompanied by the development of potential strategies. Analyzing and exploring these challenges and strategies enhances comprehension of CAR-T therapy for solid tumors and offers important perspectives for future studies.

2. Challenge of Tumor-Specific Antigens in Solid Tumors

Tumor antigens devoid of viral origins are categorized into two types based on their characteristics: (1) Tumor-specific antigens (TSA), originate from mutations.; (2) tumor-associated antigens (TAA), arising from the overexpression or aberrant expression of non-mutated proteins. TSAs, expressed exclusively by cancer cells and often playing a crucial role in tumorigenesis, are considered ideal targets for cancer therapy.

Ideal tumor-associated antigens (TAAs) should exhibit specific expression on tumor cells while being absent or minimally present in healthy cells. For example, such as CD123 or CD33 antigens demonstrate high expression levels in tumor cells while also being present in critical bone marrow stem cells[4]. Therefore, identifying a therapeutic window that allows targeted recognition of malignant cells with high CD123 expression without targeting

normal cells expressing moderate levels of CD123 is crucial. Conversely, if anti-CD123 CAR-T cells are administered directly, there is a risk that these cells may inadvertently target normal tissues with low-level expression of the target antigen while primarily recognizing tumor cells. This occurrence could result in unintended detrimental effects, potentially leading to widespread disruption of bone marrow function, known as panmyelosis[5]. For example, in 2010, in a case of ERBB2-targeted CAR-T therapy for colon cancer, CAR-T cells targeted cells lining the lungs that showed reduced levels of ERBB2 expression, resulting in progressive hypotension and bradycardia in the patient receiving CAR-T, ultimately leading to cardiac arrest[6] . Additionally, The majority of tumor cells employ mechanisms to remove immunogenic epitopes associated with tumor-associated antigens (TAAs) in order to evade recognition and attack by the host immune system[7, 8]. Hence, the identification of tumor antigens that are both specific and immunogenic is crucial for the effective treatment of solid tumors. Consequently, novel tumor recognition strategies are often required in CAR-T therapy to overcome challenges related to specificity and heterogeneity, with the aim of maximizing the therapeutic efficacy of CAR-T cells against solid tumors.

3. Feasible Strategies for CAR-T Targeting Tumor-Specific Antigens

3.1 *Selection of Target Antigens*

The identification of target antigens is paramount to the efficacy of chimeric antigen receptor T-cell (CAR-T) therapy in oncological treatments, as it dictates the ability of CAR-T cells to precisely recognize and eradicate tumor cells while sparing healthy cells. Ideally, target antigens should exhibit high expression levels on tumor cells while demonstrating minimal or no expression in normal cells. Currently, specific target antigens for solid tumors are gaining significant attention in clinical research projects involving CAR-T therapy.

3.1.1 *CEA*

CEA, as a sensitive tumor biomarker, plays a significant role in gastrointestinal cancers, particularly in tissues and serum of colorectal cancer (CRC) where its expression is prominent. Although CEA is expressed to some extent in the gastrointestinal tract, this expression is mainly limited to the apical surface of epithelial cells facing the lumen, and its expression is hardly detectable in most normal adult tissues. Importantly, immune cells do not recognize CEA expression, making CEA a highly promising therapeutic target in CAR-T therapy[9]. This innovative therapy not only demonstrates potential therapeutic effects but also shows preliminary safety through local delivery. In a study involving 10 gastrointestinal cancer patients, 7 patients who had disease progression prior to CAR-T treatment achieved stable disease control. Evaluation of treatment effects utilized advanced imaging techniques such as PET/CT and MRI. Results showed significant tumor volume reduction in two patients. Long-term observation data also indicated a significant decrease in serum CEA levels in most patients[10].

3.1.2 *Mesothelin*

Mesothelin is a membrane glycoprotein linked to sugar-phosphatidylinositol and exhibits elevated expression in most cases of malignant pleural mesothelioma, ovarian, pancreatic and certain lung cancers[11]. Its expression is limited in the normal tissues of the pericardium, pleura, and peritoneum. Mesothelin serves as a focal point for innate immune responses in cases of malignant pleural mesothelioma (MPM), ovarian, and pancreatic cancers[12]. Preclinical studies have shown CAR T cells can specifically recognize mesothelin[13]. In November 2021, MSKCC released the results of a study on CAR-T therapy for malignant pleural mesothelioma targeting MSLN, showing significant efficacy with a median survival of 17.7 months in 23 patients and a one-year survival rate of 74%. No severe adverse reactions or “off-target effects” were observed during treatment, providing a new therapeutic strategy for this disease.[14].

3.1.3 *Claudin 18.2*

In their research, Sahin and collaborators determined that Claudin 18.2, a subtype of Claudin 18, is an exceedingly precise biomarker for cells in the differentiated gastric epithelium. This marker is involved in various significant neoplasms, including those affecting the stomach, esophagus, pancreas, lungs, and ovaries, and it responds to targeting via specific monoclonal antibodies[15]. Owing to its distinct expression pattern and cellular surface localization, Claudin 18.2 represents an optimal candidate for targeted therapeutic strategies. In May 2022, The inaugural clinical investigation of CAR-T therapy CT041, targeting CLDN18.2, was commenced in China. This trial yielded significant objective response and disease control rates among 37 patients with severe gastrointestinal cancers, with notably strong results in cases of gastric cancer. The therapy was well-tolerated and demonstrated a manageable safety profile, devoid of serious adverse reactions, thus presenting a new therapeutic approach for patients with gastrointestinal cancers[16].

Furthermore, clinical trials for CAR-T therapy targeting HER2, EGFR, PSMA, GD2, CD133, and CEA are actively progressing to potentially meet broader clinical needs

for solid tumors, bringing hope to more patients.

3.2 *CAR Molecule Modification*

Precise cell targeting poses challenges due to the lack of a single surface marker that distinguishes most mammalian cell types from others. Advances in cell immunotherapy and synthetic biology have significantly altered the way we target cancer cells. Targeted therapy is no longer limited to a single biomarker; by modifying CAR-T cells to utilize multiple synthetic receptors or a specific receptor, and based on the specific combination of proteins present on the cell surface, multiple target antigens can be simultaneously bound to enhance the specificity in recognizing tumor antigens[17].

3.2.1 *Co-LOCKR*

Co-LOCKR is a novel design of AND gate and NOT gate for CAR. It involves a receptor and a protein logic circuit designed through computation to perform logical operations outside the cell. Building upon the previously developed LOCKR switch by Lajoie et al., the system was further optimized into a co-localization-dependent (Co-LOCKR) mode[18]. This advanced Co-LOCKR AND gate circuit comprises two proteins, referred to as "cage" and "key". These proteins are equipped with a domain that specifically binds to antigens. Notably,

The cage protein includes a peptide segment designed to interact specifically with chimeric antigen receptor (CAR) T cells; however, under normal circumstances, this peptide segment is obscured by the locking structure domain of the cage protein. The association of the key protein with the cage protein catalyzes a conformational alteration in the latter, which results in the exposure of a specific peptide segment. This segment is then capable of engaging with the chimeric antigen receptor (CAR), thereby initiating its activation. Importantly, the design of the cage and key proteins ensures they remain non-interactive in solution, maintaining their functional integrity. Upon their co-localization on the cellular surface, where their antigen-binding regions align, this proximity facilitates the formation of functional cage-key complexes, which is critical for the activation process. This strategic interaction underscores the sophisticated engineering behind the protein design, enhancing the efficacy of the therapeutic mechanism[19].

3.2.2 *Tandem CAR T-cell*

Researchers have successfully developed a novel tandem CAR molecule, where the extracellular binding domain ingeniously fuses two single-chain antibody fragments (scFv) targeting different tumor-associated antigens (TAA). This design strategy extends beyond mere linkage to the CD3ζ chain by incorporating additional co-stimulatory signaling domains like CD28 or 4-1BB. This strategic enhancement markedly improves the activation milieu for CAR-T cells. Notably, this modification enables tandem CAR-T cells to achieve optimal activation solely upon the concurrent recognition of two distinct tumor-associated antigens (TAAs). This sophisticated approach not only elevates the specificity and potency of CAR-T cell therapy but also underscores its potential as a robust strategy in cancer treatment. This feature greatly enhances the specificity and safety of CAR-T therapy, as CAR-T cells are triggered only when both antigens are expressed on the target cell surface and recognized[20]. Moreover, researchers have also explored a similar CAR design strategy where one CAR structure also includes two scFVs. One scFv is linked to the intracellular CD3ζ chain, providing the first signal, while the other scFv is linked to the co-stimulatory signaling domain, providing the second signal. This design also requires both antigens to be expressed and recognized on the target cell simultaneously to activate these two signals, thereby triggering the activation and anti-tumor effects of CAR-T cells[21].

3.3.3 *SynNotch-CAR-T*

A research team from the University of California cleverly applied the SynNotch receptor to CAR-T cells[22]. This SynNotch-CAR T cell has a unique dual recognition mechanism; it must simultaneously recognize the SynNotch antigen and the CAR antigen. Importantly, only after being initiated by the SynNotch ligand, can the SynNotch-CAR T cell execute the CAR-induced cytotoxic function[23]. This design effectively prevents healthy cells from being mistakenly targeted by CAR-T cells, significantly reducing the occurrence of off-target effects, and further enhancing the safety and precision of CAR-T therapy.

4. Summary

Chimeric antigen receptor T-cell therapy represents a significant breakthrough in the treatment of solid tumors, noted for its precision in targeting specific antigens within these cancers. The effectiveness of checkpoint blockade therapies has underscored the value of immunotherapeutic strategies in the management of solid tumors, further supporting the potential of this innovative approach as an effective "living drug" in oncological treatment. However, despite these advancements, the application of this therapy in solid tumors encounters numerous obstacles. Clinical trials often report response rates that are below anticipated levels, prompting a need for introspection and the pursuit of new methodologies. To overcome these challenges, researchers are actively engaged in identifying more relevant target antigens and improving the delivery and persistence of these therapeutic cells within the tumor

microenvironment. With ongoing advancements and enhancements, there is optimism for achieving groundbreaking progress in the use of this therapy for solid tumor treatment, potentially offering better outcomes and hope to a broader range of patients.

References

1. Schuster, S.J. *et al.* Chimeric Antigen Receptor T Cells in Refractory B-Cell Lymphomas. *NEW ENGL J MED* 377, 2545-2554 (2017).
2. Binnewies, M. *et al.* Understanding the tumor immune microenvironment (TIME) for effective therapy. *NAT MED* 24, 541-550 (2018).
3. Grigor, E.J.M. *et al.* Risks and Benefits of Chimeric Antigen Receptor T-Cell (CAR-T) Therapy in Cancer: A Systematic Review and Meta-Analysis. *TRANSFUS MED REV* 33, 98-110 (2019).
4. Kenderian, S.S. *et al.* CD33-specific chimeric antigen receptor T cells exhibit potent preclinical activity against human acute myeloid leukemia. *LEUKEMIA* 29, 1637-1647 (2015).
5. Gill, S.I. How close are we to CAR T-cell therapy for AML? *BEST PRACT RES CL HA* 32, 101104 (2019).
6. Morgan, R.A. *et al.* Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *MOL THER* 18, 843-851 (2010).
7. McGowan, E. *et al.* PD-1 disrupted CAR-T cells in the treatment of solid tumors: Promises and challenges. *BIOMED PHARMACOTHER* 121, 109625 (2020).
8. Jiang, Z. *et al.* HIV-1-Specific CAR-T Cells With Cell-Intrinsic PD-1 Checkpoint Blockade Enhance Anti-HIV Efficacy in vivo. *FRONT MICROBIOL* 12 (2021).
9. Nap, M., Mollgard, K., Burtin, P. & Fleuren, G.J. Immunohistochemistry of Carcino-Embryonic Antigen in the Embryo, Fetus and Adult. *Tumor biology* 9, 145-153 (1988).
10.Zhang, C. *et al.* Phase I Escalating-Dose Trial of CAR-T Therapy Targeting CEA+ Metastatic Colorectal Cancers. *MOL THER* 25, 1248-1258 (2017).
11.Chang, K. & Pastan, I. Molecular Cloning of Mesothelin, a Differentiation Antigen Present on Mesothelium, Mesotheliomas, and Ovarian Cancers. *Proceedings of the National Academy of Sciences - PNAS* 93, 136-140 (1996).
12.Thomas, A.M. *et al.* Mesothelin-specific CD8+ T Cell Responses Provide Evidence of In Vivo Cross-Priming by Antigen-Presenting Cells in Vaccinated Pancreatic Cancer Patients. *The Journal of Experimental Medicine* 200, 297-306 (2004).
13.Carpenito, C. *et al.* Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains. *Proceedings of the National Academy of Sciences - PNAS* 106, 3360-3365 (2009).
14.Adusumilli, P.S. *et al.* A Phase I Trial of Regional Mesothelin-Targeted CAR T-cell Therapy in Patients with Malignant Pleural Disease, in Combination with the Anti–PD-1 Agent Pembrolizumab. *CANCER DISCOV* 11, 2748-2763 (2021).
15.Sahin, U. *et al.* Claudin-18 Splice Variant 2 Is a Pan-Cancer Target Suitable for Therapeutic Antibody Development. *CLIN CANCER RES* 14, 7624-7634 (2008).
16.Qi, C. *et al.* Claudin18.2-specific CAR T cells in gastrointestinal cancers: phase 1 trial interim results. *NAT MED* 28, 1189-1198 (2022).
17.Mazor, Y. *et al.* Enhanced tumor-targeting selectivity by modulating bispecific antibody binding affinity and format valence. *SCI REP-UK* 7 (2017).
18.Langan, R.A. *et al.* De novo design of bioactive protein switches. *NATURE* 572, 205-210 (2019).
19.Lajoie, M.J. *et al.* Designed protein logic to target cells with precise combinations of surface antigens. *SCIENCE* 369, 1637-1643 (2020).
20.Hegde, M. *et al.* Tandem CAR T cells targeting HER2 and IL13Rα2 mitigate tumor antigen escape. *J CLIN INVEST* 126, 3036-3052 (2016).
21.Schneider, D. *et al.* A tandem CD19/CD20 CAR lentiviral vector drives on-target and off-target antigen modulation in leukemia cell lines. *J IMMUNOTHER CANCER* 5 (2017).
22.Morsut, L. *et al.* Engineering Customized Cell Sensing and Response Behaviors Using Synthetic Notch Receptors. *CELL* 164, 780-791 (2016).
23.Roybal, K.T. *et al.* Precision Tumor Recognition by T Cells With Combinatorial Antigen-Sensing Circuits. *CELL* 164, 770-779 (2016).