The function of anti-mesothelin CAR T cells is improved by hairpin RNAs.

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Abstract

Hematologic malignancies have demonstrated good results with chimeric antigen receptor (CAR) T cell treatment. CAR T cells' antitumor activity, on the other hand, needs to be improved in order to improve therapeutic efficacy in hematologic and solid malignancies. On-target, off-tumor toxicity, antigen escape, short CAR T cell persistence, limited growth, trafficking to the tumour, and T cell activity suppression by an inhibitory tumour microenvironment are all challenges to overcome. Here, we'll look at how genetic engineering can be used to improve the antitumor efficacy of CAR T cell therapy in preclinical animals by refining the design of CAR T cells. The goal of this work was to see how targeted Tim3 knockdown affected the antitumor function of anti-mesothelin (MSLN)-CAR T cells. Three distinct shRNA sequences specific to different regions of the human Tim3 gene were developed and co-inserted into lentiviral vectors with an anti-MSLN-CAR transgene to knock down Tim3 expression. Tim3 expression was measured before and after antigen stimulation to determine the efficiency of Tim3 targeting in T cells. MSLN-CAR T cells and Tim3-targeted MSLN-CAR T cells were then examined for cytotoxic effects, proliferative response, and cytokine production. Tim3 was up-regulated when T cells and MSLN-CAR T cells were activated, according to our findings. In distinct groups of MSLN-CAR T cells, knocking down Tim3 resulted in a considerable reduction in its expression. Tim3 knockdown increased MSLN-CAR T cells' cytotoxic efficacy, cytokine generation, and proliferative ability. Tim3 knockdown increased MSLN-CAR T cells' cytotoxic efficacy, cytokine generation, and proliferative ability. Our findings suggest that inhibiting Tim3 signalling allows tumor-infiltrating CAR T cells that would otherwise be inactivated to continue to grow and perform effector activities, thereby changing the tumour microenvironment from immunosuppressive to immunosupportive.

Keywords: Mesothelin, Chimeric antigen receptor, Tim3, Targeted knockdown, Tumor microenvironment.

Introduction

In addition to antigen loss in relapsed malignancies following CAR T cell therapy, tumour expression of the targeted antigen is likely to be diverse [1]. To accomplish total tumour eradication, CAR T cells must drive epitope spreading and a broader immune response, which would result in the destruction of antigen-negative tumour cells as well. Heterogeneity may be particularly important in solid tumours. In a variety of solid tumours, several targets for CAR T cell treatment have been identified. Clinical trials employing CAR T cells in solid tumours, on the other hand, have shown only modest antitumor activity [2]. Adoptive cell therapy for solid malignancies faces a number of challenges, including target antigen selection, T cell proliferation, and durability, T cell trafficking into the tumour and the ability to overcome an immune suppressive milieu are two examples [3]. Even though the requirement of two antigens for CAR T cell activation raises the risk of antigen escape, targeting a specific combination of two antigens can be used to boost specificity and diminish on-target, off-tumor adverse effects. CD19 CAR T cells attack normal B cells, causing B-cell aplasia, which can be treated with immunoglobulin infusions once a month. However, a case report of a patient treated with HER2-targeting CAR T cells who had significant toxicity and died due to low levels of HER2 on lung epithelium highlights the importance of cautious CAR construct design, as tumor-specific targets are limited.

Wendell Lim's lab recently created a new system in which two antigens are required for full CAR T cell activation: an AND-gate CAR called synNotch. A designed antigen-recognition domain, a Notch core, and an artificial transcription factor are all part of this synthetic protein, which is split off and activated when antigen stimulation occurs. Because this transcription factor only activates the CAR and thus the T cells when both antigens are present, the CAR and thus the T cells are only activated when both antigens are present [4].

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This system operates in an orthogonal manner and does not require the use of an intermediate signaling molecule, making it a versatile tool for controlling specific signal-response cascades in a range of applications. However, whether the non-human transcription factors are immunogenic has yet to be determined.

Conclusion

CAR T cell treatment has shown considerable promise in clinical trials, particularly in B-ALL. However, increasing CAR T cells' antitumor activity will be critical if they are to be successful in other hematologic and solid cancers. The use of armored CAR T cells to produce local production and release of a range of chemicals to enhance CAR T cell function, influence tumor-infiltrating immune cells, and directly target tumour cells is a strong method. It is envisaged that local synthesis of cytokines and checkpoint inhibitors will lessen the negative effects associated with systemic treatment. CARs, chimeric stimulatory receptors, and synNotch are examples of synthetic molecules that boost tumour selectivity and limit antigen escape. Furthermore, as discussed for the iCAR, switch receptor, and chimeric cytokine receptors, genetic engineering of chimeric receptors allows for the conversion of inhibitory signals into stimulating responses and vice versa, thereby increasing tumour specificity, safety, and reducing immune suppressive signaling [5]. Pre-clinical investigations in syngeneic tumour models will aid in determining which of the discussed tactics is best, which will likely vary depending on the target antigen and tumour type.

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