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Innovative pan-tumor target strategy for CAR-T therapy: cancer-specific exons as novel targets for pediatric solid and brain tumors

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Abstract

Chimeric antigen receptor T-cell (CAR-T) immunotherapy has achieved remarkable success in treating chemotherapy-refractory hematological malignancies. However, its efficacy in solid and brain tumors remains limited due to challenges such as insufficient target antigens, poor T-cell adaptability, inefficient tumor site trafficking, and the immunosuppressive tumor microenvironment. To address these challenges, Shaw and colleagues proposed an innovative strategy targeting cancer-specific exons (CSEs) in pediatric solid and brain tumors. Using RNA sequencing data from 16 tumor types, the study identified 157 highly tumor-specific targets, including both known and novel proteins. The researchers validated several targets, including FN1 and COL11A1, demonstrating their therapeutic potential in in vitro and in vivo models. The study's approach of integrating exon-level analysis with a broad search for extracellular matrix proteins offers a new frontier for CAR-T therapy, providing valuable insights for improving immunotherapy in pediatric solid tumors. Although promising, the study also highlights the need for further evaluation of tumor recurrence and CAR-T cell exhaustion. The identification of novel pan-tumor targets may revolutionize CAR-T therapy design and expand its application in pediatric cancer treatment.

Keywords CAR-T immunotherapy, Cancer-specific exons, Pediatric tumors, Solid tumors, Tumor microenvironment

Main text

In the past decade, chimeric antigen receptor T-cell (CAR-T) immunotherapy has demonstrated significant efficacy in patients with chemotherapy-refractory hematological malignancies, including lymphoma, leukemia, and multiple myeloma [1]. However, the success of CAR-T cell therapy in treating solid and brain tumors has been limited [2]. Recent clinical trials have further emphasized these limitations, including a restricted number of targetable antigens with uneven expression, limited adaptability of T cells, ineffective trafficking of T cells to tumor sites with insufficient penetration of tumor barriers, and the immunosuppressive nature of the tumor microenvironment [2–5].

Target selection is a crucial determinant of CAR-T therapy's efficacy. To ensure both safety and effectiveness, the ideal target antigen must exhibit specificity, stability, and broad malignant cell coverage. Unfortunately, most CAR-T targets for solid and brain tumors fail to meet these stringent criteria. Currently, target selection

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mainly focuses on differentially expressed antigens [6] that also exist at low levels in normal tissues, making the search for novel targets a promising solution. However, many newly identified antigens are derived from intracellular proteins, which are difficult to target using traditional CAR-T cells. Consequently, researchers are now turning to genomic and proteomic approaches to identify new CAR-T cell targets [7, 8].

Recently, Shaw and his team proposed an innovative pan-tumor targeting strategy using cancer-specific exons (CSEs) as potential targets for immunotherapy against pediatric solid and brain tumors [9]. Through RNA sequencing analysis of 16 types of pediatric tumors, they identified proteins encoded by 157 genes as highly tumor-specific, either at the gene level or as alternatively spliced (AS) isoforms. The majority of these CSE targets result from gene-level aberrations. Among the identified targets, 11 (CD83, CD276, FAP, FN1, GPC2, GPC3, IL1RAP, KDR, KIT, MET, and CD133) have already been investigated in preclinical or clinical studies, while the remaining 93% represent novel targets. Approximately 30% of these targets are highly expressed across both solid and brain tumors, underscoring the potential for pan-tumor target development.

Shaw et al. further categorized the targets into primary and secondary groups based on their expression in normal tissues and vital organs. Primary targets, which exhibit the least expression in normal tissues and bone marrow, are expected to minimize treatment-related side effects. In patient-derived xenograft models, three primary targets—FN1, VCAN1, and COL11A1—were widely expressed in pediatric solid and brain tumors. In vitro studies demonstrated that both COL11A1-CAR and FN1's alternative splicing extra domain CAR (EDB-CAR) had anti-tumor activity against pediatric sarcomas. Orthogonal experiments confirmed the antigen specificity of COL11A1-CAR. Additionally, Shaw's team used a mouse osteosarcoma model to validate the in vivo anti-tumor efficacy of COL11A1-CAR, showing a significant extension of median survival in CAR-treated mice, although tumors eventually recurred.

In conclusion, Shaw et al. demonstrated that CSEs can serve as candidate targets for CAR-T therapy through RNA-seq data mining, yielding a wealth of potential targets for future research. The team validated at least one target, COL11A1, which broadens the scope of immunotherapy options for pediatric solid and brain tumors.

The success of this research can be attributed to several key factors. First, the study employed exon-level analysis, enabling researchers to identify exons specifically expressed in tumor cells due to alternative splicing, thereby discovering more potential targets. Second, the team expanded the range of CAR-T cell targets by including proteins from the extracellular matrix, rather than focusing solely on membrane-bound proteins. Their results showed that extracellular matrix proteins, such as COL11A1, can also serve as effective CAR-T targets. Third, the team conducted an integrated analysis of large-scale RNA sequencing data from multiple databases, including contributions from St. Jude Children's Hospital, the University of Washington, and the National Cancer Institute. This expansive data set ensured the reliability and depth of their findings. Additionally, strict screening criteria were applied to ensure that CSE targets were highly cancer-specific, requiring significantly higher expression in tumor samples compared to normal tissues, and excluding targets highly expressed in critical organs such as the brain, liver, lungs, and bone marrow.

However, the study has some limitations. First, the team used normal adult tissue samples as controls to validate CSE targets in pediatric tumors, which may

(See figure on next page.)

Fig. 1 Cancer-specific exons as novel immunotherapy targets in pediatric sarcomas and solid tumors. **a** Analysis Process of Cancer-Specific Exons (CSEs): Shaw and his team followed a systematic approach to identify cancer-specific exons. First, they mapped RNA-seq data from tumor samples and normal tissues. Next, they selected exons with enriched expression in tumors but not in normal tissues. They further filtered exons based on their presence on the cell membrane or expression in the extracellular matrix. The exons were then categorized into Tier 1, representing those with minimal expression in corresponding normal tissues and essential organs, and Tier 2, based on the degree of cancer-specific expression. Targets were further divided into those specific to alternative splicing (AS) exons and gene-level targets. In this study, the team focused on three transcripts from Tier 1: VCAN1, FN1 (with its exon EDB), and COL11A1. These were validated using techniques such as flow cytometry, RT-qPCR, and immunohistochemistry (IHC) to confirm their cell surface expression. FN1 and COL11A1 were subsequently selected for further research. **b** Validation of CSEs as Immunotherapy Targets: Through a combination of in vitro, orthogonal, and in vivo experiments, Shaw's team demonstrated that COL11A1/EDB-CAR T cells exhibit robust anti-tumor activity and antigen specificity against a range of pediatric sarcomas. **c** Significance of CSEs as Novel Targets: COL11A1 has emerged as a promising new biomarker, playing a crucial role in pediatric sarcomas and various solid tumors. Additionally, its expression has been observed in certain adult cancers with poor prognosis, such as pancreatic cancer, indicating broad clinical relevance. This discovery highlights the potential for wide applicability in cancer immunotherapy. Moreover, the team developed the CSE-miner tool, enabling researchers to explore all targets identified in this study with ease. The tool offers multiple visualization methods, including scatter plots, representation maps, heat maps, and gene views, facilitating comprehensive analysis of each target. This dataset not only provides valuable resources but also outlines a roadmap for advancing pediatric cancer immunotherapy

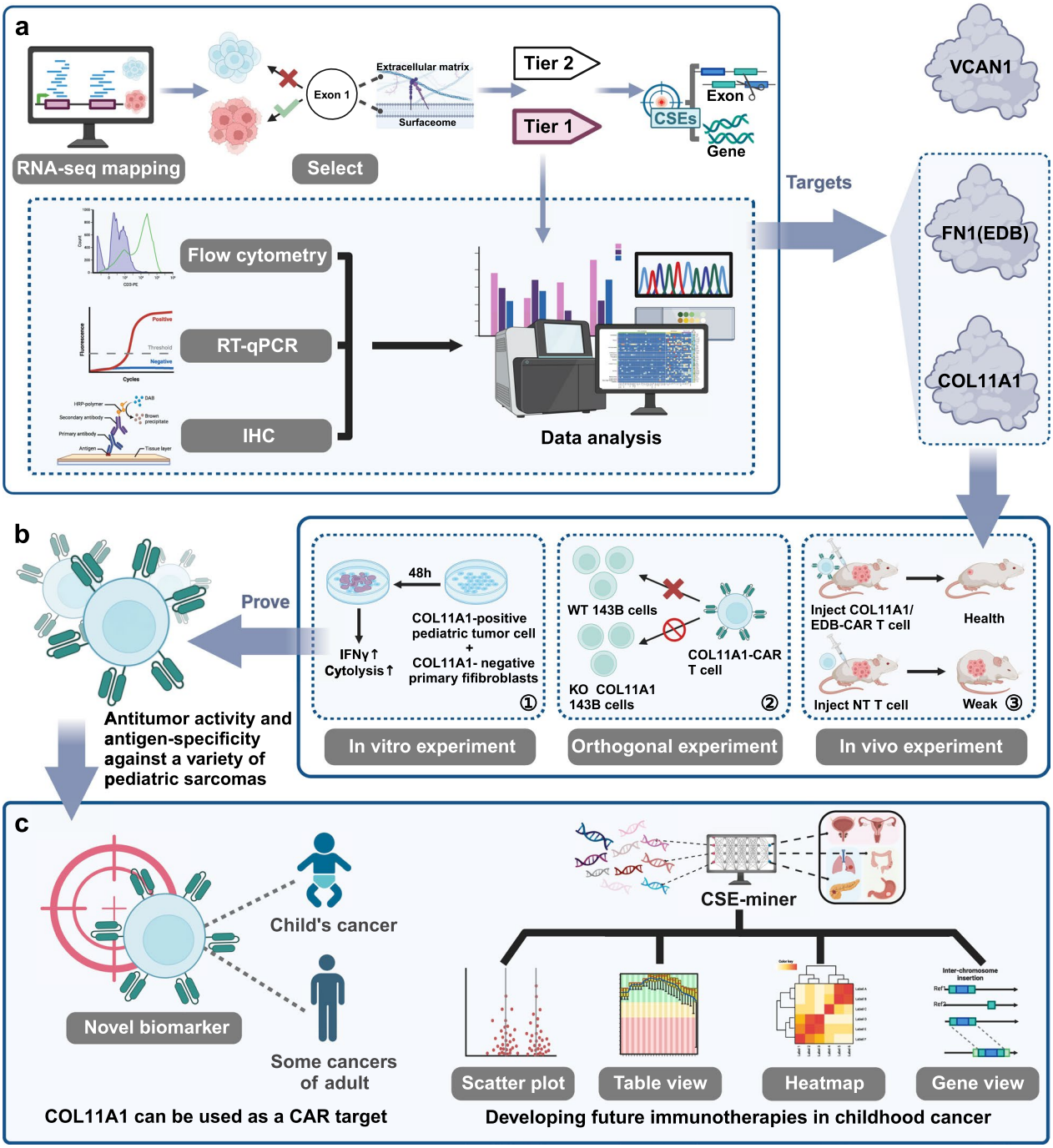

Fig. 1 (See legend on previous page.)

introduce bias due to the differences between pediatric and adult tissue expression. Additionally, pediatric tumors often arise from developmental origins and exhibit unique biological characteristics that allow them to evade treatment in unpredictable ways [10]. The recurrence of tumors in mice treated with COL11A1-CAR, accompanied by reduced COL11A1 expression, raises questions about the role of CAR-T cell exhaustion and tumor immune evasion, which require further investigation. Lastly, the occurrence of secondary T-cell tumors in some patients undergoing CAR-T therapy calls for careful evaluation of the long-term safety of Shaw's CAR-T strategy.

CAR-T therapy holds great promise for treating solid tumors. Continued exploration of suitable targets and optimization of design strategies are essential to overcoming the challenges of CAR-T therapy. Shaw et al.'s approach offers a new direction for pan-tumor target discovery, with potential applications beyond CAR-T, including immune cytokines and antibody-conjugated therapies. This research opens up vast possibilities for the future of pediatric solid tumor immunotherapy.

Abbreviations

AS	Alternatively spliced
CAR-T	Chimeric antigen receptor T-cell
CSE	Cancer-specific exon

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Author contributions
Yuqing Luo and Jianqiao Shentu analyzed the literature and wrote the manuscript. Jianqiao Shentu, Yuqing Luo, and Hening Xu drafted the figure. Yongming Xia and Jianqiao Shentu conceived the idea. Lili Fang and Shiwei Duan reviewed and revised the manuscript. All authors have read and approved the final the final manuscript.

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