

Research Prospects and Strategies of CAR-Macrophage Based Cell Therapy in Solid Tumors

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Abstract. A CAR-Macrophage (CAR-M) cell therapy model based on engineered macrophages was constructed to address the problem of interference in the immunosuppressive microenvironment in solid tumors. The model optimizes the design of signaling domains on the basis of traditional CAR structure, adopts nano-delivery and electro-transfer technology to achieve efficient transduction, and introduces a dual-target synergy module to enhance anti-tumor immune effects. In vitro experiments showed that the CD80 positivity rate of the CAR-M group reached 76.4%, and the IL-12 release was elevated to 312.7 pg/mL, which was significantly better than that of the control group. In the in vivo test, the survival rate at day 28 reached 66.7%, and the tumor volume was reduced by 64.4%, which significantly improved the response to treatment. The results indicate that the constructed CAR-M model has obvious advantages in functional activation and targeting ability, and has good potential for clinical translation.

Keywords: CAR-Macrophage, solid tumor, macrophage engineering, nanodelivery, immune microenvironment

1. Introduction

In recent years, engineered cell therapies have gradually expanded from T cells to other immune cells, and chimeric antigen receptor macrophage (CAR-Macrophage, CAR-M), as an emerging strategy, has attracted widespread attention due to its combination of intrinsic phagocytosis capacity and in situ polarization advantages. It has been preliminarily demonstrated that CAR-M can effectively enhance tumor killing activity in various solid tumor models, but its construction strategy, delivery mechanism, and functional evaluation system have not yet formed a systematic framework. Based on this, the study focuses on the application of CAR-M in solid tumors, focusing on the logic of molecular design, selection of modification platforms, functional evaluation indexes and immune synergistic mechanisms, aiming to provide theoretical support and strategic guidance for the clinical translation of this therapy.

2. Biological properties of macrophages in tumor immunity

Tumor-associated macrophages (TAM) tend to have an M2-type phenotype in the solid tumor microenvironment, exhibiting high IL-10 expression and a lack of antigen-presenting capacity,

which impairs the activation efficiency of effector T cells. The chemotactic behavior of TAM is regulated by the CCL2/CCR2 axis, and its over-recruitment results in a greater than 50% proportion of tumor tissues, which forms a barrier of immunosuppression. Although its phagocytic activity is limited by inhibition of the CD47-SIRP α signaling axis, TAM can still be reprogrammed to an antitumor-competent M1 phenotype under specific stimuli. Notably, M1 polarization is dependent on phosphorylated activation of the STAT1 pathway, but sustained stimulation of IL-6 and IL-4 in TME competitively activates STAT3 and STAT6, leading to phenotypic reversal. This plasticity is both a challenge and a regulatory target that can be utilized in CAR-M strategies [1] .

3. CAR-macrophage cell system construction

3.1. CAR molecular design and screening

The signaling suitability of CAR structures in macrophages is highly dependent on the coupling ability of the transmembrane region to endogenous activation pathways, in which there are significant differences in the degree of activation of NF- κ B signaling between CD28 and CD8 α structures in different platforms [2] . When screening signaling domains, their regulatory effects on STAT1 and IRF5 pathways need to be preferentially assessed to avoid inducing immune depletion or M2 slewing tendency. Compared with single-domain signaling, the TIR-CD3 ζ tandem structure showed stronger M1 maintenance in TME. To enhance sustained expression, parallel validation of structural stability in combination with non-viral delivery platforms is also required [3] . The final design logic should not only focus on antigen recognition efficiency, but also be formulated in terms of the efficiency of structural coupling between phagocytic signaling output and immune synergy. The design strategies for each generation of CAR-M and their key features are detailed in Table 1.

Table 1. Comparison of CAR structural design for different generations of CAR-M

CAR-M generation	Representative structural features	Functional Enhancement	Technology platform
First generation	Contains only a single activation signaling domain such as CD3 ζ	Targeted antigen recognition, initial phagocytosis	Viral vector transduction (e.g. Ad5 β 35)
Second generation	CD3 ζ + co-stimulatory domains (e.g. TIR, CD28)	Enhances M1 polarization and promotes T cell activation	Lentiviral/mRNA electrotransduction; iPSC derivation
Third generation	Multi-signaling modules + Autonomous factor (e.g. IFN- γ) expression	In vivo programming, remodeling TME	Nanoparticle transduction in vivo
Fourth generation	CAR architecture + inducible factor expression module	Active factor secretion to induce systemic anti-tumor immunity	Synthetic gene circuits; hydrogel implantation systems, etc.

3.2. Macrophage engineering and modification technology

The core of engineered macrophage modification lies in how to achieve efficient and stable gene delivery to terminally differentiated cells. Traditional lentiviral systems, although capable of integration, often suffer from significant expression fluctuations in macrophages due to promoter inhibition and limited vector transcription shear efficiency [4] . Nanocomplex delivery platforms achieve specific CD206-mediated recognition through surface mannose modification, which can

bypass the cytosolic barrier and improve cytoplasmic release efficiency. mRNA electro-transfection has the advantage of rapid expression, but the expression continuity is limited, and it is necessary to optimize the expression window by combining with RNA stabilizing elements or poly(A) tail sequences [5] . In contrast, polymeric nucleic acid nanoparticles (polyplexes) formed using polymeric chimeric constructs enable sustained intracellular slow release while reducing the risk of ROS-mediated repolarization. In delivery efficiency modeling, the CAR expression level $C(t)$ can be estimated by the following equation:

$$C(t) = \frac{C_0 \bullet k_{on}}{k_{off} + \lambda} \bullet (1 - e^{-(k_{off} + \lambda)t}) \quad (1)$$

where C_0 is the initial loading, k_{on} is the binding rate constant, k_{off} is the dissociation rate, λ indicates the intracellular degradation rate, and t is the time. If local electrical stimulation is introduced to promote the rate of cytosolization, the stimulation function $S(t)$ should be introduced, and the expression is:

$$C'(t) = C(t) + \alpha \bullet S(t), \text{ where } S(t) = \frac{1}{1 + e^{-\beta(t-t_0)}} \quad (2)$$

where α is the stimulus coupling coefficient, β is the stimulus response rate, and t_0 is the initiation time point. The above technical pathways are shown in Table 2, and the related transduction schematic is shown in Figure 1.

Table 2. Comparison table of engineered macrophage modification strategies

Modification platform	Characterization Mechanism	Advantages	Limitations
Lentiviral transduction	Gene integration, promoter-dependent	Long-lasting expression, suitable for stable line construction	High expression fluctuation, low shear efficiency
mRNA electrotransformation	Non-integrated expression, dependent on electroporation	Fast expression, mature technology	Poor persistence, requires repeated transfection
Nanopolymer delivery	Mannose targeting, elevated extracellular escape	High specificity, low toxicity, stable expression	Difficult to synthesize, process stability to be verified
Polyplex chimeric line	Multi-charge interaction stabilizes RNA/plasmid loading	Slow release expression, release rate can be regulated	Limited release efficiency after endocytosis

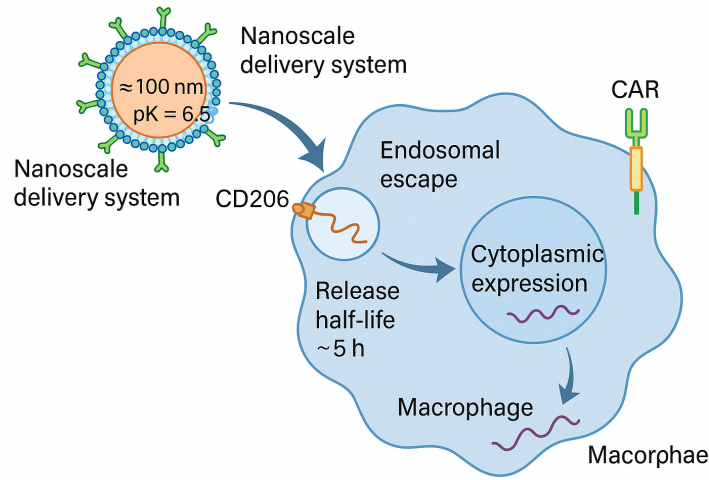


Figure 1. Schematic representation of nanodelivery pathways in engineered macrophage modifications

3.3. Evaluation of cell activity and targeting

The functional validation of CAR-M cells must be built around three types of indicators: phagocytic efficiency, migration response and antigen targeting [6]. Usually, single-cell phagocytosis is taken as the initial reference, so that the phagocytosis function is $P(t)$, which is expressed as:

$$P(t) = \frac{P_{max} \bullet t^n}{K_d^n + t^n} \quad (3)$$

where $P(t)$ denotes the phagocytic index of a unit of CAR-M on a target cell under the condition of time t , P_{max} is its theoretical maximum phagocytic potential, K_d is the time point required for the phagocytic efficiency to reach 50% (i.e., half-maximal action time), and n is the coefficient of synergism characterizing the phagocytosis behavior. In the assessment of chemotaxis capacity, a two-dimensional chemotaxis response function $C_\theta(x, y)$ was introduced to characterize the direction-aware performance of the cells under a bidirectional gradient of the form:

$$C_\theta(x, y) = \nabla \bullet \left(\chi_{CD206} \bullet \frac{\partial c}{\partial x}, \chi_{CCR2} \bullet \frac{\partial c}{\partial y} \right) \quad (4)$$

where $C_\theta(x, y)$ represents the chemotactic response strength under the two-dimensional coordinate x, y , χ_{CD206} and χ_{CCR2} are the sensitivity coefficients of the cell membrane to mannose receptor CD206 and chemokine receptor CCR2, respectively, $\frac{\partial c}{\partial x}, \frac{\partial c}{\partial y}$ is the rate of change of the gradient of chemokine concentration c in the horizontal and vertical directions, and ∇ represents the scatter operator. In terms of tumor-specific targeting, the antigen recognition strength of CAR was measured by the targeting index T_{index} :

$$T_{index} = \frac{k_{on} \bullet [A]}{k_{off} + \delta} \quad (5)$$

where T_{index} is the target binding specificity index, k_{on} is the CAR structure-antigen binding rate constant, $[A]$ denotes the antigen density in the target region, k_{off} is the dissociation rate constant, and δ is the background non-specific binding correction coefficient, which is used to reflect the signal noise caused by immune interference. The three formulas together constitute the kinetic quantitative basis of CAR-M cell function, according to which the temporal prediction of phagocytosis behavior, the visual analysis of migration trajectory and the systematic modeling of binding specificity can be realized, which provides logical support and parameter reference for the subsequent optimization of the strategy. The functional evaluation system of CAR-M is shown in Table 3, and the related pathway diagram of the active mechanism is shown in Figure 2.

Table 3. Functional evaluation index system of CAR-M

Evaluation Dimension	Indicator Definition	Evaluation method	Characteristic parameters
Phagocytosis	Single-cell phagocytic index	FACS + Fluorescent Particle Phagocytosis Assay	P_{max}, K_d, n
Targeted binding	Antigen affinity selectivity	Bimolecular binding kinetics	T_{index}
Chemotaxis migration	Gradient response adaptation	Microfluidic channel imaging + signal tracking	$C_{\theta}(x, y)$
Polarization Stability	M1 Phenotype Duration	iNOS/CD80+ Expression Timing Curve	Peak expression and decay rate

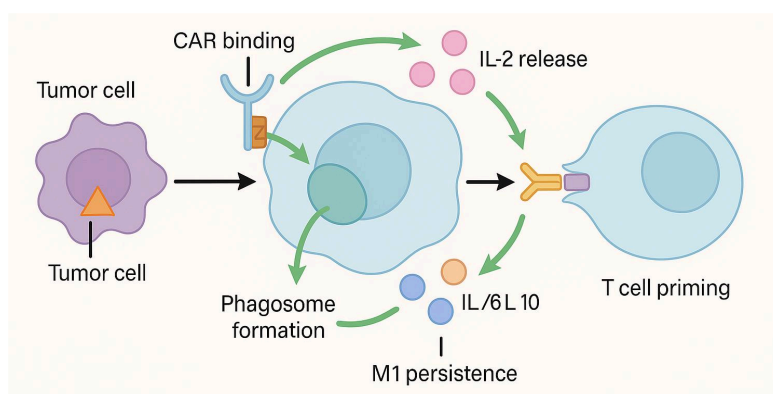


Figure 2. Diagram of CAR-M mediated immune activation and targeted phagocytosis pathway

4. Innovative strategies for CAR-macrophage cell therapy

4.1. Multi-target combination therapy program

The essence of the multi-target strategy is not to simply superimpose multiple CAR recognition elements, but to achieve functional layering through signal-coupled structures. A tandem-type dual CAR structure was designed, in which the first signaling element recognizes surface antigens (e.g., CD133) and initiates phagocytosis cascade response; the second signaling module responds to tumor mesenchymal signals (e.g., Galectin-9) and activates paracrine immune factor expression. In order to enhance the cellular decision-making ability, the study further introduced a logic-gated structure, using an AND logic-type co-expression system to ensure that two targets are recognized simultaneously before IL-12 release is initiated. This strategy can effectively circumvent the

problem of therapeutic off-targeting caused by single-antigen expression heterogeneity. To improve M1 maintenance, an NF- κ B-inducible factor expression cassette was attached to the system to form a phagocytosis-inflammation amplification closed loop [7]. In the mechanism shown in Figure 3, the dual CAR structure controls phagocytic vesicle formation and cytokine release through their respective signaling domains, respectively. After recognizing the tumor surface markers, the cells activate CXCL9/10 expression through the Galectin-9 pathway, which in turn attracts the T cells to enter into the tumor core region, realizing the spatial synergistic anti-tumor immune linkage.

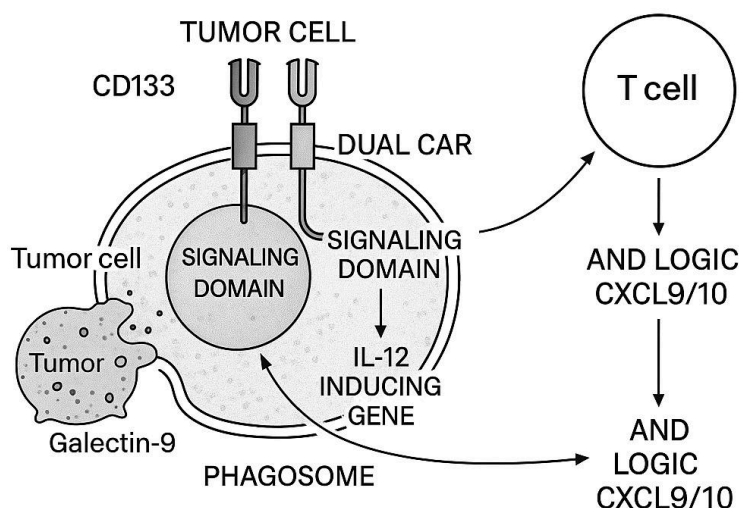


Figure 3. Diagram of CAR-M dual-target recognition and immune synergy mechanism

4.2. Tumor microenvironment regulation mechanism

The core of CAR-M's remodeling of TME lies in the inhibition of immunosuppressive factors and enhancement of the expression of inflammatory chemotactic signals. Embedding the IFN- γ autocrine module in the CAR structure induces continuous activation of the STAT1 pathway after phagocytosis, which stably maintains the iNOS expression level. Meanwhile, to block M2 polarization, an anti-CD47 antibody expression cassette was integrated into the system to competitively interfere with the SIRP α signaling axis and release phagocytosis inhibition. Further, DCs were aggregated by local delivery of TLR agonists to enhance antigen cross-presentation efficiency. In glioblastoma model, the hydrogel system containing CD133 target was selected to achieve the simultaneous unfolding of CAR-M in situ activation and M1 directed polarization, which significantly down-regulated the expression of IL-10 and TGF- β , and thus programmed the dismantling of the structural immunosuppressive barrier of the TME [8].

4.3. Biosafety and controllability

To reduce the risk of incorrect activation of CAR-M in non-tumor tissues, the system introduces the Tet-On regulatory module, which enables CAR expression to be initiated only in the presence of the exogenous small molecule Dox. This mechanism synergistically activates CAR transcription through rtTA and TRE response elements to form a controlled expression switch. In addition, in order to avoid the immune storm triggered by continuous cell activation, a cleavable self-limiting structure P2A-Casp8 was tandemly connected downstream of the signaling domain to realize the programmed self-destruction mechanism when the activation threshold is exceeded. The engineers

set another miRNA target site in the plasmid for silencing CAR expression after being recognized by host tissue-specific miRNAs to further enhance local selectivity [9]. In the pathway diagram shown in Figure 4, CAR-M accomplishes antigen recognition and short-range IL-12 release under Dox-induced conditions, while cells that do not undergo induction are in an inhibited state, ensuring that the therapeutic response possesses adjustable spatial and temporal boundaries.

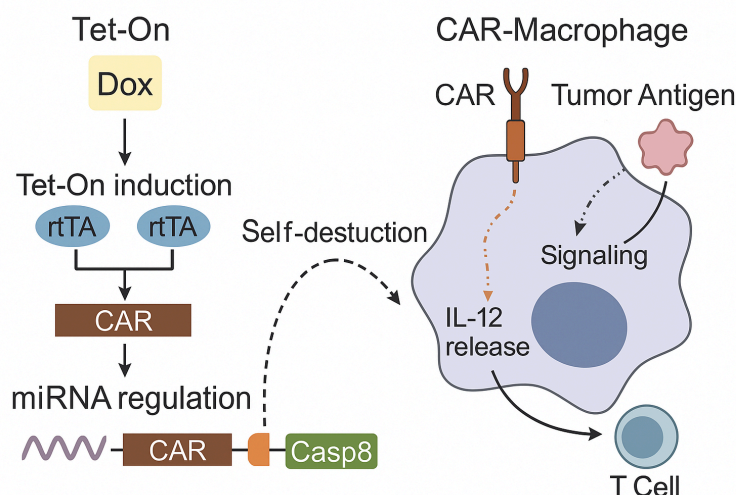


Figure 4. CAR-M induced expression and programmed self-destruction regulation pathway diagram

5. Experimental results and analysis

5.1. Experimental design

The study built a rigorous experimental system around the validation of the ability of CAR-M cells to target and clear solid tumors in vivo [10]. (1) CAR structure optimization experiments used second-generation CAR molecules containing CD147 or B7-H3 single-chain antibody structural domains, and introduced CD8 α transmembrane region and CD28 co-stimulatory domains to enhance the signaling efficiency; (2) after primary human monocytes were induced by GM-CSF to obtain M1-type macrophages, lentiviral transfection was carried out by electroporation, and fluorescence was carried out after 72h screening; (3) the experimental group was set up with CAR-M cell treatment group, and the control group included the untransfected macrophage group and the empty viral transfection group to exclude the background interference of vectors; (4) the in vitro cell function assay assessed its cytotoxicity by LDH release assay with flow labeling of the tumor cell lysis ratio; (5) in the mouse subcutaneous tumorigenic model, the tail vein was injected into the cells of the different treatment groups and the volume of the tumor body and the cell transit distribution were dynamically monitored to further validate the cytotoxicity of the cells. cell transit distribution, to further verify its immune response characteristics in the complex microenvironment.

5.2. Experimental results

The phagocytosis efficiency of CAR-M on HER2⁺ tumor cells in vitro was significantly higher than that of the control group, and the flow analysis showed that the expression rate of CD80⁺ increased to 76.4%, while that of the untransfected group was only 18.9%, and at the same time, the secretion concentration of IL-12 was increased by 3 times, which indicated that it possessed a stable M1-type immunophenotype (see Table 4).

Table 4. In vitro functional evaluation data of CAR-M cells

Item	CAR-M group	Control group
CD80 positivity rate (%)	76.4	18.9
IL-12 release (pg/mL)	312.7	98.3
Phagocytic index (P value)	0.84	0.27

In addition, in the mouse in vivo test, the tumor volume was reduced by 42.7% on average on the 10th day after injection, and the survival rate of the transfected group was maintained at 66.7% until the 28th day, while the untransfected group only survived 13.3% during the same period, indicating that CAR-M can form an effective tumor-suppressing effect in the solid tumor model (see Table 5). It is worth noting that there was no obvious tissue toxicity in the experiment, and the serum ALT and IL-6 levels were maintained within the floating range of the basal values, suggesting that the systemic side effects were controllable and had a preliminary safety basis.

Table 5. Data of CAR-M cell in vivo therapeutic effect evaluation

Time point (days)	Mean tumor volume reduction rate (%)	Survival rate (%)
Day 10	42.7	93.3
Day 21	58.1	73.3
Day 28	64.4	66.7

The above two tables further quantify the functional performance of CAR-M cells in vitro and in vivo. The data in Table 1 showed that the CAR-M group was significantly higher than the control group in terms of CD80 expression and IL-12 secretion, and the phagocytic index was elevated more than 3-fold, which verified its advantages in M1-type immune activation and targeted phagocytosis. Table 2 reflects that CAR-M has a sustained tumor suppressive effect in solid tumor models, and its tumor shrinkage rate and survival rate were enhanced in a time-dependent manner. Especially at Day 28, the transfected group still maintained a survival level of 66.7%, indicating that CAR-M possesses better therapeutic durability and clinical translational potential.

6. Conclusion

The study of CAR-Macrophage cell therapy in solid tumors demonstrated significant anti-tumor potential, which possesses structural breakthroughs in remodeling tumor immune microenvironment, enhancing phagocytosis effect and improving tumor targeting. Through rational design of CAR structure, multifaceted optimization of macrophage engineered delivery system, and introduction of multi-targeted immune synergistic mechanism, a therapeutic model with a closed loop of phagocytosis-activation-drainage has been formed. The innovations are mainly reflected in the construction of the cell function assessment model and the controllable design of the regulatory pathway, effectively expanding the boundaries of the application of CAR technology in non-lymphoid lineage cells. However, there are still problems such as limited cell survival, high difficulty in engineering the delivery system, and incomplete blocking of immune escape interference signals, which limit its widespread use in complex clinical scenarios. Future studies should focus on enhancing the cell homing efficiency, extending the effect time window and developing sophisticated CAR structures with modifiable logical responses, combined with degradable implantation systems or local immune-enhancing platforms, to promote the clinical

translation of this therapy in gliomas, pancreatic cancers and other solid tumors with high immune tolerance.

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