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HIGHLIGHT

Lyophilized lymph nodes: A paradigm shift in CAR T-cell delivery for solid tumor therapy

CAR T (chimeric antigen receptor T-cell) therapy represents a paradigm shift in cancer treatments. By empowering immune cells to target malignant cells directly, it opens another door to precision medicine, promising cures for once refractory malignancies. However, the extension of CAR T therapy to solid tumors confronts formidable obstacles[1]. The physical and biochemical barriers within the tumor microenvironment, such as dense extracellular matrices and immunosuppressive factors, impede CAR T cell infiltration and function, leading to diminished success rates in solid tumor treatment[2].

Utilizing lyophilization technology to prepare active biomaterials holds significant promise in the field of biomedical sciences[3]. The primary advantage of this technology is its capability to dehydrate tissues while maintaining their structural integrity and biological activity. This is especially crucial for intricate biological materials, where cellular and tissue architecture play a vital role in functionality. Recently, a pioneering study published in *Nature Materials* by Gu's team has demonstrated the innovative application of lyophilized lymph nodes (L-LNs) as delivery vehicles for CAR T cells[4] (Fig. 1). Through the lyophilization process, LNs underwent a transformation into highly porous structures, capable of accommodating T cells due to their enhanced hygroscopic properties following dehydration. The increased absorptive capacity of the L-LNs facilitated efficient loading of CAR T cell suspensions.

Unlike conventional methods that struggled with effective infiltration of CAR T cells into solid tumors, this strategy employed freeze-dried LNs from patients as a scaffold for delivering CAR T cells to target tumors. It harnessed the structural and molecular integrity of L-LNs, ensuring the presence of essential cytokines, chemokines, and the structural meshwork crucial for T cell survival and proliferation. Studies revealed a 3.5-fold increase of CAR T cell proliferation within L-LNs compared to those within hyaluronic acid (HA) hydrogels, along with a significant enhancement in promoting CAR T cell differentiation towards memory phenotypes[5]. Additionally, the L-LNs facilitated sustained release and activation of CAR T cells, critical for their function and efficacy in tumor suppression.

In mouse models, the effective delivery of CAR T cells *via* L-LNs demonstrated superior efficacy in preventing tumor recurrence. Upon implantation into the tumor bed post-surgery, lyophilized L-LN scaffolds acted as a reservoir and facilitated the controlled release of CAR T cells into the tumor resection site, thereby effectively maintaining an active cellular response against residual tumor cells. Notably, L-LNs exhibited a higher intratumoral CAR T cell activation and increased production of cytotoxic factors, significantly outperforming the HA hydrogel counterparts. Furthermore, the biocompatibility and safety of autologous L-LN materials were critical considerations in the development of advanced delivery systems for CAR T cell therapy. *In vivo* implantation showed minimal local and systemic inflammatory responses in subjects receiving L-LNs compared to those implanted with HA hydrogels. The reduced inflammatory profile of L-LNs suggested a higher level of biocompatibility and safety for clinical utilization in CAR T cell therapy.

LNs are integral components of the adaptive immune system, playing essential roles in T cell initiation, activation, and tolerance[6]. Their highly organized structure contains diverse cellular types that collectively detect and respond to pathogenic antigens. The concept of utilizing L-LNs as a vehicle for CAR T cell delivery presents a profoundly innovative and potentially transformative approach, addressing critical obstacles currently impeding the effective treatment of solid tumors with CAR T therapy. The prospect of enhancing efficacy in preventing tumor recurrence, especially in post-surgical scenarios where residual tumor cells remain as the source of relapse, is particularly encouraging. This strategy not only maintains the viability and efficacy of therapeutic cells, but also presents a novel method for targeted cellular therapy. By fully utilizing the inherent structural properties of LNs and combining them with the preservative capabilities of freeze-drying for biochemical components, this technique coordinates the biological activity and loading capacity

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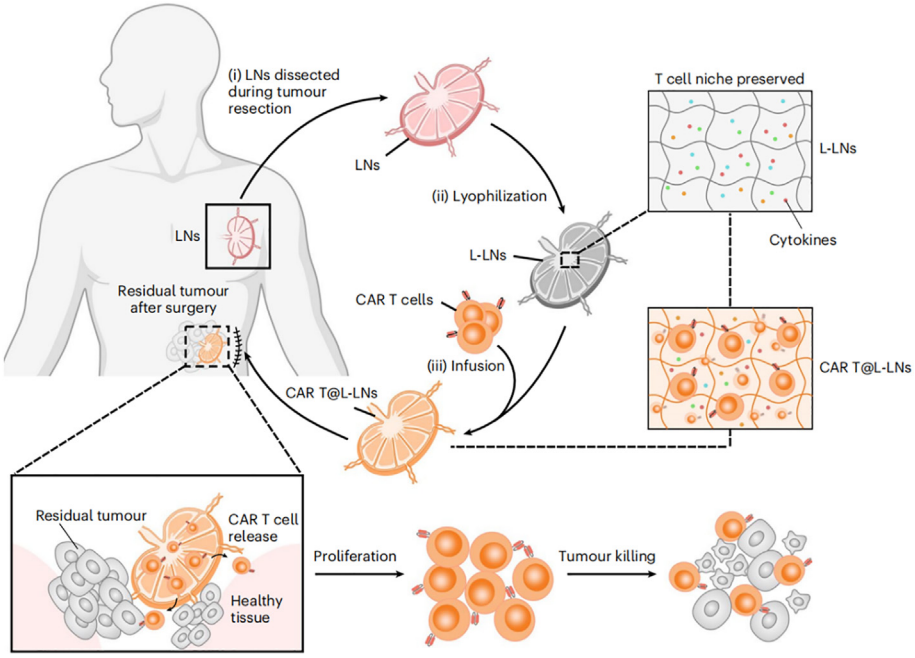

Figure 1 Schematic showing the implantation process of patient-derived L-LNs loaded with CAR T cells in the tumor bed following surgery. L-LNs maintain the extracellular porous microarchitecture and critical environment for T cell survival, proliferation and differentiation. Reproduced with permission from Ref. 4. Copyright © 2024 Springer Nature.

of LNs as an innovative delivery platform, and offers a new paradigm in the delivery mechanisms for cell-based therapies. These advancements highlight the vast potential and versatility of lyophilization technology in advancing therapeutic strategies within the biomedical field. Importantly, the use of autologous materials significantly streamlines the complexities and costs associated with CAR T cell therapies, therefore improving patient accessibility and minimizing the needs for extensive *in vitro* handling.

Looking forward, extensive researches and clinical trials are imperative to refine this technology and validate its applicability and safety in clinical settings, aiming to establish a standard platform for L-LNs based cellular immunotherapy in oncology. On one hand, more manpower and resources would be paid to expand this new approach on various tumor types, particularly for those involving critical organs and in complex anatomical regions. On the other hand, some cancer surgeries only take a couple of hours. Finding ways to dramatically shorten the lyophilization time or establishing alternative methods for implanting the L-LNs loaded with CAR T cells would be attractive. The translational potential, along with its capacity to utilize autologous tissues and integrate seamlessly into existing surgical procedures, underscores its revolutionary impact on cancer treatment. For clinical practitioners, it is necessary to receive standardized training on surgeons' skills to achieve the best therapeutic effect. Additionally, enhancing the efficacy of CAR T cell responses could accelerate the approval and adoption of CAR T therapies in clinical settings, broadening their applicability to a wider range of solid tumors. The translational significance of employing L-LNs for CAR T cell delivery lies in the potential for standardizing this treatment modality, reducing variability, and potentially lowering the costs associated with manufacturing and administration of CAR T cells. This innovative approach could significantly influence the future landscape of cancer treatment by extending the benefits of this groundbreaking therapy to a broader patient population.

Author contributions

Qiuhua Luo: Writing – original draft. Xiaojing Yan: Supervision, Investigation. Hong-Xu Liu: Conceptualization, Supervision, Writing – review & editing. Heran Li: Supervision, Investigation.

Conflicts of interest

The authors declare no conflict of interest.

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