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Commentary

Oversized liposomes boost macrophage-targeted RNA delivery to regulate macrophage polarity

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Macrophages play a key role in maintaining tissue homeostasis, regulating immune responses, and promoting tumor progression.[1] Dysregulation of macrophage polarization between the pro-inflammatory M1 and anti-inflammatory M2 phenotypes worsens inflammation, leading to the development of inflammatory-related diseases. Investigators have developed numerous strategies to regulate the balance of M1/M2 macrophage ratio and cytokine content, which may serve as a practical approach for reverse inflammatory status for various disease treatments. Exploring novel vectors to genetically regulate macrophages *in vitro* and *in vivo* is attractive. The targeted gene delivery to macrophages is a crucial challenge for boosting gene therapeutic efficacy. Incorporating ligands such as mannose and transferrin into gene transfer vectors has significantly improved delivery efficiency toward native macrophages.[2] In addition to these ligand-based approaches, various bacterial and protozoan microorganisms have been used to infect macrophages and establish chronic infections, representing a potential powerful method to transfer therapeutic DNA agents to macrophages *in vivo*.[3,4] However, utilizing these intracellular microorganisms raises biosafety concerns.

To overcome these challenges, in an article recently published in *Molecular Therapy - Nucleic Acids*, Song et al. developed size-controlled liposomes that encapsulate microRNAs (miR/MT-Lip) to facilitate the M1 to M2 polarization switch and reduce inflammation without deleterious effects on cells.[5] By delivering miR-10a to macrophages, the switch from M1 to M2 macrophages in the uterus suppressed local inflammation, thereby restoring infertility due to uterine inflammation. Compared with other active targeting techniques relying on ligands, such as antibodies or mannose, this size-dependent approach is more feasible in achieving therapeutic goals. The size control is much easier to achieve than the decoration of ligands, which generally require tedious modification processes. Moreover, this approach can reduce biosafety concerns. Overall, this approach shows great promise in boosting macrophage targeting.

The reported delivery system, MT-Lip, uses liposomes containing phospholipids and cholesterol as carriers to deliver microRNAs into macrophages. PEI is added to the liposomes to enhance RNA loading capacity and macrophage internalization. The authors also control the size of the liposomes by adjusting the proportion of cholesterol in total lipids. By obtaining larger-sized liposomes (~1.24 μm), they achieved a higher macrophage-specific internalization rate. The authors tested their delivery system by co-incubating fluorescently labeled microRNA (miRNA) with two different cell lines: HEK293T cells and RAW 264.7 cells. After evaluating cell internalization using fluorescence microscopy and flow cytometry, the authors found that MT-Lip-encapsulated miRNA was specifically internalized by RAW 264.7 cells but not HEK293T cells. In corresponding *in vivo* experiments, MT-Lip-delivered fluorescent miRNAs had signals only in splenic macrophages, with almost undetectable fluorescent signals in other immune cells such as neutrophils, natural killer (NK) cells, T cells, B cells, dendritic cells (DCs), and peripheral blood mononuclear cells (PBMCs). These findings suggest that MT-Lip can specifically target macrophages, which has great potential for treating macrophage-related diseases.

The authors achieved M2 polarization of macrophages at both cellular and *in vivo* levels using MT-Lip encapsulated with miR-10a (miR-10a/MT-Lip). In RAW 264.7 cells treated with lipopolysaccharide (LPS), miR-10a/MT-Lip converted M1 macrophages into M2 macrophages, as indicated by upregulated expression of related molecular markers. *In vivo*, the researchers analyzed splenocytes and PBMCs to evaluate macrophage-specific targeting of their miR/MT-Lip in immune cells. They found that miR-10a/MT-Lip specifically targeted macrophages without disturbing other immune cells in the spleen of mice with inflammation induced by LPS. Finally, in a mouse model of Asherman's syndrome (AS) characterized by sterile inflammation and fibrosis, the therapeutic effects of miR-10a/MT-Lip were evaluated through intravenous injection for several days. The treatment successfully decreased the M1/M2 ratio and increased the number of M2 macrophages without affecting other uterine cell types or immune cells.

In addition to miRNAs, long non-coding RNAs (lncRNAs) are a crucial class of non-coding RNAs that have functional significance

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in various ways, such as influencing chromatin function, modulating stability and translation of cytoplasmic mRNAs, and interfering with signaling pathways through several mechanisms. Recent research has demonstrated that lncRNAs can participate in M1/M2 polarization and possess multifunctional roles in disease progression. For instance, growth-arrest-specific transcript 5 (GAS5) lncRNA overexpression has been observed to inhibit JAK2/STAT3 signaling, thereby promoting M1 polarization.[6] This phenomenon was recognized in childhood lung inflammation and held significant potential for developing pneumonia-targeting therapeutic strategies. Although Song et al.'s approach is aimed at miRNA delivery, it is suggested that this system could deliver small interfering RNAs (siRNAs) targeting GAS5 lncRNA, and effectively hinder macrophage polarization to M1 by decreasing the amount of lncRNAs and mitigating related inflammatory responses.

Moreover, regulating M1/M2 macrophage polarization is applied in various medical scenarios, such as tumor therapy, tissue engineering, and inflammatory diseases. Nanomedicine is developing rapidly in tumor immunotherapy and is expected to break through the bottlenecks and challenges in immunotherapy. Deeply exploiting the role of biomaterials in macrophage action and polarization will significantly accelerate the development of novel macrophage therapies combined with immunomodulatory biomaterials. Applications in different biological scenarios must be accompanied by more relevant basic research. Analysis of cellular or tissue responses to immunomodulatory biomaterials using state-of-the-art high-throughput gene expression profiling can reveal a more complete and more evident interconnection between relevant biochemical signals in tumor immunotherapy, inflammation, and healing processes. Fascinatingly, rapidly developing artificial intelligence (AI) and big data strategies aim to find applications in medical research that combine strategies for cancer nanomedicine and immunotherapy. A shared cloud service platform for analyzing the interaction of immune cells, tissues, and organs can be established and opened in the future.

It is worth noting that the phenotypic conversion of macrophages plays a multifaceted role in different diseases or stages of the same disease, as it reflects changes in the body's immune status. Different macrophage phenotypes can determine the development and outcome of a disease to a considerable extent. Appropriately intervening in the signaling pathways and local microenvironment of macrophage polarization can steer macrophages toward an expected direction for treating diseases. However, M1 macrophages may also exert beneficial effects in some scenarios, such as clearing infections or cancer cells.[3,7] Therefore, when using macrophage polarity to treat diseases, it is necessary to consider different diseases and their physiological stages and determine when and how to perform the phenotypic conversion.

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AUTHOR CONTRIBUTIONS

D.W. and J.Z. prepared the original draft, and J.C. reviewed and edited the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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