BIO Integration

SORTing the Fate of Nanodelivery Systems

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This commentary is based on the article "Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR–*Cas* gene editing" [*Nature Nanotechnology*, volume 15, pages 313–320 (2020)].

With the rapid development of nanotechnology, the role of nanocarriers in biomedicine is becoming increasingly important. The bottleneck, however, is efficient delivery of nanomedicine to specific organs or specific lesions, and it has been a hotspot in nanomedicine circles [1]. In general, the targeting of nanocarriers can be divided into two major directions. One is passive targeting, that is, the ability of nanocarriers to target specific sites depends on nano-inherent characteristics and the physiological characteristics of the lesion site. For example, nanocarriers (~10 to 100 nm) are considered optimal for in vivo targeting delivery due to the enhanced permeability and retention (EPR) effect, which occurs only in the vicinity of tumors [2]. Increased angiogenesis leads to rapid development of blood vessels for tumor growth, which leads to significant increase in vascular permeability in the tumor area compared with healthy tissues [3, 4]. In addition, for specific microenvironmental changes in tumor or lesion sites, including weak acidity, enzyme overexpression, high levels of redox, etc., the design of targeted responsive-nanomaterials under the above special conditions has also been a focus of research in recent years [5–7]. The second direction is active targeting, that is, modifying special ligands or antibodies on the surface of nanocarriers to actively target organs and tissues expressing the corresponding receptors. For example, due to the high expression of alpha V beta III ($\alpha_V\beta_3$) integrin in tumor blood vessels, the attachment of the RGD peptide, which can specifically target $\alpha_V\beta_3$ on the surface of nanocarriers, can significantly increase the tumor targeting ability of nanodrugs [8, 9].

On the whole, to improve the targeting of liposomes, nano-micelles, or other nanocarriers, their stability and targeting should be improved through physical or biochemical methods. PEGylated nanocarriers greatly prolong the stability of nanocarriers and solve the nanocarriers' inability to circulate in the blood for a long time [9, 10]. However, how to prevent nanocarriers from targeting other organs or tissues besides the liver is an unsolved problem. According to our current understanding, nanomedicines exhibit distinct liver affinity after systematic injection because of their natural physiological advantages, including slow blood flow [11, 12] and discontinuous vasculature in hepatic sinusoids [13]. This targeting mechanism is similar to the tumor EPR effect mentioned above, which takes advantage of the discontinuity of vascular endothelium and the retention of nano-sized substances.

It is exciting to see new advances in the targeting of other organs and tissues besides the liver. James E. Dahlman previously reported a system called Fast Identification of Nanoparticle Delivery (FIND), which is capable of simultaneously quantifying >100 lipid nanoparticles (LNPs) delivering mRNA to multiple cell types in vivo [14]. They quantified more than 250 LNPs in vivo and identified two formulations that deliver RNA to endotheliocytes. The screening system uses designed DNA barcodes and the Cre-Lox animal model. After intravenous administration, flow cytometry and high-throughput sequencing systems are required to determine the cell distribution of different LNPs. Although this is a good screening platform, it is expensive and time consuming, and ordinary researchers may not have the capability to set up such a laboratory. Therefore, a predictable and efficient system to enhance the targeting ability and performance of nanocarriers to target organs is urgently needed.

In another study, Cheng et al. [15] proposed an intelligent approach called Selective Organ Targeting (SORT), which targets different organs, including the liver, spleen, and lung, based on charge regulation and transformation within the nanoparticles itself. Traditional LNPs are composed of cationic lipids, amphipathic phospholipids,

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cholesterol, and PEG-lipids. Effective intracellular delivery depends on internal ionizable amines equilibrium and external hydrophobicity. Therefore, the authors tried to add additional components (called a SORT molecule) to alter the internal charge of LNPs, thereby regulating their predictable organ targeting properties in vivo. LNPs containing nucleic acid therapy are specifically bioengineered to induce liver-, spleen-, and lung-specific gene regulation. Specifically, on the premise of not destroying the original four component proportions of LNPs (5A2-SC8/DOPE/Chol/PEG=15:15:30:3), the proportion of 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) was adjusted from 0 to 100% to make a series of LNPs.

As a highly cationic lipid, DOTAP is one of the most widely used lipids for gene transfection applications and has been proven to be very be efficient for both in vitro and in vivo transfection applications; however, the latter is debatable due to its toxicity issue in systemic circulation [16]. Therefore, the percentage of cationic DOTAP is a key factor in adjusting tissue target specificity. As the percentage of DOTAP increased, LNP-loaded mRNA expression was gradually transferred from the liver to the spleen and then to the lungs, showing an obvious and precise trend of selective organ delivery. To test the limits of the SORT method, the researchers evaluated the effectiveness of a variety of cations, anions, and ionizable cations as lipids in SORT technology, which is widely applicable to a variety of LNP types and molecular categories. Results show that SORT is controllable and predictable, becoming a modular general strategy for achieving targeted organ delivery.

As researchers, we are thrilled with the results. The discovery of SORT not only enables the predictable targeting of nanoparticles to specific organs but is also expected to advance the development of protein replacement and gene correction therapies. The technique can be widely applied to many existing LNPs and other nanoparticle systems. SORT-optimized LNPs enable safe and efficient delivery of organ-specific mRNAs and CRISPR/*Cas9*-mediated gene editing therapy, and SORT is expected to open new avenues for the development of gene therapy.

Nevertheless, further improvement of the SORT system is still warranted. As discussed here, in order to increase the targeting of LNPs, one often tends to choose ligands or antibodies that are specific to surface modification. The availability of these overexpressed receptors (often in malignancies) should be taken into consideration. Could it be possible that the combination of this active targeting strategy further improves the editing effect of target cells? Furthermore, this research provides a contour prediction system, and further research should focus on precise mechanisms that explain how SORT mechanistically enables tissue targeting. This could only be achieved by the collaboration of various experts in different fields in exploring the possibilities of SORT through multiple angles [17], and we look forward to the realization of that day.

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