Nanoparticles: The new promise for region-specific targeting of microglia

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Q5 Researchers working on microglia are constantly looking for new targeting tools, more precise in time, and space. In this article, Guo et al. present the results of their latest study in which they develop nanoparticles capable of targeting microglia with high spatial specificity and delivering siRNAs to alter the transcriptome Q6 profile of the cells. Although certain obstacles remain, this study is very promising in the race for new tools to manipulate microglial activity locally in the brain.

Q2Q3 Main text

The field of microglia research is desperately lacking tools to target the activity of these cells in a region-specific manner. The *Cx3cr1-Cre*^{ERT} - *LoxP* system is the most commonly used at present to generate an inducible microglial gene deletion in the brain.[1] On the other hand, the manipulation of region-specific microglial genes in the brain is also not feasible by viral approaches, as most viral vectors that have proven effective in assisting gene manipulation in neurons and astrocytes have substantial difficulties in transducing microglial cells, probably due to the natural immune protective behavior of microglia.[2] Researchers are looking for new methods to overcome this obstacle.

A recent study by Guo et al. (Dr. Chun-Xia Yi's lab) established a novel method, using lipid-polymer-hybridized nanoparticles (LPNPs) to deliver siRNA in targeted brain regions to modify microglial gene expression in rodents. This method is based on the fact that microglial cells are the professional phagocytes in the brain, which makes them the Q7 most powerful cells to endocytose the nanoparticles. Because of this, the LPNPs are prioritized to be taken up by microglia but not by other non-phagocytic cells in the brain. More importantly, the materials constructing the Q8 nanoparticles in this study are all "microglial friendly." Except for the Rhodamine B or the gold-Alexa555 that are Q9 packed inside the LPNPs for tracing purposes in this study, the LPNP is formed with phospholipids, cholesterol, Poly-L-Lysine, and mPEG-PLGA, materials which are all biocompatible and biodegradable. After being digested in Q10 lysosomes, the broken-down nanomaterials can be largely recycled within microglia and serve as substrates for fueling or for cell components. Eventually no "nano-debris" is left in microglia.

To prove *in vivo* that these nanoparticles are specific to microglia, the authors used degradable fluorescent dye Rhodamine B and non-degradable gold-Alexa555 to fate-map the LPNPs upon injection into the rat hypothalamus. Both labeling methods showed microglia are the dominant cell type in the brain that take up the LPNPs. Their next Q11 question is whether the LPNPs-carried siRNA can survive from "eat-me and digest-me"–a physiological process of microglia upon phagocytizing "foreign substrates"—and being released from lysosomes into cytosolic area for silencing the targeted gene expression. The authors proved that significant amounts of the CD11b or TLR4 siRNA carried by LPNPs can survive from phagolysosome processes *in vivo*, as indicated by a clear down-regulation of CD11b protein expression, or less microglial response to the endotoxin lipopolysaccharide (LPS) due to knocking down the LPS Q12 receptor TLR4, both in the hypothalamus. This method provides an ideal genetic switch allowing the reversible and

brain-site-specific control of targeted gene activity in microglial cells *in vivo*. In addition, with the hydrophilic and hydrophobic layers formed by the hydrophilic mPEG and hydrophobic PLGA, these LPNPs can be developed further to co-pack compounds (either water soluble or water resistant) for pharmacological interventions.

Q13 This study raises several questions. A complete phagocytosis involves three main steps: "find me, eat me, digest me." The exact intracellular pathways that control these steps are still unclear. For taking up nanoparticles, currently known potential signals include TMEM2, CD14 receptor, Tyro3-Axl-Mer (TAM) receptors, and two pathways identified by recent studies, i.e. the leptin receptor[3] and lipoprotein lipase.[4] Further studies on these pathways by, for example, using uptake inhibitors to block the phagocytosis or other form of endocytosis might help to elucidate the precise mechanism for microglia taking up certain nanoparticles.

Q14 Regarding how exactly nanoparticles or nanoparticle-carrying molecules escape from lysosomes, one hypothesis is the "molecular cone-shape" theory: the cationic lipid in the DSPC interacts with the anionic phospholipids in the phagolysosome, forming the "cone shape" to disrupt the bilayer structure of the membranes, thus releasing the siRNA into the cytosol.[5] Another possible mechanism is the "proton sponge effect" theory: the *amido group* in the LPNP nanoparticles can absorb large amounts of proton, causing consistent chloride ion efflux into the lysosome, and finally making the lysosome swell and burst.[6,7]

Q15 Several steps must still be taken before this tool can be used systematically or even considered for use in clinical research. First, a solution must be found to deliver the LPNPs into the brain following peripheral administration. Moreover, it is possible that this tool favors the targeting of phagocytic microglial cells, at the expense of cells that are less phagocytic or not at all. This may be an advantage or a problem depending on the subpopulation targeted. It is also necessary to further increase the cellular specificity of action of the LPNPs, as scarce labeling has been observed in non-microglial cells. Finally, a strategy must be defined in order to target them to a specific brain region, after having verified that this tool works in the same way in regions other than the hypothalamus. But despite all this, the new tool developed by the Yi lab represents a very important and promising step in the development of a microglial cell targeting strategy with a very high spatial resolution.

Q16 Acknowledgments

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Declaration of interests

The author declares no competing interests.

References

The corrections made in this section will be reviewed and approved by a journal production editor. The newly added/removed references and its citations will be reordered and rearranged by the production team.

[1] Zhao X.-F., Alam M.M., Liao Y., Huang T., Mathur R., Zhu X., et al. Targeting microglia using Cx3cr1-Cre lines: revisiting the specificity. eNeuro 2019;6. doi:10.1523/eneuro.0114-19.2019. ENEURO.0114-19.

[2] Maes M.E., Colombo G., Schulz R., Siegert S. Targeting microglia with lentivirus and AAV: recent advances and remaining challenges. Neurosci. Lett. 2019;707:134310. doi:10.1016/j.neulet.2019.134310.

[3] Gao Y., Vidal-Itriago A., Milanova I., Korpel N.L., Kalsbeek M.J., Tom R.Z., et al. Deficiency of leptin receptor in myeloid cells disrupts hypothalamic metabolic circuits and causes body weight increase. Mol. Metab. 2018;7:155–160. doi:10.1016/j.molmet.2017.11.003.

[4] Gao Y., Vidal-Itriago A., Kalsbeek M.J., Layritz C., Garcia-Caceres C., Tom R.Z., et al. Lipoprotein lipase maintains microglial innate immunity in obesity. Cell Rep. 2017;20:3034–3042. doi:10.1016/j.celrep.2017.09.008.

[5] Semple S.C., Akinc A., Chen J., Sandhu A.P., Mui B.L., Cho C.K., et al. Rational design of cationic lipids for siRNA delivery. Nat. Biotechnol. 2010;28:172–176. doi:10.1038/nbt.1602.

[6] Wojnilowicz M., Glab A., Bertucci A., Caruso F., Cavalieri F. Super-resolution imaging of proton sponge-triggered rupture of endosomes and cytosolic release of small interfering RNA. ACS Nano 2019;13:187–202. doi:10.1021/acsnano.8b05151.

[7] Boussif O., Zanta M.A., Behr J.P. Optimized galenics improve in vitro gene transfer with cationic molecules up to 1000-fold. Gene Ther. 1996;3:1074–1080.

eToc Blurb

Researchers working on microglia are constantly looking for new targeting tools, more precise in time, and space. In this article, Guo et al. present the results of their latest study in which they develop nanoparticles capable of targeting microglia with high spatial specificity and delivering siRNAs to alter the transcriptome profile of the cells. Although certain obstacles remain, this study is very promising in the race for new tools to manipulate microglial activity locally in the brain.

Queries and Answers

Q1

Query: In the author list, please check that all names are spelled correctly and that surnames (highlighted) have been identified appropriately. First and middle names are abbreviated in the table of contents, whereas surnames are not (e.g., “X.Y. Zhang”). Please review this section carefully, and either confirm that it is approved for publication or indicate any necessary changes.
Answer: ok

Q2

Query: If applicable, please confirm that, for any figures that contain error bars, scale bars, and/or p values, these elements have been defined in the corresponding figure legends (not simply in the main text). If any have not been defined, please indicate how and where to add definitions.
Answer: no figures

Q3

Query: If any figures require simple text changes, please mark them in the proofs. If more substantial changes are needed, please upload a revised file and explain what changes have been made.
Answer: no figure

Q4

Query: Can you please add the city and country for affiliation 2?

Answer: the IUF is an institution without walls. So no city

However, we can add "France" for the country

Q5

Query: I am unsure of the meaning of the sentence starting with “Researchers working on microglia…” Would “Researchers working on microglia are constantly looking for new targeting tools and more time and space.” be an appropriate revision? If not, can you please suggest another one?

Answer: Change for "Microglia researchers are constantly looking for new targeting tools that are more precise in time and space."

Q6

Query: The sentence starting with “Although certain obstacles…” has been edited for clarity. Can you please confirm the revision is okay?

Answer: OK

Q7

Query: The sentence starting with “This method is based on…” has been edited for clarity. Can you please confirm the change still preserves your intended meaning?

Answer: OK

Q8

Query: The sentence starting with “Except the Rhodamine B…” has been edited for clarity. Can you please confirm this change still reflects your intended meaning?

Answer: OK

Q9

Query: Please note that I have changed the spelling of “cholestorol” to “cholesterol.” Can you confirm this change is correct?

Answer: OK

Q10

Query: I am unsure of the meaning of the sentence starting with “After being digested in lysosomes…” Would “After being digested by lysosomes, the broken-down nanomaterials can be largely recycled within microglia and serve as substrates for fueling or for cell components.” be an appropriate edit? If not, can you please suggest another revision?

Answer: Yes, perfect, thank you

Q11

Query: I am unclear on the phrase “LPNPs-carried siRNA.” Do you mean “siRNA that is carried by LPNPs”? If so, may I suggest revising the text to read “LPNP-carried siRNA”?

Answer: ok

Q12

Query: The sentence starting with “The authors proved that significant amounts…” is a little unclear. The last clause “…both in the hypothalamus” seems incomplete. Can you please review and suggest a revision?

Answer: The authors demonstrated that significant amounts of CD11b siRNA or TLR4 carried by LPNPs can survive phagolysosome processes in vivo, as indicated by a clear downregulation of CD11b protein expression in the hypothalamus. Furthermore, the microglial response to the lipopolysaccharide (LPS) endotoxin is reduced due to the neutralization of the LPS TLR4 receptor in this structure.

Q13

Query: Per journal guidelines, preview articles should not contain any other heading other than the “Main text” heading. Therefore, I have removed “Questions raised:” and created a sentence to preface the following paragraph. Can you please confirm this still reflects your intended meaning? If not, can you suggest another revision?

Answer: ok

Q14

Query: If your paper uses acronyms that are not standard in your field, please add definitions for each at their first mention in the text. Here are some abbreviations that may need defining: DSPC.

Answer: DSPC=Distearoylphosphatidylcholine

Q15

Query: The sentence starting with “Finally, a strategy must be defined…” has been edited for clarity. Can you please confirm that the change still reflects your intended meaning?

Answer: ok

Q16

Query: Have we correctly interpreted the following funding source(s) and country names you cited in your article: Institut Universitaire de France, France; University of Bordeaux, France?

Answer: Yes