ACS Chemical Neuroscience

Viewpoint

pubs.acs.org/chemneuro

Chemical Biology Probes for Extracellular Vesicles Facilitate Studies of Neuroinflammation

Lei Yan, Jiaqi Liang, and Hang Yin*

Center of Basic Molecular Science, Department of Chemistry, Tsinghua University, Beijing 100082, China

ABSTRACT: Neuroinflammation has been conceived as an important cause for or contributor to neurological diseases. With major strides in new technology, scientists can use chemical biology tools developed in non-neuronal systems to research neuroinflammation. Extracellular vesicles (EVs) play a vital role in mediating neuroinflammation via carrying pathogenic misfolded proteins as well as nucleic acids, suggesting important biological functions. Nonetheless, it is a daunting goal to study these ultramicroscopic EVs in part due to the technical hurdle of specific labeling and preparation. Therefore, development of new detection methods of EVs will promote further understanding of EVs in the nervous system, thereby expediting the diagnosis and therapy development for neurological disorders. Recent progress toward a new class of chemical biology probes simultaneously targeting the highly curved surface and the particular lipid compositions of EVs may offer an alternative strategy for their detection, isolation, and purification, which not only will facilitate research on their mechanism in neuroinflammation and neurological diseases, but also may lay the groundwork for the next generation of diagnostics and prognostics.

KEYWORDS: *Neuroinflammation, extracellular vesicles, membrane curvature, lipidomics, detection technology, drug discovery*

Neuroinflammation resulted from abnormal activation of neuroglia leads to cytokines release, which may contribute to the development of various neurodegenerative diseases. Ever-increasing evidence has shown that extracellular vesicles (EVs) play a crucial role in mediating this process. Although previously thought to be sheer extracellular debris, EVs secreted by cells under both normal and pathologic conditions have been suggested to carry out important biological functions. Exciting new research of EVs has emerged focusing on drug delivery, detection, and labeling method, as well as biomarker development. In particular, specific and selective chemical probes for these EVs have facilitated in-depth studies both in vitro and in vivo, which may shed new lights on the molecular mechanism of neuroinflammation and disease development.

By and large, the actual function and secretion/take-up mechanism of EVs in the nervous system remain unknown. Recent reports in the literature have shown that exosomes (EVs with diameters from 30 to 100 nm) carry pathogenic misfolded proteins, propagating inflammatory signals in neighboring cells. Moreover, pathogenic factors of gene expression of recipient cells are attributed to exosomes, such as amyloid β-peptides of Alzheimer's disease, prion protein scrapie of prion disease, and α-synuclein of Parkinson's disease.[1] One of the major difficulties in treating these diseases is the lack of efficient ways allowing drugs to cross the blood-brain barrier (BBB). Statistics show that 98% of potent drugs for various nervous diseases cannot be used in clinic due to poor permeability through the BBB.[2] Considering the relationship between EVs and neuroinflammation, drugs carried by EVs may have wide applications. For instance, Haney and co-workers developed an efficient method to load catalase into exosomes without significantly changing their structures, showing the accumulation of loaded exosomes in neurons and microglia in the brain producing a potent neuroprotective effect.[2] Nevertheless, more studies about the behavior and mechanism of EVs are required. On the other hand, the diagnosis of neurodegenerative diseases is increasingly important. Brain tissue samples from schizophrenic patients and bipolar disorder patients have significantly elevated levels of exosomal miR-497 and miR-29c, respectively.[1] Based on this finding, it is promising to detect the level of specific proteins or nuclear acids in EVs as a novel strategy for diagnosis of prognostic development.

Accurate detection of EVs is a key step to research the relationship between EVs and neuroinflammation. Some detecting technologies, based on the physical properties (e.g., sizes and the diffusion coefficient) of EVs, include electron microscopy, flow cytometry, dynamic light scattering (DLS), and nanoparticle tracking analysis (NTA).[3] Electron microscopy can directly show the vesicles of various sizes (30−1000 nm), but the fixation process may impact the shape of EVs. Flow cytometry is a popular detection method for vesicles, but it is typically limited to the identification of particles greater than 100 nm, preventing the detection of exosomes. The low refractive index of vesicles and a bias toward detection of heterogeneous solutions restrict application of DLS. NTA is an emerging technology for nanoparticles and not depending on the refractive index of the vesicles. Nonetheless, the isolation method (e.g., ultracentrifugation) for reducing impurity of biological samples is also often required prior to using NTA. Recently, scientists have developed new detection methods based on chemical and biological characteristics of EVs.[3] EVs commonly contain lipids, membrane proteins, and nucleic acids (DNA, mRNA, and microRNA). Proteomic signatures used to identify EVs include MHC class I and II, tetraspanins (CD9, CD63, CD81, and CD82), and cytosolic proteins (e.g., Hsp70

Special Issue: Neuroinflammation

Received: November 27, 2015
Accepted: December 2, 2015
Published: December 10, 2015

DOI: 10.1021/acschemneuro.5b00310
ACS Chem. Neurosci. 2016, 7, 418−419

and Hsp90). Detection of the EV proteins is relatively straightforward with analytical techniques like Western blotting or enzyme-linked immunosorbent assay (ELISA). However, soluble antigens can also be detected, and it is impossible to measure vesicles sizes or concentrations with these techniques. EVs also present enriched phosphatidylserine (PS) in the outer membrane leaflet, so fluorescently labeled PS-binding protein (e.g., Annexin V) is appropriate to determine the PS levels of EVs, combined with flow cytometry. Considering the drawbacks of detection methods above, there is an urgent need for an efficient noninvasive tool to detect EVs in the nervous system.

Most recently, membrane curvature and lipid compositions have emerged as new targets for peptide probes for EVs (Figure 1). Yin's group developed a macrocyclic peptide sensor, C2BL3C, derived from the membrane-inserting loop of the membrane fusion protein synaptotagmin I.[4] C2BL3C is a 12-residue cyclic peptide with rigid structure prepared by click chemistry. It can bind highly curved liposomes (diameter <100 nm) and isolated exosomes from plasma. Another example, MARCKS-ED, a 25-residue peptide sensor, derived from myristoylated alanine-rich C kinase substrate (MARCKS), can selectively bind synthetic liposomes and biological EVs with highly curved surfaces as well.[5] As mentioned above, EVs are submicrometer-sized membrane sacs consisting of different lipids such as PS, PE and PG. Traditional methods cannot detect specific lipid compositions, whereas MARCKS-ED can selectively identify PS via electrostatic attraction between positively charged lysine residues and negatively charged PS lipid head groups. Experimental demonstration and computational simulation also suggest that insertion of five bulky hydrophobic phenylalanine residues into lipid-packing defects contributes to the special membrane curvature sensing character of MARCKS-ED.[6] MARCKS-ED can offer an efficient method to detect EVs by recognizing their small sizes and lipid compositions.

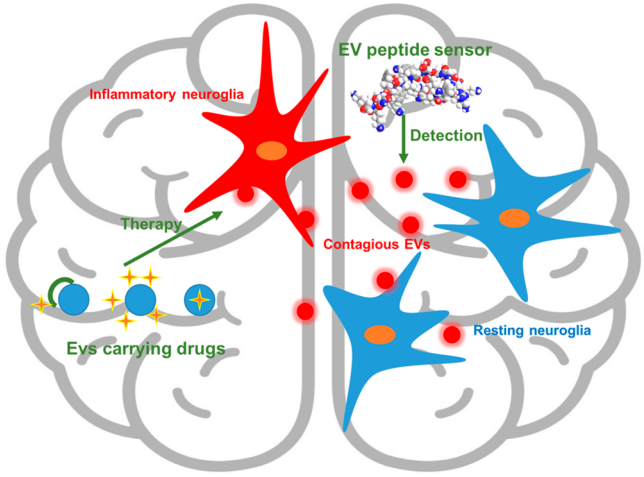

Figure 1. Peptide sensor detecting EVs and drug-carried EVs. Inflammatory neuroglia (red star) secrete contagious EVs (red circle) to infect resting neuroglia (blue star). The EV peptide sensor (globular model) can detect these EVs based on membrane curvature and lipid compositions. EV peptide sensor (green curve bar) conjugated pharmacophore (orange star), surface modifying of EVs (blue circle), or drug-packing EVs may be the potential therapy targeting inflammatory neuroglia.

As an emerging biomarker for neurological disorders, EVs have caught eyes of more neurochemical biologists. These membrane curvature peptide sensors may shed light on the correlation between EVs and neuroinflammation, with some remarkable advantages, such as wide detection ranges of sizes (30−400 nm), being able to bind to specific lipid compositions, convenient synthesis as requirement, appropriation for bioassays, and low cost. However, for clinical application, researchers must develop more specific and selective EVs sensors, based on a more explicit mechanism. In addition to the diagnosis marker, EVs also can be used as drug carriers due to the ability of crossing the BBB. Drug-packing EVs, surface modifying of EVs, or membrane curvature sensors conjugated pharmacophore may be the potential ways to target diseased abnormal cells in the nervous system (Figure 1). Studies of various nervous diseases may be enhanced greatly by these new EVs chemical biology probes.

AUTHOR INFORMATION

Corresponding Author

*E-mail: yin_hang@tsinghua.edu.cn.

Funding

We thank the National Natural Science Foundation of China (NSFC Grant No. 21572114) and Tsinghua University for the financial support of this work.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Gupta, A., and Pulliam, L. (2014) Exosomes as mediators of neuroinflammation. *J. Neuroinflammation 11*, 68.

(2) Haney, M. J., Klyachko, N. L., Zhao, Y., Gupta, R., Plotnikova, E. G., He, Z., Patel, T., Piroyan, A., Sokolsky, M., Kabanov, A. V., and Batrakova, E. V. (2015) Exosomes as drug delivery vehicles for Parkinson's disease therapy. *J. Controlled Release 207*, 18−30.

(3) Kastelowitz, N., and Yin, H. (2014) Exosomes and microvesicles: identification and targeting by particle size and lipid chemical probes. *ChemBioChem 15*, 923−928.

(4) Saludes, J. P., Morton, L. A., Ghosh, N., Beninson, L. A., Chapman, E. R., Fleshner, M., and Yin, H. (2012) Detection of highly curved membrane surfaces using a cyclic peptide derived from Synaptotagmin-I. *ACS Chem. Biol. 7*, 1629−1635.

(5) Morton, L. A., Yang, H., Saludes, J. P., Fiorini, Z., Beninson, L., Chapman, E. R., Fleshner, M., Xue, D., and Yin, H. (2013) MARCKS-ED peptide as a curvature and lipid sensor. *ACS Chem. Biol. 8*, 218−225.

(6) Morton, L. A., Tamura, R., de Jesus, A. J., Espinoza, A., and Yin, H. (2014) Biophysical investigations with MARCKS-ED: dissecting the molecular mechanism of its curvature sensing behaviors. *Biochim. Biophys. Acta, Biomembr. 1838*, 3137−3144.

DOI: 10.1021/acschemneuro.5b00310
ACS Chem. Neurosci. 2016, 7, 418−419