

# Extracellular vesicles and ceramide: new mediators for macrophage chemotaxis?<sup>1</sup>

Natalie J. Török<sup>2</sup>

Department of Medicine, Gastroenterology and Hepatology, University of California, Davis, Sacramento, CA, and Northern California Veterans Administration, Mather, CA

Intercellular communication is a vital process in the function of all multicellular organisms. Communication between liver cells is known to occur through multiple pathways including secreted mediators, direct cell-cell contact, and by membrane-surrounded particles referred (variably) to as extracellular vesicles (EVs) or exosomes. Although the term exosome was initially reserved to describe vesicles that were released after the fusion of the multivesicular endosomes with the plasma membrane, it is likely that circulating EVs represent a heterogeneous population of exosomes, microparticles, or microvesicles in a size range of 40–100nm, yet which are difficult to resolve using current purification methods (1). Exosomes are originally formed in the multivesicular endosomes whereas microvesicles are released by direct budding of the cell membrane (2). EVs have been shown to be shed from most cells types under both physiological conditions and in disease states. This process of shedding EVs from injured cells may have a pathogenic role in communicating danger or stress signals. EVs can interact with recipient cells by fusion, ligand-receptor interaction, or internalization via receptor-mediated endocytosis or micropinocytosis thus promoting uptake of their cargo (2). From the perspective of fatty liver disease, it is important to note that serum biomarkers to detect progressive nonalcoholic steatohepatitis (NASH) are still lacking, and so clinical and basic studies that advance our understanding of EVs in NASH patients and which may lead to diagnostic EV signatures that reflect histological progression are of substantial interest (3, 4).

In this issue of the *Journal of Lipid Research*, Kakazu et al. (5) studied the role of palmitate-induced endoplasmic reticulum (ER) stress and IRE1( $\alpha$ ) activation in the generation of C16:0 ceramide-enriched EVs from hepatocytes. In addition, the authors provide novel insights into the mechanisms of macrophage-associated chemotaxis elicited by pro-inflammatory EVs (5). Why are these findings so timely and important? First, macrophage recruitment is a key feature of progressive NASH, leading to a cascade of pro-inflammatory events and accelerating the progression

of hepatic fibrosis (6). Second, egress of bone marrow-derived macrophages and recruitment to the liver is known to involve complex chemokine signaling, but local, lipid-derived mediators of chemotaxis have not been previously described in the context of NASH. These mediators and the signaling cascades elicited by specific lipid metabolites could be central to macrophage migration/activation, and targeting these events could be a potential therapeutic approach in NASH. Kakazu et al. show that the ceramide metabolite sphingosine-1-phosphate (S1P) contained within lipotoxic EVs triggered S1PR1 signaling in macrophages, and furthermore, demonstrated that inhibiting S1PR1 expression could mitigate macrophage migration. Further studies will be required to study the precise mechanisms of S1P recognition in macrophages and to resolve the question of whether other receptors could be involved. Interestingly, a recent study demonstrated that hepatocyte exosomes that transfer sphingosine kinase 2 (SK2) to target hepatocytes can cause an increase in S1P and cell proliferation in models of ischemia/reperfusion or partial hepatectomy (7). Therefore, it is important to further delineate target specificity of EVs and whether there is differential signaling in other cell types of the liver (such as Kupffer cells, stellate cells, or other nonparenchymal cells).

A major advance in the work by Kakazu et al. is the depiction of lipotoxicity/ER stress in the generation of lipotoxic EVs. Palmitate is a known inducer of all three arms of unfolded protein response pathways (8). Therefore, selectivity of EV production to the ER stress sensor IRE1 $\alpha$  is intriguing and would merit further studies linking the IRE1 $\alpha$ /XBP1 axis to generation of multivesicular endosomes. Another question is how ceramide or its metabolites are sorted to the EVs. Sequestration of the cargo within EVs is thought to be highly regulated, although the precise mechanisms involved are yet to be discovered. Sphingolipid-containing raft membrane microdomains were previously shown to be required for cargo segregation in an alternative pathway of sorting, independent of the

<sup>1</sup> See referenced article, *J. Lipid Res.* 2016, 57: 233–245.

<sup>2</sup>To whom correspondence should be addressed.  
e-mail: njtorok@ucdavis.edu

endosomal sorting complex required for transport (9), and these vesicle were enriched in ceramides. Thus, it is possible that EVs generated from lipotoxic hepatocytes are actually derived from these membrane constituents. To confirm the in vitro findings, the authors showed that EV C16 ceramide and EV SIP were significantly increased in patients with NASH compared with obese patients or those with simple hepatic steatosis. These data may indicate that EVs containing C16 ceramide or SIP play a role in the pathogenesis of NASH progression and/or could reflect disease stage.

Developing serum markers to detect disease progression in NASH is of major importance. Circulating EVs have gained much attention in recent years as potential biomarkers in NASH (10, 11). Extensive characterization of serum EVs in animals with NASH led to the discovery of specific microRNA signatures with miR-122 and 192 (10) that was later confirmed in studies with NASH patients (11, 12). In the current study, for the first time, EVs enriched in C16 ceramide/SIP were found in patients with early NASH. If confirmed in future clinical studies; these markers could become important tools for identifying patients at risk of necroinflammation and fibrosis.

The work by Kakazu et al. has provided important information regarding a novel pathway by which lipotoxic hepatocytes signal recruitment of bone marrow derived myeloid cells via C16 ceramide/SIP containing EVs. Future studies should evaluate how this subpopulation of EVs contribute to inflammation/fibrosis and whether they are indeed suitable and predictive serum biomarkers that might be used to detect progressive nonalcoholic fatty liver disease.

## REFERENCES

1. Bobrie, A., M. Colombo, G. Raposo, and C. Thery. 2011. Exosome secretion: molecular mechanisms and roles in immune responses. *Traffic*. **12**: 1659–1668.
2. Yoon, Y. J., O. Y. Kim, and Y. S. Gho. 2014. Extracellular vesicles as emerging intercellular communicasomes. *BMB Rep*. **47**: 531–539.
3. Julich, H., A. Willms, V. Lukacs-Kornek, and M. Kornek. 2014. Extracellular vesicle profiling and their use as potential disease specific biomarker. *Front. Immunol*. **5**: 413.
4. Arrese, M., A. Eguchi, and A. E. Feldstein. 2015. Circulating microRNAs: emerging biomarkers of liver disease. *Semin. Liver Dis*. **35**: 43–54.
5. Kakazu, E., A. S. Mauer, M. Yin, and H. Malhi. 2015. Hepatocytes release ceramide-enriched pro-inflammatory extracellular vesicles in an IRE1 $\alpha$ -dependent manner. *J. Lipid Res*. **57**:233–245
6. Seki, E., and R. F. Schwabe. 2015. Hepatic inflammation and fibrosis: functional links and key pathways. *Hepatology*. **61**: 1066–1079.
7. Nojima, H., C. M. Freeman, R. M. Schuster, L. Japtok, B. Kleuser, M. J. Edwards, E. Gulbins, and A. B. Lensch. 2016. Hepatocyte exosomes mediate liver repair and regeneration via sphingosine-1-phosphate. *J. Hepatol*. **64**: 60–68.
8. Malhi, H., and R. J. Kaufman. 2011. Endoplasmic reticulum stress in liver disease. *J. Hepatol*. **54**: 795–809.
9. Trajkovic, K., C. Hsu, S. Chiantia, L. Rajendran, D. Wenzel, F. Wieland, P. Schwille, B. Brügger, and M. Simons. 2008. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science*. **319**: 1244–1247.
10. Povero, D., A. Eguchi, H. Li, C. D. Johnson, B. G. Papouchado, A. Wree, K. Messer, and A. E. Feldstein. 2014. Circulating extracellular vesicles with specific proteome and liver microRNAs are potential biomarkers for liver injury in experimental fatty liver disease. *PLoS One*. **9**: e113651.
11. Pirola, C. J., T. Fernandez Gianotti, G. O. Castano, P. Mallardi, J. San Martino, M. Mora Gonzalez Lopez Ledesma, D. Flichman, F. Marshahi, A. J. Sanyal, and S. Sookoian. 2015. Circulating microRNA signature in non-alcoholic fatty liver disease: from serum non-coding RNAs to liver histology and disease pathogenesis. *Gut*. **64**: 800–812.
12. Becker, P. P., M. Rau, J. Schmitt, C. Malsch, C. Hammer, H. Bantel, B. Mullhaupt, and A. Geier. 2015. Performance of serum microRNAs -122, -192 and -21 as biomarkers in patients with non-alcoholic steatohepatitis. *PLoS One*. **10**: e0142661.