

## Extracellular Vesicles Act as Messengers of Macrophages Sensing Atherogenic Stimuli

Taras Afonyushkin, Christoph J. Binder

The release of vesicles by cells into the extracellular space is found in many aspects of biology, and such extracellular vesicles (EVs) are gaining increasing attention in diverse areas of biomedical research.<sup>1,2</sup> EVs represent a heterogeneous population differing in size, number, and composition that are dependent on the type and activation state of the parental cells that release them. Based on size, EVs can be classified into apoptotic bodies (>1  $\mu\text{M}$ ), smaller microvesicles (100 nm–1  $\mu\text{m}$ ), and exosomes (<125 nm), which are derived from multivesicular bodies. The biological properties of EVs depend on their composition and the specific cargo that is carried by them, including proteins, lipids, and nucleic acids. Their ability to transfer these contents to other cells makes them important contributors to intercellular communication. In particular, the transfer of different types of non-coding RNAs by EVs has been shown to modulate target cell functions.<sup>3</sup>

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The function of EVs is being investigated in the context of several pathological conditions, including cardiovascular disease, in which EVs derived from various vascular cells have been studied extensively.<sup>2</sup> They are proposed to contribute to disease pathogenesis by promoting inflammation and thrombosis via different mechanisms. On the other hand EV-associated miRNAs have also been shown to mediate protective effects on endothelium.<sup>4,5</sup> Therefore, circulating EVs of different cellular origin are considered as candidates for biomarkers of disease activity and risk.<sup>6</sup> Moreover, different classes of EVs have been identified in atherosclerotic plaques of different stages. However, little is known about the effects of EVs in cardiovascular disease in vivo, and their potential contribution to disease pathogenesis is largely based on the interpretation of data from in vitro experiments. Indeed, many studies characterize the effects of EVs using cell-based assays in vitro or analyze the function of isolated circulating EVs ex vivo. Only few investigators have addressed the role of EVs in atherosclerosis and vascular inflammation in vivo by administering exogenously generated EVs to animal models. Thus,

a better understanding of the role of EVs in the context of cardiovascular disease is of great interest.

A study in this issue of *ATVB* investigates macrophage-derived EVs. Given the central role of macrophages in atherogenesis, Ngyuen et al asked the question whether stimulation of macrophages with an atherogenic stimulus, such as oxidized low-density lipoprotein (OxLDL), results in the release of EVs that in turn would modulate the function of naive macrophages.<sup>7</sup> The authors found that OxLDL-treated macrophages release EVs that are enriched in a set of microRNAs, including miR-146a, which has previously been shown to play a role in vascular inflammation and atherosclerosis development. In a set of elegant experiments, the authors could further demonstrate active transfer of EV content to naive macrophages. Because bioinformatic analyses revealed a central role of EV-associated miRNAs in the expression of genes that regulate cell migration, the authors evaluated the effect of OxLDL-induced EVs in modulating macrophage migration. They could convincingly demonstrate that the treatment with OxLDL-induced EVs inhibits chemokine-dependent macrophage migration in vitro and in vivo. In the second part of their study, Ngyuen et al then focus on the role of miR-146a in mediating the response of OxLDL-induced EVs. Using anti-miR-146a and a miR-146a mimic, they could clearly show a nonredundant role for miR-146a in macrophage migration. Moreover, they demonstrated 2 RNA-binding proteins, IGF2BP1 (insulin-like growth factor 2 mRNA-binding protein 1) and HuR (human antigen R), which are involved in cell migration via modulating  $\beta$ -actin expression, as targets of miR-146a that could mediate this effect. The expression of both genes was found to be upregulated in lesions of *Ldlr*<sup>-/-</sup> mice reconstituted with bone marrow of miR-146a ko mice. The authors suggest that OxLDL-induced EVs could promote atherosclerotic lesion formation by inhibiting emigration of lesional macrophages via the transfer of miRNAs that modulate the expression of key genes controlling migration.

The identification of macrophage-derived EVs as novel modulators of macrophage migration in the presence of atherogenic stimuli adds to a growing list of possible retention signals that modulate macrophage migration under hyperlipidemic conditions. Modulating the release or the clearance of EVs in atherosclerotic plaques may offer additional ways to interfere with atherogenesis. Still little is known about the pathways involved in this. The data presented here identify OxLDL as a trigger for specific EV release. This study provides novel insights into the understanding of the proatherogenic activity of OxLDL. It remains to be shown whether similar mechanisms are triggered by other inflammatory stimuli, such as cytokines or microbial ligands. In this regard, regulatory function of EVs may present a general principle in the control of inflammation.

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The authors propose a model in which OxLDL-derived EVs mediate their action in a paracrine manner. However, inside atherosclerotic plaques, all macrophages are likely exposed to OxLDL to a similar extent. This raises the questions whether EVs released at sites of high local concentration of OxLDL also have the capacity to influence the function of myeloid cells at distant anatomic sites, such as the bone marrow or the spleen, where cells are not directly exposed to OxLDL. Functionally, it is also not clear whether OxLDL-induced EVs would only inhibit emigration or whether they could also limit recruitment of monocytes/macrophages into the plaque.

Although the activities of OxLDL-induced EVs seem to be dependent on >1 miRNA or even other EV components, the authors focus on the role of miR-146a as one factor controlling the ability of EVs to inhibit macrophage migration. Whether this EV-associated miRNA effect translates into accelerated atherogenesis or even atheroprotection is unclear. Polymorphisms of miR-146a have been suggested to be associated with a higher atherosclerosis risk.<sup>8–10</sup> Moreover, recent studies in mice suggest a dual role of miR-146 in atherosclerosis. On one hand, ablation of endothelial miR-146a promotes endothelial cell inflammation in vitro and administration of miR-146a to mice inhibits atherosclerotic lesion formation.<sup>11,12</sup> On the other hand, genetic deletion of miR-146a has been found to paradoxically reduce atherosclerosis in mice.<sup>13</sup> Increased expression of miR-146a in lesions does not necessarily identify it as proatherogenic. It may also serve as part of a negative feedback mechanism by limiting NF- $\kappa$ B signaling and overt inflammation.<sup>14</sup> Therefore, it will be important to show how and to which extent EV-associated miR-146 are distributed in vivo and how these are taken up by the different cell types contributing to plaque formation. Is there EV-mediated exchange of miR-146a between different cell types, such as macrophages and endothelial cells? Which cells are the major source and the primary targets of EV-associated miR-146a?

The article of Nguyen et al identifies a novel mechanism by which OxLDL can exert proatherogenic effects and adds novel information on the potential role of EVs and miR-146a in atherogenesis. Clearly, additional research is needed to understand the exact contribution of macrophage-derived EVs carrying miR-146a to atherogenesis. Combining genetic deletion and active administration of miR-146a containing EVs

may provide useful insights (Figure). A direct demonstration of a function of endogenous EVs and of EV-associated cargo, such as miRNA, in modulating cardiovascular disease will be a major challenge for the future.

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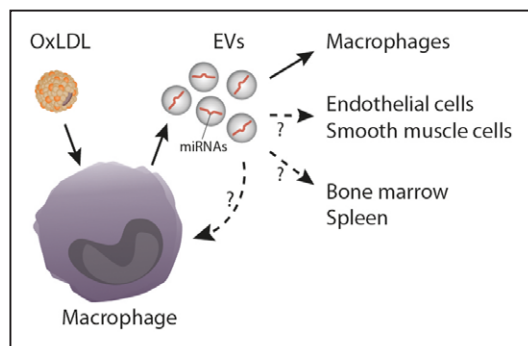
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None.

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**Figure.** MicroRNA-carrying extracellular vesicles (EVs) that are released by oxidized low-density lipoprotein (OxLDL)-sensing macrophages may transfer information to various cells during atherogenesis.

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