Effects of Ozone-Generated Microvesicles and MV-MiR-199a-3p on Inflammatory Lung Responses in Alveolar Macrophages

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Ozone is an urban air pollutant and highly reactive oxidant which generates free radicals and oxidizes cellular components. In addition to direct ozone-induced alveolar damage, evidence suggests that lung macrophages have displayed either a protective or destructive role in the lungs following ozone exposure. Extracellular vesicles (EVs) play an essential role in intercellular communication via the transfer of EV cargo. Accumulating evidence suggests that EVs regulate a variety of human diseases through their effects on cell-cell crosstalk. The purpose of this study was to investigate the role of ozone-induced EVs in macrophage activation and lung inflammation. Microvesicles (MVs), a sub class of EVs, are the main type of EV detected in broncho-alveolar lavage fluid. Using nanoFACS, we found that both air- and ozone-generated MVs are primarily derived from epithelial cells. Through functional assays, we found that treatment of mice *in vivo* with ozone-induced MVs upregulates the expression of multiple inflammatory cytokines. To delineate the mechanisms by which ozone-induced MVs induce macrophage classical activation or lung inflammation, we focused on the regulatory mechanism of miR-199a-3p, a microRNA which was significantly increased in expression within MVs after ozone exposure. To introduce miR-199a-3p mimics into MVs, a modified method of calcium chloride transfection was used. We found that MV-miR-199a-3p induces expression of IL-1β in macrophages. Previously, we have reported that epithelium-derived MVs can be taken by lung macrophages and transfer MV-miRNAs into the recipient cells. Interestingly, miR-199a-3p level in ozone-stimulated macrophages was highly upregulated compared to macrophages obtained from air-control mice, however, the level of precursor miR-199a-3p was not significantly increased. This result suggests that elevated mature miR-199a-3p level in ozone-induced macrophages results from MV-mediated delivery of mature miR-199a-3p. Collectively, ozone-induced MVs potentially lead to acute upregulation of inflammatory cytokines in both mice lung tissue and macrophages. We also uncovered that mature miR-199a-3p may be selectively-packaged into MVs after ozone exposure and transferred to recipient macrophages, ultimately resulting in pro-inflammatory activation of macrophages and lung inflammation.