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2. Title
   A critical role of neutrophils in the pathogenesis of inflammation-mediated atherosclerosis caused by smoking habit

3. Period
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4. Summary
   [Background] Growing body of evidence suggest that neutrophil extracellular traps (NETs) promote inflammasome activation in macrophages, which, in turn promoting atherogenesis. It is also known that macrophage autophagy inhibits inflammasome activation, thereby suppressing atherogenesis. However, it remains unknown the association between NETs and macrophage autophagy in the progression of atherosclerosis. The aim of this study is to investigate whether the smoking-promoted Neutrophil Extracellular Traps (NETs) formation and concomitant suppression of the macrophage autophagy activity play an important role in the pathogenesis and progression of the atherosclerosis.
   [Methods] NETs induced by a nicotine stimulation of the HL-60 cell-derived neutrophil-like cells were co-cultured with the THP-1 cell-derived macrophages to investigate the effects and mechanisms of the autophagy activity. We next generated a neutrophil-specific Pad4-deficient *ApoE–/–* (*ApoE–/–*; *Neu-Pad4–/–*) mice by crossing *Pad4fl/fl* mice, *Tg-Mrp8-Cre* mice, and *ApoE–/–* mice for analyzing the development and progression of the atherosclerotic lesions in an atherosclerotic mouse model in which the NETs are not produced. Nicotine stimulation of a neutrophil-like cells induced NETs in a similar manner to PMA stimulation and 7KC-induced autophagosome formation in the macrophages was suppressed, and inflammasome activity was activated in the presence of NETs. *In-vivo* experiments revealed that the abolishment of NETs formation resulted in the attenuation of atherosclerotic plaques in a nicotine-administered HFD-fed *ApoE–/–* mice. *In-vitro* studies demonstrated that the NETs promote EGFR activity, thereby suppressing the Beclin-1-associated PI3 kinase activity by enhancing Beclin-1 phosphorylation of the tyrosine residues of Beclin-1 in the macrophages. In addition, we found that the NETs negatively regulate an autophagosome-lysosome fusion as well, possibly through upregulating the Rubicon expression.
   [Conclusions] These results suggest that NETs-mediated EGFR-Beclin-1 signaling in the macrophages promotes atherogenesis by an autophagy inhibition-mediated inflammasome activation. We will further investigate the role of nicotine-induced NETs formation in the pathogenesis of atherosclerosis.