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COMMENT

Self-DNA sensing in cigarette smoke-induced vascular inflammation: the role of mitochondrial DNA release in vascular endothelial cells

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Cigarette smoking is an independent and modifiable risk factor for atherosclerotic cardiovascular disease (ASCVD), including coronary heart disease and ischemic stroke [1, 2]. All forms of smoking, such as electronic cigarettes, shisha, cigars, cigarettes and passive smoking are associated with increased risk of ASCVD [1]. Smoking cessation leads to rapid decreases in risk of ASCVD and reduces mortality rates due to ASCVD even in individuals after many years of heavy smoking or patients with diagnosed ASCVD [1]. While epidemiologic studies and clinical trials have shown a strong relationship between cigarette smoking and ASCVD, the underlying mechanisms, including the exact components of cigarette smoke (CS) responsible for this association, remain largely unclear [2].

CS consists of two phases: the tar phase containing nicotine, the addictive substance of CS, and the gas phase. Components of the tar phase, the sizes of which range from 0.1 to 1 µm in diameter, do not pass through the lung alveolar walls and thus exert local toxicity. On the other hand, components of the gas phase can pass through the lung alveolar epithelium to reach the circulating blood and cause systemic effects, although the half-lives of most components of the gas phase is extremely short. Recently, relatively stable components in the gas phase, such as acrolein (ACR), methyl vinyl ketone (MVK) and 2-cyclopenten-1-one (CPO), have been reported to have cytotoxic effects on vascular cells [3]. However, how these components contribute to the pathogenesis of ASCVD remains unelucidated.

In this issue of *Hypertension Research*, Kobayashi et al. provided important information on the role of ACR, MVK and CPO in proinflammatory responses in vascular endothelial cells [4]. Endothelial cell dysfunction and vascular inflammation play critical roles in the pathogenesis of ASCVD [2]. The authors found that the combination of ACR, MVK and CPO at their sublethal doses induces expression of proinflammatory cytokines, such as interleukin (IL)-6 and IL-1α, oxidative stress, mitochondrial damage, DNA damage response and cytosolic accumulation of nuclear and mitochondrial DNA in human umbilical vein endothelial cells (HUVECs), whereas ACR, MVK or CPO alone causes only DNA damage response without the increase in cytosolic DNA fragments. Treatment with a reactive oxygen species (ROS) scavenger, N-acetyl-L-cysteine, or depletion of mitochondrial DNA (mtDNA) with ethidium bromide suppressed oxidative stress, accumulation of cytosolic mtDNA and IL-6 expression that were caused by the combination of ACR, MVK and CPO, while N-acetyl-L-cysteine, but not ethidium bromide, inhibited IL-1α production. Although the detailed mechanism remains unclear, this study suggests that the combination of ACR, MVK and CPO exerts additive or synergistic effects on proinflammatory cytokine expression through ROS production and mtDNA release in HUVECs.

ACR, MVK and CPO are unsaturated carbonyl compounds that cause DNA damage and depletion of glutathione, an endogenous ROS scavenger [5]. Interestingly, in this study, each compound did not induce oxidative stress and mitochondrial damage at the maximum concentration that did not cause cell death of HUVECs, while the combination of these compounds at the same concentrations induced oxidative stress and mitochondrial outer membrane permeabilization that are below the threshold necessary to trigger apoptosis. These findings suggest that the effect of the combination of these compounds might be attributable not to augmentation of the common mechanisms of

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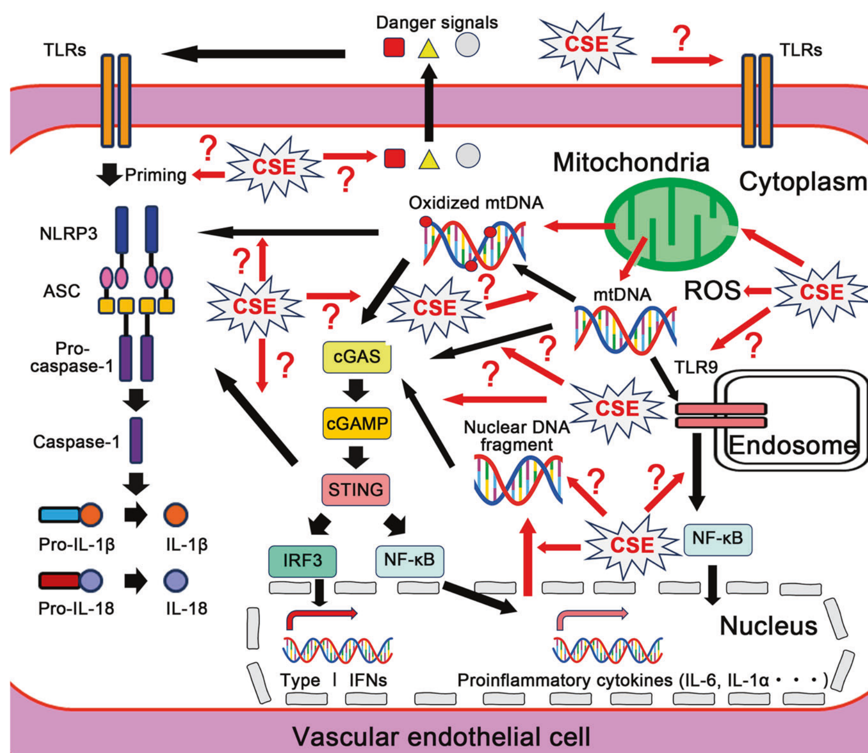

Fig. 1 Self-DNA sensing mechanisms and cigarette smoke extract (CSE)-induced inflammation in vascular endothelial cells. The main components of CSE (i.e., acrolein, methyl vinyl ketone, and 2-cyclo-penten-1-one) synergistically cause mitochondrial DNA (mtDNA) release and reactive oxygen species (ROS) production in vascular endothelial cells, which results in proinflammatory cytokine expression such as interleukin (IL)-6 and IL-1α. MtDNA or oxidized mtDNA can be sensed by various pattern-recognition receptors (PRRs), including stimulator of interferon genes (STING), inflammasomes and toll-like receptors (TLRs), which leads to activation of caspase-1 and transcription factors, such as interferon regulatory factor 3 (IRF3) and nuclear factor-κB (NF-κB), and subsequent production of type I interferons (IFNs) and proinflammatory cytokines. The potential interaction was suggested between mtDNA-induced activation of the cyclic GMP-AMP synthase (cGAS)-STING pathway and NLRP3 inflammasome activation. These PRR-mediated signaling pathways can be modulated by extracellular danger signals released from stressed cells through TLRs in an autocrine/paracrine manner. How CSE modulates accumulation of cytosolic mtDNA and nuclear DNA fragments and activates self-DNA sensing mechanisms in vascular endothelial cells remains largely unelucidated. ASC, the apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain; cGAMP, cyclic GMP-AMP

unsaturated carbonyl compounds, but to the combination of compound-specific mechanisms. Indeed, the combination of the three compounds induced IL-1α production in mtDNA-depleted HUVECs, although oxidative stress was suppressed in these cells. Further studies are warranted to elucidate compound-specific mechanisms and their interactions.

Inflammation is initiated by sensing exogenous or endogenous molecules, including nucleotides, through pattern-recognition receptors (PRRs). MtDNA can induce inflammation through activating various PRRs, including stimulator of interferon genes (STING), inflammasomes and toll-like receptors (TLRs) (Fig. 1) [6–9]. PRRs activate their specific downstream proinflammatory signaling pathways in a cell-type-specific manner. Detailed assessment of each PRR-mediated signaling pathway would help understand how ACR, MVK and CPO in combination synergistically induce vascular inflammation.

STING is a cytosolic DNA sensor that is activated by cyclic GMP-AMP synthesized through the interaction between cyclic GMP-AMP synthase (cGAS) and cytosolic DNA fragments. Activation of the cGAS-STING pathway leads to activation of downstream transcription factors, such as interferon regulatory factor 3 and nuclear factor-κB (NF-κB), to produce type I interferons and proinflammatory cytokines [9]. STING was shown to recognize cytosolic mtDNA to promote tumor necrosis factor-α and interferon-β production in macrophages, which accelerates atherosclerosis in apolipoprotein E-deficient mice fed with a western-type diet [6]. It was reported that CS extract activates the cGAS-STING pathway to induce IL-6 expression in HUVECs [10]. In HUVECs treated with the three compounds in combination, cytosolic mtDNA might activate proinflammatory responses through the cGAS-STING pathway. This study showed that cytosolic nuclear DNA did not induce proinflammatory responses in HUVECs,

although STING can detect cytosolic nuclear DNA [9]. Further studies will be necessary to clarify the role and mechanism of cytosolic DNA sensing through the cGAS-STING pathway in HUVECs under treatment of the three compounds. To this end, it is of great interest to examine the impact of cytosolic DNA oxidation on activation of the cGAS-STING pathway, considering the significance of ROS production in proinflammatory responses in this study.

Inflammasomes are cytosolic multiprotein complexes that consist of PRRs, such as nucleotide-binding domain and leucine-rich repeat receptors (NLRs) and absent in melanoma (AIM) 2-like receptor, the apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain, and procaspase-1. Inflammasome activation follows a two-step process: priming and activation. Priming is initiated by TLRs, which trigger NF-κB-induced transcription of inflammasome components. In the activation process, inflammasome assembly and caspase-1 activation upon activation of PRRs lead to maturation and secretion of IL-1β and IL-18. It was reported that oxidized mtDNA activates the NLRP3 inflammasome for IL-1β production in macrophages [7]. The NLRP3 inflammasome has been shown to play important roles in cardiovascular disease [11]. Interestingly, mtDNA was shown to activate the NLRP3 inflammasome in a STING-dependent manner, indicating a potential interaction between the cGAS-STING pathway and the inflammasome signaling [12].

CS was reported to activate NLRP3 and AIM2 inflammasomes in immune cells [13]. However, inconsistent conclusions have been reported on the role of CS in inflammasome activation in macrophages and vascular endothelial cells, which might result from different concentration and components of CS extract [13]. Importantly, extracellular signals, including neural signals, have been shown to play important roles in NLRP3 inflammasome activation in vivo for both priming and activation [11]. It would be of great importance to examine whether the combination of the three compounds activates inflammasomes in vascular endothelial cells in vivo. In addition, assessment of the impact of mtDNA oxidation on inflammasome activation would be necessary to clarify the mechanism of action of the three compounds in vascular endothelial cells. Furthermore, it would be interesting to examine whether a flap-structure-specific endonuclease 1 inhibitor, which suppresses oxidized mtDNA release that leads to activation of the NLRP3 inflammasome and cGAS-STING pathway, mitigates CS-induced vascular inflammation [7].

TLRs are type I integral membrane glycoprotein that are expressed on the cell surface or in intracellular compartments. TLR signaling induces proinflammatory responses by recruiting adapter proteins within the cytoplasm. TLR9, expressed in endosomes, was reported to recognize cell-free DNA released from degenerated cells in macrophages, which promotes vascular inflammation and atherosclerosis in apolipoprotein E knockout mice [14]. TLR9 also recognizes mtDNA [8]. Electronic cigarette vapor was reported to increase cytoplasmic mtDNA in macrophages, which induces the expression of proinflammatory cytokines through TLR9 activation and promotes atherosclerosis [8]. On the other hand, it was reported that TLR9 might not contribute to proinflammatory responses in CS extract-treated HUVECs [10]. It is of great interest to examine whether ACR, MVK or CPO directly modulates TLR9 signaling. Endogenous danger signals secreted from cells under pathological stimuli, including cell-free DNA, modulate intracellular proinflammatory signaling in an autocrine/paracrine manner through TLRs on the cell surface [15]. Further studies will be necessary to investigate whether the three compounds directly contribute to this mechanism.

In conclusion, ACR, MVK and CPO, the long-lived components in the gas phase of CS, might synergistically induce proinflammatory responses in vascular endothelial cells through mitochondrial damage and subsequent accumulation of mtDNA in the cytoplasm. Further studies are needed to clarify target molecules of these components and the downstream signaling pathways involved in mtDNA-induced vascular inflammation, which would help identify potential therapeutic targets for the treatment of ASCVD associated with CS.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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