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EDITORIAL

Extracellular RNA and Endothelial TLR3 Link Inflammation and Venous Thromboembolism

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Venous thromboembolism is a common and multifactorial disease that increases the risk of morbidity and mortality.[1] This condition is strongly related to risk factors, endogenous and exogenous, which are known to induce an inflammatory process. Venous thromboembolism involves endothelial cell activation, triggering inflammation and activation of the coagulation system. Although much progress has been made in recent years,[2] the exact mechanisms underlying venous thromboembolism are not fully clear. In this issue of the *Journal of the American Heart Association* (*JAHA*), Najem and colleagues[3] evidenced a relationship between venous thromboembolism and inflammation. They elegantly demonstrate a release of eRNA (extracellular RNA) from damaged endothelial cells; circulating eRNA regulates cellular inflammatory response through binding TLR3 (Toll-like receptor 3), known for recognizing various RNA species, including self-RNA fragments.[4] Overall, these processes are proven to be involved in the pathophysiology and progression of venous thromboembolism. Using animal models and cell cultures, the authors observed that eRNA exacerbated thrombus formation through TLR3 activation, promoting neutrophil recruitment and secretion of C-X-C Motif Chemokine Ligand 5 (CXCL5), a chemokine involved in inflammation. Treatment with RNase I reduced thrombus size, highlighting the role of eRNA in the pathogenesis of venous thromboembolism through TLR3-mediated mechanisms. These findings clarify how eRNA is released by damaged cells and is involved in inflammation and coagulation, suggesting targeting eRNA-TLR3 interactions as a potential therapeutic strategy for managing venous thromboembolism.

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Several observations suggest that eRNA expression is dysregulated in venous thromboembolism.[5,6] We can consider eRNA like a type of DAMP (damage-associated molecular pattern); DAMPs are recognized by TLRs ((Toll-like receptors), and TLR activation triggers an inflammatory response including production of cytokines and leukocyte recruitment, which are 2 main steps heavily involved in venous thromboembolism.[7,8] Importantly, endogenous ligands of TLRs have been associated with thrombosis; the most representative TLR involved in eRNA recognition is TLR3.[9]

Earlier research has established that eRNA plays a role in various tissues, including the nervous system.[10] For instance, in astrocytes, eRNA interacts with certain DAMPs,

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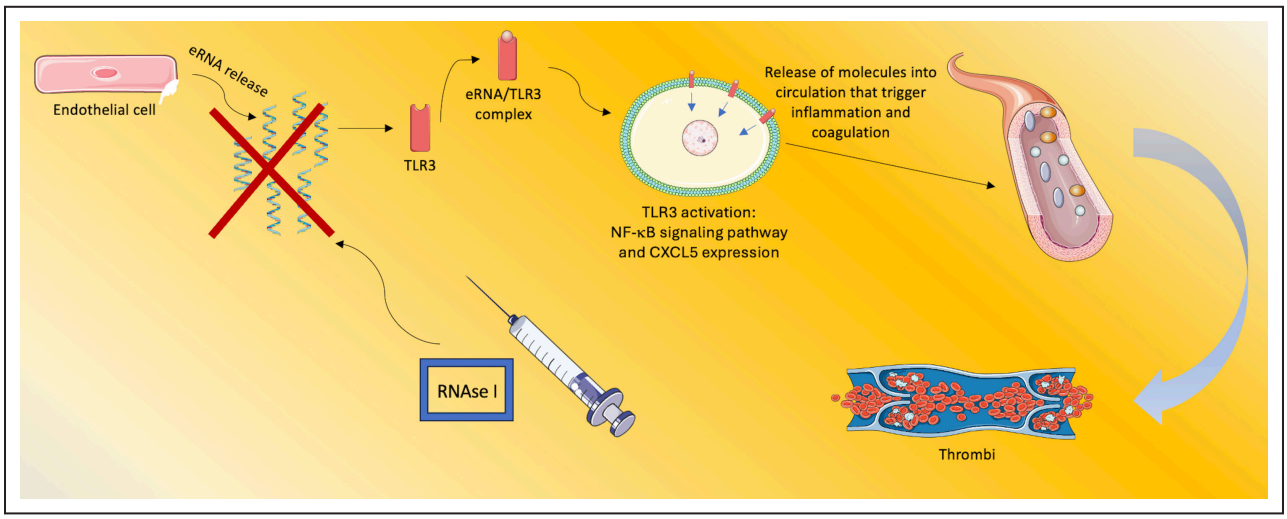

Figure. Schematic representation of the functional roles of eRNA and endothelial TLR3 in linking inflammation and venous thromboembolism.
CXCL5, C-X-C Motif Chemokine Ligand 5; eRNA, extracellular RNA; NF-κB, Nuclear Factor Kappa B; RNAse, Ribonuclease; TLR3, Toll-like receptor 3.

contributing to inflammation and neurological disorders; the DAMP signal from eRNA sensitizes astrocytes to act as key components of cerebral innate immunity, responding synergistically to both external and internal triggers of inflammation through TLR2/NF-κB (Nuclear Factor Kappa B) -dependent signaling pathways.[11]

ENDOTHELIAL DYSFUNCTION AND THROMBOSIS

The endothelium serves as a vital barrier between blood and vessels, acting as both a semipermeable physical shield and a biological interface. It responds to diverse chemical and mechanical signals from nearby tissues.[12] Moreover, endothelial cells play a pivotal role in regulating vascular tone and hemostasis.[13] Endothelial dysfunction, often characterized by diminished NO production, primarily results in vasodilatory impairment, underscoring its importance in maintaining vascular balance and making it a crucial target for therapy. Reduced availability of NO may stem from insufficient NO synthesis or, indirectly, from heightened reactive oxygen species production, which deactivates NO sources.[14,15]Whenever endothelium damage occurs, endothelial cells act, releasing molecules that signal the damage, which triggers an inflammatory response and eventually procoagulation pathways.[16] A long-time inflammatory process predisposes patients to a thrombotic diathesis. Activation of the proinflammatory pathway occurs when eRNA binds to TLR3. Furthermore, eRNA and TLR3 play key roles in triggering the expression of tissue factor and activating proteases involved in the contact phase of blood coagulation, including factors XII and XI. Notably, eRNA can impact endothelial function by influencing permeability, cytokine production (eg, Tumor Necrosis Factor Alpha [TNF-α], IL [interleukin]-1β, and IL-6), and leukocyte adhesion both in vivo and in vitro, primarily through promoting the expression of VCAM-1 (vascular cell adhesion molecule-1). Activation of TLR3 was associated with increased thrombus burden, neutrophil recruitment, and formation of NETs (neutrophil extracellular traps), supporting a role for TLR3 in thrombotic mechanisms; eRNA treatment increased thrombus size and neutrophil recruitment, with effects abolished in TLR3-deficient mice, indicating TLR3 dependence. In their study, Najem et al[3] used the $FeCl_3$-induced thrombosis murine model[17] to demonstrate the presence and functional significance of eRNA in venous thrombosis. This model, associated with free radical generation and endothelial cell activation, was deemed suitable for studying early thrombus events related to cell stress and eRNA release. Moreover, the study indicates that $FeCl_3$-induced thrombosis correlates with circulating eRNA levels, because RNAse I treatment reduced thrombus size and decreased neutrophil recruitment to the thrombotic site. Furthermore, in vitro experiments on endothelial cells revealed the potential of eRNA to induce tissue factor expression, reduce plasma clotting time, and enhance IL-8 and CXCL5 secretion, potentially contributing to neutrophil recruitment.

These observations suggest that eRNA impacts venous thrombosis through TLR3 signaling, potentially mediated by NF-κB activation and CXCL5 expression. However, further investigations are warranted to fully characterize the effects of eRNA on thrombosis,

including dose–response studies and examination of endothelial cell procoagulant properties. Taken together, these findings provide valuable insights into the mechanistic role of eRNA in the pathogenesis of venous thromboembolism.

In summary, the activated pathway (Figure) includes eRNA release by cells (necrotic or damaged cells), binding to TLR3, formation of eRNA/TLR3 complex, activation of NF-κB, and Interferon Regulatory Factors (IRF-3/7), and expression of type I Interferon (IFN) genes, including IFNβ, in addition to other cytokines. Interestingly, eRNA was no longer capable of inducing NF-κB phosphorylation when TLR3 expression had been knocked down by small interfering RNA (siRNA).

Polyinosinic:polycytidylic acid is an immunostimulant similar to eRNA in morphology and is known to interact with TLR3.[18] It can be considered a synthetic analog of double-stranded RNA and is a common tool for scientific research on the immune system. To determine whether eRNA affects the thrombotic diathesis, Najem and coworkers[3] performed in vitro experiments in human endothelial cells, demonstrating that these cells incubated with eRNA or polyinosinic:polycytidylic acid displayed significant amplification of fibrin network formation and thrombotic diathesis. In parallel, the expression of TFPI (tissue factor pathway inhibitor) was significantly reduced in endothelial cells treated with eRNA compared with the control.

Lastly, Najem and collaborators[3] have provided promising insights into the therapeutic potential of RNase I. Specifically, their research indicates that pretreating rodents with RNase I markedly reduces the likelihood and size of thrombi. Injection of RNase I has been demonstrated to diminish the expression and activity of tissue factor (TF), as well as decrease fibrin deposition in vivo.[19]

These experimental observations have underscored a compelling molecular pathway centered on the potential for developing novel treatments involving RNase I. RNase I, a highly catalytic pancreatic ribonuclease, has recently been implicated in both inflammation and blood coagulation processes.[20] These enzymes play essential roles in degrading various RNA substrates and mediating diverse biological functions. A prospective therapeutic strategy could involve administering medications containing this enzyme to patients at elevated risk of venous thromboembolism. Such treatments have the potential to mitigate morbidity and mortality, thereby enhancing patients' quality of life.

ARTICLE INFORMATION

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Disclosures

None.

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