European Review for Medical and Pharmacological Sciences 2022; 26: 7605-7615

Role of HMGB1 in the formation of chronic thromboembolic pulmonary hypertension after acute pulmonary embolism

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Abstract. – Acute pulmonary embolism (PE) may be a common but fatal condition in several countries; in untreated or inadequately therapeutic PE patients, is a commonly occurring long-term complication affecting patient survival treatment and prognosis, contributing to right heart disease and may even be fatal. To date, the pathogenesis of chronic thromboembolic pulmonary hypertension (CTEPH) due to acute pulmonary embolism remains unclear; hence, there is an immediate demand for medications that are directly aimed at both preventing and managing the progression of CTEPH. Previous studies have shown that the inflammatory response is associated with thrombosis and the development of pulmonary cardiovascular disease. High-mobility Group B 1 (HMGB1), a damage-associated molecular pattern (DAMP), is involved in deep vein thrombosis and inflammatory reactions, vascular remodeling, and thrombosis in pulmonary hypertension. Therefore, we hypothesized that HMGB1 participates in the process of CTEPH development after acute PE. This paper details the dynamic changes in HMGB1 and the relationship between HMGB1 and the advancement of CTEPH after acute PE to better understand the pathogenic mechanisms and potential clinical applications.

Key Words:
HMGB1, PE, CTEPH, Neutrophil extracellular traps, Inflammation.

Abbreviations

HMGB1: high-mobility group B 1; PE: acute pulmonary embolism; CTEPH: chronic thromboembolic pulmonary hypertension; DAMP: damage-associated molecular pattern; PAH: pulmonary arterial hypertension; TLR4: toll-like receptor 4; RAGE: receptor for advanced glycation end products; NF-κB: factor-kappa B; VTE: venous thromboembolic; DVT: deep vein thrombosis; WT: wild-type; NETs: neutrophil extracellular traps; TLR2: toll-like receptor 2; t-PA: tissue-type plasminogen activator; sRAGE: soluble RAGE; NLSS: two localization signals; ER: endoplasmic reticulum; HUVECs: human umbilical vein endothelial cells; LPS: lipopolysaccharide; ROS: active oxygen; RNS: active nitrogen; PASMC: pulmonary artery smooth muscle cells; TNF-α: tumor necrosis factor-alpha; IL-1β: interleukin-1β; IL-18: interleukin-18; PRRs: pattern recognition receptors; dsHMGB1: disulfide HMGB1; frHMGB1: complete reduction of HMGB1; IL-6: interleukin-6; Ang II: angiotensin II; MCT: monocrotaline; cfDNA: cell-free DNA; MPO: myeloperoxidase; TFPI: tissue factor pathway inhibitor; NE: neutrophil elastase; LPA; lysophosphatidic acid; sICAM: soluble intercellular adhesion molecule 1; sVACM: soluble vascular cell adhesion molecule 1; HPAEC: human pulmonary artery endothelial cells; HPASMC: human pulmonary artery smooth muscle cells; PAMPs: pathogen-related molecular patterns; COPD: chronic obstructive pulmonary diseas; IPAH: idiopathic pulmonary arterial hypertension.

Introduction

PE is a group of diseases with increased local vascular resistance of the pulmonary artery caused by sudden embolism of the thrombus to the pulmonary artery. At present, there are no clear guidelines for clinical nursing patients[1]. PE without a definite cause or delayed treatment easily develops into chronic thromboembolic pulmonary hypertension (CTEPH), increasing pulmonary vascular resistance and pulmonary vascular remodeling due to incomplete thrombus lysis or repeated pulmonary vascular embolism. This in turn, leads to pulmonary vascular endothelial dysfunction and an imbalance in cytokine secretion, eventually developing into CTEPH. Studies have shown that the incidence of CTEPH is approximately 3% after PE[2]. Prior studies have shown that HMGB1, as a DAMP, participates in endothelial disorders, inflammation,

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in situ thrombosis, and other pathophysiological processes[3]. Serum HMGB1 concentrations in patients with PAH significantly correlate with pulmonary artery pressure measurements and pulmonary vascular resistance and may be positively associated with the severity of pulmonary hypertension. They are significantly and positively correlated with the prognosis of pulmonary hypertension and the severity of thromboembolic complications[4-7]. Therefore, we review the pathophysiological mechanisms of HMGB1 in diseases associated with pulmonary hypertension and speculate on its role in the development of CTEPH after acute PE to provide a theoretical basis for clinical prevention and treatment

Dynamic Changes in HMGB1 After Acute PE

Studies have shown that platelet activation is present in patients with acute PE, platelet-derived HMGB1 recruits and activates neutrophils at the thrombus site, and modulates platelet surface CD62p expression and soluble CD62p release, promoting neutrophil extracellular trap (NET) formation and thrombus formation[6]. Myeloid leukocytes and platelets mediate HMGB1 release through Toll-like receptor 2 (TLR2) and RAGE receptors promoting monocyte activation, and secretion of tissue factors and cytokines, leading to further platelet activation, promoting HMGB1 oxidation, releasing its prothrombotic activity leading to platelet aggregation, further amplifying the inflammatory response and promoting thrombosis[8]. The mean concentration of HMGB1 in the plasma of PE patients before anticoagulation was 2.54-fold higher than that in controls and 1.99-fold higher than that post-treatment patients, indicating the involvement of HMGB1 in thrombus formation in PE patients[9]. Progressive development of recurrent venous thromboembolism or CTEPH after PE is due to nondissolution of thrombus, presence of inflammation, or endothelial damage. High expression of HMGB1 and RAGE was found in myofibroblasts from endarterectomy tissue of CTEPH patients, and serum soluble RAGE (sRAGE) and HMGB1 (1141.1 ± 173.1 pg/ml) concentrations were significantly increased, suggesting that the HMGB1/RAGE pathway is meaningful in the pathophysiology of CTEPH[10]. In addition, the HMGB1/TLR4/NLRP3 inflammasome signaling pathway can mediate pulmonary artery endothelial cell ferroptosis activating an inflammatory response that triggers pulmonary vascular remodeling and mediates the formation of pulmonary hypertension[11]. Therefore, we may deduce that HMGB1 has an important function in the evolution of CTEPH after acute PE and review possible mechanisms of occurrence to explore potential clinical treatments for CTEPH (Figure 1).

HMGB1 Structure and Function

In 1973, a nonhistone protein with high mobility in polyacrylamide gel electrophoresis was extracted from the calf thymus and named "high

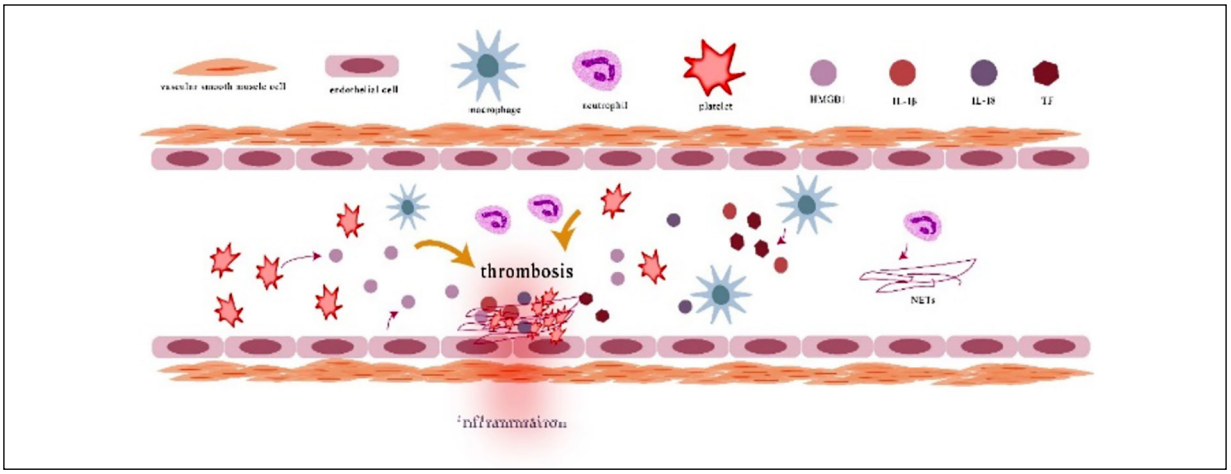

Figure 1. Role of HMGB1 in thrombosis. Platelet-derived HMGB1 can act on itself to make platelets aggregate; HMGB1 acts on TLR4 and RAGE receptors on macrophages, polarizes macrophages, produces inflammatory factors and cytokines, and promotes thrombosis; When HMGB1 acts on RAGE receptor on neutrophils, the release of NETs increases and acts as a scaffold to promote thrombosis; In addition, HMGB1 can interact with lysosomes in endothelial cells, cause lysosomal damage and release cathepsin B, damage endothelial cells and promote coagulation and thrombosis.

mobility group protein"[12]. HMGB1 is made up of 215 amino acid residues, including Box A, Box B, C-tail, and N-terminus; its stable localization in the nucleus depends on two localization signals (NLSS): NLS1 (amino acids 28-44) and NLS2 (amino acids 179-185) (Figure 2)[13]. HMGB1 performs different functions in different subcellular locations; in the nucleus, HMGB1 acts as a non-histone protein and binds to chromatin. However, when cells are stimulated externally, HMGB1 phosphorylation reduces its affinity to bind to the nucleus and is released into the cytoplasm or intercellular matrix in a nonendoplasmic reticulum (ER)/Golgi network manner as it does not possess a signal peptide[14]. In the cytoplasm, poly (ADP-ribose) polymerase (PARP-1) regulates the level of HMGB1 acetylation and cytoplasmic translocation[15]. Extracellularly, cells release HMGB1 in response to environmental changes such as lipopolysaccharide (LPS), active oxygen (ROS), active nitrogen (RNS), and Ca^{2+} to exert proinflammatory effects or cause tissue damage[16] (Figure 3).

HMGB1 exerts different biological functions by acting on different receptors, and the more widely studied ones include TLR4 and RAGE. In experimental pulmonary hypertension studies, activation of apoptosis-associated speck-like protein containing CARD (ASC) by dsRNA-dependent protein kinase (PKR) increased the release of HMGB1 and IL-1β in endothelial cells of mice. This regulates pulmonary vascular remodeling and promotes the progression of pulmonary hypertension[17]; however, TAK-242 as a TLR4 inhibitor delays PASMC migration, pulmonary vascular remodeling and the development of pulmonary hypertension[18]. In hypoxic pulmonary hypertension, HMGB1 and TLR4 expression are significantly upregulated. The HMGB1/TLR4 pathway-mediated inflammatory response can cause tissue and organ damage and disease progression, therefore studying the mechanism of the HMGB1/TLR4 pathway in

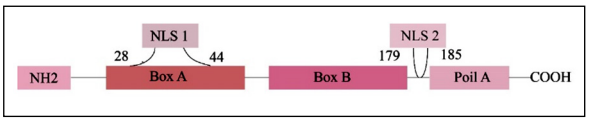

Figure 2. HMGB1 structure. HMGB1 includes two DNA binding domains: Box A (1-79 amino acids) and Box B (89-162 amino acids); a C-terminal and an N-terminal. Box A plays an anti-inflammatory role and contains NSL1; Box B plays a pro-inflammatory role and contains NSL2. NSL, nuclear localization signal.

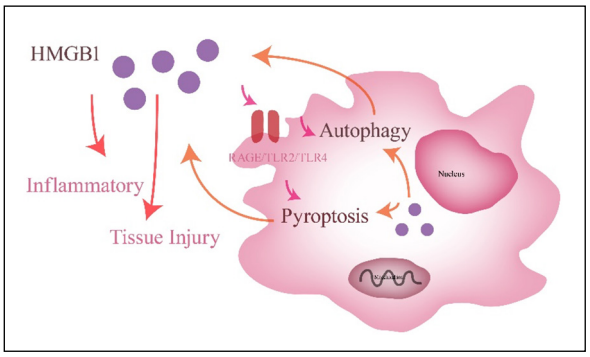

Figure 3. HMGB1 function. In the nucleus, HMGB1 is involved in DNA replication, transcription, and DNA repair as a non-histone protein. In the cytoplasm, HMGB1 can cause autophagy and cell death. Extracellular HMGB1, as a DAMP, is involved in the inflammatory response and tissue damage.

disease and finding effective blocking agents are crucial for the clinical intervention of disease[19].

In vitro, cytological studies have shown that HMGB1 binding to the RAGE receptor can produce inflammatory cytokines[20]. Overexpression of RAGE in pulmonary artery smooth muscle cells (PASMCs), as well as pulmonary artery endothelial cells (PAECs) in pathological states, is associated with inflammation and pulmonary vascular injury[21]. In addition, RAGE expression on the surface of activated platelets was upregulated and platelet-derived HMGB1 mediates the secretion of various proinflammatory agents, such as interleukin-8 (IL-8), interleukin-1β (IL-1β), and tumor necrosis factor-alpha (TNF-α), by triggering the downstream inflammatory pathways MAPK and NF-κB[22]. This finding demonstrates that the activation of the HMGB1/RAGE pathway has a significant impact on the development of inflammatory diseases *in vivo*.

The Role of HMGB1 in the Occurrence and Development of Acute PE

The underlying lesions of acute PE include endothelial cell injury, pulmonary vascular remodeling, and thrombosis, with multiple factors existing independently or coexisting with each other leading to disease development[23]. After the onset of PE, increased plasma levels of IL-1β, TNF-α and HMGB1 mediates the local inflammatory response due to local hypoxia in pulmonary artery endothelial cells caused by thromboembolism; in addition, HMGB1 can significant-

ly promote the proliferation and migration of PASMCs and accelerate the formation of pulmonary hypertension[24]. Furthermore, HMGB1 is associated with thrombosis, providing a scaffold for platelet and red blood cell activation by stimulating neutrophils to produce neutrophil extracellular traps(NETs)[25]. However, because the signs and symptoms of acute PE are not specific, it is highly susceptible to clinical misdiagnosis and underdiagnosis. Due to delayed treatment or inappropriate use of therapeutic drugs, acute PE is highly susceptible to developing into CTEPH or recurrent thromboembolism, which can develop into right heart failure or even death in severe cases[26-28]. Current studies have shown that the pathological mechanisms of acute PE and CTEPH include inflammation[29], a blood hypercoagulable state[30], and endothelial cell injury[31], and we speculate that HMGB1 is involved in the formation of CTEPH after acute PE.

HMGB1 and Inflammation

As a member of the DAMPs, HMGB1 is passively released extracellularly in response to inflammatory stimuli or cell death and interacts with pattern recognition receptors (PRRs) on immune cells to mediate the host inflammatory response to pathogens.

HMGB1 and Macrophage Polarization

HMGB1 in different redox states has different biological functions. It has been shown that disulfide HMGB1 (dsHMGB1), but not fully reduced HMGB1 (frHMGB1), could have a proinflammatory effect by triggering macrophage polarization and secreting inflammatory molecules such as TNF-α, and interleukin-6(IL-6) through TLR4[32]. In chronic hypoxic environments, macrophage infiltration in rat lung tissue is significant, the lumen of small pulmonary arteries is narrowed, the wall is thickened, and polarized M1 macrophages are involved in vascular remodeling in pulmonary hypertension. In a retrospective study of a 5-year cohort of patients with acute pulmonary embolism, dynamic changes in angiotensin II (Ang II) were found in patients with PE[33]. Ang II induces HMGB1 nuclear translocation via the JAK/STAT pathway, promotes HMGB1 hyperacetylation and release, and induces M1 macrophage polarization[34]. In addition, M1 macrophages were found to induce endothelial cell apoptosis and participate in the inflammatory response in monocrotaline (MCT)-induced pulmonary hypertension[35].

HMGB1 and NETs

Pulmonary embolism (PE) is part of venous thrombus embolism (VTE), and recent studies have shown that platelets in VTE mice release HMGB1, which promotes neutrophil aggregation, and increases the release of NETs, exerts procoagulant effects, and mediates thrombosis[6]. Increased HMGB1-induced NET production exists in thrombotic diseases, such as ischemic stroke[25], coronary atherosclerosis[36], and acute lung injury induced by ischemia-reperfusion[37]. NETs are DNA reticulations modified by granule proteins released upon neutrophil activation, and their anti-inflammatory properties are thought to be associated with an important role in thrombosis. Studies have shown that NETs provide a scaffold for platelets, erythrocytes, leukocytes, and procoagulant factors to attach to the surface of the injured endothelium. In addition, the nucleosome component of NETs can inhibit fibrinolysis by degrading tissue factor pathway inhibitor (TFPI) through neutrophil elastase (NE). After PE, plasma levels of lysophosphatidic acid (LPA) and NETs are significantly elevated, and studies have shown that LPA promotes NETs to produce thrombi with anti-t-PA properties and stimulates thrombus fibrosis. These are difficult to degrade by the endogenous fibrinolytic system, forming mechanized thrombi leading to recurrent thromboembolism or CTEPH[38]. In addition, to high levels of cell-free DNA (cfDNA) in the plasma of VTE patients, studies have shown that cfDNA supports the stability of platelets and red blood cells in thrombi, activates the endogenous coagulation pathway, and inhibits fibrinolysis[39]. One year after the acute onset of VTE, patients were found to have higher levels of myeloperoxidase (MPO)-DNA complexes in plasma than healthy controls and increased neutrophil chemotaxis, expression of soluble intercellular adhesion molecule 1 (sICAM) mediated adhesion to endothelial cells, and soluble vascular cell adhesion molecule 1 (sVACM) activated endothelial cells to produce cytotoxicity and accelerate thrombosis[40]. In addition, NETs promote thrombotic fibrosis by promoting the differentiation of monocytes into fibroblasts and enhancing TGF-β activity; increased neutrophil activation and NET production, reduced fibrosis after targeting NETs by DNase1, and increased thrombolysis were found in CTEPH patients[41]. NETs play a procoagulant and antifibrinolytic role in the acute phase of thrombosis and also act as upstream activators of TGF-β and promote vascular thrombotic fibro-

sis. Fibrosis, as an important marker of chronic thrombosis, is used in the study of chronic thrombosis. However, although inferior vena cava ligation has been widely studied as a model of CTEPH, it still cannot fully replicate the progression from acute PE to CTEPH in humans, and the establishment of an animal model of CTEPH is still a problem to be solved.

HMGB1 and Vascular Remodeling

PE leads to abnormal per pulmonary small vessel remodeling causing arterial lumen occlusion, resulting in pulmonary hypertension; the mechanisms of pulmonary artery remodeling include pulmonary artery endothelial damage, smooth muscle cell regeneration, and inflammatory cell infiltration.

HMGB1 and Endothelial Cell Damage

PE leads to pulmonary vascular obstruction and local lung tissue hypoxia under hypoxic conditions. On the one hand, HMGB1 can act as an inflammatory factor to cause direct tissue damage; on the other hand, it induces the secretion of inflammatory agents to aggravate the inflammatory cascade response. It has been specifically shown that HMGB1 is involved in the mechanism underlying endothelial damage in several diseases, such as atherosclerosis[42] and ischemic stroke. In a hypoxic environment, human pulmonary artery endothelial cells (HPAECs) release HMGB1 with human pulmonary artery smooth muscle cells (HPASMCs), which inhibits HPAEC proliferation, causing endothelial cell injury through the HMGB1/RAGE pathway, and induces HPASMCs to produce inflammatory factors that promote pulmonary vascular remodeling. Interestingly, stimulation of HPAECs and HPASMCs cultured *in vitro* at different HMGB1 concentrations (0.1 μg/ml, 1 μg/ml, and 10 μg/ml) did not promote cell proliferation and migration after 12 or 24 hours, which appears to contradict reports in the literature[43,44]. The reason for the different results may be because that HMGB1 acts through different signal transduction pathways, HMGB1 is internalized in endothelial cells; induces lysosome activation and histone B activation, promotes endothelial cell proliferation, migration, and tube-forming capacity, and promotes vascular regeneration in mouse hind limb ischemia[45]. Interestingly, HMGB1 inhibits HPAEC migration in a TLR4/interferon regulatory factor 3 (IRF3)-dependent manner, suggesting that the HMGB1/TLR4 pathway inhibits pulmonary vascular remodeling[46]. However, whether HMGB1 promotes proliferation and migration of pulmonary artery smooth muscle cells in CTEPH patients, promoting the mechanism of pulmonary hypertension formation remains to be further investigated.

HMGB1 and Pyroptosis

Reducing inflammatory cell death in the vascular endothelium, and thus improving vascular endothelial function, has become a priority in the prevention and treatment of CTEPH after acute PE. Recent studies have reported the involvement of pyroptosis as an intrinsic immune component in the progression of multiple diseases. "Pyroptosis" was proposed in 2001 as a type of programmed cell death mediated by inflammasomes in dependence on caspase-1 or caspase-4/5/11. Gasdermin D is then split into the N-terminal hole forming domain and a C-terminal inhibition zone. The N-terminal hole forming domain is introduced into the cell membrane, forming a pore, causing the cell membrane to swell and rupture, releasing inflammatory factors such as IL-18 and IL-1β, which are involved in the inflammatory response. It can be activated by a variety of substances such as bacteria, DAMPs, pathogen-related molecular patterns (PAMPs), and DNA, and activates inflammasomes such as NLRP3, pyrin, and AIM2 to mediate the pyroptosis pathway through the action of intracellular RHOA, histone protease and dsDNA[47,48].

Factors related to the pyroptosis pathway are involved in the development of thrombosis-related diseases and PAH. It is worth mentioning that the role of ASC in aseptic disease is currently controversial. Conflicting reports suggest that platelet NLRP3 inflammasome activation regulates platelet activation and aggregation and accelerates thrombosis, whereas ASC is involved in the thrombogenic process as an important component of the NLRP3 inflammasome[49]. A series of additional studies have shown that $ASC^{-/-}$ mice enhance their thrombosis by stimulating platelet P-selectin and GPIIb/IIIa expression. Although some differences in multiple animal studies can be explained by experimental design, it is necessary to further explore mechanisms regulating abnormal cytokine secretion in thromboembolic diseases to explain these contradictory results. Furthermore, caspase-8 mediates IL-1β secretion by promoting M1 macrophage polarization and activating the NLRP3 inflammasome to induce the proliferation of pulmonary artery smooth

muscle cells and accelerate the formation of pulmonary hypertension[50]. Pulmonary artery endothelial cell ferroptosis can mediate the development of pulmonary hypertension in MCT rats via the HMGB1/TLR4/NLRP3 pathway[11]. The difficulty in preventing and diagnosing CTEPH after acute PE is due to its complex inflammatory pathophysiological mechanisms, and previous studies have shown that important pathological mechanisms reported for PE include inflammation and endothelial cell injury. Although some mechanisms of endothelial injury, such as endothelial cell ferroptosis and apoptosis, have been demonstrated, the role of pyroptosis mechanisms in the development of CTEPH after acute PE is unclear; in addition, CTEPH is accompanied by significant endothelial cell dysfunction and cytokine production, such as IL-1β, suggesting that pyroptosis may be involved in the pathophysiological process of CTEPH (Figure 4).

HMGB1 and Proliferation of Pulmonary Artery Smooth Muscle Cells

Pulmonary artery vascular remodeling involving smooth muscle cells (DUSP-1) inhibits PASMC proliferation[51]. However, the proliferation and migration of PASMCs appear to be key players in the distant complications of PE, with thrombotic events being one of the pathological mechanisms of CTEPH and thrombin, a key part of the coagulation cascade reaction. These are involved in the progression of CTEPH by activating the Akt/mTOR pathway to induce PASMC proliferation[52]. Furthermore, it has been shown that serum levels of HMGB1 are elevated in people with chronic obstructive pulmonary disease (COPD) combined with idiopathic pulmonary arterial hypertension(IPAH), and in *in vitro* experiments, HMGB1 stimulation of PASMCs promoted cell migration and proliferation[53]. However, the mechanism of action of HMGB1 in vascular remodeling in CTEPH is unclear, which provides new insights into the prevention and treatment of CTEPH.

Potential Targets for Post-PE CTEPH Treatment

The current long-term prognosis for PE remains poor as the current treatment for PE is based on anticoagulation, thrombolysis, and surgery. In a single-center retrospective study, anticoagulation in patients with PE in the presence of hemodynamic disturbances was found to be accompanied by progressive right heart failure within 24 h in some patients[54]. In addition, pulmonary endarterectomy is considered to be the treatment of

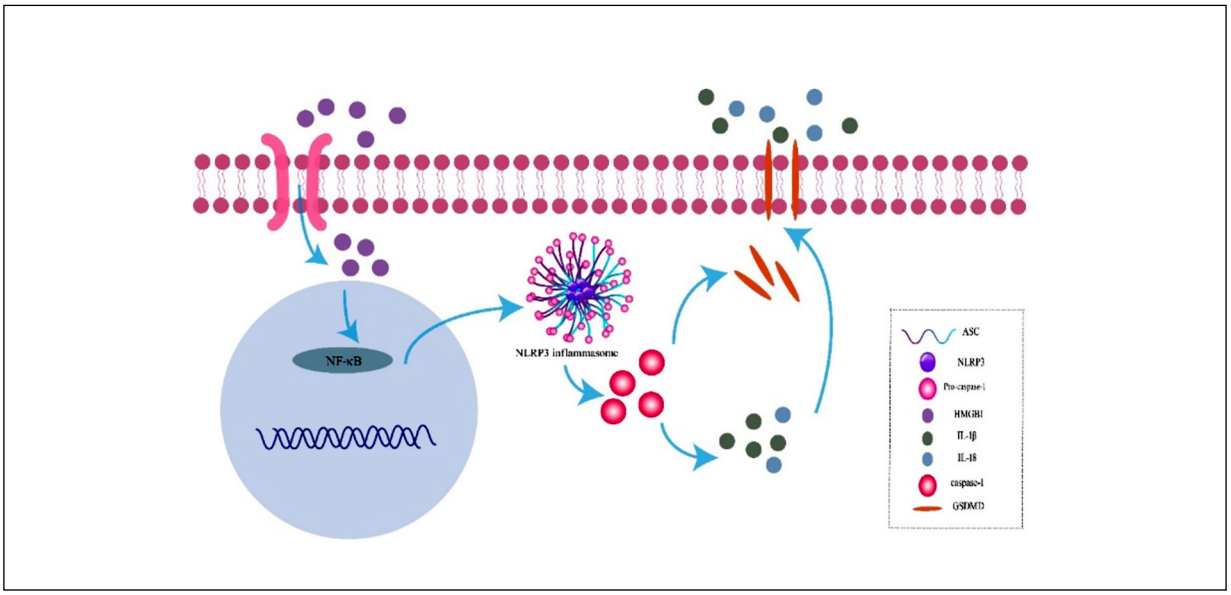

Figure 4. HMGB1 mediates endothelial cell pyroptosis. HMGB1 acts on RAGE/TLR4/TLR2 receptors on endothelial cells to activate intracellular NF-κB, which activates pro-caspase-1, ASC, NLRP3, and then they assemble into NLRP3 inflammasome. NLRP3 inflammasome promotes cleavage of pro-caspase-1 into mature caspase-1. Mature caspase-1 plays two roles, on the one hand, cleaves GSDMD to form GSDMD-NT, which perforates the cell membrane and mediates pyroptosis; on the other hand, it promotes the cleavage of pro-IL-1β and pro-IL-18 to mature IL-1β and IL-18, which mediates the inflammatory response.

choice for CTEPH, but the procedure is difficult in patients with microvascular embolism. In the follow-up of patients with CTEPH, no significant differences were found in survival over 2 years in the targeted drug treatment group compared to the pulmonary endarterectomy group. Therefore, the development and exploitation of targeted agents is a priority for CTEPH.

The inflammatory response remains a key factor in the development of acute PE to CTEPH, and HMGB1 released by activated platelets can act on itself to cause platelet aggregation, activate platelets leading to platelet aggregation and release the inflammatory factor HMGB1[55,56]; stimulate monocytes to release tissue factors to initiate the body's coagulation cascade[57,58]; activate neutrophils, and release NETs to provide thrombotic scaffolds for platelets and leukocytes via NETs[59]. In addition, it can act on endothelial cells to release inflammatory factors such as ICAM, VCAM-1, VWF, IL-6, and TNF-α, exacerbating the inflammatory response[60,61], promoting the proliferation and migration of pulmonary artery endothelial cells and pulmonary artery smooth muscle cells that induce arterial vascular remodeling in the lungs, and promoting the development of CTEPH. HMGB1-mediated cellular scorching can also produce inflammatory elements, such as IL-1β and IL-18[62]. Thus, immunotherapy against inflammatory factors remains a huge problem at present. Anti-HMGB1 has been an emerging new therapeutic strategy in a rat model of MCT-induced pulmonary arterial hypertension, HMGB1 injection revealed increased lung inflammation, thickened pulmonary artery vessel walls, and increased end-systolic pressure in the right ventricle, but significant improvement was achieved with the application of HMGB1 antibodies, suggesting that blocking HMGB1 could be a promising therapeutic approach for PAH[4]. In addition, elevated levels of HMGB1 and sRAGE were found in the serum of CTEPH patients or in lung tissue resected from PEA, suggesting that our anti-HMGB1 treatment may improve lung tissue damage due to inflammation[10]. Clinical evidence indicates that indomethacin, a commonly used NSAID, administered rectally 15-20 minutes before endoscopic retrograde cholangiopancreatography (PEP) significantly downregulates HMGB1 levels and significantly reduces the risk of PEP[62]. Heparin functionalized adsorbents (Seraph-100) have been shown to adsorb positively charged HMGB1, activate platelets and reduce platelet counts by 75% within 120 minutes. Importantly, these are approved to attenuate early thrombotic complications in patients with critical novel coronavirus pneumonia. Some studies have shown that Seraph-100 clears circulating pathogens in the circulation but has limitations in the clearance of intracellular pathogens. Therefore, the clinical efficacy of Seraph-100 as a novel agent needs to be further evaluated. With the in-depth study of HMGB1, the pharmacological effects of glycyrrhizin as a plant extract have been demonstrated in diseases such as tumor and hepatitis. It was shown that glycyrrhizin attenuates sepsis-induced lung injury by inhibiting HMGB1-mediated release of inflammatory factors[63]; additionally, it can reduce caspase-11-mediated cellular scorch death, which has a protective effect against sepsis-induced tissue damage[64]. However, no studies have shown the pharmacological effects of glycyrrhizic acid in CTEPH, which provides new ideas for the treatment of CTEPH. Fennel brain as an extract of fennel cress and sweet anise exerts anti-inflammatory effects via the HMGB1/TLR4/MYD88/NF-κB pathway, is a protective agent against ischemia-reperfusion kidney injury[65], and has also been shown to antagonize the progression of nicotine-induced hypertension[66]. Gegen Qinlian Pills (GQPs) are traditional Chinese medicines, and GQP treatment protects against inflammatory thrombosis by inhibiting the HMGB1/NF-κB/NLRP3 pathway to reduce inflammatory factor release and thrombosis, providing a theoretical basis for the clinical application of GQPs[67]. Currently, HMGB1 inhibitors are relatively widely studied and can antagonize the development of a variety of inflammatory diseases through their anti-inflammatory effects, but there are still some limitations. Despite the good results of HMGB1 anti-inflammatory effects in basic studies, to our knowledge, these inhibitors have not been applied to the clinic and clinical data are, therefore, still lacking. Moreover, the lack of subjects in clinical studies may lead to potentially biased results, thus, larger, neutral combined studies are needed to investigate the anti-inflammatory and antithrombotic effects of HMGB1 inhibitors.

In addition, the role of other inflammatory factors in pulmonary hypertension and thrombotic disease cannot be ignored. In DVT mice, FXII activation of the PI3K/AKT signaling pathway was found to promote inflammatory factor expression, and serum IL-6 and TNF-α levels were significantly downregulated in FXII knockout

mice. Meanwhile, LY 294002 (PI3K inhibitor) pretreatment significantly reduced inflammatory factor secretion and inhibited thrombosis; therefore, FXII may be a therapeutic target in thrombophilia[68]. MiR-181b-5p can inhibit MCT-induced progression of pulmonary hypertension by targeting endothelin and TGF-BR1 to inhibit endothelial cell mesenchymal transition, suggesting that antagonizing TGF-BR1 and endothelin helps to delay the progression of pulmonary hypertension and that the clinical combination of TGF-BR1 antagonists may largely attenuate the development of CTEPH after PE. Further *in vivo* experiments or clinical trials are needed to test the efficacy[69]. While etanercept has been extensively applied as a TNF-α monoclonal antibody for the clinical treatment of PE and DVT[70], the potential complications of anti-TNF therapy still need to be fully recognized during clinical treatment to prevent the reoccurrence of thromboembolic time. Fondaparinux, as an anti-FXa drug, is superior to low-molecular-weight heparin in preventing venous thrombosis and is used clinically for the prevention and treatment of DVT[71]. Thrombolytic therapy, as well as cardiopulmonary resuscitation, should be given promptly in the event of cardiac arrest due to acute pulmonary embolism, and studies have shown that TFPI can positively contribute to thrombolysis, providing a theoretical basis for the treatment of cardiac arrest after PE[72]. Therefore, targeting inflammatory factors provides the rationale for CTEPH treatment after acute PE.

Conclusions

HMGB1, as a cell nuclear component, is actively released by activated immune cells to trigger an inflammatory response and is one of the key factors in thrombotic disease. Clinical data suggest that increased serum HMGB1 levels are correlated with progressive pulmonary hypertension, right heart failure, and ischemia–reperfusion liver injury; because the pathogenic mechanisms of HMGB1 have not been clearly investigated, it remains unclear whether anti-HMGB1 therapy alone antagonizes the innate immunity of patients. Therefore, by discussing the relevance of HMGB1 receptors and their function in disease progression, we hope to provide a prospective therapeutic goal for the advancement of acute PE and a further idea for clinical studies of targeted drugs.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (81970419) and Graduate Innovation Fund of Harbin Medical University (YJSS-JCX2019-37HYD).

Funding

This study was supported by the National Natural Science Foundation Project of China (Grant Number: 81970419).

Ethics Approval

This article does not contain any studies with human participants or animals performed by any of the author.

Authors' Contribution

All authors contributed to the study conception and design. Data collection and analysis were performed by Miao Li. The manuscript was prepared by Miao Li, Xinyi Zhao, and Yan Kou. All authors have read and approved the final manuscript.

Data Availability Statement

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

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