Review

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Multiple Roles of Histidine-Rich Glycoprotein in Vascular Homeostasis and Angiogenesis

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Histidine-rich glycoprotein (HRG) is a 75 kDa plasma protein that is synthesized in the liver of many vertebrates and present in their plasma at relatively high concentrations of 100-150 µg/mL. HRG is an abundant and well-characterized protein having a multidomain structure that enable it to interact with many ligands, function as an adaptor molecule, and participate in numerous physiological and pathological processes. As a plasma protein, HRG has been reported to regulate vascular biology, including coagulation, fibrinolysis and angiogenesis, through its binding with several ligands (heparin, FXII, fibrinogen, thrombospondin, and plasminogen) and interaction with many types of cells (endothelial cells, erythrocytes, neutrophils and platelets). This review aims to summarize the roles of HRG in maintaining vascular homeostasis and regulating angiogenesis in various pathological conditions.

Key words: histidine-rich glycoprotein, vascular biology, coagulation, angiogenesis

Histidine-rich glycoprotein (HRG) is a 75 kDa single polypeptide chain protein that was first purified from human plasma almost half a century ago [1,2]. HRG is mainly synthesized in the liver [3] and has been shown to be present in the plasma of many vertebrates (humans, mice, cows, rats and rabbits) [4] as well as in aquatic invertebrates [5]. It is relatively abundant, circulating at a concentration of approximately 100-150 µg/mL in human blood [6]. Human HRG has been analyzed as having 507 amino acids [3] in multiple domains: two cystatin-like regions at the N-terminus and a histidine-rich region (HRR) flanked by two proline-rich regions (PRRs) and a C-terminal domain [7]. HRG can simultaneously interacts with multiple ligands, including heparin [2], heme [8], Zn^{2+} [9], plasminogen [10], fibrinogen [11], thrombospondin (TSP) [12], tropomysin [13], vasculostatin [14], immunoglobulin G [15], complement components [16] and phospholipids [17] through several independent binding sites [18], thereby regulating numerous biologic processes. HRG maintains homeostasis of the vascular system to provide immediate responses to foreign pathogens and vascular damage and has functions in modulating coagulation [19], fibrinolysis [10] and angiogenesis [4,20] and helping to kill pathogens [21-23]. In our study, we also found that HRG interacts with many different cell types in blood vessels, including endothelial cells, erythrocytes, neutrophils and platelets, to help prevent vascular dysfunction (Fig. 1). In this review, we focus on the roles of HRG in the maintenance of vascular homeostasis and regulation of angiogenesis.
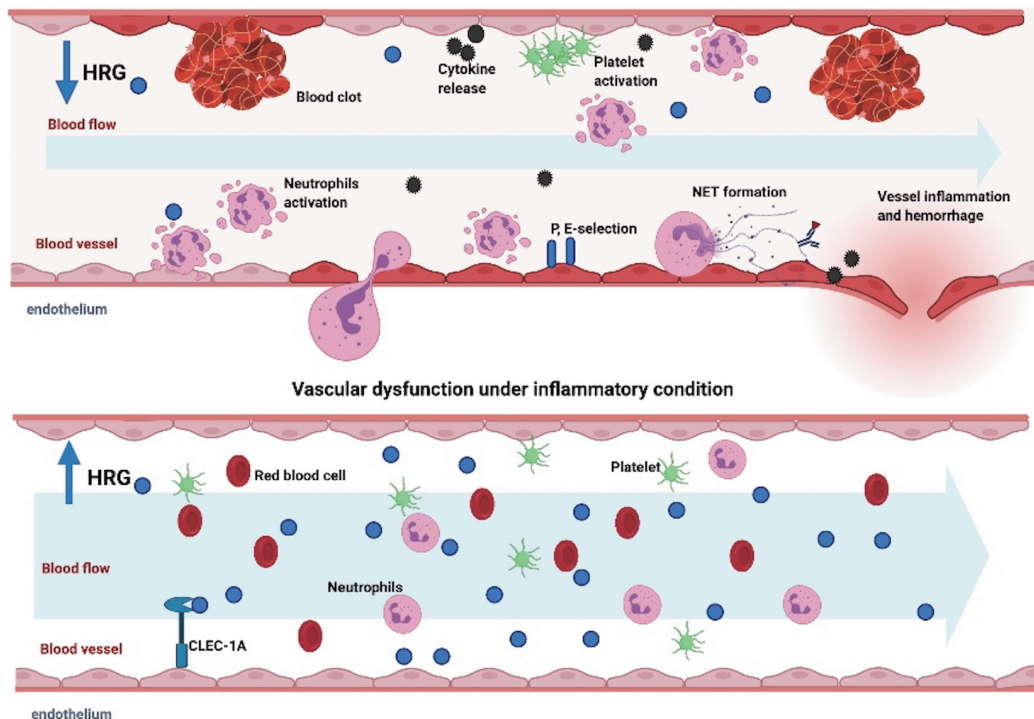
Fig. 1   HRG helps to maintain the vascular homeostasis.

The Role of HRG on Maintaining Vascular Homeostasis

Plasma proteins are essential for a variety of normal physiologic processes, such as coagulation, fibrinolysis, angiogenesis, tissue remodeling and transporting of nutrients, as well as the detection and clearance of unwanted materials such as pathogens, immune complexes (ICs) and dying/dead cells. The pathologies of many diseases like sepsis include vascular dysfunction associated with coagulopathy and dysregulated inflammation/immune responses. These pathologies are likely related to dysregulation of neutrophils, activation and damage of vascular endothelial cells, enhanced platelet aggregation, immune paralysis, and procoagulant activity. HRG's location in plasma as well as its multiple ligands within hemostatic pathways have implicated this protein as a modulator of hemostasis.

HRG regulates both coagulation and fibrinolysis. In previous studies, HRG was found to modulate various components of the coagulation cascade through its binding with molecules related to coagulation and fibrinolysis. HRG inhibits the procoagulant activity of monocytes by blocking the normal interaction between heparin and antithrombin III [24-26]. In addition, it has been shown that HRG can act as an antifibrinolytic agent due to its high-affinity interaction with plasminogen, by which it inhibits the interaction of plasminogen with fibrin clots, thus indirectly inhibiting plasmin-mediated fibrinolysis [27, 28]. This proposed antifibrinolytic effect of HRG would be expected to potentiate clot formation and persistence within the vasculature and in tissues. In contrast, other studies have demonstrated the pro-fibrinolytic role of surface-immobilized HRG [29]. Immobilized HRG binds plasminogen with a high affinity, resulting in an approximately 30-fold increase in the conversion of plasminogen to plasmin by tPA, with theoretically a concomitant increase in the fibrinolytic potential in the microenvironment of fibrin clots. Collectively, these studies suggest that HRG is an important agent in the regulation of coagulation and fibrinolysis.

HRG interacts with several blood cells. The initial step of the disruption of vascular homeostasis is the injury of vascular endothelial cells. It has been demonstrated that HRG can protect vascular endothelial cells from strong activation and apoptosis induced by lipopolysaccharide (LPS) or TNF-α *in vitro* [30, 31]. HRG

also can protect against the vascular barrier dysfunction of sepsis in mice and can prevent LPS/TNF-a-induced cytokine release from endothelial cells *in vitro*, thus helping to maintain the barrier integrity of the vascular endothelium [31]. Moreover, HRG was also shown to inhibit release of the damage-associated molecule-high mobility group box-1 (HMGB1) from endothelial cells through its binding with the C-type lectin domain family 1 member A (CLEC 1A) receptor expressed on endothelial cells [32].

HRG plays important roles in neutrophils, maintaining their round shape and smooth cell surface and suppressing the spontaneous production of reactive oxygen species (ROS). Plasma HRG maintains circulating neutrophils in the quiescent state, thereby facilitating the ease of their passage through capillaries and limiting damage to vascular endothelial cells, which may be induced by the ROS released from neutrophils [30]. Likewise, decreased plasma levels of HRG in sepsis can result in the adhesion of neutrophils on vascular endothelial cells, with subsequent intravascular NETosis and immunothrombosis [30].

Platelets are small cellular fragments derived from the budding of megakaryocytes in the bone marrow. They play an important role in the regulation of blood hemostasis as well as other processes such as immunity, tumor metastasis and angiogenesis. At sites of blood vessel injury, platelets are activated and aggregate to prevent hemorrhage. HRG has been found within platelets and has also been reported to be released following platelet activation with thrombin [33]. There are studies showing that HRG binds to platelets in a specific saturable manner dependent on Zn^{2+}. Binding is potentiated by thrombin-induced platelet activation [34,35]. $HRG^{-/-}$ mice display a shorter bleeding time than $HRG^{+/+}$ mice, indicating a role of HRG as a negative regulator of platelet function [36]. The underlying mechanisms of action are unclear but could involve the recruitment of other plasma proteins to the platelet surface. Candidates that are involved in platelet function and binding to HRG are TSPs and vitronectin [37]. Taken together, the above-mentioned findings suggest a role for HRG both as an anticoagulant, an antifibrinolytic modifier and a regulator of platelet function [36].

Since red blood cells (RBCs) play significant functions in blood clotting and related disorders [38], the RBC rheology has been widely studied in different disease conditions. Numerous studies have reported on the dysregulated adherence of erythrocytes to endothelial cells in sickle cell disease (SCD), malaria and diabetes. This adhesion plays a pivotal role in initiating thrombus formation after the activation of neutrophils and platelets in immunothrombosis. HRG also strongly inhibits the Zn^{2+}-induced aggregation of erythrocytes and the adhesion of erythrocytes to the damaged vascular endothelium: two more of its antithrombotic effects [39].

Functions of HRG in Angiogenesis

In healthy adults, angiogenesis is rare. The vasculature is tightly regulated by pro- and anti-angiogenic factors, keeping it quiescent during normal conditions. Excessive angiogenesis has been implicated in numerous pathological conditions such as rheumatoid arthritis, retinopathy and tumor growth, and the possibility of inhibiting the formation and remodeling of blood vessels is therefore of great clinical interest [40,41]. This section focuses on the role of HRG in the regulation of both pro- and anti- angiogenic properties.

Pro-angiogenesis activity. There have been reports on both the enhancing and inhibitory effects of HRG on angiogenesis, including both specific and more general effects on cellular function. One group reported that HRG inhibits the antiangiogenic effect of the angiogenesis inhibitor TSP-1 and -2 via the HRG N-terminal and C-terminal interfering with the binding of TSP to its receptor CD36 (Fig. 2) [42]. HRG can also tether plasminogen/plasmin to the surface of endothelial cells, thereby potentially enhancing cell migration and invasion, which is important during angiogenesis among other processes [43].

Anti-angiogenesis activity. Conversely, some researchers have demonstrated HRG's negative role in angiogenesis by various mechanisms [44-46]. HRG can bind to heparan sulphate on the extracellular matrix (ECM) and mask its heparanase cleavage sites, thereby preventing the release of growth factors from the ECM [47]. HRG has also been reported to inhibit endothelial cell tube formation and proliferation *in vitro* [45]. These results were later extended with *in vivo* data, where HRG was shown to inhibit tumor growth and vascularization in a subcutaneous fibrosarcoma mouse model, with the antiangiogenic effect mediated by the His/Pro-rich domain [45,46].
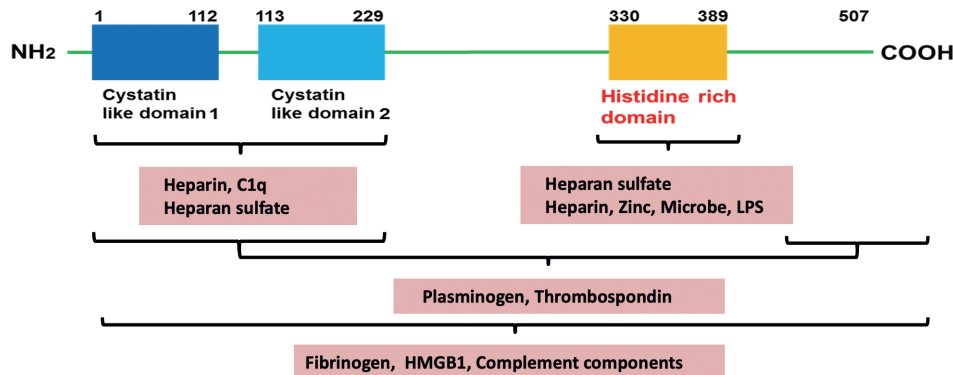
Fig. 2　　Domain structure of HRG and its binding molecule.

Concluding Remarks

Vascular dysfunction occurs as a common pathological process in many severe diseases and is accompanied by abnormal coagulation, fibrinolysis, inflammation and activation of various cells in blood. In this review, we revealed that HRG can protect endothelial cells, neutrophils, RBC and platelets from overwhelming activation in pathological processes, thus regulating coagulation and angiogenesis and maintaining homeostasis of the vascular endothelium. Although HRG has been shown to interact with a variety of ligands and to regulate numerous biological activities, much more remains to be discovered about this intriguing molecule.

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