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Role of the *growth arrest-specific gene 6* (*gas6*) product in thrombus stabilization

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Abstract

Growth arrest-specific gene 6 (*gas6*) product enhances the formation of stable platelet macroaggregates in response to various agonists. To determine whether Gas6 amplifies the response to known platelet agonists through one or more of its receptor tyrosine kinases of the Tyro3 family, mice deficient in any one of the Gas6 receptors (Gas6-Rs: Tyro3, Axl, or Mer) were submitted to thrombosis challenge and their platelet function was examined. The loss of any one of the Gas6-Rs protects mice against thromboembolism induced by collagen-epinephrine and stasis-induced thrombosis. Importantly, these mice do not suffer spontaneous bleeding and have a normal bleeding time but a tendency to repetitively re-bleed after transient hemostasis. Re-bleeding in mice lacking any one of the Gas6-Rs is not due to thrombocytopenia or coagulopathy but to a platelet dysfunction characterized by a lack of the second wave of platelet aggregation and an impaired clot retraction, at least in part by reducing outside-in $\alpha_{IIb}\beta_3$ signaling and platelet granule secretion. The early release of Gas6 by agonists perpetuates platelet activation through its three receptors, reinforcing outside-in $\alpha_{IIb}\beta_3$ signaling by activation of PI3K and Akt signaling and stimulation of tyrosine phosphorylation of the β_3 integrin. Furthermore, "trapping" Gas6 prevents pathological thrombosis, which indicates that blocking this novel cross-talk between the Gas6-Rs and $\alpha_{IIb}\beta_3$ integrin may constitute a novel target for antithrombotic therapy.

Keywords: Thrombus stabilization; Thrombosis; Embolism; Platelet; β3 integrin; Gas6; Tyro3; Axl; Mer

Introduction

Thrombosis, which causes life-threatening conditions such as heart attacks, strokes and pulmonary embolism, is responsible for about 50% of mortality in industrialized countries. Formation of a platelet plug initiates arrest of bleeding at sites of vascular injury but also triggers inopportune thrombi within atherosclerotic arteries. After initial formation of a single platelet monolayer, additional platelets are recruited into the growing hemostatic plug. Without further stabilization, platelet plugs prematurely disaggregate. The molecular mechanisms controlling platelet plug stabilization are incompletely characterized; however, the activated $\alpha_{IIb}\beta_3$ integrin plays a central role in this process by transmitting signals that guarantee persistent platelet activation, irreversible platelet aggregation and clot retraction. Recently, models targeting perpetuation of platelet activation revealed that additional signaling molecules exist. The present review will focus on the role of Gas6, a vitamin K-dependent protein, in thrombus stabilization.

Structure and general functions of Gas6 and its receptors

Gas6 is the product of the *growth arrest-specific gene 6* (*gas6*) which was originally identified in fibroblasts as a gene whose expression is upregulated in growth arrest [1]. Gas6 is a member of the vitamin K-dependent protein family and shares significant homology with anticoagulant protein S, both in module organization and amino-acid residues composition. Protein S is a cofactor for activated protein C, a serine protease which inactivates the coagulation factors Va and VIIIa. Genetic deficiency of protein S in man is one of the most common inherited risk factors for thrombosis. Gas6 comprises an N-terminal γ-carboxyglutamic acid (Gla) domain followed by four

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epidermal growth factor (EGF)-like domains and a large C-terminal region homologous to the sex hormone binding globulin (SHBG) (Fig. 1).

Gas6 is a ligand for three receptors belonging to the Tyro3 receptor tyrosine kinase (RTK) subfamily: Tyro3 (also called Sky, Rse, Brt, Tif, Dtk, Etk2), Axl (Ufo, Ark, Tyro7) and Mer (c-Eyk, Nyk, Tyro12). These single transmembrane RTKs share a characteristic structural organization with an extracellular domain composed of two immunoglobulin-like domains followed by two fibronectin type III-like motifs, a transmembrane domain and a C-terminal cytoplasmic tyrosine kinase domain (Fig. 2A). Ligand binding within the extracellular domain results in dimerization of the RTKs and subsequent autophosphorylation of their intracellular tyrosine kinase domains (Fig. 2B). The relative affinities of the receptors Tyro3, Axl and Mer for Gas6 vary over 100-fold, with Axl presenting the highest affinity, followed by Tyro3 and Mer [2]. Gas6 binds to its receptors through its SHBG-like domain [2–4], and a possible modulatory effect of the Gla domain in receptor binding has been reported [5,6].

Besides their role of mere ligand-induced RTKs, Gas6 receptors (Gas6-Rs) possibly function as cell adhesion receptors in a ligand-independent manner. Indeed, the architecture of their extracellular domain resembles that of cell adhesion molecules, such as intercellular adhesion molecules (ICAM) and vascular cell adhesion molecules (VCAM) that also present the same tandem of Ig domains at their N termini [7]. Recent observations made at the molecular level demonstrated that Ig domains of Tyro3 form dimers in vitro, both in the crystal and in solution [8], therefore reinforcing previous data obtained at the cellular level proposing cell adhesion as a possible mode of action for Axl [9]. For cell adhesion to occur, monomeric Gas6-Rs displayed on the surfaces of opposing cells, dimerize through homophilic interactions (Fig. 2C). Although the contribution of a single homophilic interaction is weak, a large cluster of molecules might be sufficient to promote stable cell adhesion. Such a model has been proposed for a variety of cell adhesion molecules of the Ig superfamily [10,11].

Another mechanism which might contribute to dimerization of Gas6-Rs is their glycosylation state. Tyro3 family receptors are expressed in a great variety of tissues where differently glycosylated forms of these receptors might exist. Glycosylation-dependent dimerization could then explain why Axl overexpression causes cell adhesion in some cell lines [9] but not in others [12].

Gas6 is expressed in many tissues, including endothelial cells [1], vascular smooth muscle cells [13] and bone marrow cells [14]. Consistent with this wide distribution, Gas6 has been

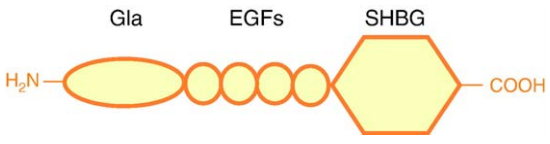

Fig. 1. Schematic representation of the Gas6 protein. The vitamin K-dependent protein Gas6 is composed of an N-terminal Gla domain rich in γ-carboxyglutamic acids, followed by four EGF-like domains, and a large C-terminal sex hormone binding globulin (SHBG)-like domain.

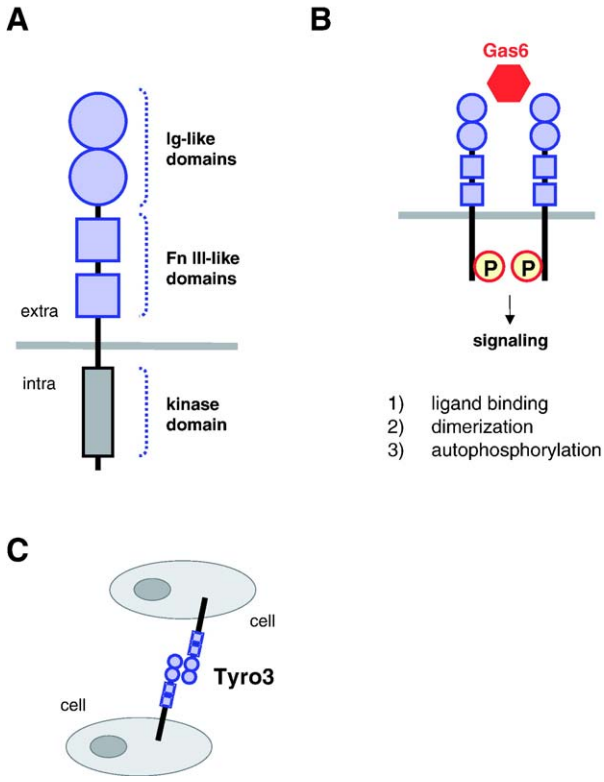

Fig. 2. Gas6 is a ligand for receptor tyrosine kinases of the Tyro3 family. (A) The extracellular domain of Tyro3, Axl and Mer receptors comprises two immunoglobulin (Ig)-like modules and two fibronectin type III (FN III-like) domains, whereas their intracellular domain is composed of a kinase domain. (B) Binding of Gas6 to its receptors triggers their dimerization and the subsequent phosphorylation of tyrosine residues within their intracellular domain. Gas6 signaling is then transmitted to the cell. (C) Homophilic binding of Gas6 receptors. In addition to ligand-induced signal transduction, Gas6 receptors might participate in cell adhesion through homophilic interactions between one receptor on one cell and one receptor on an adjacent cell, as reported for Tyro3.

involved in various crucial cell functions including reversible cell growth arrest [1], survival [15], proliferation [13,15,16], cell adhesion [12,17,18] and cell migration [18,19]. Recently, studies of mice deficient in Gas6 or in any one of its three receptors demonstrated a role for Gas6 pathway in platelet function and thrombus stabilization [20–22].

Models targeting the Gas6 pathway

Inactivation of the *gas6* gene prevents venous and arterial thrombosis in mice and protects them against fatal thromboembolism induced by a mixture of collagen and epinephrine [20]. However, *gas6*-deficient ($Gas6^{-/-}$) mice do not suffer spontaneous bleeding and have normal bleeding after tail clipping. Inhibition of Gas6 is also attainable by the use of antibodies directed against the C-terminal part of Gas6 that is responsible for binding of Gas6 to its receptors [4,5]. Those Gas6-neutralizing antibodies inhibit platelet aggregation in vitro [21,22] and protect mice against fatal thromboembolism without causing bleeding in vivo [21]. Purified recombinant murine Gas6 (mrGas6) is able to restore normal aggregation and