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Blood coagulation dissected

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ABSTRACT

Hemostasis is the physiological control of bleeding and is initiated by subendothelial exposure. Platelets form the primary vascular seal in three stages (localization, stimulation and aggregation), which are triggered by specific interactions between platelet surface receptors and constituents of the subendothelial matrix. As a secondary hemostatic plug, fibrin clot formation is initiated and feedback-amplified to advance the seal and stabilize platelet aggregates comprising the primary plug. Once blood leakage has been halted, the fibrinolytic pathway is initiated to dissolve the clot and restore normal blood flow. Constitutive and induced anticoagulant and antifibrinolytic pathways create a physiological balance between too much and too little clot production. Hemostatic imbalance is a major burden to global healthcare, resulting in thrombosis or hemorrhage.

1. Hemostasis overview

Hemostasis is the physiological watch-dog for vascular damage. To avert blood loss, clot is produced through a cascade of molecular events where the activation of upstream proteins results in acquisition of downstream enzyme and cofactor function. Since clotting is critical for health, imbalances in clot formation, stability or degradation may result in pathology. Excessive clot formation leads to thrombosis and related diseases, and is responsible for the largest number of annual deaths compared to any other disease globally [1]. Conversely, inadequate clot formation causes clinical bleeding and is relatively rare. Bleeding disorders are a challenge to manage and the recombinant therapeutics prescribed to treat specific deficiencies are among the costliest of all drugs. In the current review, fundamental molecular principles of clot formation are overviewed to assist the clinical practitioner to improve diagnostic approaches, optimize patient management and reduce healthcare delivery costs.

The control of bleeding has many molecular components comprised mainly of proteins that circulate in plasma and several proteins that are integral to the cell membrane. In addition to proteins, anionic phospholipid (aPL) on the cell surface and metal ions (e.g. calcium) are necessary for effective clot formation. The clot is a molecular mesh made of polymerized fibrin. Most of the proteins involved in hemostasis require cleavage to express their various activities, and fibrin is no exception. Its precursor is fibrinogen, which is directly cleaved by thrombin. Unlike fibrinogen, fibrin monomers spontaneously polymerize to form the insoluble clot. Thrombin generation from its inactive precursor, prothrombin, and the numerous functional effects of thrombin are strictly regulated and restricted to the local environment of damaged vasculature. Knowledge of these physiological check-points provides opportunities for intervention.

Fig. 1 organizes hemostasis into functional pathways that sequentially control clot formation (coagulation) and subsequent dissipation (fibrinolysis). Numerous circulating proteins constitutively survey the vasculature to prevent unnecessary clot formation (anticoagulation) or its premature degradation (antifibrinolysis). The function of these is constitutive and does not require proteolytic activation. Vascular damage initiates coagulation to stop bleeding, which is amplified predominantly by thrombin to overcome the intrinsic anticoagulant threshold of plasma. The accumulation of thrombin induces an additional anticoagulant pathway to prevent further clot formation and an inducible antifibrinolytic system to ensure the clot persists. Fibrin ultimately provides the primary trigger for its own degradation to restore normal blood flow after the clot has adequately sealed the vascular leak and tissue repair has been initiated.

2. The platelet plug

2.1. Adhesion

Platelets circulate as small, anucleate cells derived from megakaryocytes (MKs) in the bone marrow. Following endothelial injury, the

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Fig. 1. Hemostasis Overview. Inhibitors of coagulation and fibrinolysis constitutively survey blood. Their antagonistic thresholds must be surpassed by accumulation of activated hemostatic proteins for induction of the respective pathways. Vascular damage and the ensuing bleed initiates clot formation through the coagulation pathway. After the local build-up of thrombin and consequent deposition of clot have averted blood loss, an enhanced anti-coagulant response and clot dissolution are induced.

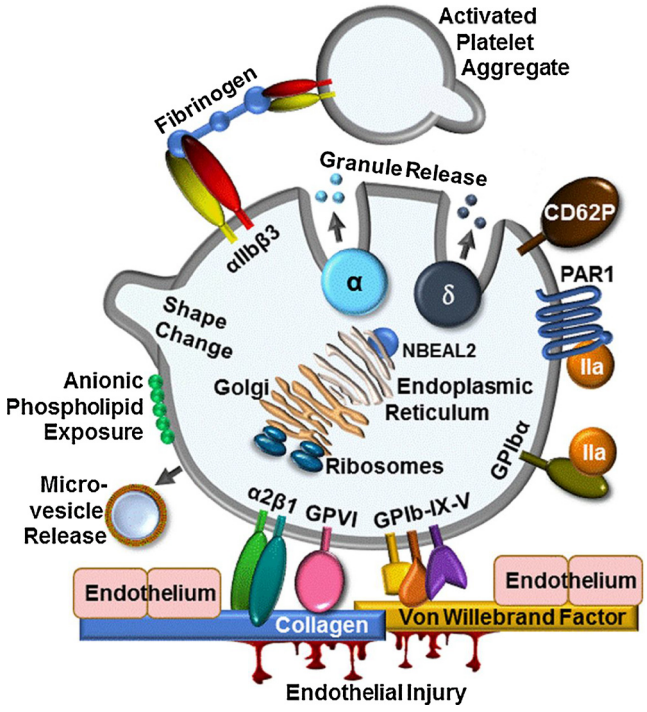

Fig. 2. The primary hemostatic plug. Vascular damage localizes and activates platelet activation, which is facilitated by engagement of specific platelet surface receptors with constituents of the subendothelial matrix. Numerous platelet changes occur including: shape change, degranulation (α- and dense (*δ*)-granules), microparticle release, αIIbβ3 integrin activation, anionic phospholipid flipping to the membrane surface and CD62P extracellular accessibility. After platelet activation, fibrinogen binding to αIIbβ3 is facilitated, which aggregates platelets by bridging cells. Platelet activation induces degranulation, which activates neighboring cells. Consequent anionic phospholipid exposure provides a localized surface for procoagulant enzyme complex assembly. This contributes to thrombin generation, which activates more platelets and other cells through PAR1 (and PAR4 not shown) and GPIbα, adding to clot formation and platelet plug stabilization.

initial vascular seal is facilitated by aggregated platelets, which is formed in three general stages, each mediated by discrete receptors on the platelet surface (Fig. 2). In the first step, exposure of adhesive molecules within the subendothelial matrix causes platelet recruitment. Subendothelial collagen and von Willebrand Factor (vWF) bind to platelet α2β1 integrin and glycoprotein (GP) VI, and GPIb-IX-V, respectively. These interactions localize circulating platelets to the site of vascular damage.

2.2. Activation

The second stage of primary vascular plug formation is platelet activation. Platelets contain two types of secretory granules. Alpha (α)-granules are the most abundant and store various functional molecules, including: the platelet hemostatic agents P-selectin and platelet activating factor 4; clotting factor (F) V; the antifibrinolytic protein, type I plasminogen activator inhibitor (PAI-1); and cell modulators, interleukins and chemokines [2]. Dense (*δ*)-granules contain inorganic and small organic molecules (e.g. calcium, serotonin, adenosine diphosphate, polyphosphate) [3,4]. Numerous agonists induce platelet activation, including collagen and vWF, constituents released from granules and thrombin. These serve to activate and recruit more platelets to the local environment, and stimulate other neighboring cell types. The latter is an aspect of the important involvement of platelets in host inflammatory and innate immune responses to injury or pathogen infection [5] and contribute to induction of neutrophil extracellular traps [6]. These platelet functions have been discovered relatively recently and are comprehensively reviewed elsewhere.

2.3. Aggregation

The third stage of primary vascular seal deposition is platelet aggregation. In response to stimulation, platelets undergo striking morphological changes to maximize subendothelial surface coverage, which involves reorganization of cytoskeletal components resulting in pseudopod extension and surface-spreading. These shape changes allow the normally discoid platelets to fit tightly together as they aggregate. The contact between platelets is mediated by fibrinogen after platelet stimulation activates its cell surface receptor, αIIbβ3 integrin. Fibrinogen has 2-fold symmetry, consisting of a dimer of three gene products forming the α-, β- and γ-subunits. This configuration enables fibrinogen to bridge αIIbβ3 on different platelets causing aggregation and alleviation of bleeding.

2.4. Disorders

Highlighting the importance of platelets in hemostasis, clinical bleeding occurs when the platelet number falls. This thrombocytopenic condition may be inherited [7] or acquired [8]. Numerous inherited platelet deficiencies that do not necessarily result in thrombocytopenia may cause dysfunction. Defining the affected molecular defect has helped to ascribe specific function to individual platelet proteins. Classic examples of inherited platelet diseases are: Bernard–Soulier syndrome, where platelets fail to adhere to vWF due to GPIb-IX-V mutations [9]; gray platelet syndrome, which is typified by platelets having an α-granule deficiency due to mutations in the endoplasmic reticulum protein, NBEAL2 [10]; and Glanzmann thrombasthenia, which is consistent with abnormal αIIbβ3 leading to impaired platelet aggregation [11,12]. While important strides have been made utilizing state-of-the-art proteomics and deep genomic sequencing technologies, multiple functions have been assigned to most platelet proteins, currently creating a barrier to accurately predicting phenotype from genotype.

3. Clot initiation

3.1. Roles of cells

The platelet plug requires protection against the sheer stress of