Connexin 37 Counteracts Clotting

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The thrombotic response elicited by arterial injury or atherosclerosis varies widely between individuals; in some, diffuse coronary artery disease never triggers thrombotic occlusion, whereas others with limited disease experience myocardial infarction (MI) due to arterial thrombosis. Understanding the predictors for arterial thrombosis will improve tailored therapy for preventing and treating the complications of atherosclerosis and vascular injury. Extensive effort has been invested in developing accurate tools for risk stratification based on clinical features, but interactions between genetic and environmental factors will also influence an individual’s risk for thrombosis. We are only beginning to appreciate how genetic factors may have an impact on the function of platelets, which play a key role in arterial thrombosis by forming an initial plug at sites of arterial injury or atherosclerotic plaque rupture or erosion. Ex vivo assays of platelet function reveal substantial interindividual heterogeneity, suggesting the hypothesis that intrinsic platelet reactivity may predict propensity to thrombosis. In animal models, the targeted deletion of any one of a number of proteins involved in platelet activation and/or aggregation protects from experimental thrombosis. Likewise, in the case of pharmacologically targeting P2Y12 receptors, more potent antagonists reduce clinical outcomes such as acute stent thrombosis. However, whether heightened platelet reactivity, in the absence of antiplatelet therapy, is causally associated with arterial thrombosis remains unknown. This is due, in part, to a lack of understanding of the pathways that may normally check platelet activation. In the current issue of Circulation, Angelillo-Scherrer et al 1 add connexin 37 (Cx37) to the small, but growing, list on membrane proteins that negatively regulate platelet function and implicate the Cx37 genotype as a risk marker for thrombosis.

The findings of Angelillo-Scherrer et al are important for several reasons. The work provides the first evidence that aggregating platelets can form gap junctions. Gap junctions serve as a conduit to connect neighboring cells functionally by electrochemical coupling and exchange of small molecules, typically <1000 Da in size. Connexins are tetraspanning membrane proteins that self-associate with 6 homomeric or heteromeric connexin complexes, forming a connexon hemichannel. Two connexon hemichannels on adjacent cells interact in a head-to-head manner, thereby allowing cytoplasmic exchange between cells. Gap junction proteins may also affect cellular functions via adhesive actions or through intracellular signaling effects of their cytoplasmic domains. Angelillo-Scherrer et al demonstrate that aggregating platelets exchange neurobiotin, an amino acid derivative of biotin, and that platelet-platelet exchange of neurobiotin is attenuated in the absence of Cx37. These results establish that platelets form functional gap junctions and communicate with one another via cytoplasmic exchange.

Second, the work from Angelillo-Scherrer and colleagues focuses attention on Cx37 as an intrinsic negative regulator of platelet function. Mice genetically engineered to lack Cx37 (encoded by Gja4) have shortened bleeding times after tail transection and faster time to thrombosis following application of ferric chloride to the mesenteric vessels. In keeping with these in vivo observations, Cx37-deficient platelets show heightened stimulatory responses and aggregation to low-dose agonists. Together, their findings suggest that Cx37 normally checks platelet activation.

The pathways known to negatively regulate function can be divided into 2 types: those that involve production or degradation of a soluble mediator, such as nitric oxide, prostacyclin, or adenosine diphosphate, by other vascular cells and those that may be triggered by cell-cell contact (Figure). Platelet endothelial cell adhesion molecule-12 and carboxyembryonic antigen cell adhesion molecule-13 members of the immunoglobulin superfamily containing the immunotyrosine-based inhibitory motif, and junctional adhesion molecule-Å4 and endothelial cell specific adhesion molecule,3 non-immunotyrosine-based inhibitory motif-containing receptors, inhibit platelet function, possibly by homotypic binding that initiates intracellular signaling. Thus, there is ample precedent to envision that contact-dependent events between platelets may serve to halt the continued enlargement of growing platelet thrombi. Cx37, by promoting interplatelet connections, may propagate signaling between closely aligned platelets (Figure). The platelet gap junctions formed by Cx37 may allow small molecules to diffuse through aggregating platelets to limit thrombus growth, or to disperse out of highly active platelets to limit their potential. However, whether the inhibitory function of platelet Cx37 requires the formation of gap junctions between platelets and/or diffusion of signaling molecules remains to be established. Nonetheless, the ability of platelets to limit their own reactivity may provide an important restraint on thrombus growth and may explain why platelet surfaces eventually passivate, that is, lose their capacity to recruit new platelets.
Finally, the observations of Angelillo-Scherrer et al may provide mechanistic insight into the observation that Cx37 polymorphisms predict complications of atherosclerosis. The Cx37-C1019T allele results in a proline to serine substitution at amino acid 319. In several studies, the Cx37–1019C (319proline) polymorphism strongly associates with the development atherosclerosis in carotid arteries and coronary arteries and is an independent risk factor for MI. However, other studies found that the Cx37–1019T (319serine) allele predicts acute MI. Until the present work, the association of the genetic polymorphism in Cx37 with coronary artery disease was thought to reflect a role for Cx37 in regulating monocytes and vascular cells. Gap junctions may serve an important role in promoting cellular communication between endothelial cells and from the endothelium to smooth muscle cells in response to mechanical stress and circulating stimuli. Connexin-mediated interactions between endothelial cells may allow the cells to migrate en masse as a sheet to cover denuded or damaged vessels rapidly. Cx37 hemichannels also promote monocyte adhesion and vascular inflammation. Indeed, the absence of Cx37 in Apoe−/− mice enhances monocyte adhesion to endothelium and accelerates the development of atherosclerosis.

The work by Angelillo-Scherrer et al indicates that platelets from individuals with the Cx37–1019C allele have heightened platelet reactivity ex vivo in response to low doses of arachidonic acid, ADP, or collagen. Furthermore, cells expressing Cx37–319 proline display attenuated gap junction exchange in comparison to cells expressing Cx37–319 serine. Together, these results suggest the possibility that the reduced cytoplasmic exchange associated with the Cx37–1019C allele attenuates the inhibitory effects of Cx37 on platelet function. One might speculate that the heightened platelet reactivity associated with the Cx37–1019C allele would predict an increase in propensity for thrombosis and MI. If true, these observations could provide a mechanistic explanation for the MI risk associated with the Cx37–1019C allele. Whether heightened platelet reactivity contributes to increase in the development of experimental atherosclerosis in Apoe−/− mice lacking Cx37 or to clinical complications in humans with the reduced function Cx37–1019C variant is not known.

In summary, Angelillo-Scherrer and colleagues not only identify Cx37 as a novel protein in platelets that negatively regulates platelet activation, but they also provide a potential explanation for high platelet reactivity associated with a polymorphism in Cx37. The work sets the stage for future investigations to establish that platelets communicate through gap junction-mediated cytoplasmic exchange, to determine whether cytoplasmic exchange is require for the inhibitory effects of Cx37, and if so, to discover the identity of the molecules exchanged. Finally, Cx37 and accompanying platelet hyperreactivity needs to be functionally validated as risk for MI. Ideally, studies will demonstrate that Cx37 genotype can be used to stratify patients to treatment strategies aimed to lower platelet reactivity and improve outcomes.

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References
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