Ju (2013) — "Single-molecule study on GP1bα and vWF-mediated platelet adhesion and signal triggering"

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1 Summary

- Platelet adhesion is initiated in a two-step cascade:
  1. Fast tethering to immobilized vWF by GP1bα recruits platelets to the site of vascular injury.
  2. Stable adhesion is then mediated by integrins. For example, $\alpha_{IIb}\beta_3$ binds to immobilized vWF and fibronectin, and also immobilizes plasma proteins like vWF and fibrinogen.
- This is the general picture of thrombus formation, however the details still remain to be ironed out. In particular, they are interested in how a mechanical stimulus is translated into biochemical signals in this two-step cascade.
- They used a biomembrane force probe (BFP) to measure force-dependent lifetimes of molecular bonds, to estimate the association and dissociation rates of individual bonds.
- They also combined the BFP with fluorescence imaging to observe kinetics and Ca^{++} signaling together.
- They made the following new insights on platelet adhesion:
  1. The vWF-GP1bα displays a catch-bond behavior, where the off rate decreases with increasing shear force
  2. GP1bα has a high on rate, and is therefore critical in mediating cell tethering to substrate at higher shear rates.
  3. There was a strong correlation between bond lifetimes and Ca^{++} levels. This supports a model where force and strutural variation regulate platelet signaling through the lifetime of the A1-GP1b bond.
  4. They found GP1b-triggered integrin priming. Following a calcium signal, $\alpha_{IIb}\beta_3$ displayed an intermediate binding affinity. Notably, platelet shape does not change, suggesting that this priming is activation independent. Thus it may reinforce adhesion following initial GP1b-vWF tethering.

2 Introduction

- Regulation of the initial adhesion process is important, as insufficient adhesion cannot stop bleeding well enough to maintain hemostasis, while too much adhesion results in thrombosis.
- Traditional methods of studying platelet adhesion (flow chambers and microfluidics) lack temporal and spatial resolution, and are unable to characterize kinetics of single bonds. They used single-bond experiments using a BFP to characterize adhesion and signaling functions of GP1b.
- Three specific aims:
  1. Study how vWF regions surrounding A1 regulates the vWF-GP1bα interaction under force.
     - vWF requires an extensional force to uncover the A1 domain for binding with GP1b.
     - They used a BFP to analyze the regulatory role of vWF regions surrounding the A1 domain, in particular looking at the N-terminal flanking region. They used a BFP to analyze the single-bond dissociation of GP1bα from vWF, and to examine the effect of structural variation on vWF-GP1bα kinetics. They also used a flow chamber to measure the rolling velocity of washed platelets over immobilized vWF.
  2. Characterize physical reulation of vWF-GP1bα 2D association.
     - Two possible mechanisms for flow-enhanced platelet adhesion to vWF: transport-dependent on rate, and a force-dependent off rate.
     - They used a BFP to address these two hypotheses

3. Study how force triggers platelet calcium signaling via GP1bα.
   - They wanted to demonstrate that mechanical force regulates platelet signaling. More specifically they defined the vWF-GP1bα catch bond as responsible for optimal Ca^{++} signaling, and verified the hypothesis that the Ca^{++} signal triggered by vWF-GP1bα is responsible for priming integrins.

3 Chapter 4: The interplay of force and the A1 domain N-terminal flanking region on regulating the vWF-GP1bα catch bond

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Article Evaluation

Reference

Ju, L. (2013). *Single-molecule study on GP1bα and von Willebrand factor-mediated platelet adhesion and signal triggering*. PhD thesis, Georgia Institute of Technology.