Recycling Controls Procoagulant and Cell Signaling Functions of Tissue Factor

Kenneth M. Brinkhouse Young Investigator Prize in Thrombosis Competition

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Presenter Disclosure Information Elements

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FINANCIAL DISCLOSURE:
No relevant financial relationship exists
P2X7 receptor couples inflammation and coagulation

• High levels of extracellular ATP, released by stressed and injured cells, activate P2X7 receptors on macrophages, key orchestrators of inflammation and thrombosis in cardiovascular disease

• P2X7 receptor activation promotes the release of pro-inflammatory IL-1β and procoagulant MP carrying TF and PS

Rothmeier et al, 2015 JCI
Thrombo-inflammatory MP induce fibrin strands

Whole Blood Flow:

TF\(^+\) macrophages

Fibrin (red)

ATP

TF\(^{-/-}\) macrophages

ATP

Rothmeier et al, 2015 JCI
P2X7 receptor couples inflammation and coagulation

- P2X7 receptor activation is coupled to extracellular reductive changes

- We identified by proteomics of thiol-modified surface proteins the activation of the thioredoxin reductase/thioredoxin system and inflammasome activation as central to P2X7 receptor signaling

Rothmeier et al, 2015 JCI
Trafficking of TF onto filopodia critical for MP release

control

Filopodia

PS

TF

TF+/PS+ MP

P2X7

TRX-TRXR-system

caspase 1

Il-1β

caspase 1 is central to the coupling of inflammation and thrombosis

Rothmeier et al, 2015 JCI
Injury Signals activate Cell Surface TF

- Antibody blockade demonstrates that cell surface TF is activated by ATP stimulation of macrophages
- Release of procoagulant MP secondary event downstream of TF activation on cell surface
- Cell adhesion controls TF cell surface activity

Rothmeier et al, 2015 JCI
Internalization prevents TF activation

- Cell surface TF is constitutively internalized
- P2X7 receptor activation with ATP prevents TF internalization
- Inhibition of dynamin-dependent internalization with the inhibitor Dynasore also retains TF on the cell surface

unpublished data
Inhibition of receptor uptake promotes TF release on MP

Blockade of internalization activates cell surface pools of TF on cells and generates TF+/PS+ procoagulant MP

Inhibition of internalization with Dynasore does not induce cytoskeleton remodeling

unpublished data
P2X7 receptor regulates the small Rho GTPase arf6

Differentially released proteins from ATP and Dynasore treated cells

Arf6 is associated with Dynasore generated procoagulant TF+ MP

Dysasore ATP

P2X7 receptor signaling inactivates arf6

unpublished data
Integrin-associated arf6 controls TF

- TF interacts with integrin $\alpha 4\beta 1$ and $\alpha 5\beta 1$ (Dorfleutner et al, 2004)

- Integrin $\alpha 4$ S988A (S/A) mutant favors paxillin and ADP-ribosylation factor GTPase-activating protein (Arf-GAP) recruitment leading to decreased arf6 activity in the context of cell adhesion

unpublished data
TF coagulation and signaling function

- Tracking of FVIIa on epithelial cells using fluoro-labeled antibodies
- arf6 was inhibited by pre-incubation with the small molecule Secin H3 (SH3)

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(TF:FVIIa:FX) → coagulation → FXa

TF:FVIIa → PAR2 cleavage → signaling → IL8 induction

unpublished data
Arf6 regulates TF function

epithelial cells    melanoma cells

SLIGRL-mediated PAR2 activation

TF:FVIIa-mediated PAR2 activation

FXa generation

unpublished data
Arf6 controls TF coagulation-signaling switch

- MP-triggered Thrombosis
- Coagulation

- Cell Signaling

- arf6-GTP

- P2X7 receptor activation
Acknowledgements

The Scripps Research Institute
Ruf lab:
- Pablito Tejada
- Cynthia Biazak
- Jennifer Royce
- Maki Kitano

Ruggeri lab:
- Zaverio Ruggeri
- Patrizia Marchese

University of California San Diego
- Mark Ginsberg
- Brian Petrich
- Joe Cantor

Funding
Deutsche Forschungsgemeinschaft
NIH
National Heart, Lung, and Blood Institute
novo nordisk®