Extracellular Signal-regulated Kinase 5 Associates with Casein Kinase II to Regulate GPIb-IX-mediated Platelet Activation via PTEN/PI3K/Akt Pathway

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**OBJECTIVES**

To reveal the function and mechanisms of Extracellular Signal-regulated Kinase 5 (ERK5) in glycoprotein (GP) Ib-IX-mediated platelet adhesion, aggregation, and activation.

**METHODS**

The functions of ERK5 in GPIb-IX-mediated human platelet activation were assessed using botrocetin/VWF or platelet adhesion to VWF under shear stress in the presence of a specific inhibitor of ERK5. ERK5-associated proteins were pulled down from Chinese hamster ovary (CHO) cells transfected with HA-tagged-ERK5, identified by mass spectrometry, and confirmed in human platelets. Roles of ERK5-associated proteins in GPIb-IX-mediated platelet activation were clarified using specific inhibitors.

**RESULTS**

1. ERK5 inhibitor XMD8-92 suppressed the second wave of human platelet aggregation induced by Botrocetin/VWF (Fig. 1A, B), and inhibited human platelet adhesion on immobilized VWF under shear stress (Fig. 1C, D).
2. Casein kinase II (CKII) was identified as an ERK5-associated protein in human platelets (Fig. 2A). CKII inhibitor TBB, similar to XMD8-92, specifically restrained PTEN phosphorylation in human platelets treated with Botrocetin/VWF (Fig. 2B).
3. The aggregation experiments confirmed that both PI3K inhibitor wortmannin and Akt inhibitor SH6 dose-dependently inhibited Botrocetin/VWF-induced human platelet aggregation (Fig. 3A). The results presented in Fig. 3(B) showed the phosphorylation levels of Akt were significantly suppressed in human platelets stimulated by Botrocetin/VWF in the presence of ERK5 inhibitor XMD8-92, CKII inhibitor TBB and PI3K inhibitor wortmannin respectively.
4. These results suggested that ERK5/CKII regulate GPIb-IX-mediated platelet activation via the PTEN/PI3K/Akt signaling pathway (Fig. 4A). Furthermore, results showed that the average size of the platelets that spread on immobilized Fg was 247.5 ± 17.23 pixels for XMD8-92 pre-incubated platelets vs 446.5 ± 57.69 pixels for DMSO pre-incubated platelets, demonstrated that ERK5 may play critical roles in integrin αIIbβ3-mediated human platelet spreading (Fig. 4B).

**CONCLUSIONS**

The interacting protein of ERK5 in human platelet is identified as casein kinase II (CKII). We identified a new Src-Raf-MEK5-ERK5/CKII-PTEN pathway, which play critical role in GPIb-IX-mediated platelet activation via regulation of PI3K/Akt activation. And blockage of ERK5 association with CKII may serve as a promising anti-platelet targets.

**References**