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POSTER ABSTRACTS

301.VASCULATURE, ENDOTHELIUM, THROMBOSIS AND PLATELETS: BASIC AND TRANSLATIONAL

Mice Lacking α-Actinin-1 in Megakaryocytes Display the Feature of Thrombocytopenia and Impair Platelet Functions

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Cytoskeleton remodeling and mitochondrial bioenergetics play important roles in platelet biogenesis and functions. α-Actinin-1, a filament crosslinker, was widely expressed in nonmuscle cells, including megakaryocytes (MKs) and platelets. The cytoskeleton is essential for normal mitochondrial morphology, motility, and distribution. In recent years, the accumulated evidence suggests that inherited mutations in α-actinin-1 are implicated in congenital macrothrombocytopenia. However, the role and underlying mechanism of α-actinin-1 in platelet biogenesis and platelet functions remain poorly studied. In this study, we generated an MK-specific α-actinin-1 knockout mouse model (referred to herein as PF4- *Actn1* $^{-/-}$) to investigate the contribution of α-actinin-1 in platelet biogenesis and functions.

Blood count tests showed that PF4- *Actn1* $^{-/-}$ mice exhibited reduced platelet counts. The decreased platelet number in PF4- *Actn1* $^{-/-}$ mice was not attributed to accelerated platelet clearance or impaired TPO generation *in vivo*. Platelet count changes observed in an anti-GPIbα antibody-induced thrombocytopenia model and splenectomy indicated that the diminished platelet counts in PF4- *Actn1* $^{-/-}$ mice might be due to defects in platelet biogenesis. Compared to control mice, PF4- *Actn1* $^{-/-}$ mice had normal numbers of MK progenitors, including in LSK, MPP, CMP, GMP, MEP, and PreMegE. H&E staining and flow cytometry revealed a decrease in the number of MKs in BM, but an increase in the number of MKs in the spleen. The absence of α-actinin-1 significantly increased the proportion of 2N-4N MKs and decreased the proportion of 8N-32N MKs. CFU-MK colony formation and MK migration in response to SDF-1 signaling was inhibited in PF4- *Actn1* $^{-/-}$ mice. Furthermore, *in vitro* studies showed a reduced ratio of PPF-bearing MKs in fetal liver-derived PF4- *Actn1* $^{-/-}$ MKs. Collectively, these data suggest that α-actinin-1 deficiency in mouse MKs could impair platelet biogenesis, resulting in thrombocytopenia in PF4- *Actn1* $^{-/-}$ mice.

Platelet adhesion and spreading on immobilized fibrinogen and collagen, thrombin-stimulated clot retraction, and platelet aggregation in response to various concentrations of ADP, thrombin, and collagen was significantly decreased in PF4- *Actn1* $^{-/-}$ platelets. PF4- *Actn1* $^{-/-}$ platelets also exhibited decreased integrin αIIbβ3 activation and reduced P-selectin exposure in response to various concentrations of ADP, ADP+EPI, thrombin, collagen, and convulxin. Notably, PF4- *Actn1* $^{-/-}$ platelets showed inhibited calcium mobilization, reactive oxygen species (ROS) generation, and actin polymerization in response to collagen and thrombin. Furthermore, PF4- *Actn1* $^{-/-}$ mice demonstrated significantly impaired hemostasis. No differences were observed in the PT, APTT, TT, and fibrinogen concentrations between PF4- *Actn1* $^{-/-}$ mice and *Actn1* $^{f/f}$ mice. However, tail, liver, and brain bleeding tests revealed that PF4- *Actn1* $^{-/-}$ mice had significantly prolonged bleeding time and increased bleeding volume. Kaolin-activated whole thromboelastography (TEG) mapping assay indicated that there were no differences in the R-time, K-time, and α-angle values, but the maximum amplitude was decreased in PF4- *Actn1* $^{-/-}$ mice.

To explore the mechanism of α-actinin-1 in platelet biogenesis and platelet functions. Low- (2-4N) and high-ploidy (≥8N) *Actn1* $^{f/f}$ and PF4- *Actn1* $^{-/-}$ MKs were sorted for quantitative proteomics analysis. The results of 4D label-free quantitative proteomics revealed that protein expression was significantly decreased in high-ploidy MKs following α-actinin-1 knockdown. Functional enrichment analysis of differentially expressed proteins indicated that α-actinin-1 deletion reduced platelet activation and mitochondrial functions. PF4- *Actn1* $^{-/-}$ platelets exhibited reduced mitochondrial membrane potential, mitochondrial ROS generation, mitochondrial respiration and glycolysis, suggesting alterations in mitochondrial function in platelets.

In this study, we report that mice with α-actinin-1 deficiency in MKs recapitulate the features of thrombocytopenia and exhibit impaired platelet functions.

Disclosures No relevant conflicts of interest to declare.

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