Check for updates

ELSEVIER

Blood 142 (2023) 2564–2565

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

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

Non-Activating Integrin β3(R734C) Mutation Associated with Macrothrombocytopenia and Impaired Platelet Function

Keiichi Nakata, MD[1,2], *Keigo Akuta, MD PhD*[3,1], *Takaya Endo, MD*[1], *Midori Koike, MD*[1,2], *Hisashi Kato, MD PhD*[1], *Yoshiaki Tomiyama, MD PhD*[1], *Naoki Hosen, MDPhD*[1], *Hirokazu Kashiwagi, MD*[2]

[1]Department of Hematology and Oncology, Osaka University Graduate School of Medicine, Suita, Japan
[2]Department of Blood Transfusion, Osaka University Hospital, Suita, Japan
[3]Department of Signal Transduction, Research Institute for Microbial Diseases, Osaka University, Suita, Japan

Introduction

We and others identified several mutations in *ITGA2B* or *ITGB3* associated with congenital macrothrombocytopenia. These mutations are located around transmembrane domains of αIIb or β3, and basically induce constitutive activation of αIIbβ3. Recently, we identified a mutation, β3(R734C), which is located in cytoplasmic tail of β3 without inducing αIIbβ3 activation in a Japanese family with macrothrombocytopenia. We analyzed the effect of the mutation on platelet production and function using knock-in (KI) mice.

Cases and Transfection assay

The proband was a 14-year-old Japanese girl, who showed macrothrombocytopenia with 60-90 x 10^9/L platelet counts and mild bleeding tendency. Her mother, her maternal aunt and a cousin also showed macrothrombocytopenia. The expression of αIIbβ3 on their platelets was decreased to ~50% of healthy control. GPVI expression was decreased, whereas GPIb expression was increased probably due to the increase in platelet size. Genetic analysis revealed that all affected subjects were heterozygous of β3(R734C) mutation. No PAC-1 binding was observed to the platelets of the affected subjects or αIIbβ3(R734C)-transfected 293T cells, indicating that the mutation does not cause αIIbβ3 activation.

Methods and Results of mouse studies

We generated β3(R734C) KI mouse (C57BL6/J background) by CRISPR/Cas9-mediated genome editing. The β3(R734C) KI mice were viable with no apparent bleeding tendency. Platelet counts of heterozygous (Hetero), and homozygous (Homo) KI mice were decreased relative to those of wild-type (WT) mice [WT: 1095 ± 52 (x10^9/L), Hetero: 834 ± 93**, Homo: 445 ± 30** (mean±SD, **P<0.01 compared with WT, n=6 respectively)], with an increase in platelet size, indicating that β3(R734C) leads to macrothrombocytopenia. Moreover, the expression of αIIbβ3 and GPVI in platelets was decrease in KI mice like human subjects. Platelet aggregations were impaired in KI mice. JON/A binding was not detected on non-stimulated platelets and agonist-induced P-selectin and JON/A binding were impaired in homo KI mice, particularly in PAR4-AP stimulation. Platelet spreading on fibrinogen (Fgn) was impaired in KI mice with or without ADP and thrombin stimulation. Next, we analyzed megakaryocytes (Mgk) derived from bone marrow after 5 days incubation with murine thrombopoietin. Interestingly, the expression of αIIbβ3 in Mgk of KI mice was compatible with WT and the rate of filopodia and/or lamellipodia formation of Fgn-adhered Mgk was decreased in KI mice [WT: 78(%), Homo: 32(%) (P<0.01)]. RhoA activation was assessed by ELISA and it was significantly decreased in Fgn-adhered Mgk of KI mice [WT: 0.33±0.039, Homo: 0.16±0.029 (mean OD 490nm Δblank±SD, P<0.01)]. Finally, we observed impaired proplatelet formation of fetal liver derived-Mgk in KI mice [WT: 26.2±2.7(%), Homo: 15.2±1.9(%) (P<0.01)] with abnormal morphology (Figure 1). These results suggest that impaired αIIbβ3 outside-in signaling and cytoskeletal remodeling in KI mice lead to macrothrombocytopenia.

Conclusions

We revealed that a non-activating β3(R734C) is a causal mutation of macrothrombocytopenia in human and mouse. This mutation leads to impaired inside-out and outside-in signaling of αIIbβ3, which results in abnormal platelet production with impaired cytoskeletal reorganization. Our results also suggest that abnormal signaling induced by β3(R734C) causes reduced expression of GPVI, which has not been reported in other αIIbβ3-related macrothrombocytopenia.

Disclosures Tomiyama: *Sysmex:* Consultancy; *Kissei Phamaceutical:* Honoraria; *Novartis:* Honoraria; *Kyowa Kirin:* Honoraria. **Hosen:** *MOCHIDA PHARMACEUTICAL CO.,LTD.:* Research Funding; *Nippon Shinyaku Co., Ltd.:* Research Funding; *ONO*

Downloaded from http://ashpublications.net/blood/article-pdf/142/Supplement 1/2564/2205284/blood-9166-main.pdf by guest on 08 June 2024

© 2023 by The American Society of Hematology

PHARMACEUTICAL CO., LTD.: Research Funding; *Eisai Co., Ltd.:* Research Funding; *Kyowa Hakko Kirin Co., Ltd.:* Research Funding; *TEIJIN LIMITED:* Research Funding; *CHUGAI PHARMACEUTICAL CO., LTD.:* Research Funding; *Otsuka Pharmaceutical:* Research Funding; *Shionogi Pharma Co., Ltd.:* Research Funding; *ASAHI KASEI PHARMA CORPORATION:* Research Funding. **Kashiwagi:** *Sysmex:* Honoraria, Research Funding; *Novartis:* Honoraria; *Kissei Phamaceutical:* Honoraria; *Argenix Japan:* Honoraria.

Figure 1

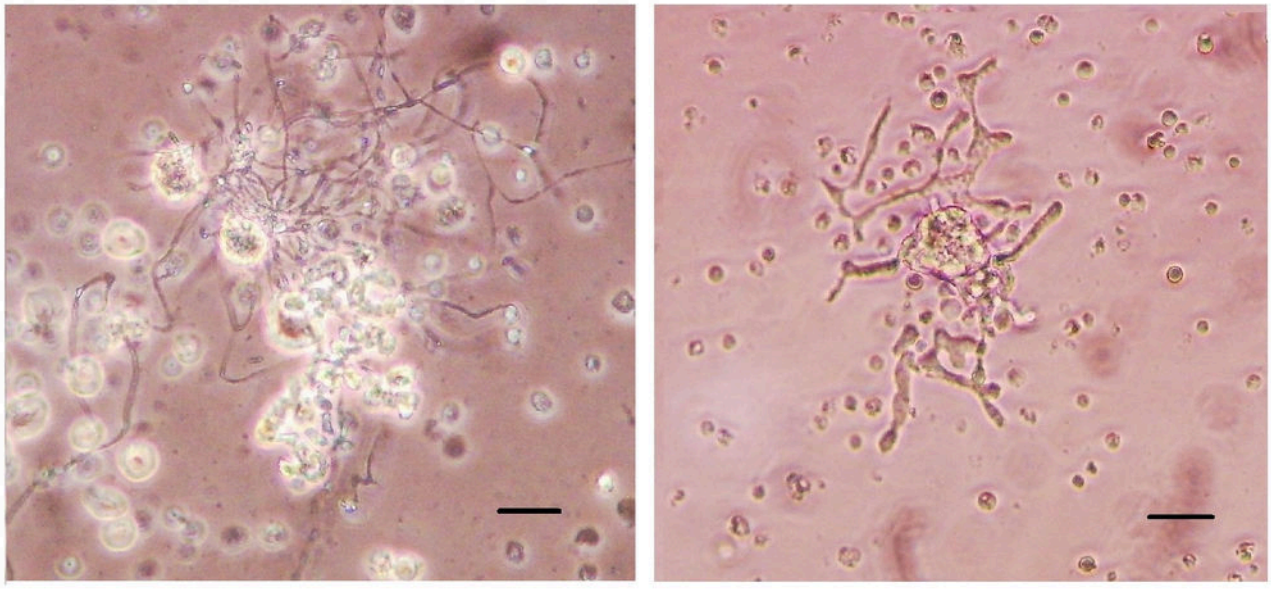

Figure 1.
Representative microscopic images of murine proplatelet formation of fetal liver derived-megakaryocytes (left: wild type (WT), right: homozygous (Homo) knock-in mice). Scale bars represent 50μm.

Figure 1

https://doi.org/10.1182/blood-2023-178475

Downloaded from http://ashpublications.net/blood/article-pdf/142/Supplement 1/2564/2205284/blood-9166-main.pdf by guest on 08 June 2024