Critical role of TRPC5 for platelet activation and in vivo recovery

Aastha Chauhan[1], Anastasiia Stratiievska[1], S. Lawrence Bailey[1], Moritz Stolla[1,2]

1 Bloodworks Northwest Research Institute, Seattle, WA
2 University of Washington Medical Center, Department of Medicine, Division of Hematology, Seattle, WA

Contact: achauhan@bloodworksnw.org

INTRODUCTION

Temperature sensitivity and storage-induced platelet changes have been studied for decades, however, the molecular mechanisms behind these changes are still poorly understood. This study focuses on the temperature-sensitive ion channel transient receptor potential cation channel (TRPC5) in platelets. TRPC5 is a multi-pass membrane protein that is activated as a hetero-multimeric assembly with several other mammalian transient receptor potential channel proteins: TRPC1, TRPC3, and TRPC4. The main function of the TRPC5 channel is to control the transportation of calcium ions across the membrane and it is believed to be mediated by a phosphatidylinositol second messenger pathway that is triggered by either receptor tyrosine kinases or G-protein coupled receptors. Lack of the TRPC5 channel or inhibition can prevent the rise of the cytosolic free calcium concentration, leading to the prevention of platelet activation.

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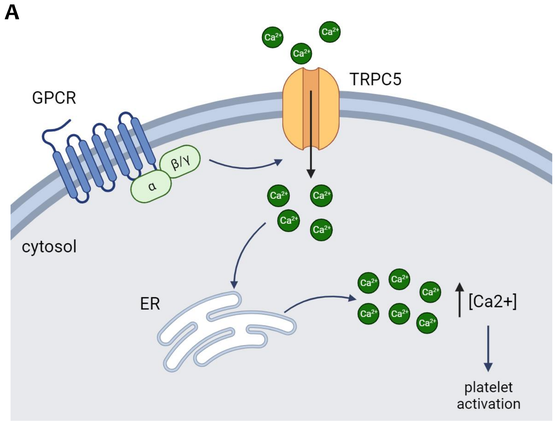

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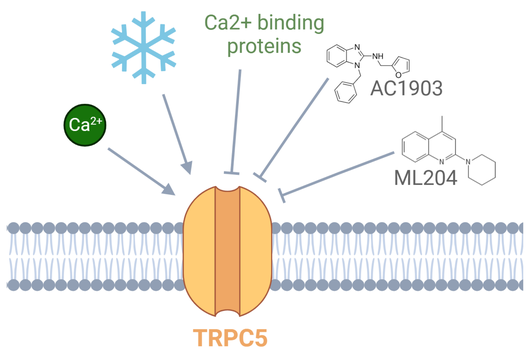

Figure 1: (A) TRPC5 channel signaling pathway. **(B)** Known activators and inhibitors of the TRPC5 channel.

METHODS

CBC analysis was performed by collecting whole blood in ACD-A and then run on a hematology analyzer. We compared platelet activation using flow cytometry on freshly collected platelets from wild-type (WT) (SvlmJ) and TRPC5-/- mice from the same background. Fresh samples were prepped by collecting whole blood in ACD-A and generating PRP. For storage experiments, whole blood was obtained in ACD-A and PRP was generated and placed on an agitator at RT for 24h. The stored PRP was washed, and those washed platelets were then used for platelet activation using flow cytometry. Recovery and survival were assessed by transfusion of 24h, RT-stored, biotin-labeled WT and TRPC5-/- platelets into C57Bl/6J mice.

The TRPC5-/- mice have a deletion of the transmembrane 6 ion channel domain and transmembrane 5 pore in the protein. The deletion was performed with the help of two loxP sites—one inserted upstream of exon 5 and the other downstream of exon 5— that were present in the targeted vector. The construct was electroporated into embryonic stem (ES) cells obtained from 129S6/SvEvTac. Blastocysts were then injected with ES cells that were correctly targeted. After breeding the resulting chimeric animals, the progeny was then mated with B6.Cg-Tg(Sox2-cre)1Amc/J mice in order to eliminate exon 5. In order to create a homozygous colony, offspring were interbred. Litter from the F3 generation was genotyped to find homozygous female and hemizygous male knock-out mice. These were the TRPC5-/- mice which were then interbred to obtain more TRPC5-/- mice.

Wild –type mice SvlmJ and C57BL/6J were obtained from Jackson Laboratory along with the TRPC5-/- mice. The SvlmJ and TRPC5-/- mice share the same background.

CBC ANALYSIS

	WT(SvlmJ)	TRPC5-/-	p value
WBC (L 10^3/mm^3)	3.605	3.41	0.489
RBC (10^6/mm^3)	4.7285	5.3585	**0.013**
HGB (L g/dl)	7.34	8.635	**0.001**
HCT (L %)	22.08	24.645	0.136
PLT (H 10^3/mm^3)	464.05	244.1	**2.24E-12**

Figure 2: Assessment of WT vs TRPC5-/- CBC. *Overview of the mean WBC, RBC, HGB, HCT, PLT values, and p value from an unpaired t-test. TRPC5-/- mice have significantly higher RBC and HGB values, and significantly lower platelet count compared to WT mice. The values were measured on an ABX (human CBC analyzer), so all the samples were diluted 1:1 with ACD-A to maintain consistency and flag gates were adjusted for counting mouse blood samples. n=20.*

IN VITRO ACTIVATION

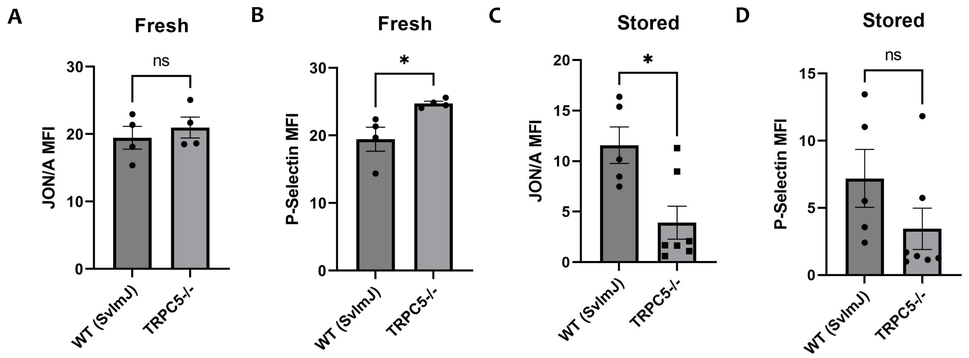

Figure 3: Reduced *in vitro activation TRPC5-/- platelets compared to WT (SvlmJ).* *Platelets were collected from WT(SvlmJ) and TRPC5-/- mice.* ***AB*** *Activation levels of fresh PRP to 1.5µg/mL collagen-related peptide was measured using flow cytometry.* ***(A)*** *Fresh JON/A (PE) MFI* ***(B)*** *Fresh P-selectin (FITC) MFI* ***CD*** *Activation levels of platelets stored at RT for 24 hours and washed to 100ng/mL convulxin were measured using flow cytometry.* ***(C)*** *Stored JON/A (PE) MFI* ***(D)*** *Stored P-selectin (FITC) MFI. Data shown as mean ± SEM and individual data, n=4-7, ns = not significant, *p<0.05, **p<0.01.*

IN VIVO RECOVERY

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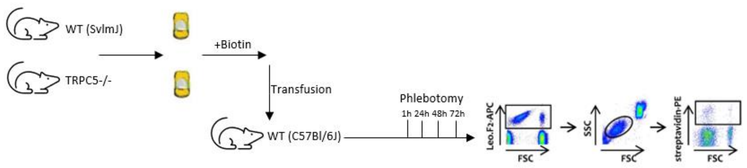

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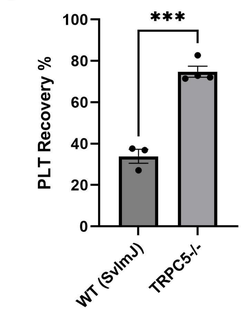

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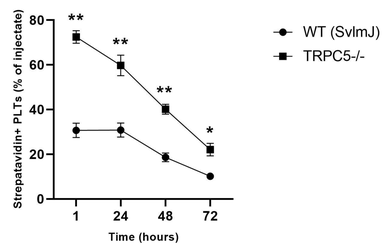

Figure 4: Increased in vivo recovery of TRPC5-/- platelets compared to WT (SvlmJ). ***(A)*** *Outline of experimental approach. Whole blood was collected from WT (SvlmJ) and TRPC5-/- mice and the platelets were labeled with biotin. Biotin labeled platelets were then transfused into WT (C57Bl/6J) mice. Retroorbital bleeds were performed at 4 different time points post-transfusion to examine the survival and recovery of the platelets.* ***(B)*** *Recovery of fresh platelets* ***(C)*** *WT (SvlmJ) (circles) and TRPC5-/- (squares) platelets (streptavidin positive events by flow cytometry) over 72 hours, plotted as percentage of initial injectate count. Data shown as mean ± SEM, n=3-4, **p<0.01, *p<0.05*

CONCLUSIONS

TRPC5 is critically involved in platelet activation at RT as seen with the decrease in platelet activation after storage. The higher platelet recovery in TRPC5-/- mice compared to WT (SvlmJ) indicates that TRPC5 also plays a role during platelet in vivo recovery. Our focus for future studies will be on the mechanism of reduced platelet activation in stored TRPC5-/- platelets.