Investigating Red Blood Cell phenotypes in the absence of Tropomyosin 1

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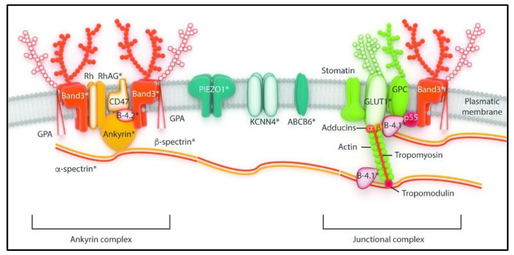
Abstract

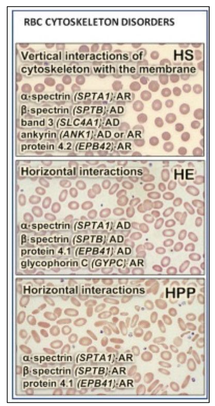
Altering erythrocyte membrane components can be clinically relevant. Red blood cell cytoskeletons contain many different components, and altering them can result in many mutations. This project focuses on Tropomyosin 1 (TPM1), a protein stabilizing actin in red blood cells and other cells in the body. Mutations in almost all other genes in the red blood cell have been studied, but not for TPM1. Through a series of breedings, a Gene Trap Reporter mouse model was created, as well as a conditional knockout mouse model to remove TPM1 in red blood cells in order to study its effects in blood cells. A total knockout of TPM1 is embryonically lethal due to cardiac defects. With each mouse model, tropomyosin levels and blood phenotypes were analyzed, displaying a general correlation between decreased levels of tropomyosin 1 and increased levels of elliptocytosis and abnormal red blood cell phenotypes.

1. Introduction

The red blood cell cytoskeleton is intricate and important, and contains many components. An important aspect of the red blood cell cytoskeleton is that RBC F-actins are stabilized along their lengths by an equimolar ratio of two tropomyosin isoforms, Tpm1.9 and Tpm3.1. They are also capped by Tmod1 and alpha/beta-adducin. Mutations in genes and proteins in the red blood cell can cause many disorders. We know clinical consequences for mutations in almost all of these genes located in the red blood cell membranes, but not for TPM1.

Tropomyosin 1 is an actin regulatory protein with significant roles in blood, cardiac, skeletal muscle, and ocular system development and function. In humans, TPM1 mutations can cause severe dilated cardiomyopathy. Murine TPM1 knockout (KO) Is lethal in embryonic life due to severe cardiac malformation. However, embryonic lethality has precluded investigation of TPM1 roles in most organ systems. For example, single nucleotide polymorphisms (SNPs) associated with TPM1 have been linked with altered blood counts, and TPM1 deficiency enhanced blood cell formation in an *in vitro* model of human hematopoiesis—but how TPM1 impacts this process *in vivo* is still unknown.





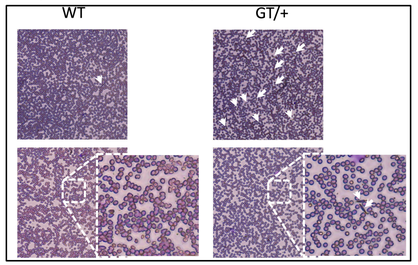
2. Gene Trap Reporter Mouse Model

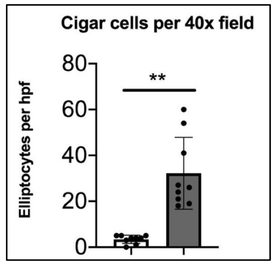
In order to test the effects of reduced TPM1 levels on blood cells, a Gene Trap Reporter mouse model for TPM1 was initially created to knockout all expression of TPM1 from all cells in the mouse. A gene trap cassette was inserted between the first and second TPM1 exons, containing an En2 Splice Acceptor as well as a LacZ reporter. The mouse construct proved to be an efficient gene trap as no knockouts were produced, due to lethality through cardiomyopathy in the murine embryo.

The green triangles are FRT sites, and the red triangles are LoxP sites. The LoxP sites are recombined with cre, and FRT sites are recombined with FLP to excise that part of the gene. The En2SA is a very strong splice acceptor and should capture all the transcript and act as a reporter for LacZ.

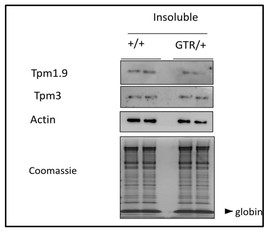
Gene Trap Mice Phenotypical Results:

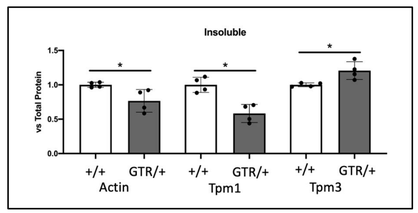
The following data was collected from heterozygous Gene Trap (GTR) mice, where one allele was Gene Trap and one allele was wild type. An evident physical difference was the presence of more elliptocytes in the Gene Trap heterozygous mice.





TPM1 decreased 50% in the heterozygous Gene Trap as compared to the wild type mouse, and there was a resulting compensatory increase in the amount of TPM3. Additionally, there was a decrease in actin, likely because actin is stabilized by TPM1. TPM1 binds actin around two times more strongly than TPM3, and thus a lack of TPM1 would cause the membrane to be destabilized regardless of the compensatory increase of TPM3.



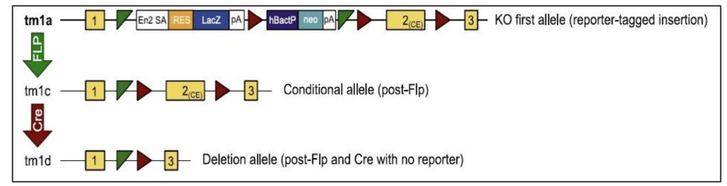


4. Conclusions & Directions for Further Research

- Successfully created a conditional mouse knockout of TPM1 in red blood cells
- Based on current research progress, knockout of TPM1 causes increased levels of elliptocytosis.
- There is a compensatory mechanism that stabilizes overall TPM levels in blood cells.
- We have two new mouse models to study TPM1 function (Gene Trap and CKO)
- Look into human implications and see if there are related human phenotypes; lack of research on TPM1 in blood development due to heart conditions.
- Obtain human data from PennMed BioBank to see if these mice findings correlate with humans, further research in that direction.

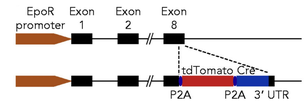
3. Creating a Conditional Knockout of TPM1 in Murine RBCs

To further study the effects of a full knockout of TPM1 without the barrier of embryonic lethality, it was necessary to create a conditional knockout of TPM1 in just the red blood cells, as opposed to every single cell in the body as in the Gene Trap (GTR).

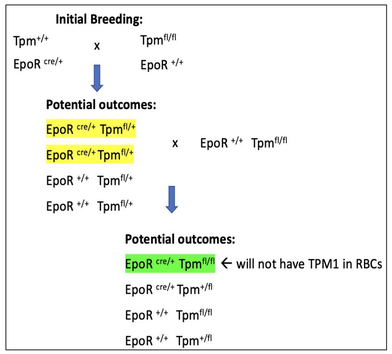


The GTR construct (tm1a in the diagram above) was then combined with a mouse with flippase recombinase (Flp) to recombine sequences between the flippase recognition target (FRT) sites. This was our tm1c allele.

We also had an allele with an EpoR Cre construct, which was inserted in the 8th TPM1 exon.



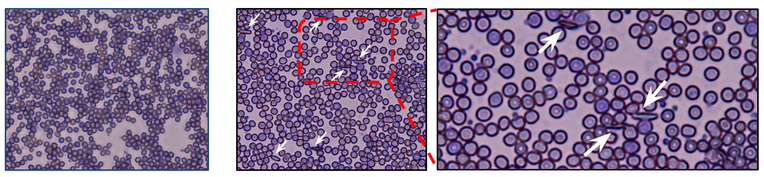
The diagram below details the breedings and potential offspring of each breeding to get the desired genotype.



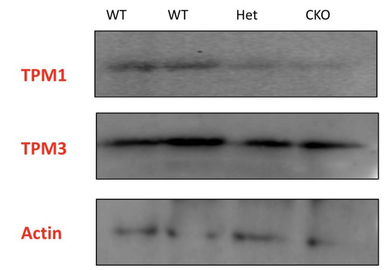
Out of a litter of 4 mice from the EpoR cre/+ TPM fl/+ x EpoR +/+ TPM fl/fl breeding, one mouse displayed the desired genotype of EpoR cre/+ TPMfl/fl. This meant that there was no TPM1 in any of the red blood cells, as the EpoR cre was specific to RBCs.

NEXT STEPS AND RESULTS:

Next steps with the TPM1 knockout mouse were to visualize any morphological changes. In the GTR/+ mouse, elevated levels of elliptocytosis were seen with just one GTR allele and a WT allele, since a homozygous GTR mouse was embryonically lethal. Thus, a full knockout of TPM1 would theoretically produce an even greater amount of elliptocytes.



In addition, it was necessary to verify that the knockout mouse indeed had no more TPM1 in its red blood cells. Blood samples were obtained from each of the 4 mice in the litter and a western blot was run for TPM1. Results were convincing, depicting a strong band of TPM1 in the wild types, a weaker band in the heterozygous mouse, and a lack of functional TPM1 in the knockout mouse.



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