

“Storage gene signature from the single red cell transcriptome analysis”

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Project Overview

The blood doping is a significant challenge in our fight to ensure fair athletic competition. The blood doping typically involved the transfusion of red blood cells which have been stored outside of human body. We have found that red blood cells have significant amount of RNAs which are not well characterized. Therefore, we will employ cutting edge single cell technology to identify whether subsets of RBCs and/or RNA will undergo storage-associated changes to distinguish these stored red cells from fresh red cells. We will develop single cell RNA-FISH methods identify individual stored red cells as a novel way to detect blood doping.

Results and Conclusions:

1. During blood storage, we have extensively validated the induction of miR-720 during storage. The miR-720 induction are consistent in all 15 tested samples and likely to comprise the storage signature that can be used to detect ABT.
2. miR-720 is a cleave product from threonine-tRNA. During storage, there is concordant increase in miR-720 and remaining tRNA fragments based on Northern blots.
3. However, we noted that increased tRNA cleavage is not a general phenomenon. When we probed the small RNA Northern with different tRNA probes, we did not see the increased tRNA fragments seen for the miR-720. In addition, when we perform small RNA-Seq of fresh vs. stored RBC, we did not observe a general increase in tRNA fragments.
4. Small RNA-Seq of the fresh vs. stored RBC samples further reproduced the induction of miR-720 by ~16 folds. In addition, we identified additional transcripts which were increased by at least 10-fold during storage. We have reproduced the RNA-Seq in 4 additional paired samples and in the process of analyzing the data. Therefore, these storage-enriched transcripts can serve as robust storage signatures.
5. The stored RBC lysates contain the cleavage activities that include angiogenin, a stress-responsive nuclease. Angiogenin in the storage solution is increased during in vitro storage. The immune-depletion of angiogenin significantly reduced the miR-720 cleavage and addition of the recombinant angiogenin enhance the miR-720 induction during storage.

6. We have also optimized the single cell RNA-FISH methods to validate the increased miR-720 positive population during storages. This detection method can be used to detect small portion of RBC cells which have been stored and mixed with fresh RBCs.
7. It is also possible to separate fresh vs. stored RBC cells based on optical volume changes stressed in microfluidic devices using quantitative phase imaging. Such methods can detect single stored red cells without labeling.