Role of CARM1 in Regulating Qki-mediated Brain Cholesterol Biosynthesis

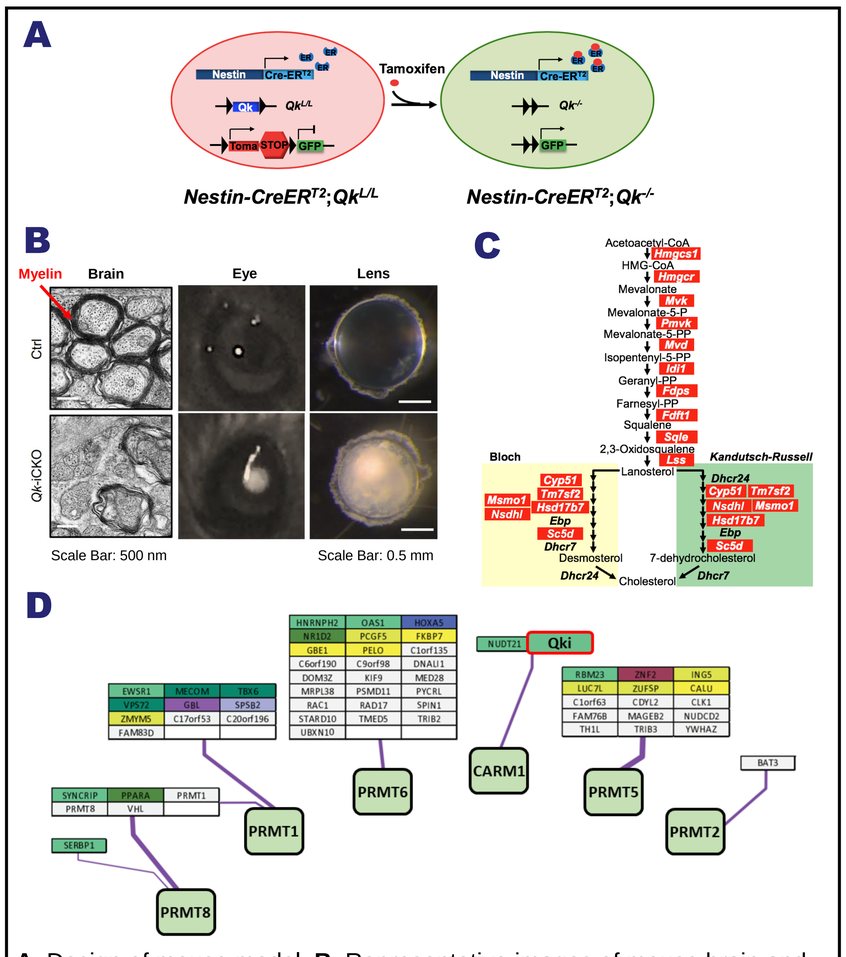
Yannie Guo[1], Seula Shin[2], Jianji Chen[3], Mark Bedford[3], Jian Hu[2]

Department of Bioengineering, Rice University, Houston, TX, USA.[1]
Department of Cancer Biology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA. [2]
Department of Epigenetics and Molecular Carcinogenesis, The University of Texas MD Anderson Cancer Center, Houston, TX, USA. [3]

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Introduction

- Cholesterol-rich organs such as brain and eye lens depend extensively on de novo cholesterol biosynthesis, in which Quaking (Qki) serves as a master transcription regulator.
- In early development, *Qk*-deficient mice displayed myelination defects and cataracts due to reduction of Qki-mediated cholesterol biosynthesis.[1,2]
- Studying mechanisms underlying the enhancement of Qki activity can aid in developing potential therapeutic targets. However, key upstream regulators of Qki's role in gene transcription remain unclear.
- Protein arginine methyl transferases (PRMTs), are known to regulate gene expression through methylation of transcription regulators.
- PRMT interactome mapping has shown that PRMT4 (CARM1) interacts with Qki,[3] but the effect of interaction on cholesterol biosynthesis is not well understood.
- **We hypothesize that Qki methylation by CARM1 downregulates the gene expression of Qki-mediated cholesterol biosynthesis.**



A. Design of mouse model. **B.** Representative images of mouse brain and eye lens to show phenotypic effects of Qki depletion in these tissues.[1,2] **C.** Cholesterol biosynthesis pathway with downregulated genes upon Qki deficiency in the brain and eye lens labeled in red. **D.** Interactome mapping of various PRMTs shows CARM1 interaction with Qki.[3]

Methods

Part 1: Qki Methylation By CARM1

- Purification of recombinant Qki wild type and arginine mutants
  - WT, R242A, R242A/R256A
- *In vitro* reactions: Qki proteins + CARM1 + S-adenosyl-L-[methyl-3H]-methionine (SAM)
- SDS-PAGE and radiolabeled film exposure

Part 2: Effect of CARM1 Inhibition on Qki

- Treatment of neural stem cells (NSCs) with TP-064, a CARM1 specific inhibitor
- Protein expression of Qki targets including Hmgcs1 were analyzed using immunoblotting

Results: Part 1

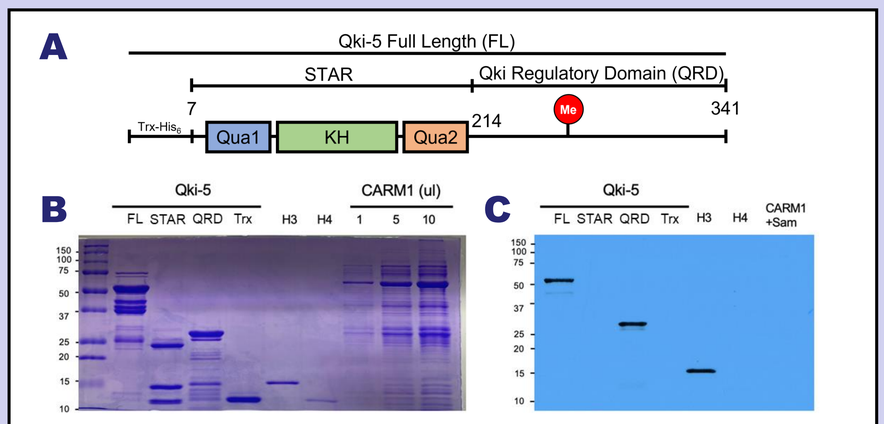


Figure 1. CARM1 Methylates Qki on its Qki Regulatory Domain (QRD). **A.** Schema of the full length Qki protein and its component domains. **B.** Purified Qki proteins visualized via Coomassie Blue staining. **C.** Methylation assay of purified Qki proteins shows CARM1 methylates Qki on its QRD.

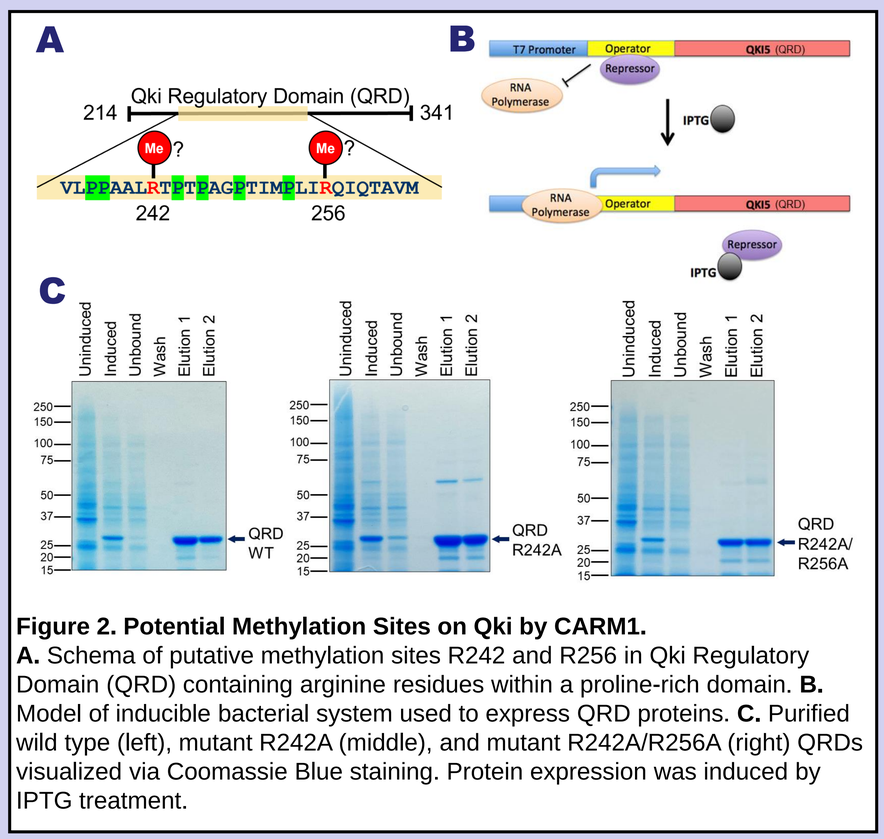


Figure 2. Potential Methylation Sites on Qki by CARM1.
A. Schema of putative methylation sites R242 and R256 in Qki Regulatory Domain (QRD) containing arginine residues within a proline-rich domain. **B.** Model of inducible bacterial system used to express QRD proteins. **C.** Purified wild type (left), mutant R242A (middle), and mutant R242A/R256A (right) QRDs visualized via Coomassie Blue staining. Protein expression was induced by IPTG treatment.

Results: Part 2

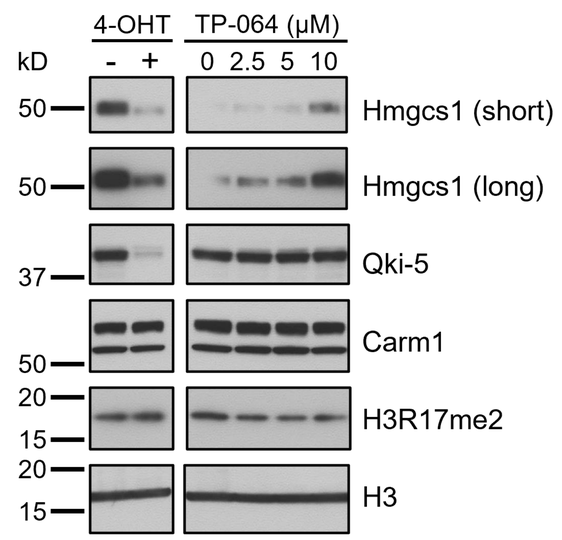
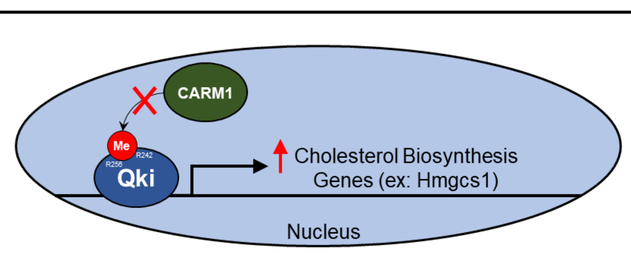


Figure 3. CARM1 Inhibition Increases Qki Target Gene Expression.
Mouse neural stem cells (NSCs) were treated with 4-OHT to deplete Qki. Treatment of NSCs with increasing doses of TP-064 (CARM1 inhibitor) shows increase in expression of Hmgcs1, a Qki target that is involved in brain cholesterol biosynthesis.

Conclusions

- We found that methylation of Qki by CARM1 occurs in the Qki Regulatory Domain (QRD). This methylation negatively regulates Qki-mediated cholesterol biosynthesis.
- To continue this project, it would be valuable to test additional Qki target genes in cholesterol biosynthesis to study the effect of the CARM1-Qki axis on this pathway.
- Immunofluorescence co-staining of CARM1 and markers for neural cells such as oligodendrocytes, astrocytes, and neurons in brain tissues would be useful to investigate the types of cells in the brain that the CARM1-Qki axis affects.



Proposed Model of Qki-Mediated Cholesterol Biosynthesis Regulated by CARM1
Methylation of Qki by CARM1 negatively affects the gene expression of Qki targets in cholesterol biosynthesis.

Acknowledgements

Many thanks to Marites Melancon, Chandra Bartholomeusz, and Nancy Strange for their organization of MD Anderson Cancer Center Summer Experience and Summer Undergraduate Research Program.

References

1) Shin S, Zhou H, He C, Wei Y, Wang Y, Shingu T, et al. Qki activates Srebp2-mediated cholesterol biosynthesis for maintenance of eye lens transparency. *Nat Commun.* 2021;12(1):3005.
2) Zhou X, Shin S, He C, Zhang Q, Rasband MN, Ren J, et al. Qki regulates myelinogenesis through Srebp2-dependent cholesterol biosynthesis. *eLife.* 2021;10:e60467.
3) Weimann M, Grossmann A, Woodsmith J, Özkan Z, Birth P, Meierhofer D, et al. A Y2H-seq approach defines the human protein methyltransferase interactome. *Nat Methods.* 2013;10(4):339–42.

For more information, please contact Yannie Guo at ysg1@rice.edu