CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)
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Project Number
S0512

Project Title
Solving the Mysteries of Reversible Messenger RNA Methylation

Abstract

Objectives/Goals
The objective was to determine whether ALKBH5 is a new m6A demethylase in vitro and in vivo and to determine whether its demethylation effects had impacts on cellular processes.

Methods/Materials
siRNA was used to selectively knock out ALKBH5 expression in HeLa cells. Western Blot analysis was performed to confirm the change in ALKBH5 expression levels compared to control cells. Antibodies were used to separate mRNA into two fractions, one containing m6A and one absent of m6A. qRT-PCR was used to analyze the abundance of genes believed to be substrates of ALKBH5 in each of the fractions.
Different oligonucleoutides were synthesized to determine the substrate-specificity of ALKBH5, QQQ-LC/MS was used to analyze demethylation activity.
Finally, flow cytometry coupled with the knockout of ALKBH5 in HeLa cells was used to analyze if ALKBH5's demethylation activity had impacts on cellular processes such as nuclear export.

Results
qRT-PCR analysis showed that the knockout of ALKBH5 in HeLa cells increased m6A enrichment in all experimental genes while the control gene remained relatively constant.
QQQ-LC/MS analysis of the synthesized oligonucleotides showed that ALKBH5 has strong preference for ssDNA however, as DNA is located mainly in the nucleus, and ssDNA rarely exists in vivo, ssRNA such as mRNA is the most likely substrate of ALKBH5. Furthermore ALKBH5 demonstrates sequence specificity as it prefers the known consensus sequence for m6A as opposed to a randomly generated sequence.
Finally, in the flow cytometry experiment, knocking out ALKBH5 significantly increased RNA export.

Conclusions/Discussion
My experiment further validates the significance of the mRNA epigenetic regulatory system. I demonstrated ALKBH5's demethylation capabilities in vivo. Furthermore I discovered that single stranded RNA is the most likely substrate of ALKBH5. Finally I showed that these effects impact cellular processes such as nuclear export. These findings demonstrate the significance of the mRNA epigenetic regulatory system and may prove significant in studying heritable diseases.

Summary Statement
My experiment seeks to further validate and shows the significance of the newly discovered mRNA epigenetic regulatory system by investigating the effects of a new m6A demethylase, ALKBH5, linked to cancer, in vivo and in vitro.

Help Received
I performed research at the University of Chicago under the supervision of Professor Chuan He and Ms. Guanqun Zheng