Inhibiting Histone Methyltransferase G9a by Addition of Small Molecules

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Introduction

Nucleosomes are the fundamental building blocks of chromatin. Nucleosomes consist of segments of DNA wrapped tightly around an octamer of four core histones. Each histone contains an unstructured N-terminus that is subjected to post-translational modification by chromatin modifying enzymes. One of the most characterized forms of chromatin modification is methylation. In general, histone methylation is correlated with the activity of genes and regulation of transcription.

Histone H3 lysine 9 (H3K9) methylation is an epigenetic signal that correlates with gene silencing in a variety of organisms. In general, H3K9 methylation occurs when a methyl group is transferred from S-adenosylmethionine (SAM) to a histone protein on the lysine. This reaction is catalyzed by one of three histone methyltransferase (HMTase) G9a. Several types of cancer have been shown to have an over expression of G9a in cells. The knockdown of G9a through RNA interference leads to a decrease in proliferation of cancer cells.

Methods and Results

Protein Production: Producing G9a Enzyme for in vitro Assay

In vitro DELFIA assay: Testing for G9a Inhibition

In vivo assay: Testing Effects of Inhibitors on Cholesterol Levels

Conclusions & Future Plans

Protein Production: Producing G9a Enzyme for in vitro Assay

Other HMTase inhibitors that bind to G9a & GLP exist

Future Plans:
- Test compound’s legitimacy by ordering new sample and rerunning DELFIA
- Run DELFIA with higher concentrations of BRD-XXX
- Test compounds selectivity through assays developed by the Chemical Biology Platform at the Broad Institute

In vitro DELFIA assay: Testing for G9a Inhibition

• BIX-01294 showed increased expression of cholesterol biosynthesis genes
• BRD-XXX showed no effect on cholesterol biosynthesis genes
• H3K9 methylation may not have an effect on cholesterol biosynthesis

Future Plan: Test global lysine 9 methylation levels when cells interact with BRD-XXX by using mass spectrometry, and test local levels of lysine 9 methylation by using chromatin immunoprecipitation

In vivo assay: Testing effects of Inhibitors on Cholesterol Levels

• BIX-01294 showed increased levels of cholesterol in cells
• BRD-XXX showed no increase in cholesterol levels
• Future Plan: Test other phenotypic responses to treating cells with BRD-XXX

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References