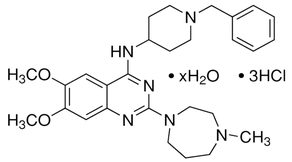

Inhibiting Histone Methyltransferase G9a by Addition of Small Molecules

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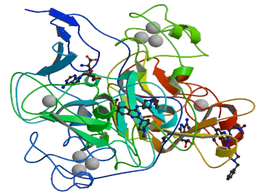
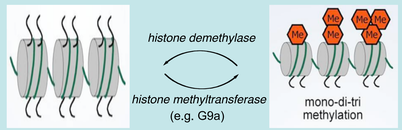


Introduction

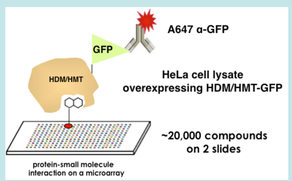
Nucleosomes are the fundamental building blocks of chromatin. Nucleosomes consist of segments of DNA wrapped tightly around an octamer of four core histones. Each histone contains an unstructured tail that is subjected to post-translational modification by chromatin modifying enzymes. One of the most 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 transfered from S-adenosylmethionine (SAM) to a functional group on the lysine. This reaction is catalyzed by one type of 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.



Inhibition of G9a has been observed when the enzyme is in contact with BIX-01294. This compound binds to G9a and G9a-like protein (GLP) *in vitro* and impairs their function. Likewise, BIX-01294 has been shown to hinder the levels of H3K9 methylation *in vivo*. The introduction of BIX-01294 into cells has been linked to alterations in global lysine 9 methylation and increased levels of cholesterol in cells.

It is believed that other small molecules can reproduce similar results observed with BIX-01294. Additional small molecules that bind to G9a were identified via small molecule microarrays (SMM).



Goals & Hypothesis

Goal #1: Identify novel inhibitors of G9a *in vitro* by testing compounds from SMM for inhibition

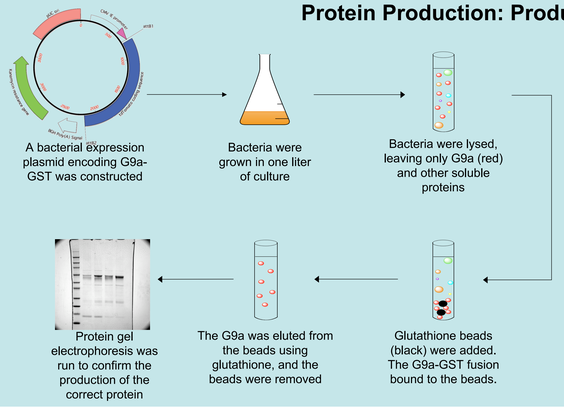
Goal #2: Test inhibitors' activity *in vivo*

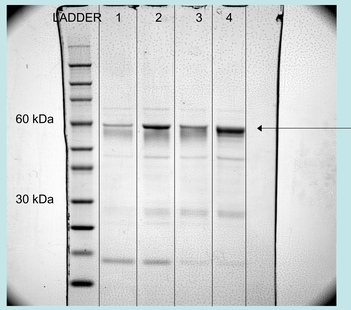
- Observe known changes in gene expression associated with G9a inhibitors
- Test for increased levels of cholesterol in cells exposed to small molecules

Hypothesis: A small molecule that was shown in the SMM to bind to G9a will also inhibit its function

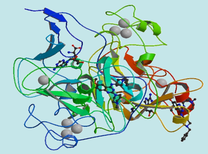
Methods and Results

Protein Production: Producing G9a Enzyme for *in vitro* Assay





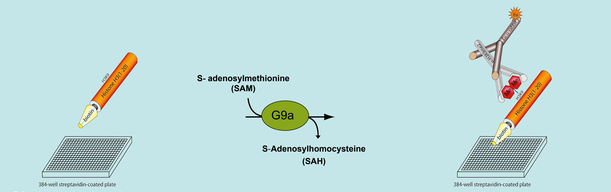
Coomassie Stained Protein Gel



G9a is 58kDa

Lane 1: Positive Control: G9a protein
Lane 2: Fraction 1 of produced G9a
Lane 3: Fraction 2 of produced G9a
Lane 4: Fraction 4 of produced G9a

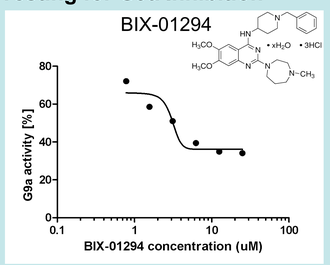
In vitro DELFIA assay: Testing for G9a Inhibition

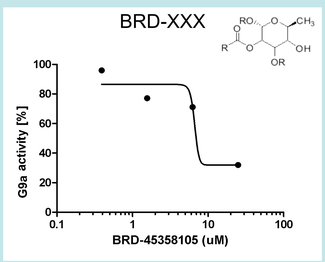


- G9a was added to plate
- Compounds were added via pin transfer
- Peptide and SAM added to plate

- G9a was incubated with compounds and peptide

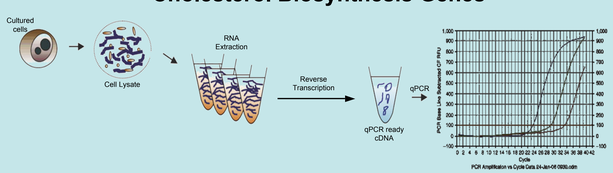
- Primary antibody against H3K9me2 added
- Fluorescent tagged secondary antibody against primary antibody added

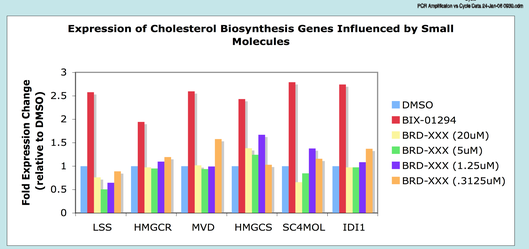




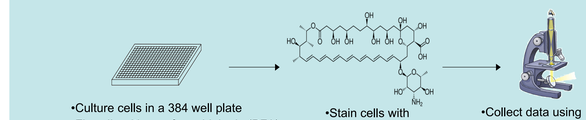
NOTE: compound was shown to only have selectivity for GLP

qPCR: Testing Effects of Inhibitors on Expression of Cholesterol Biosynthesis Genes





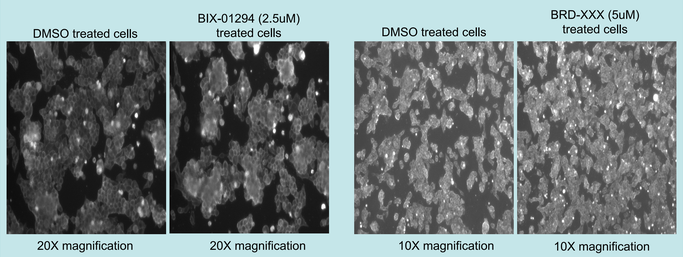
In vivo assay: Testing Effects of Inhibitors on Cholesterol Levels



- Culture cells in a 384 well plate
- Fix cells with paraformaldehyde (PFA)
- Quench PFA with glycine

- Stain cells with filipin in PBS

- Collect data using fluorescence microscope



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 vitro 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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I would first like to acknowledge my mentor, Drew Adams, for helping me with all of my experiments and being an excellent teacher and guide. I would also like to acknowledge Megan Rokop for giving me the opportunity through her outreach program for high school students, along with Allison Martino and Rachel Woodruff, who helped Megan make my time at the Broad fun and exciting. I would also like to thank Qiu Wang for her donations of compounds, and Chioma Madubata for the donation of her cell line. I would like to acknowledge Stefan Kubicek for the use of his graphics. I would finally like to acknowledge Angela Koehler for helping to collect the SMM data used in this investigation.

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