MicroRNA Analysis Paired with a Novel Live Cell Viability Assay: A Complete Epigenetic Workflow in Human Cancer Cell Lines

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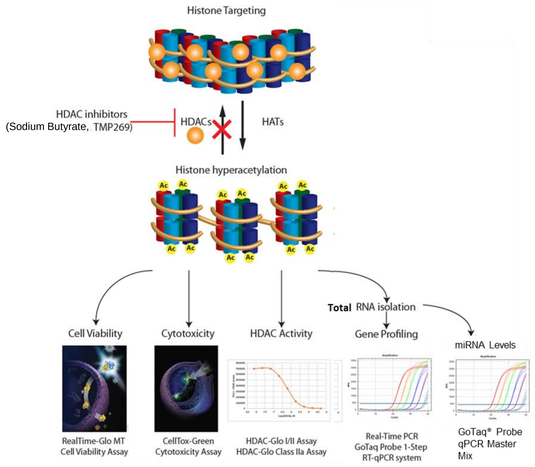
Abstract #1957

ReliaPrep™ miRNACell, Tissue MiniprepSystem, MiniprepSystem RealTime-Glo™ Cell Viability Assay, CellTox™ Green Cytotoxicity Assay and the HDAC-Glo™Assay are **For Research Use Only. Not for Use in Diagnostic Procedures**.

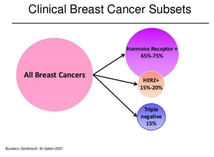
1. Introduction

The requirements for nucleic acid purification for use in RNA profiling have expanded with the growing interest in the role of microRNAs (miRNAs) and other small non-coding RNAs in cancer cell growth and metastasis. This evolution of expression analysis highlights the need for more sophisticated tools for total RNA isolation beyond traditional mRNAs. Here we describe tools developed to isolate total RNA, for mRNA and miRNA analyses, in the context of an epigenetic workflow with cancer cell lines. Using this workflow with two breast cancer cell lines, with distinct responses to histone deacetylase (HDAC) inhibition, we can measure cell health, cytotoxicity, HDAC activity, and mRNA plus miRNA expression profiling in a single experiment. The manual total RNA isolation method we describe can also be used with 3D cell cultures.



2. Materials and Methods

Compare responses of two common breast cancer cell lines to HDAC inhibitor treatment

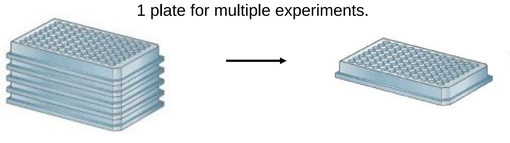


Breast Adenocarcinoma Cell Lines Tested:
MCF7: HER low, ERα+, PR+
MDA-MB-231: "Triple Negative"

HDAC Inhibitors
TMP269: HDAC class IIa Inhibitor
Sodium Butyrate: Pan HDAC Inhibitor

Efficient multiplexed workflow

1 plate for multiple experiments.

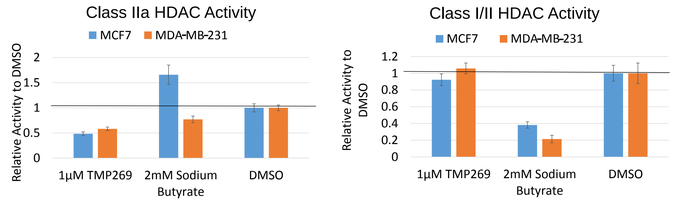


RealTime-Glo™ Cell Viability Assay
CellTox ™ Green Cytotoxicity Assay
- Enables viability and cytotoxicity at multiple time points in live cell and is compatible with downstream RNA isolation

ReliaPrep™ miRNA Cell and Tissue Miniprep System
- Total RNA isolation including miRNA, mRNA, and other small non-coding RNAs

3. A Luciferase Based Measure of HDAC Activity Confirms HDAC Inhibition in Breast Cancer Cells

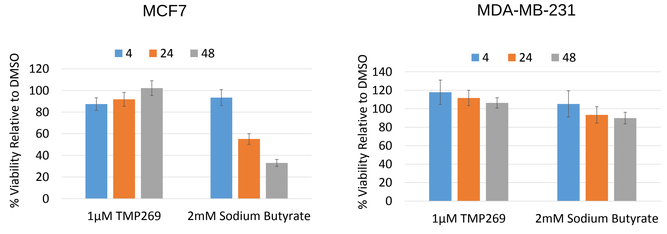
HDAC activity decreases with TMP269 and Sodium Butyrate treatment



HDAC activity was measured with HDAC-Glo Assay at 48 hours after compound treatment. N=2 for each condition.

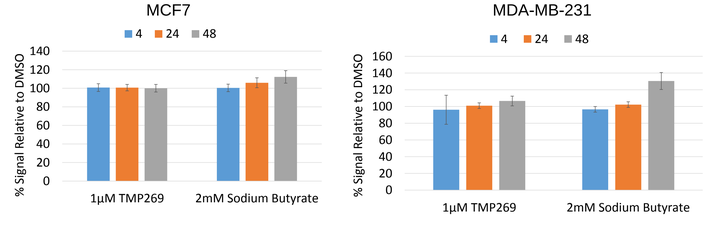
4. MCF7 and MDA-MB-231 Cells Exhibit Differential Responses to HDAC Inhibition

Cell viability monitored during HDAC inhibitor treatment



Cell viability was measured with RealTime-Glo™ MT Cell Viability Assay at 4, 24, and 48 hours after compound treatment. N=8 for each condition.

Cytotoxicity monitored during HDAC inhibitor treatment



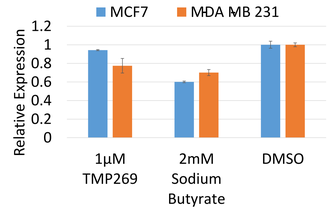
Cell death was measured with CellTox™ Green Cytotoxicity Assay at 4, 24, and 48 hours after compound treatment. N=8 for each condition.

MCF7 :
- TMP269
  - No Effect on Viability
  - No Effect on Cell Death
- Sodium Butyrate
  - Decrease in Cell Growth
  - Minimal Cell Death

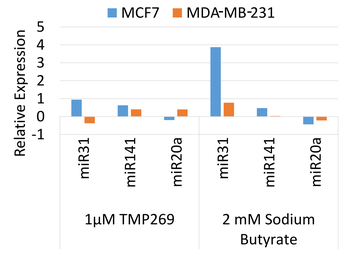
MDA-MB-231 :
- TMP269
  - No Effect on Viability
  - No Effect on Cell Death
- Sodium Butyrate
  - Minimal Decrease in Growth
  - Minimal Cell Death

5. RNA Expression Profiling After HDAC Inhibition

mRNA Expression (CDK2)



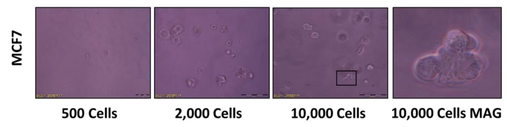
miRNA Expression



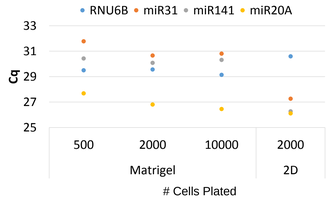
- CDK2 mRNA expression is decreased with sodium butyrate treatment in both cell lines
- miR31 expression is increased with sodium butyrate treatment in MCF7 cells

6. miRNA Expression Profiling from MCF7 Cells Grown in Matrigel

MCF7 cells grown in Matrigel



miRNA Expression



- MCF7 cells were grown in Matrigel for 48 hours
- ReliaPrep™ miRNA Cell and Tissue Miniprep System isolates total RNA from cells in Matrigel
- miRNA and small RNA expression was measured by TaqMan® Assay.

7. Conclusions

In these studies we demonstrated the isolation of total RNA, mRNA and miRNA, for applications aimed at understanding the role of these small or non-coding RNAs in cancer cell growth and metastasis. Prior to RNA isolation, we used a suite of cell-based assays to measure cell viability, cytotoxicity, and HDAC activity all within the context of an epigenetic workflow.

- TMP269 and sodium butyrate inhibition of HDAC activity in MCF7 and MDA-MB-231 cells could be detected using the luciferase-based HDAC-Glo™ Assay .
- Sodium butyrate decreased MCF7 cell viability but did not stimulate cytotoxicity. This decrease in cell growth was coupled with a decrease in CDK2 mRNA and a dramatic increase in miR31.
- Data collection was maximized from a single plate by multiplexing the RealTime-Glo™ Cell Viability Assay and the CellTox™ Green Cytotoxicity Assay followed by RNA purification using the ReliaPrep ™ miRNA Cell and Tissue Miniprep System.
- miRNA could be isolated from 3D cultures of cells grown in Matrigel using the ReliaPrep miRNA Tissue and Cells Kit. The miRNA expression profile varied compared to cells grown in 2D culture.

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