The Loss of Silence is the Song of Death

Knockout of DROSHA increases sensitivity of HCT 116 cells to death induced by Pladienolide B and DRB

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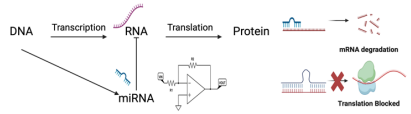
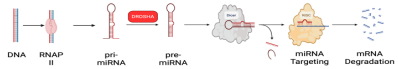
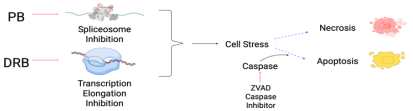
Introduction



- miRNAs are Post-Transcriptional Gene Regulators
- Silencing mechanism work by degradation of mRNA or complementary binding
- Buffer System maintaining order
- Removal create chaos in Transcriptome-Proteome Interface



- DROSHA is a Nuclease key to canonical miRNA Processing



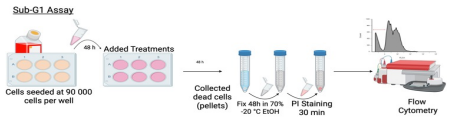
- PB is a Spliceosome inhibitor, blocking mRNA maturation
- DRB is a CDK inhibitor, halting transcription elongation
- ZVAD is a broad caspase inhibitor, blocking Apoptosis
- Previous Work in McKay Lab showed increased apoptosis in HCT 116 cells with Dro -/- relative to WT when stressed with ACT-D

Hypothesis & Objective

Hypothesis: Dro -/- Cells will display **increased apoptosis** relative to WT Cells

Objective: Show lack of miRNA buffer system weakens Colon Cancer Cells to Cytotoxic Stress

Methods

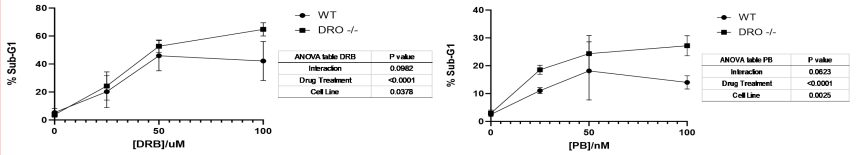


Treatment 1 consisted of No Drug Control, DMSO Vehicle Control, DRB (25, 50, 100 uM) or PB (25, 50, 100 nM). **Treatment 2** was controls, DRB or PB at 50 and 100, uM and nM respectively, with or without ZVAD

Immuno-Blotting was done in 3 biological replicates
1° Ab was Rabbit Anti-Drosha [Incubated overnight at 4°C]
2° Ab was GAR [Incubated 2h]

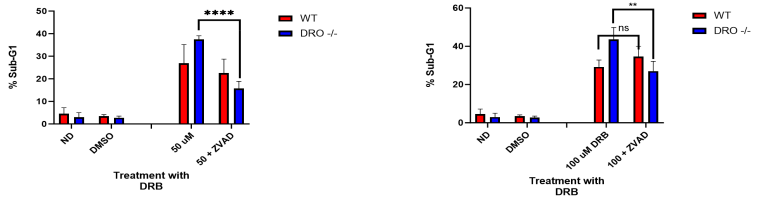
Results

DRB and PB Both Show increased Cell Death in Absence of DROSHA



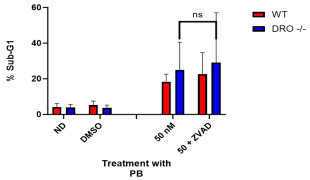
Cell Death was assessed after treatment by PI staining to measure sub-G1 DNA load corresponding to fragmented DNA strands typical of Apoptosis

Caspase Inhibitor Rescues DROSHA null Cells from DRB-Induced Cell Death

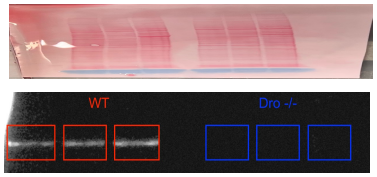


Caspase Inhibition cause minor change in death in WT but a significant change in Dro -/-
Relative to control, both cell line displayed increased death. Presence of miRNA suggest damping of apoptotic signal causing death to occur through a different pathway

PB Death is Caspase Independent

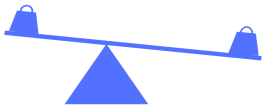


High Variance prevented evaluation of statistical significance



Top Panel: Ponceau S Stain Control
Bottom Panel: Validation of DROSHA Knock out by Immunoblot. ECL indicate presence of DROSHA protein in WT and absence in k/o.
Experiment was carried out for 3 biological replicates.

Summary



- miRNAs act as negative feedback circuit stabilizing internal cellular dynamics.
- In HCT 116 colon cancer cells, miRNAs help stabilize cells in presence of transcription stress
- Removal of miRNA chaos-buffer system does not prevent proliferation.
- However, sensitivity to cytotoxic stress is heightened

- In absence of miRNAs, cell death due to Transcription stress induced by DRB proceeds by mainly Apoptosis. [Both Exp]
- Death in presence of miRNAs is suggested to be caspase independent given no significant difference in WT with and without ZVAD.
- miRNAs presents itself to be a buffer system against transcription-stress induced Apoptosis
- Cells displayed increased death in presence of spliceosome stress by PB
- Lack of miRNAs enhanced this effect [Both Exp]
- Caspase inhibition cause no change in death both with and without miRNAs
- Findings suggest PB triggers cell death in an alternate pathway to apoptosis
- Implies translational stress is buffered by miRNAs
- Lack of miRNAs make cells globally more vulnerable.

Conclusion

DROSHA K/O sensitize cells to transcriptional and splicing stress. However, mode of cell death is dependent on stressor. Transcription elongation inhibition leads to apoptosis, which is protected by ZVAD caspase inhibition. PB cause splicing stress death independent of caspase.

Future Directions

- Re-run PB Experiments
- Testing for other forms of cell death like Necrosis, Autophagy, Ferroptosis, Necroptosis, Parthanatos
- RNA-Seq on WT and Dro -/- with and without DRB to identify the genes involved in the observed response
- We are looking for genes suppressed by miRNA in WT but upregulated in Dro -/-
- Also test p53 activity to check apoptosis pathway with DRB
- Repeat experiment with other stressors of transcription
- Map Integrated Stress Response's connection to miRNAs
- PB cause Splicing stress, leading to ER stress, monitor ISR-Mediated death
- This would correlate to increase in ATF4 expression

References

Knockout of DROSHA increases the sensitivity of HCT116 cells to apoptosis in response to actinomycin-D treatment
By Gavin Sharpe