The Mouse Mammary Tumor Virus Mediates the Antineoplastic Action of Decitabine

Andrew Brola, Cade Wycoff, Richard Tuttle, William Wycoff, Ryan Johnson, Dr. Yingguang Liu

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Endogenous Retroviruses

- Compose over 8% of the human genome[1]
- Activated and overexpressed in human breast cancer
- Previous research shows that HERV-K knockdown hinders breast cancer growth, migration, and invasion

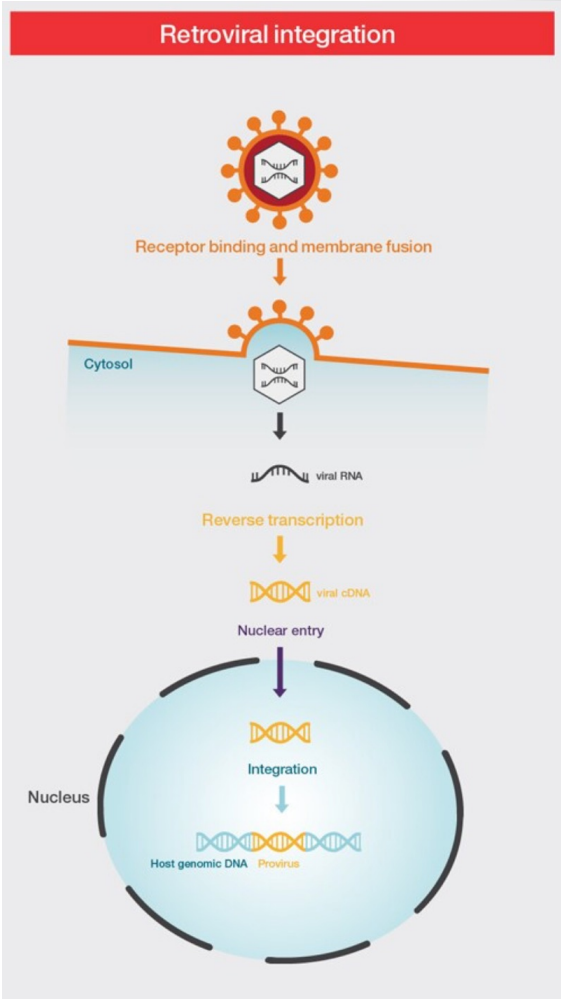

Image from T*hermoscientific*

Mouse Mammary Tumor Virus

- MMTV is a complex retrovirus known to initiate mammary cancers[2]
- Exogenous infection begins in GI tract and eventually spreads to mammary tissue

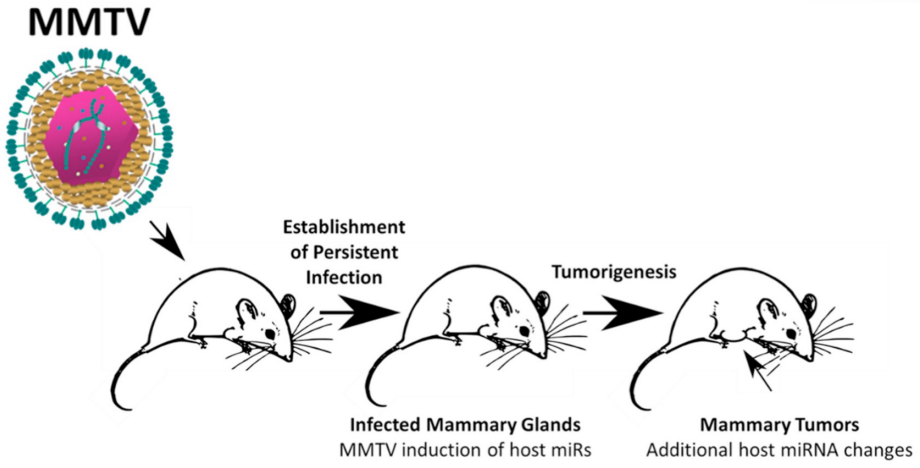

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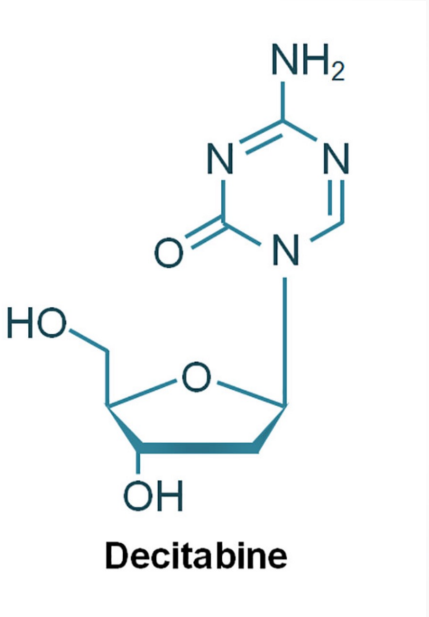
Image from *Virology*

Decitabine (DAC)

- Cytidine analog that is activated intracellularly[2]
- DAC is incorporated into DNA
  - DNA methyltransferase 1 (DNMT-1) binds irreversibly
  - Stimulates endogenous retrovirus (ERV) expression[3]
  - Stimulates expression of interferon beta (IFN-β)



Decitabine

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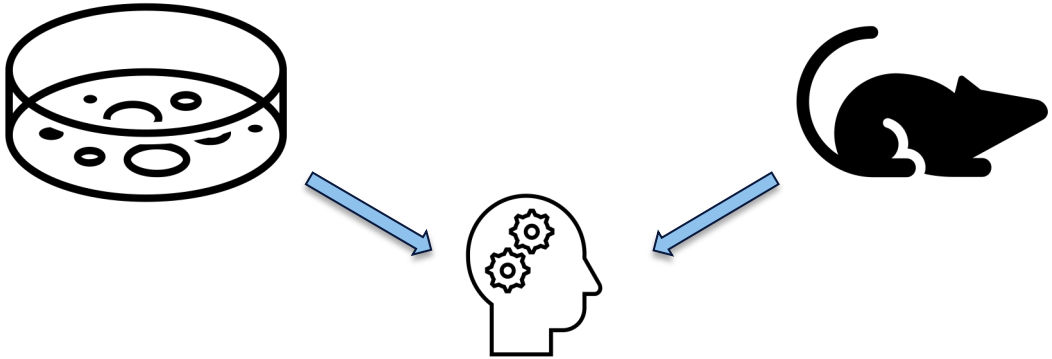
Image from Wong et. al

Specific Aim

This project endeavors to clarify the role of MMTV in the pharmacodynamic mechanisms of decitabine using in vitro and in vivo models.



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Methods

Engineered 4T1 cells with MMTV or IFN-β knockdown

BALB/c mice inoculated with engineered 4T1 cells

Mice injected every other day with DAC or phosphate buffered saline (PBS)

Tumors measured twice per week

Mass of harvested tumors recorded after sacrifice

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Methods

Supernatant of 4T1 cells used to hyperinfect MC38 cells with MMTV

Expression of MMTV *env* and *pol* genes was quantified with RT-PCR and western blot analysis

Quantification hyperinfected MC38 cell survival after treatment with DAC

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Effect of MMTV or IFN-β Knockdown on DAC Resistance

Figure 1. **Effect of MMTV Knockdown on DAC Resistance**

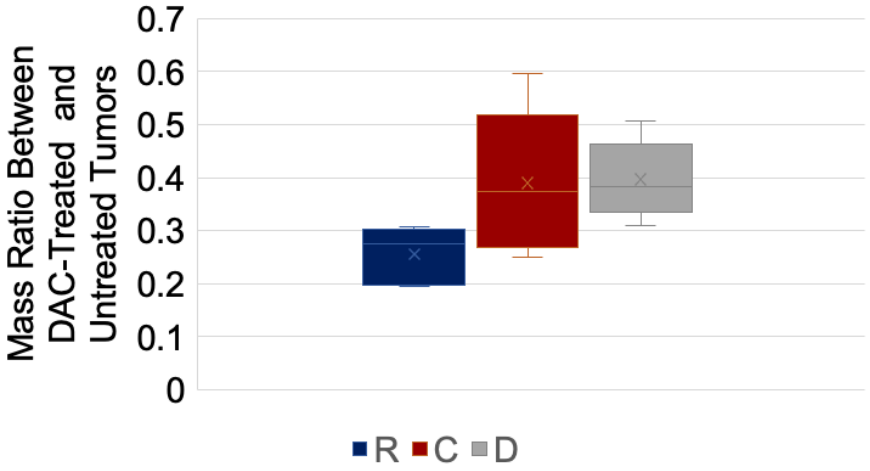
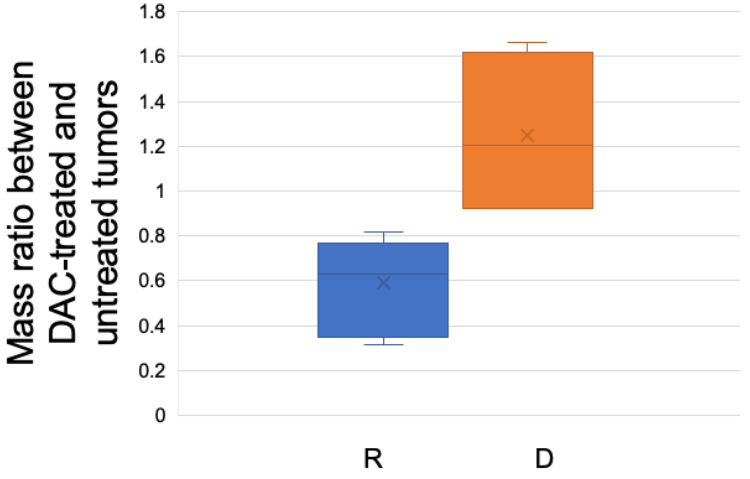


Figure 2. **Effect of INF-β Knockdown on Decitabine Resistance**



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Effect of DAC on MMTV and IFN-β RNA Expression

Figure 3. **Effect of DAC on MMTV *env* RNA in Control and Knockdown Tumors**

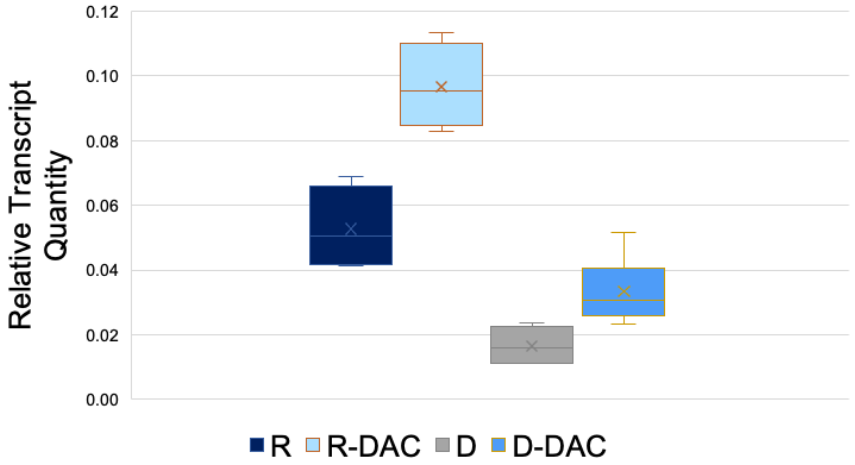
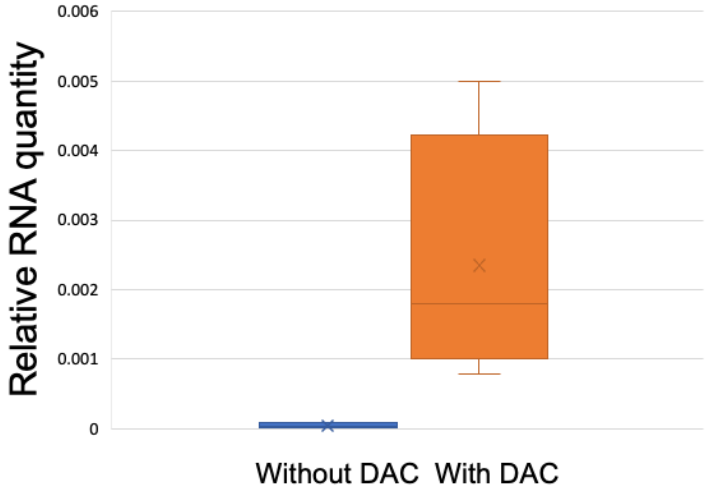


Figure 4. **Effect of Decitabine on IFN-β Gene Expression**



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Effect of MMTV Knockdown on IFN-β Protein Expression Indicates MMTV Induces IFN-β

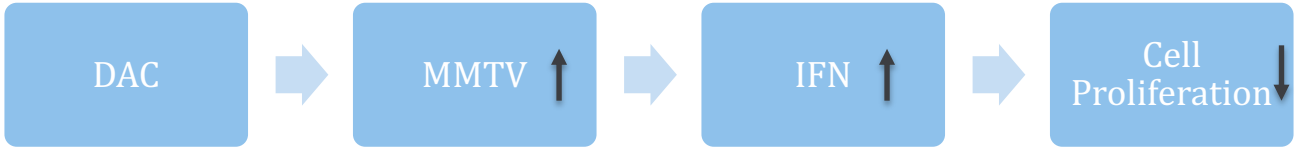
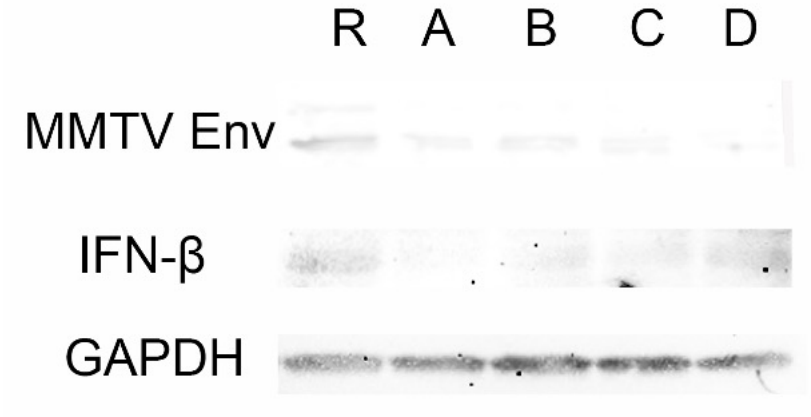


Figure 5. **Effect of MMTV Knockdown on IFN-β Expression**



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Effect of IFN-β Knockdown on MMTV Protein Expression Indicates IFN-β Suppresses MMTV

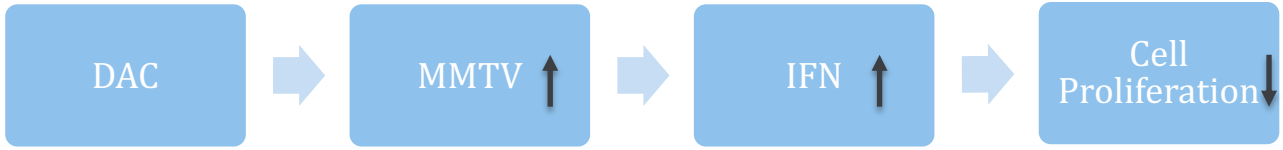
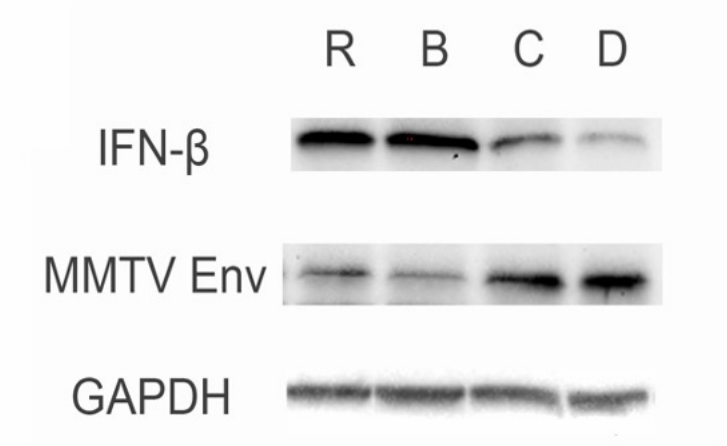


Figure 6. **Effect of IFN-β Knockdown on MMTV Expression**



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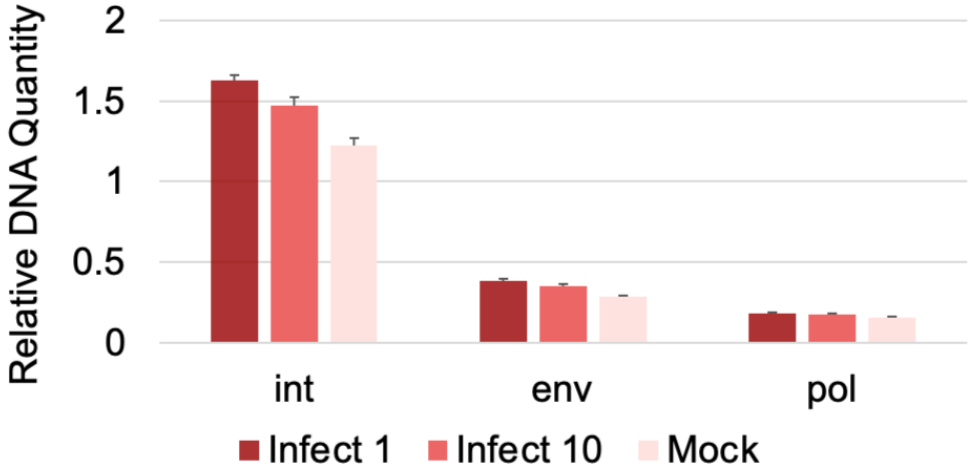
Hyperinfection of MC38 Cells

Figure 7. **Effect of MMTV Hyperinfection on Genomic MMTV DNA Contents of MC38 Cells**



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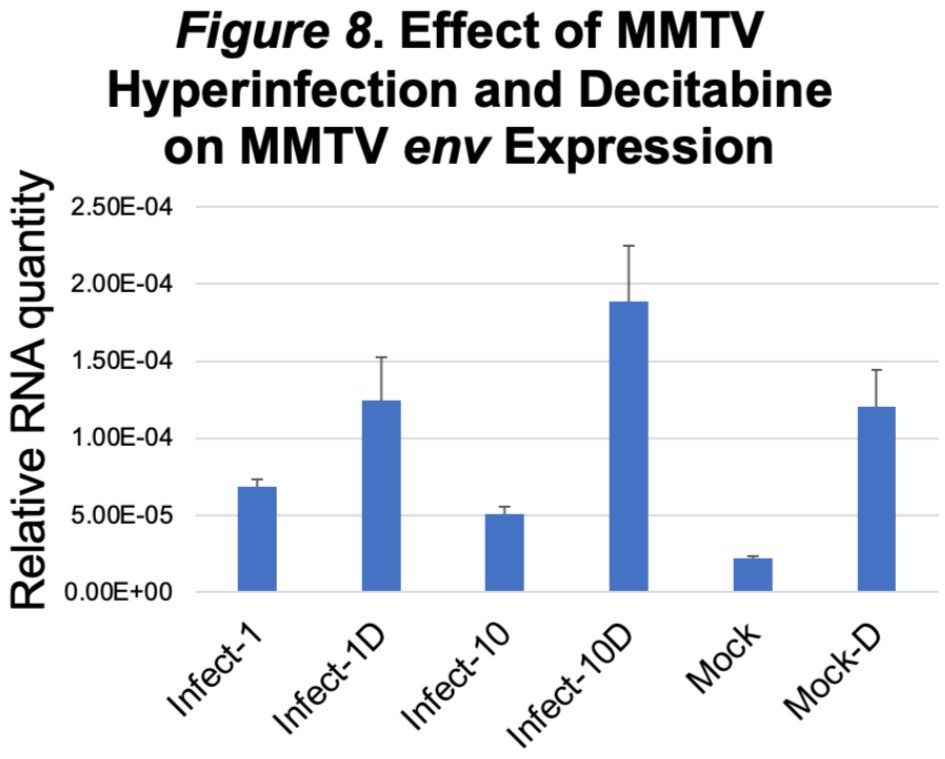
Hyperinfection of MC38 Cells



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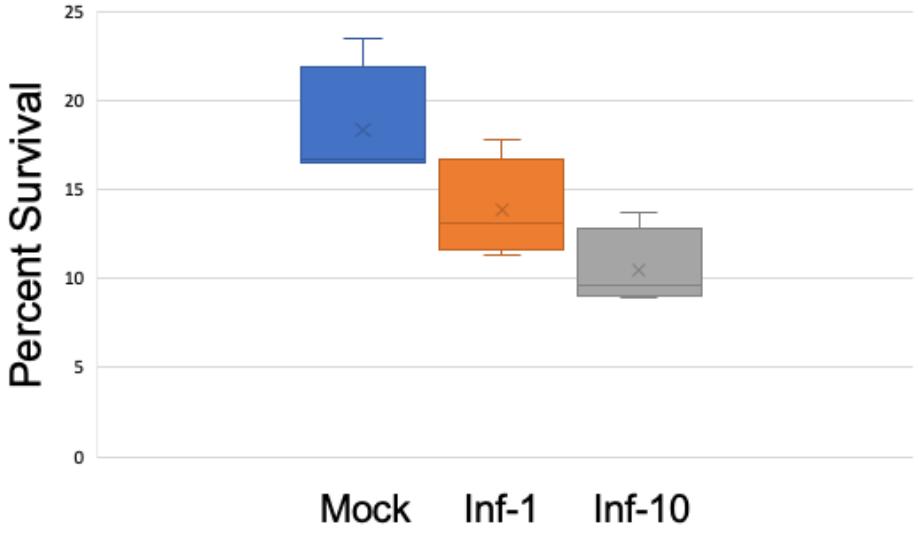
Hyperinfection of MC38 Cells

Figure 9. **Effect of MMTV Hyperinfection on Decitabine Resistance**



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Conclusions

- Higher resistance of knockdown tumors against DAC suggests that this drug acts through MMTV and IFN-β
- Enhanced DAC sensitivity of hyperinfected MC38 cells confirms this relationship
- These data support viral mimicry hypothesis as the mechanism of DNA demethylating agents

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Future Applications

- Use exogenous virus to infect cancer cells alongside the administration of a DNA demethylating agent
- Potential to expand the range of chemotherapeutic options for patients with various malignancies

References

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2. Ross SR. Mouse Mammary Tumor Virus Molecular Biology and Oncogenesis. *Viruses*. 2010;2(9):2000-2012. doi:https://doi.org/10.3390/v2092000
3. Sorrentino VG, Thota S, Gonzalez EA, Rameshwar P, Chang VT, Etchegaray JP. Hypomethylating Chemotherapeutic Agents as Therapy for Myelodysplastic Syndromes and Prevention of Acute Myeloid Leukemia. *Pharmaceuticals*. 2021;14(7):641. doi:https://doi.org/10.3390/ph14070641
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