The Mouse Mammary Tumor Virus Mediates the Antineoplastic Action of Decitabine

LIBERTY UNIVERSITY

Andrew Brola, Cade Wycoff, Richard Tuttle, William Wycoff, Ryan Johnson, Ying Liu PhD

Abstract

The purpose of this study is to clarify the role of the mouse mammary tumor virus (MMTV) in the antineoplastic effect of decitabine, a DNA demethylating agent. *In vitro* studies have shown that decitabine inhibits cancer cell proliferation by stimulating endogenous retroviruses which in turn activates the production of cytostatic interferon beta (IFN-β). We investigated the potential involvement of endogenous MMTV and the mouse IFN-β in the pharmacodynamics of decitabine using stable knockdown cell lines and MMTV hyper-infection. For the first time, we show that knockdown of MMTV or IFN-β in a mouse mammary cancer cell line (4T1) both rendered the cancer cells more resistant to decitabine in mice bearing 4T1-derived mammary cancer. Mechanistically, decitabine enhanced MMTV expression on the RNA level in both control tumors and tumors with MMTV knockdown, although the degree of enhancement was limited in knockdown tumors. Conversely, infection of a mouse colon cancer cell line (MC38) with MMTV released from 4T1 cells rendered the colon cancer cells more sensitive to decitabine. Knocking down MMTV reduces expression of IFN-β in 4T1 cells while knocking down IFN-β increases the expression levels of MMTV Env. Decitabine enhanced expression of the IFN-β gene in all cell culture samples. These data confirm the viral mimicry hypothesis as the mechanism of DNA demethylating agents. Practically, our research suggests the possibility of combining the use of an exogenous virus and a DNA demethylating agent as a new approach to cancer treatment.

Introduction

- Endogenous retroviruses (ERV) are remnants of retroviruses imbedded in cellular chromosomes that are actively expressed in many cancers.[2]
- The mouse mammary tumor virus (MMTV), which is transmitted endogenously and exogenously, is known to initiate mammary cancer.[2]
- Decitabine, an antineoplastic drug whose common brand name is Dacogen (DAC), is a DNA demethylating agent and has been shown to stimulate ERV expression in cancer cells, which induces expression of interferon beta (IFN-β), thereby halting cell proliferation; this hypothesis, based on *in vitro* studies, is called viral mimicry is still debated.[1]

Methods

- Cancer cell line, 4T1, was engineered to knockdown MMTV or IFN-β using the pGFP-C-shlenti vector expressing 29-bp short hairpin RNA. The cells were inoculated in mice to mimic mammary cancers.
- A randomized 29-mer RNA sequence was used as control for off-target effects. Mice were injected either with 7.5-10 µg of DAC or same volume of phosphate-buffered saline (PBS) every other day.
- Tumor volume was measured twice a week, and tumor mass was measured once the mice were sacrificed.
- A supernatant of 4T1 cell culture was used to infect MC38 colon cancer cells, which have a low amount of genomic MMTV copy numbers to produce cells of hyperinfection.
- Expression levels of the MMTV *env* and *pol* genes were quantified through reverse transcription polymerase chain reaction and Western blot analysis using polyclonal anti-MMTV Env.
- Survival of MC38 cells with varying levels of MMTV expression was quantified.

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

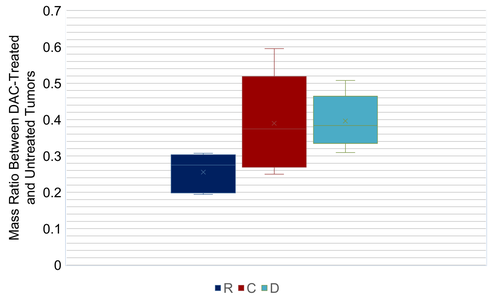


Figure 2. **Effect of INF-β Knockdown on Decitabine Resistance**

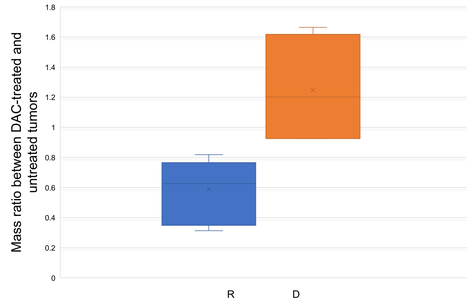


Figure 3. **Effect of DAC on MMTV *env* RNA in Control and Knockdown Tumors**

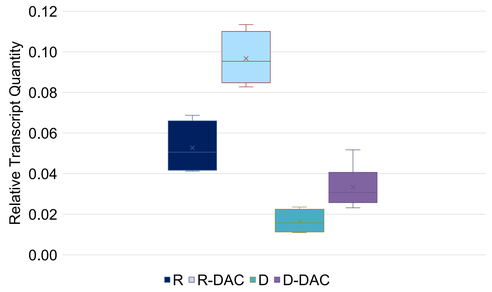


Figure 4. **Effect of Decitabine on IFN-β Gene Expression**

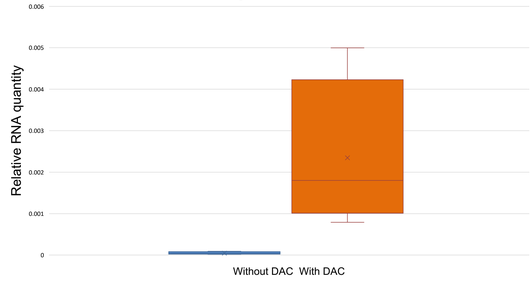


Figure 5. **Effect of MMTV Knockdown on IFN-β Expression**

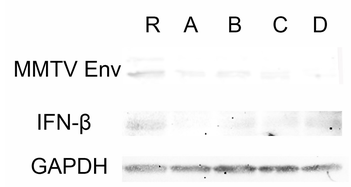


Figure 6. **Effect of IFN-β Knockdown on MMTV Expression**

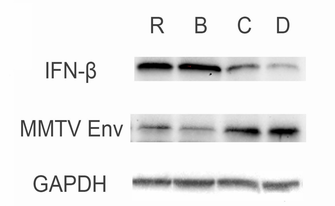


Figure 7. **Effect of MMTV Hyperinfection on Genomic MMTV DNA Contents of MC38 Cells**

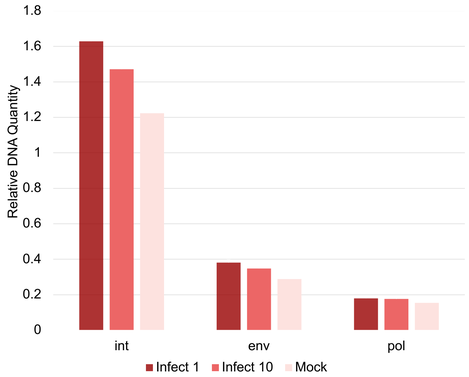


Figure 8. **Effect of MMTV Hyperinfection and Decitabine on MMTV *env* Expression**

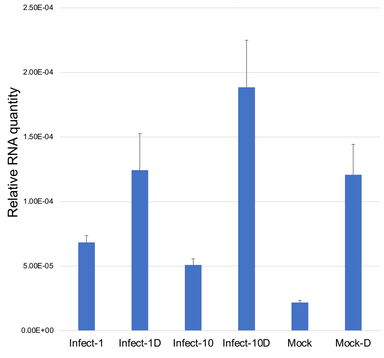
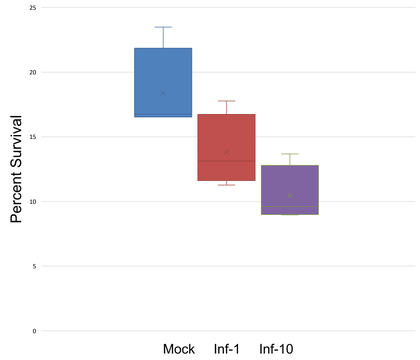


Figure 9. **Effect of MMTV Hyperinfection on Decitabine Resistance**



Results and Conclusion

Results

- When the masses of DAC-treated tumors of each cell line were normalized against the corresponding untreated tumors, the knockdown tumors were shown to be more resistant to DAC. DAC resistance between vector control and one of the knockdown cell lines (D) is statistically significant (Figure 1).
- Knockdown of IFN-β in 4T1 cell line rendered the cancer cells more resistant to DAC in mice bearing 4T1-derived mammary cancer (Figure 2), and the difference is statistically significant.
- Mechanistically, DAC increased MMTV expression in both knockdown cell lines and vector control although the degree of enhancement was limited in the knockdown tumor (Figures 3). DAC-treated samples show higher expression of the IFN-β gene expression (Figure 4). Knocking down MMTV resulted in lower expression of IFN-β on the protein level (Figure 5), while knocking down IFN-β resulted in enhanced MMTV expression (Figure 6).
- Hyperinfection of MC38 with MMTV released from 4T1 cells rendered the colon cancer cells more sensitive to DAC (Figures 7- 9).

Conclusions

- Higher resistance of knockdown tumors against DAC suggests that the drug acts through MMTV and IFN-β. This is confirmed by enhanced DAC sensitivity of hyperinfected MC38 cells.
- Furthermore, our data support the viral mimicry hypothesis as the mechanism of DNA demethylating agents.

Future Work

- Infection of tumor cells using an exogenous virus may be used as a therapeutic strategy when combined with a DNA demethylating agent. While the mechanism of this process has not been elucidated, our hope is to contribute to the expansion of chemotherapeutic options for future patients.

References

1. Chiappinelli, K. B., Strissel, P. L., Desrichard, A., Li, H., Henke, C., Akman, B., Hein, A., Rote, N. S., Cope, L. M., Snyder, A., Makarov, V., Budhu, S., Slamon, D. J., Wolchok, J. D., Pardoll, D. M., Beckmann, M. W., Zahnow, C. A., Merghoub, T., Chan, T. A., Baylin, S. B., … Strick, R. (2015). Inhibiting DNA methylation causes an interferon response in cancer via dsRNA including endogenous retroviruses. Cell, 162(5), 974–986. https://doi.org/10.1016/j.cell.2015.07.011
2. Zhou, F., Li, M., Wei, Y., Lin, K., Lu, Y., Shen, J., Johanning, G. L., & Wang-Johanning, F. (2016). Activation of HERV-K Env protein is essential for tumorigenesis and metastasis of breast cancer cells. Oncotarget, 7(51), 84093–84117. https://doi.org/10.18632/ oncotarget.11455