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CHARACTERIZATION OF THE POTENTIAL OF MOUSE MAMMARY TUMOR VIRUS (MMTV) TO DISRUPT MAMMARY EPITHELIAL CELL GENE EXPRESSION AND ITS CONSEQUENCES

by

Neena G. Panicker

Faculty Advisor

Dr. Farah Mustafa, Department of Biochemistry & Molecular Biology

College of Medicine & Health Sciences (CMHS)

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Abstract

The mouse mammary tumor virus (MMTV) is a *betaretrovirus* known to induce breast cancer in mice. Unlike most other tumorigenic retroviruses that target hematopoietic cells, MMTV primarily targets mammary epithelial cells for infection and transformation. This makes it a valuable model for studying human breast cancer, which also originates from mammary epithelial cells. MMTV is transmitted through milk, with the highest expression occurring in lactating mammary glands due to the mammary gland enhancer and hormone-responsive elements (HREs) in its promoter. However, not much is known about how MMTV affects these cells at the molecular level. Since elevated hormonal induction during pregnancy and lactation enhances MMTV expression, we used the HC11 mouse mammary epithelial cells, differentiable by prolactin, to investigate how MMTV impacts its gene expression under normal and differentiated states. The hypothesis under test proposed that *MMTV enhances mammary cell differentiation to increase its expression and viral titers for transmission to the progeny.*

To test this hypothesis, we established a stable HC11 cell line constitutively expressing MMTV and successfully differentiated these cells through various protocols. mRNAseq analysis of undifferentiated and differentiated cells revealed MMTV's surprising ability to induce global downregulation of ~ 74 & 70% of host genes, respectively. Quantitative reverse transcriptase PCR (RT-qPCR) validated the disruption of the expression of key differentiation markers, β-casein and whey acidic protein (WAP), which was very puzzling since we observed a near complete inhibition of β-casein in the differentiated state and activation of WAP in the undifferentiated state. In fact, MMTV downregulated the prolactin signaling pathway itself, and notably the prolactin receptor expression, regardless of the differentiation state of the cells. To delve deeper into MMTV's impact on prolactin receptor expression, the three known prolactin receptor promoters were cloned upstream of the *luciferase (Luc)* gene and tested in HC11 and HC11 MMTV cells. These assays revealed a downregulation of Luc expression by the promoters in the presence of MMTV. Test of individual genes of MMTV identified the MMTV *envelope (env)* gene responsible for suppressing the prolactin receptor promoter, an inhibition that was reversed upon *env* deletion, confirming its role in this process. Given the global downregulation of gene expression induced by MMTV, we explored MMTV's potential modulation of epigenetic marks in HC11 cells to repress gene expression. Interrogation of our mRNAseq data indicated a significant upregulation of the *de novo* DNA methyltransferase, DNMT3L, confirmed by RT-qPCR and western blot analysis. siRNA knockdown of DNMT3L restored prolactin receptor promoter activity, suggesting its involvement in MMTV-induced inhibition of global gene expression.

Based on these observations, we speculate that downregulation of the prolactin pathway induced by MMTV in mammary epithelial cells is used by the virus to alter the protein composition of mice breast milk so that more of the cell resources can be used to make virus particles than β-casein; yet the whey content is kept constant to provide sufficient nutrition, ensuring efficient virus transmission to the pups without compromising their health. Interesting, a similar observation has been reported in another milk transmitted retrovirus, the bovine leukemia virus, which inhibits casein synthesis in mammary epithelial cells, irrespective of their bovine or mouse origin. Additionally, our data suggests that the extensive suppression of gene expression induced by MMTV Env via DNMT3L predisposes HC11 cells to cell transformation, broadening the current notion that insertional activation of cellular protooncogenes is the primary mechanism by which MMTV induces tumorigenesis, while supporting the role of *env* as a potential MMTV oncogene.

Significance: Together, our study sheds new light into how a cancer-causing virus like MMTV manipulates host gene expression to its advantage by: 1) altering breast milk composition for enhanced viral transmission to the progeny, and at the same time 2) disrupting global molecular pathways responsible for epithelial cell growth and differentiation using epigenetic modifications like DNA methylation to potentially induce mammary cell transformation in mice. Considering the possibility that MMTV may be crossing the species barrier into humans, if proven true, our study can have direct implications for human breast cancer as well.

Keywords: Retroviruses; Mouse mammary tumor virus (MMTV); Differentiation; HC11 cells; Prolactin; Prolactin receptor; β-casein; Whey acidic protein; DNMT3L.