**Comparison of the Keratin 18 Gene DNA Methylation between Regular and Brain Metastasis Triple-Negative Breast Cancer Cells**

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### Introduction

- DNA methylation is an epigenetic mechanism used by cells to control gene expression. Hypermethylation of numerous tumor suppressor genes has been identified in multiple cancer types including breast cancer, suggesting that DNA hypermethylation may contribute to the initiation and/or development of cancer.

- DNA hypomethylating agents (e.g., azacitidine, AZA) can inhibit DNA methylation and are approved by the U.S. FDA to treat hematological malignancies such as myelodysplastic syndromes (MDS).

- Cytokeratins, such as keratin 18, are important epithelial markers. The expression of cytokeratins is decreased during the epithelial–mesenchymal transition (EMT) process, which may contribute to breast cancer metastasis.

### Preliminary Studies

- Studies of our in vivo cell and in vitro mouse models suggested AZA has better anti-tumor effects on brain metastasized triple negative breast cancer cells (referred to as "MDA-MB-231 Br") compared to their parental regular cancer cells (MDA-MB-231).

- The keratin 18 gene is present in both regular MDA-MB-231 and brain metastasized MDA-MB-231 Br cells, but its mRNA and protein levels are significantly decreased in MDA-MB-231 Br cells compared to MDA-MB-231 cancer cells.

### Hypothesis

- Decreased expression of the keratin 18 gene in brain metastasized cells is due to DNA hypermethylation, leading to metastatic characteristics and sensitivity upon AZA treatment.

### Materials and Methods

- Cells and chemicals: The parental regular triple negative breast cancer MDA-MB-231 cell line was purchased from ATCC. The brain metastasized counterpart MDA-MB-231 Br cells were generated in Dr. Lockman’s laboratory at West Virginia University.

- MTT assay: Was used to calculate IC50 values of AZA.

- Flow cytometry: Was used to measure percent of apoptotic (Annexin-V positive) cells.

- PCR and real-time PCR assays: Were used to measure the presence of genes and their mRNA levels.

- Western blotting assay: Was used to measure protein expression.

- Statistics: Statistical significance was analyzed by the Student t-test (two groups) and one-way ANOVA with a Tukey post-test (more than two groups). Significance levels were set at p<0.05 (*), p<0.01 (**), and p<0.001 (***)

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**Epithelial–Mesenchymal Transition (EMT)**

**Epithelial markers**

- E-cadherin
- Claudin
- Occludin
- Desmoglein
- Desmocollin
- Cytokeratins

**Mesenchymal markers**

- N-cadherin
- Vimentin
- Fibronectin
- Snail 1/2
- FSP1
- Smooth muscle actin

**Characteristics**

- Cobblestone
- Non-motile
- Non-invasive

**Characteristics**

- Elongated
- Motile
- Invasive

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**DNA Methylation**

Methylation the cytosine of a CpG motif silences genes

**Azacitidine (AZA)**

[![AZA structure](https://example.com/azacitidine-structure.jpg)](https://example.com/azacitidine-structure.jpg)

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**Diagram**


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**Legend**

- MDA-MB-231
- MDA-MB-231 Br

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**References**

- Benjamin Wolff, OMS II; William Kohler, OMS II; Christopher Butler; Ben Straight; Gabor Szalai; Paul Lockman; Tuoen Liu (2022). *Comparison of the Keratin 18 Gene DNA Methylation between Regular and Brain Metastasis Triple-Negative Breast Cancer Cells*. West Virginia School of Osteopathic Medicine, 400 Lee Street North, Lewisburg, WV 24901.
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Cell Growth

IC50 of AZA

Apoptosis

Results

Angiogenesis

VEGF mRNA

Wnt Signaling Pathway

Cell Metastasis

VEGF ELISA
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**Results**

**Keratin 18 Gene**

**Keratin 18 mRNA**

**Keratin 18 Protein**

**Conclusions**

- Brain metastasized breast cancer (MDA-MB-231 Br) cells have different oncological phenotype compared to their parental regular breast cancer (MDA-MB-231) cells.
- MDA-MB-231 Br cells are more sensitive to AZA treatment: (1) IC50 value of AZA in MDA-MB-231 Br cells is significantly lower than that in MDA-MB-231 cells; (2) AZA treatment triggers higher percentage of apoptotic cells in MDA-MB-231 Br cells compared to MDA-MB-231 cells.
- AZA treatment induces a higher inhibitory extent of Wnt signaling transduction pathway and angiogenesis in MDA-MB-231 Br cells compared to MDA-MB-231 cells.
- Expression of keratin 18 is significantly decreased in MDA-MB-231 Br cells.
- AZA inhibited the expression of DNMT3a enzyme in MDA-MB-231 Br cells.
- The DNA hypomethylating agent AZA may represent as a new class of chemotherapeutic agents and a novel therapy for treatment of brain metastasis of breast cancer.

**Future Studies**

- Based on our data, we hypothesize that decreased expression of the keratin 18 gene in MDA-MB-231 Br cells is due to DNA hypermethylation, which contributes to the brain metastatic characteristics in these cells.
- To elucidate the molecular mechanism of the effectiveness of AZA in treating brain metastasis breast cancer, we will compare the status of keratin 18 gene DNA methylation between the regular and brain metastasized breast cancer cells.

**References**


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