Epigenetic Regulation of microRNA Expression in Breast and Ovarian Cancer Cells
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Background

What is epigenetics?
Epigenetics describes changes in gene expression that are not due to the DNA sequence itself. Examples include gene silencing through methylation of CpG sites and regulation of protein expression by microRNA. The effects of CpG methylation are well-described in the literature. CpG sites often exist upstream from promoter regions in clusters referred to as CpG islands (CGIs). When CGI become highly methylated, this is a silencing of the downstream gene. This phenomenon and its effects have been described extensively for genes coding for DNA and proteins, but it is less understood how methylation regulates expression levels of microRNA.

What is miRNA, and how does it function as a cellular regulator?
MicroRNAs (miRNA) are small (~22 nucleotides), noncoding RNA molecules that negatively regulate the expression of protein products. They typically function by binding the 3'-untranslated region (3'UTR) of their target's messenger RNA (mRNA) and inducing degradation of the mRNA or blocking its translation into protein.

Objective

1. To confirm a change in expression for four miRNAs of interest in breast and ovarian cancer cells.

Methods

Figure 1. Normal miRNA Pathways Altered in Breast/Ovarian Cancer

(A) miR-10b, (B) miR-145, (C) miR-200c

Figure 2. SKOV-3 Ovarian Cancer Cells: Inhibition by HDACi and Calpeptin

Results

Figure 3. Changes miRNA gene expression in Breast Cancer Cells

(A) MDA-MB-231 Breast Cancer Cells

Figure 4. miRNA Expression Levels in Breast and Ovarian Cancer Cells

(A) MDA-MB-231 Breast Cancer Cells

(B) SKOV-3 Ovarian Cancer Cells

Figure 5. miRNA Expression in Treated Breast and Ovarian Cancer Cells

(A) MDA-MB-231 Breast Cancer Cells

(B) SKOV-3 Ovarian Cancer Cells

Summary

• Initial exploratory analysis showed variations in miRNA expression in MDA-MB-231 breast cancer cells after HDACi (SAHA) treatment (Figure 3). From this gene array four miRNAs known to be aberrantly expressed in breast/ovarian cancer were selected to investigate further.

• A qPCR analysis of transcript expression levels of four miRNAs of interest (10b, 145, 155, 200c) in MDA-MB-231 breast cancer cells and SKOV-3 ovarian cancer cells (Figure 4) showed:

- HDACi treatment upregulated the expression of miR-155 in both breast and ovarian cancer cells, except for miR-155 in ovarian cancer cells, which was slightly downregulated.
- Calpeptin treatment slightly downregulated the expression of miR-155 in both breast and ovarian cancer cells, except miR-155 in ovarian cancer cells, which was slightly upregulated.
- The combination of HDACi and calpeptin slightly reduced the expression levels achieved by only HDACi treatment, but overall miRNA expression levels after combination treatment were higher than the control group miRNA expression levels.
- Methylation levels of all four miRNAs are decreased after drug treatments (Figure 5).

Conclusions

• The demethylation of all four miRNAs aids their increased expression. Interestingly, expression levels increased with treatment for all:

- Anti-cancer miRNA: upregulation of miR-145 and miR-200c during a successful treatment regimen is consistent with their role in breast and ovarian cancer cell growth inhibition (i.e., they are tumor suppressor).
- Pro-cancer miRNAs: Surprisingly, two miRNAs that are over-expressed during carcinogenesis, miR-10b and miR-155, were also upregulated following anti-cancer treatment.
- These findings raise concerns that epigenetic drug treatment may not be ideal, because it upregulates some oncogenes, including oncogenic miRNAs. However:
  - Our results show that this combination of drugs is capable of killing cancer cells within a few days, which suggests that the pro-death mechanism outcompetes the mechanism that involves promoting gene expression.
- For example, HDACi re-expresses estrogen receptors in breast cancer cells, which could be exploited using combination treatment with tamoxifen.
- The methylation-demethylation pattern of miRNAs suggests that the expression of miRNAs is reversibly regulated during carcinogenesis and the death of cancer cells.

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References