Epigenetic Regulation of Imprinted Tumor Suppressor Gene ARHI in Breast and Ovarian Cancer Cells

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Abstract

Epigenetic modifications regulate gene expression by histone acetylation/demethylation and modification of promoter-associated and intragenic CpG island (CGI) dinucleotides. CGI methylations silence transcription of genes by modulating the binding of activating/depressing transcription factors and DNA polymerase III to regulatory elements. Many tumor suppressor genes, including cell cycle regulators p16 and p21, and the retinoic acid-mediated differentiation gene, RARα, undergo epigenetic silencing in different cancers. Pro-apoptotic gene ARHI is a maternally-imprinted gene that exhibits loss of heterozygosity by aberrant silencing of the paternal allele in 40% of breast and ovarian cancers, as well as by frequent mutation and deletion. The functional loss of ARHI expression contributes to uncontrolled cell growth and cancer. CGI islands (genomic regions containing a high frequency of CpG sites) of silenced genes often exhibit high methylation levels. Treatments that re-express silenced tumor suppressors mediate demethylation of specific CGI residues in CGI islands that regulate gene expression. Our laboratory is developing a combination therapy involving histone deacetylase inhibitors (SAHA) and a calpain protease inhibitor (calpeptin) for breast and ovarian cancers. Our hypothesis is that silencing of ARHI is mediated by HDAC binding to sites that are demethylated after drug-induced re-expression. Bisulfite sequencing revealed that specific ARHI-associated promoter and intragenic CGI residues are demethylated subsequent to drug treatment, and that this demethylation correlates with re-expression. We developed a ChIP-PCR assay to investigate the interaction of HDAC with the ARHI locus and observed reversible HDAC binding to specific CGI island loci that correlated with our sequencing results and re-expression.

Results

1. MeCP2 binds to methylated CGI to recruit HDAC and silence transcription
2. HDAC inhibitors are proposed to directly inhibit HDACs and indirectly inhibit methylation by DNMT
3. To determine whether HDAC1 interacts with the CGI II site in SKOV3 ovarian cancer cells
4. SAHA SKOV3 cells were treated with 10 μM SAHA or 10 μM SAHA + 10 μM Calpeptin for 48 hours
5. RNA extracted and DNA purified
6. qPCR performed to determine increase in ARHI expression relative to control
7. Ovarian cancer SKOV3 cells were treated with 10 μM SAHA or 10 μM SAHA + 10 μM Calpeptin for 48 hours
8. Genomic DNA was isolated, treated with bisulfite, and purified
9. Methylation-specific PCR was performed using methylation-specific primers of ARHI
10. ARHI methylation was inhibited by HDACi

Discussion

Epigenetic drug treatment (SAHA and Calpeptin) of MDA-MB-231 breast cancer cells resulted in selective demethylation of the CGI II site (Fig 5A)
Epigenetic drug treatment (SAHA and Calpeptin) of SKOV3 ovarian cancer cells resulted in significant demethylation of both the CGI I and CGI II sites (Fig 5B)
HDAC1 was not observed to associate with the CGI I and CGI II sites in SKOV3 ovarian cancer cells as previously reported (Fig 7A)
HDAC1 interacted selectively with the CGI I site in SKOV3 ovarian cancer cells in a manner reversible by epigenetic drug treatment (Fig 7A)
Epigenetic drug treatment (SAHA and Calpeptin) of SKOV3 ovarian cancer cells efficiently re-expressed silenced transcript (Fig 8)
Demethylation of ARHI by indirect DNMT inhibitors resulted in monosaccharide re-expression of ARHI (Fig 8B)
We hypothesize that the monosaccharide re-expression pattern exhibited by ARHI upon treatment with demethylating drugs indicates variation in the mechanisms of carcinogenesis and imprinting-mediated gene silencing
We hypothesize that the selective demethylation pattern exhibited by ARHI upon treatment with epigenetic drugs indicates variation in the regulation of drug-induced demethylation that may influence responsiveness to this treatment by different cancers

Future Studies

1. Develop a ChIP-sequencing protocol that will allow confirmation of results and greater resolution of the precise HDAC1 binding site(s) across several breast and ovarian cancer cell lines
2. Confirm (via ChIP-PCR or ChIP-seq) the suspected role of MeCP2 methyl-binding protein as the mediator for HDAC1 recruitment to methylated loci
3. Integrate the allelic expression assay with the ChIP-seq protocol to ascertain any allelic variation in binding of HDAC1 to ARHI
4. Evaluate the potential involvement of the newly reported 5-hydroxymethylcytosine epigenetic modification in the demethylation and re-expression processes

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