Sulforaphane Decreases Ezh2 Expression in PC3 Prostate Cancer Cells

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Background

Epigenetics refers to heritable changes in gene activity without a change in the underlying DNA sequence. Alterations in gene activity can result from modifications in the structure of chromatin. Chromatin, a complex of DNA and proteins found within the cell nucleus, functions in part to influence gene expression. Epigenetic changes in gene activity occur due to posttranslational modifications made to chromatin. These modifications influence chromatin structure and can thus alter gene activity. Specific enzymes control modifications made to chromatin and are often dysregulated in human diseases such as cancer. This is of great interest to researchers because, while epigenetic changes are heritable, they are also reversible.

Certain epigenetic marks on chromatin may act as a biomarker for disease severity. While there are many forms of epigenetic alterations to chromatin, the focus for the purposes of this study will be on histone methylation. Histones—the primary protein components of chromatin—function to package DNA and play roles in gene regulation. Histone methylation is a process in which methyl groups are added to lysine residues on histones. These modifications in the structure of chromatin alter the accessibility and activity of underlying DNA sequence. Alterations in gene activity can result from changes to histone methylation.

Prostate cancer is one of the most prevalent types of cancer in the U.S. and is among the leading causes of death in men of all races.1 One predictor of advanced prostate cancer and aggressive tumor behavior is the overexpression of the histone methyl transferase Ezh2.2 Ezh2 functions in the nucleus by catalyzing the tri-methylation of histone H3 lysine 27 (H3K27me3) and mediating the silencing of target genes involved in essential cellular processes such as cell cycle regulation and cell identity.3 While Ezh2 is also found in the cytoplasm of advanced cancer cells, its function here is not yet well understood. In the context of prostate cancer, dysregulated Ezh2 represses the transcription of the tumor suppressor genes promoting tumor cell growth.4-6 Inhibition of Ezh2 has been found to block prostate cancer cell growth, making Ezh2 a valuable target for cancer therapy.5 Epidemiological data suggests that eating cruciferous vegetables such as broccoli and cauliflower lowers risk of prostate cancer. Studies have found that sulforaphane—a bioactive compound found in cruciferous vegetables—decreases prostate cancer cell proliferation.6 The mechanism by which sulforaphane exerts its effect is still under investigation. A study examining the effects of sulforaphane on SCC-13 skin cancer cells found that treatment with sulforaphane decreased Ezh2 protein expression and trimethylation of lysine 27 of histone H3 in PC3 prostate cancer cells.7

Methods

PC3 prostate cancer cells, a cell line of advanced prostate cancer were plated and treated with DMSO (control) or 15µm sulforaphane (SFN). Cells were treated for 18 hours before they were lysed for protein collection. Proteins were quantified, separated by SDS-PAGE, and transferred to a nitrocellulose membrane. The membranes were then probed for the proteins of interest. The membranes were then incubated with chemiluminescent reagent and developed using an Alpha Imager to determine whether treatment with SFN resulted in a decrease in Ezh2. For cell fraction analysis, nuclear and cytoplasmic fractions were isolated at treatment endpoint. Gene expression was determined by RT-PCR using standard protocols.

Results

The decrease in Ezh2 that we saw cannot be explained by a decrease in gene expression. This suggests that SFN is working on Ezh2 on a non-transcriptional level. Figure 4 illustrates that gene expression remained the same after treatment with SFN.

Discussion

We hypothesized that sulforaphane treatment decreases Ezh2 protein expression and H3K27me3 in PC3 cells. Western blotting revealed that treatment with SFN decreased Ezh2 protein expression in PC3 cells after 18 hours. This decrease was seen in all three treatment wells. Actin was used as a reference to ensure that the observed decrease was not due to differences in total protein load between treatment and control wells.

Although treatment with SFN resulted in a significant decrease in Ezh2, there was not a consistent decrease in H3K27me3—the histone methyl mark catalyzed by Ezh2. Figure 3 reveals that the overall level of H3K27me3 does not reflect a decrease in Ezh2.

Conclusions

Sulforaphane treatment decreases Ezh2 protein expression in PC3 cells after 18 hours. Sulforaphane treatment did not decrease H3K27me3—the histone mark catalyzed by Ezh2. Treatment with sulforaphane did not alter Ezh2 at the gene expression level, suggesting that sulforaphane works to decrease Ezh2 on a non-transcriptional level.

Future Research

Further research is needed to determine what the function of Ezh2 is in the cytoplasm of these cells. One study suggests that Ezh2 in the cytoplasm of prostate cancer cells may regulate actin polymerization and thus promote the invasiveness and motility of prostate cancer cells.3 Because the function of Ezh2 outside of the nucleus is not yet well understood, there is a great need for more research in this area. Additionally, more research is needed in the area of SFN and Ezh2 in PC3 cells to better understand how SFN is working to decrease Ezh2 protein expression. It is possible that SFN may be working to inhibit advanced prostate cancer by decreasing metastasis.

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References