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Skin cancer is a significant public health issue in the United States; over three million new cases are diagnosed annually. [1] Although many cases result from lack of skin protection, genetic predisposition is another factor to be considered. Xeroderma pigmentosum (XP) is a rare genetic disorder that causes extreme sensitivity to sunlight, with symptoms including severe burning and blistering with minimal exposure, and a predisposition to skin cancer. [2] Although skin protection is important for the general population, it is especially important for individuals with this disorder to use cancer-preventive measures.

Preventive measures for cancer can include lifestyle adaptations, such as a well balanced diet. A well balanced diet can include cruciferous vegetables, such as broccoli, as they are said to contain many anti-carcinogenic compounds. Of these compounds, sulforaphane is an important chemical component that is said to reduce cancer risk. [3] One possible cause of cancer can result from damage by oxidative stress that occurs in a normal cell. Sulforaphane helps prevent damage from oxidative stress by acting in an intermediate step in a pathway that removes oxidative damage. In this intermediate step, sulforaphane releases zinc from a dominant protein in the pathway; without this release, the pathway cannot continue**. *It is still unclear if sulforaphane is able to release zinc from other pathways in the cell.*** Zinc is a key component in many biological molecules, including the xeroderma pigmentosum protein, XPA. XPA is a part of a DNA repair pathway that removes damage caused by UV light. The role of XPA in DNA repair is to recognize and bind to damaged DNA, and this binding is done by the protein's zinc finger domain. The zinc finger domain also binds to another DNA repair protein, ERCC1, which allows the cell to recognize DNA damage and recruit subsequent repair proteins in the pathway. If XPA does not bind to ERCC1, additional repair proteins cannot be recruited, and the DNA repair process cannot continue.

I will test the hypothesis that sulforaphane can inhibit XPA from binding to ERCC1 and repairing DNA damage. Recent studies show that targeting XPA with sulforaphane can decrease DNA repair efficiency. [4] The results suggest that sulforaphane is capable of removing zinc from the zinc finger domain; this removal would render the protein incapable of its binding abilities. I will test the binding efficiency and the subsequent genomic and proteomic effects in a mouse model with four variants: wild type, wild type exposed to sulforaphane, mutant, and mutant exposed to sulforaphane. **The primary goal of my study is to identify how sulforaphane interacts with and affects DNA repair machinery.** I will test my hypothesis with the following aims:

Aim 1: To determine if sulforaphane eliminates binding between XPA and ERCC1. A **yeast-2-hybrid approach** will be used to test this aim; plasmids will be constructed using sequences from the wild type and mutant mouse model. It is expected that wild type + sulforaphane and mutant will exhibit weak binding as compared to wild type, and mutant + sulforaphane binding will be completely abolished.

Aim 2: To identify the transcripts that show decreased expression as a result of sulforaphane. RNA-seq will be used to measure transcript expression in mouse cells after DNA repair is activated by UV light exposure. Similar results are expected in wild type + sulforaphane and mutant cells (with and without sulforaphane): DNA repair transcripts that are expressed in the repair pathway **prior** to XPA will show increased expression, and transcripts that are expressed **following** the XPA binding step will show decreased expression.

Aim 3: To quantify the change in expression of XPA and other DNA repair proteins in the presence of sulforaphane over time. Proteins will be quantified using a **quantitative proteomic approach with stable isotope labeling.** It is expected that DNA repair proteins will decrease in quantity over time in both wild type and mutant when exposed to sulforaphane as compared to wild type. Mutant + sulforaphane expression will decrease more rapidly compared to the wild type and other variants, and protein expression will be undetectable after completion of the assay.

If sulforaphane is in fact a chemical inhibitor of XPA, this will provide enormous insight to the mechanisms behind DNA repair, as well as give specific details behind the mechanisms of XPA. Investigating DNA repair processes is important to understanding the underlying mechanisms behind various cancers and genetic diseases, such as Xeroderma Pigmentosum.

References

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