Lynch syndrome or hereditary nonpolyposis colorectal cancer is a hereditary cancer syndrome that leads to the development of primarily colorectal and endometrial cancers [1]. Lynch syndrome can be caused by mutation of MSH6, a protein involved in the DNA mismatch repair pathway, which leads to an accumulation of mutations contributing to the development of cancer [2]. Estrogen signaling in the endometrium increases cell proliferation and greater exposure to estrogen is found to contribute to the development of endometrial cancer [3]. Estrogen receptor alpha (ERα) is known to interact with proteins in DNA repair pathways including MSH2 and PCNA, both of which interact with MSH6 [4,5]. *It remains unknown whether ERα also interacts directly with MSH6 and how this interaction affects endometrial cell proliferation and influences the development of endometrial cancer.*

Hormone therapy can be useful in the prevention and treatment of endometrial cancer, but determining the specific mechanism by which estrogen may be influencing the development of endometrial cancer in the context of MSH6 mutation can help create more targeted approaches [6]. My **long-term goal** is to determine if specific hormone therapies will be effective in prevention and treatment of endometrial cancer due to MSH6 mutation. My **primary goal** is to determine whether and how ERα signaling in cells with an MSH6 mutation contributes to the development of endometrial cancer. I will use mice as a model organism as they have a similar reproductive system to humans, and mice with MSH6 mutation have been shown to develop endometrial cancer [7]. My **hypothesis** is that MSH6 is involved in the activation of ERα as a transcription factor leading to endometrial cell proliferation.

**Aim 1: Identify ERα interaction motifs in MSH6**

**Approach:** MSH6 sequences of different species will be aligned, and sequences between species with and without estrogen will be compared to find areas where they are divergent. Bioinformatic tools such as MEME will be utilized to predict potential sites of ERα binding. An immunoprecipitation assay will be used to detect ERα binding at the predicted locations by mutating them. CRISPR/Cas9 will be used to create knockout mice in these sites and it will be observed whether they develop endometrial cancer. **Rationale:** Many proteins that MSH6 interacts with including MSH2 and PCNA interact with ERα. The sites of ERα interaction are likely sites that are conserved within species that have estrogen, but that are not conserved with species that do not have estrogen. **Hypothesis:** ERα will interact with MSH6 at sites not conserved between species with and without estrogen.

**Aim 2: Determine how estrogen interaction with MSH6 affects the transcriptome**

**Approach:** RNA-seq of wild-type and MSH6 knockout mouse endometrial cells exposed to and not exposed to estrogen will be performed. Oophorectomy will be performed on the mice to control the amount of estrogen to which they are exposed. RNA-seq results will be analyzed using gene ontology to determine what biological processes are affected by MSH6 interaction with ERα and how MSH6 mutation affects this. **Rationale:** ERα acts as a transcription factor to stimulate cell proliferation in the endometrium and MSH6 may be involved in regulating ERα activity. **Hypothesis:** ERα interaction with MSH6 will upregulate genes associated with cell proliferation. This will become dysregulated in MSH6 knockout.

**Aim 3: Determine how estrogen interaction with MSH6 impacts protein expression**

**Approach:** Protein levels of wild-type and MSH6 knockout mouse endometrial cells with and without estrogen will be measured via mass spectrometry. Oophorectomy will be performed on the mice to control the amount of estrogen to which they are exposed. The mice will be compared to wild-type SILAC mice in order to determine how ERα interaction with MSH6 affects protein expression levels and how this is affected by MSH6 mutation. **Rationale:** MSH6 interacts with many RNA binding and splicing proteins so it may be post-transcriptionally regulating mRNAs in response to ERα signaling.

**Hypothesis:** ERα interaction with MSH6 will result in an increase in expression of proteins driving the cell cycle. This will become dysregulated in MSH6 knockout.

If ERα interacts with MSH6 to regulate cell proliferation, this process is likely dysregulated when MSH6 is mutated. Understanding how this process is contributing to develop of endometrial cancer allows us to determine if hormone-based therapies can be used in the prevention and treatment of MSH6-associated endometrial cancer as an alternative to a hysterectomy.

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