

PHF8 Promotes Prostate Cancer Metastasis by Epigenetically Upregulating RIPK2

OVERVIEW/ABSTRACT

Despite advancements, metastatic prostate cancer (PC) remains incurable and is responsible for the vast majority of approximately 35,000 deaths in the United States and about 375,000 deaths globally each year.^{1,2} Thus, there is an urgent need for research that can lead to effective therapies to prevent the formation of lethal metastatic PC.

Recently, we identified receptor-interacting protein kinase 2 (RIPK2) as a highly promising drug target in PC metastasis, as its targeting led to up to a 92% reduction in metastatic progression in intact animals (*Nat. Commun.*, 2022).³ Mechanistically, RIPK2 functions through a non-canonical RIPK2/MKK7/c-Myc signaling pathway.³ The frequencies of RIPK2 overexpression correlate with PC progression, reaching 45% in metastatic PC.³ However, the upstream regulatory mechanisms of RIPK2 remain poorly understood, highlighting a critical knowledge gap.

Through computational analysis and experimental validation, we have identified the histone lysine demethylase PHF8 as a major upstream regulator of RIPK2 expression. Accumulating evidence has shown that PHF8 is associated with PC progression and shorter survival and promotes metastasis.⁴⁻⁶ However, it remains unclear how PHF8 regulates RIPK2 expression and whether PHF8's pro-metastatic functions are largely mediated through RIPK2. Here, the proposed research aims to address these immediate knowledge gaps by testing the central hypothesis that PHF8 promotes PC metastasis by epigenetically upregulating RIPK2 in PC cells. Our Specific Aims are as follows:

Aim 1. Determine how PHF8 regulates RIPK2 expression in PC cells. We hypothesize that PHF8 increases RIPK2 promoter activity through the demethylation of histones at specific sites (H3K9, H3K27, and/or H4K20). We will conduct rescue experiments and employ quantitative RT-PCR, western blotting, dual-luciferase reporter assays, and chromatin immunoprecipitation to test this hypothesis.

Aim 2. Determine to what extent RIPK2 contributes to PHF8-dependent PC metastasis. We hypothesize that RIPK2 is a major mediator of PHF8's promotion of PC metastasis. We will conduct rescue experiments and employ *in vitro* cell assays and *in vivo* experimental metastasis and xenograft tumor growth assays to test this hypothesis.

The proposed research is significant because it will not only enhance our understanding of the upstream regulatory mechanism of RIPK2 expression but also nominate RIPK2 as an effective drug target in PC metastasis promoted by PHF8, whose direct inhibition may cause significant side effects. Given that PHF8 promotes the metastasis of several other cancer types, the mechanisms uncovered by the study will have clinical implications beyond the PC space.

This research is conceptually innovative because it will be the first to detail the regulatory relationship between PHF8, histone lysine methylation, and RIPK2 transcription. It will explore RIPK2's functions in PHF8-dependent PC metastasis and lead to novel targeted therapies to combat this form of metastasis.

This proposal aligns well with the seed grant objectives, as the research topic is directly in line with the Department of Defense (DoD) Prostate Cancer Research Program's priority to "develop new treatments or improve upon existing therapies to improve outcomes for men with lethal prostate cancer." It also meets the NCI MCTB study section's priority of "evaluation of the mechanism of action of established or repurposed cancer therapeutics." Successful completion of this project will provide vital preliminary data and new PC cell line models, enhancing our future funding applications for DoD Translational Science Awards and NCI R01 grants, areas where I have previously demonstrated success (see my Biosketch and Other Support documents).