SPOP Mutations Promote Prostate Cancer Progression and Metastasis

Jingwen Hu, Chenling Meng Ph. D., Di Zhao Ph.D.

University of Texas MD Anderson Cancer Center, Department of Experimental Radiation Oncology

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Background

Prostate cancer is the most common cancer in American men and is second worldwide causing over 250,000 deaths per year.[1] Cancer whole-genome and exome sequencing studies have shown that Speckle-type POZ (pox virus and zinc finger protein) protein (SPOP) is the most frequently mutated gene in primary prostate cancer (PCa).[1] It is also reported that SPOP is characterized to play a role in tumorigenesis. [2] SPOP mutations in prostate cancer occur at a high frequency in a few specific residues (or so-called “hotspots”) in the MATH domain, such as F133V, W131G, and F102C.[3] Moreover, SPOP mutations have been identified as an early event in the development of genomic instability and tumorigenesis in PCa.[2]

Method

1. Mice – For the DX1 B6 subQ model, we subcutaneously (subQ) injected DX1 mouse prostate cells into B6 mice and measured the tumor volume twice a week and the tumor weight at the end point. For the PTEN SPOP F133V mouse model, we crossed PTEN KO mice (P) with SPOP mutant F133V mice to generate PTEN SPOP mutant mice (PSP) and then crossed with Pb-Cre mice to generate PSP prostate cancer specific PTEN KO and SPOP mutations prostate cancer genetic engineering mouse model (GEMM) with green fluorescent protein reporter.

2. Transwell assay – We performed transwell assay using 8um pore size on DU145 WT, F102C, W131G, and F133V mutant cells to determine cell migration ability.

3. Immunohistochemistry (IHC) – We performed IHC on FLAG F10 (CST 14793S, 1:200), P-AKT (CST 4060S, 1:100), H3K36 (CST 4909S, 1:1600), H3K4 (CST 9751S, 1:5000) to examine protein expression levels and localization on mouse prostate tumors.

Result

1. We performed DX1 (mouse prostate cell lines) subQ mouse model with SPOP WT and SPOP F133V mutation and found that SPOP mutation increase tumor volume and weight.

2. We performed transwell assay on DU145 WT, F133V, W131G, and F102C mutant cells and found SPOP mutations promote DU145 prostate cancer cell migration in vitro.

3. We successfully generated PSP mouse model and the IHC data showed that H3K36me3 and H3K4me3 were robustly upregulated in PSP mice compared to P KO mice.

Fig 1. **Tumor Progression**

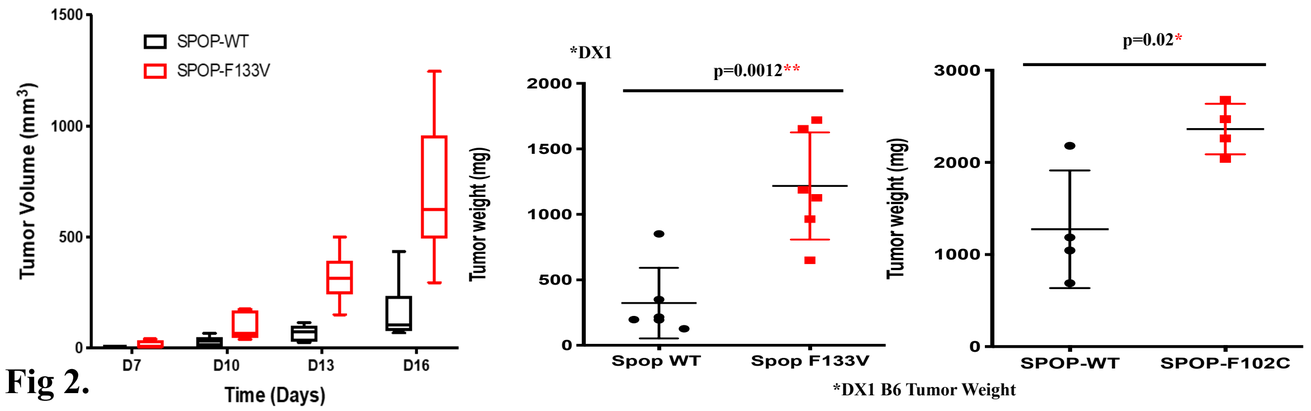


Fig 2.

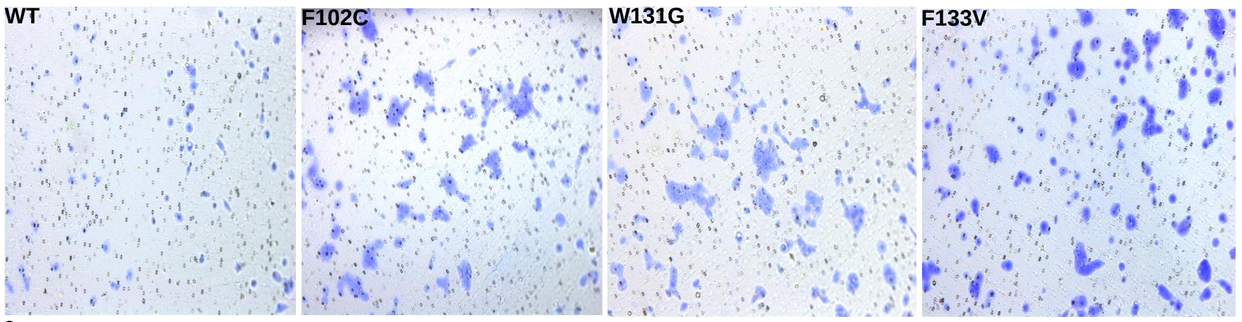
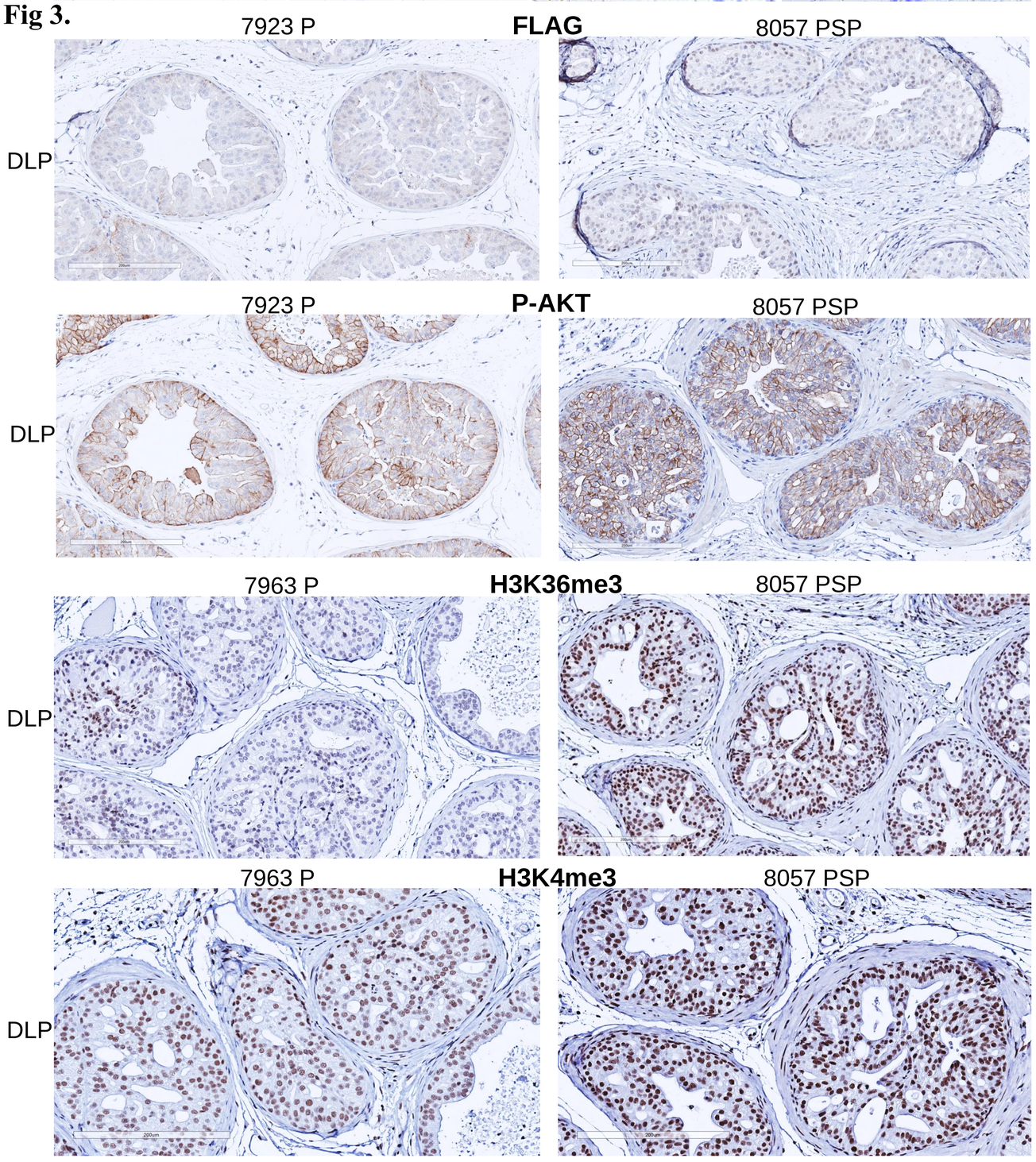


Fig 3.



Conclusion

SPOP mutation frequently occurs in prostate cancer, here we used DX1 subQ mouse model showing that mutant SPOP drives prostate tumor progression in vivo, and the in vitro data shows SPOP mutations promote tumor migration. Thereafter, we generated SPOP mutation GEMM and found that the transcription factors related activate H3K36me3 and H3K4me3 were drastically upregulated in PSP mice.

Future Goal

Our next goal is to perform RNA-sequencing to identify the downstream transcriptional factor of SPOP which regulates histone modification.

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Reference

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