FXYD5 plays diverse roles in immune evasion and tumor progression in prostate cancer

Ru M Wen[1], G. Edward W Marti[2], Neeladrisingha Das[3], Zhengyuan Qiu[1], Eric E. Peterson[1], Zenghua Fan[5], Fernando Jose Garcia Marques[4], Abel Bermudez[3], Hongjuan Zhao[1], Lawrence Fong[5], Guillem Pratx[3], Sharon J Pitteri[4], James D Brooks[1]

1 Department of Urology, 2 Department of Molecular and Cellular Physiology, 3 Department of Radiation Oncology, 4 Department of Radiology, School of Medicine, Stanford University, CA 94305
5. Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, California, United States
Correspondence: r.wen@stanford.edu

Background:
FXYD5 is involved in various biological processes, including inflammation, tumor progression, drug resistance, and hypertension. It has been suggested as a potential biomarker for several cancers, with elevated levels found in ovarian and colon cancer. However, the role of FXYD5 in prostate cancer has not yet been elucidated.

Methods:
The expression levels of FXYD5 were detected by qPCR and western blot. CRISPR/Cas9 sgRNA and cDNA were used to generate FXYD5 knockout and overexpression prostate cancer cell lines, respectively. A CRISPRi screen was conducted to identify the Siglec-7 ligand. Prostate cancer cell proliferation was assessed using cell growth curves and colony formation assays. Cell migration was evaluated through Matrigel migration and scratch assays. Cell invasion was measured using a transwell migration assay. TMRE dye was used for evaluating mitochondrial membrane potential. Lactate and LDH assays were used to evaluate the lactate and LDH levels in cancer cells. A humanized mouse model was utilized to examine the role of FXYD5 in tumor growth and immune evasion. Single-cell RNA (sc-RNA) sequencing was used to assess FXYD5 transcript levels in tumor cells within prostate cancer tissues.

Results:
The expression levels of FXYD5 were higher in androgen receptor (AR)-negative prostate cancer cells compared to AR-positive prostate cancer cells. A CRISPRi screen identified FXYD5 as a potential Siglec-7 ligand. In a humanized mouse model, 22Rv1 tumor growth was inhibited with human immune cell modulation. However, when FXYD5 was overexpressed in 22Rv1 cells, tumor growth was not impacted by immune cell-based therapy. This was due to the interaction of FXYD5 on cancer cells with Siglec-7 on immune cells, thereby inducing immunosuppressive signals. Intrinsically, FXYD5 knockout promoted PC3 cell proliferation, migration, and invasion. *In vivo* studies showed that FXYD5 knockout promoted PC3 tumor growth, while overexpression of FXYD5 inhibited PC3 tumor progression. However, in AR-positive 22Rv1 cells, overexpression of FXYD5 promoted tumor progression. FXYD5 expression was detected in tumor cells by sc-RNA sequencing of human metastatic prostate tumor tissues. In vitro assays indicate that overexpression of FXYD5 in PC3, C42B, and 22Rv1 cells reduces mitochondrial membrane potential and reactive oxygen species. Additionally, FXYD5 overexpression decreases lactate dehydrogenase and lactate levels in PC3 cells but does not impact these levels in 22Rv1 cells. In PC3 cells, FXYD5 overexpression leads to the formation of tight cell-cell interactions by downregulating membrane markers, while it does not affect cell-cell interactions in 22Rv1 cells.

Conclusion:
FXYD5 suppresses tumor growth in AR-negative prostate cancer cells and promotes tumor growth in AR-positive prostate cancer cells. Overexpression of FXYD5 inhibits immune cell-mediated tumor suppression via a Siglec-7-dependent mechanism. These findings suggest potential therapeutic strategies targeting FXYD5-based mechanisms.

Funding
This work was supported by a Department of Defense (DoD) Young Investigator Award to RMW. (W81XWH2110195), ChEM-H postdoc seed grant to RMW, and NCI grants U01CA226051 to SJP, and JDB.

Conflict of Interests
RMW receives a travel grant from Bon Opus Biosciences.