Investigating the epigenetic role of SPDEF in prostate cancer through identification of proteins involved in SPDEF function

Margaret E. Larsen[1]; Virginia Ronchi[1]; Mousa Vatanmakanian[1]; Jennifer Klein[1]; Hari K. Koul[1]

[1]Department of Biochemistry and Molecular Biology and the Stanley S. Scott Cancer Center, Louisiana State University Health Sciences Center, New Orleans, LA.

Introduction

Background:

- Prostate cancer (PCa) is the most common non-cutaneous cancer diagnosed in men (ACS, 2021).
- Therapy resistant PCa, known as castration-resistant prostate cancer (CRPC), does not fully respond to commonly used therapeutics (Chandraseker et al., 2015).
- SAM pointed domain containing ETS transcription factor (SPDEF) has been shown to play a key role in inhibiting prostate cancer metastasis (Steffan et al., 2012).
- The mechanism for how SPDEF regulates metastasis is still poorly understood.

Significance:

- Most of the 30,000 PCa deaths each year in the U.S. are due to metastatic CRPC (Chandraseker et al., 2015).
- There is still no cure for CRPC indicating the importance of studying PCa to create more effective therapies.

Hypothesis: We hypothesize that there are several proteins whose function is modified by the overexpression of SPDEF and understanding the role of these proteins in modulating the anti-metastasis effects of SPDEF in PCa may help design novel therapies for patients with CRPC.

Methods

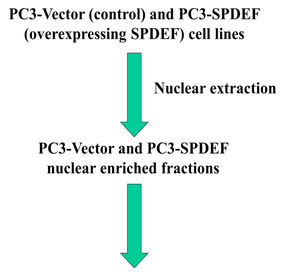


Figure 1. Schematic representing the methods for narrowing down the MS data to focus on specific proteins and function of their regulation.

Results

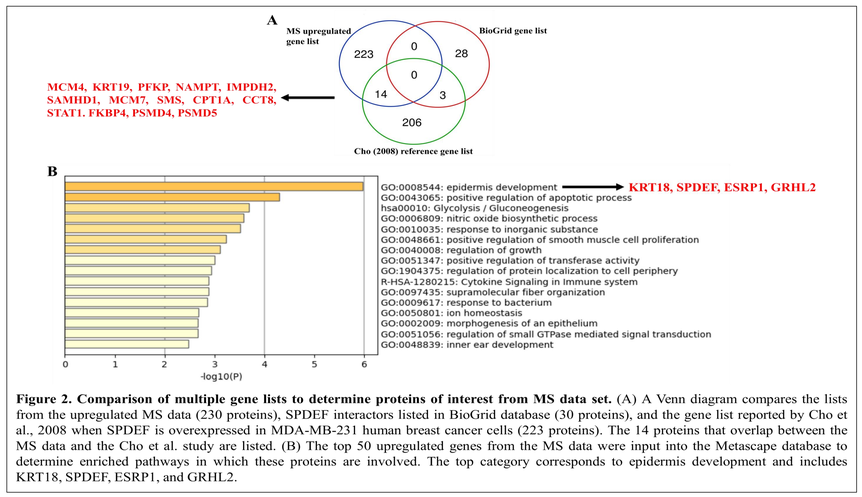


Figure 2. Comparison of multiple gene lists to determine proteins of interest from MS data set. (A) A Venn diagram compares the lists from the upregulated MS data (230 proteins), SPDEF interactors listed in BioGrid database (30 proteins), and the gene list reported by Cho et al., 2008 when SPDEF is overexpressed in MDA-MB-231 human breast cancer cells (223 proteins). The 14 proteins that overlap between the MS data and the Cho et al. study are listed. (B) The top 50 upregulated genes from the MS data were input into the Metascape database to determine enriched pathways in which these proteins are involved. The top category corresponds to epidermis development and includes KRT18, SPDEF, ESRP1, and GRHL2.

Gene	Description	MS fold change (Abundance ratio: (SPDEF)/(Vector))	Biological function
ESRP1	epithelial splicing regulatory protein 1	14.47	mRNA splicing factor regulating the formation of epithelial cell isoforms
GRHL2	grainyhead-like protein 2 homolog	8.11	A transcription factor that plays a role in primary neurulation and epithelial development
SPDEF	SAM pointed domain-containing ETS transcription factor	7.75	Plays a role in the regulation of prostate gland and prostate cancer development
KRT18	Keratin, type I cytoskeletal 18	4.60	Plays a role in filament reorganization
HAT1	histone acetyltransferase type B catalytic subunit	2.59	Coordinates histone production and acetylation
MCM4	DNA replication licensing factor MCM4	2.24	Member of the MCM complex which is necessary for DNA replication and initiation

Figure 3. List of proteins to further analyze and their respective fold change determined with MS. Six proteins (ESRP1, GRHL2, SPDEF, KRT18, HAT1, and MCM4) were chosen to be further analyzed. The fold change for these proteins was higher than 2-fold when SPDEF was overexpressed in a prostate cancer cell line, PC3, which normally has minimal SPDEF expression.

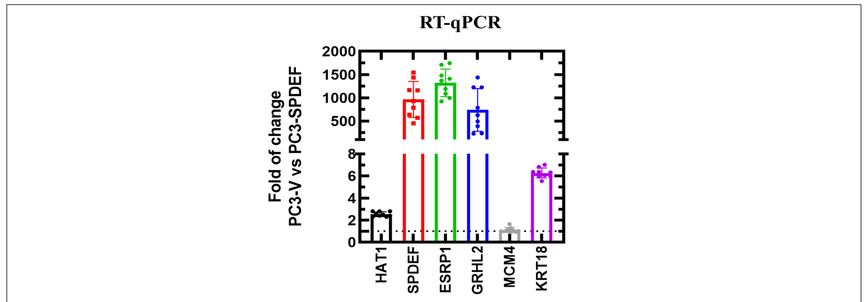
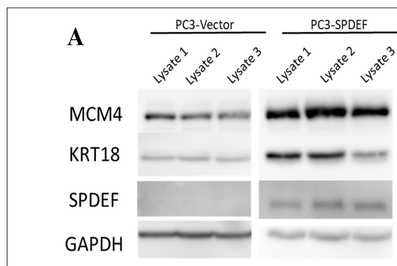


Figure 4. RT-qPCR data representing the fold change in gene expression level for the proteins of interest. RNA was extracted (Qiagen kit) from triplicates with approximately $1.2x10^6$ cells in each, and cDNA was made (BioRad protocol) using 1 µg of RNA per sample. Sybr green was used to perform the qPCR, and 4 µL of the respective cDNA was used (triplicate reaction). To determine the fold change, the PC3-Vector versus PC3-SPDEF were normalized to GAPDH. The data show a large fold change (>500-fold) for ESRP1, GRHL2, and SPDEF, indicating their expression levels are affected by overexpression of SPDEF. In contrast, HAT1 and KRT18 have a small fold change (<10-fold) in mRNA expression, and MCM4 does not have a significant fold change.



B

Protein	Average fold change: SPDEF/Vector (normalized to GAPDH) ± SD
MCM4	5.53±1.08
KRT18	6.00±1.70

Figure 5. Protein levels for MCM4 and KRT18 were modified by overexpression of SPDEF in PC3 cells. (A) Representative Western blot showing protein expression levels in lysate samples (n=3). 30 µg lysate samples were resolved on a 12% SDS-PAGE gel under reducing conditions. The proteins were transferred onto a PVDF membrane and incubated with the corresponding antibodies (MCM4: Abcam- ab4459; KRT18: ThermoFisher- MA1-80807; SPDEF: SantaCruz- sc-166846; GAPDH: Sigma-Aldrich- G8795). The bands were immunodetected by chemiluminescence using ECL reagent. (B) Bands were quantified using Amersham Imager 600 software. The fold change was then determined based on the GAPDH (loading control) levels. MCM4 shows a 5.53±1.08-fold change, and KRT18 shows a 6.00±1.70-fold change. SPDEF was used as a positive control.

Conclusions

- Altogether these results indicate that ESRP1 and GRHL2 are strongly upregulated when SPDEF is overexpressed as supported by MS and RT-qPCR data.
- mRNA expression of MCM4, HAT1, and KRT18 shows only 2-4 fold increase; however, relative protein expression levels show a 6-fold change when SPDEF was overexpressed, which is in line with the MS data.

Future directions:

- In order to corroborate this preliminary data, the MS analysis must be repeated using nuclear enriched fractions to validate these initial findings.
- Future studies will be aimed at understanding the role of these proteins in modulating the anti-metastasis effects of SPDEF in PCa to help design novel therapies to tame CRPC.

References

"Cancer Facts & Figures 2021." *American Cancer Society*, 2021, www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2021.html.

Chandrasekar, Thenappan et al. "Mechanisms of resistance in castration-resistant prostate cancer (CRPC)." *Translational andrology and urology* vol. 4,3 (2015): 365-80. doi:10.3978/j.issn.2223-4683.2015.05.02

Cho, Je-Yoel et al. "Proteomic analysis of a PDEF Ets transcription factor-interacting protein complex." *Journal of proteome research* vol. 8,3 (2009): 1327-37. doi:10.1021/pr800683b

Steffan, Joshua J et al. "The transcription factor SPDEF suppresses prostate tumor metastasis." *The Journal of biological chemistry* vol. 287,35 (2012): 29968-78. doi:10.1074/jbc.M112.379396

The UniProt Consortium. UniProt: the universal protein knowledgebase in 2021. Nucleic Acids Res. 49:D1 (2021)

Zhou, Yingyao et al. "Metascape provides a biologist-oriented resource for the analysis of systems-level datasets." *Nature communications* vol. 10,1 1523. 3 Apr. 2019, doi:10.1038/s41467-019-09234-6

This research project was supported through the LSU Health Sciences Center, School of Medicine (ML) and in part by NIH/NCI-7R01CA242839 (HK) and unrestricted funds from the Stanley Scott Cancer Center, and School of Medicine -LSUHSC, New Orleans (HK). For proteomics results, Mr. Jessie Guidry, LSUHSC Proteomics Core, was consulted.