Validation of Valosin-Containing Protein (VCP) as a Therapeutic Target for Triple Negative Breast Cancer

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Introduction

There are few effective targeted therapies available for triple-negative breast cancers (TNBCs), the most aggressive form of breast cancer (1, 2). A better understanding of critical molecular regulators of TNBC is necessary to develop effective targeted therapies. We previously determined that the transcription factors SOX9 is highly expressed in TNBCs and is required for TNBC cell survival and metastasis (3, 4). However, SOX9 is difficult to target directly for therapeutics development, so it is of great interest to evaluate more druggable SOX9 binding proteins as potential targets. Valosin-containing protein (VCP) is a member of the AAA-ATPase superfamily whose elevated expression is correlated with increased metastatic potential and poor prognosis in some cancers (5). VCP has been identified as a SOX9 binding protein through immunoprecipitation (IP)/mass spectrometry.

Hypothesis

VCP knockdown disrupts the function of SOX9 and reduces cancer cell growth and survival in TNBC

Methods

- Oncomine Analysis: VCP mRNA expression and 5-year overall survival rates were compared for six different breast cancer datasets (6).
- qRT-PCR and Western Blotting Assay: mRNA levels and protein level of indicated factors were examined by qRT-PCR and Western blotting assays.
- siRNA transfection for VCP knockdown: Transfection of cells was performed with a pool of three independent siRNA duplexes targeting VCP for 48 hours.
- Cell Growth Assay: After treatment with siRNA for 48 hours, cells were harvested and reseeded into 24-well plates. Cell number was recorded by Countess Automated Cell counter (Invitrogen, Life Technologies, Grand Island, NY) on Day 1, 3, 5, 7.
- Immunoprecipitation: used to detect VCP as a SOX9 binding protein
- Bioinformatic Analysis: mRNA-seq data were analyzed by Drs. Ganiraju Manyam and Wenyi Wang from the Department of Bioinformatics and Computational Biology, Department of Biostatistics, The University of Texas MD Anderson Cancer Center.

Results

VCP Plays a Critical Role in TNBC

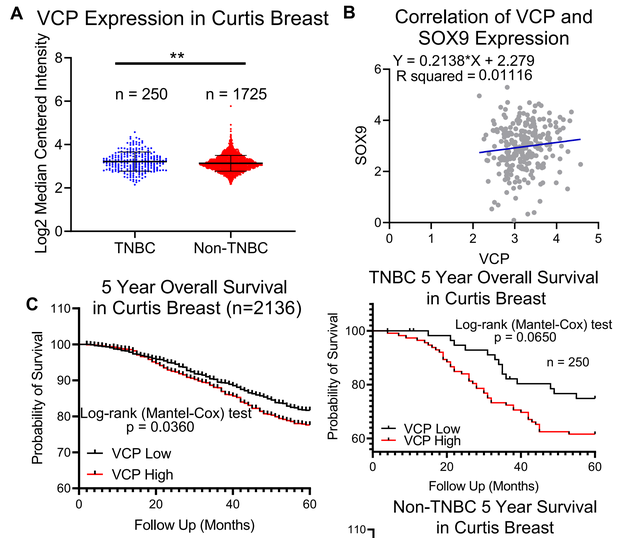

Figure 1. VCP plays critical role in TNBC. **A.** VCP presents relatively higher expression level in TNBCs than non-TNBCs. **B.** Expression of VCP correlated with SOX9 expression **C.** VCP high expression is associated with worse survival rate in Curtis breast cancer dataset. **: p< 0.01

SOX9 and VCP Expression

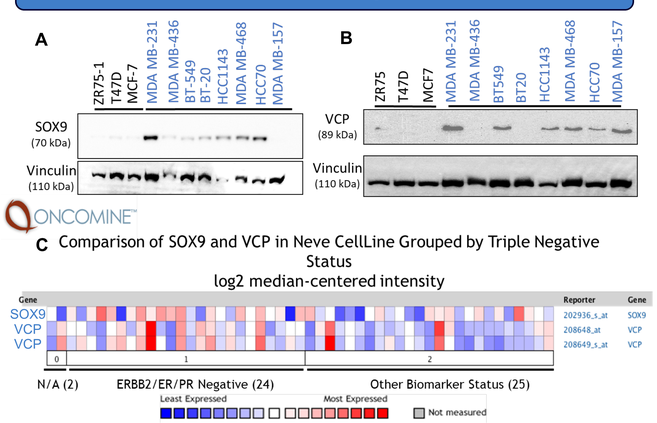

Figure 2. Analysis of SOX9 and VCP expression in 3 Non-TNBC cell lines and 8 TNBC cell lines. **A.** Western blotting analysis shows that SOX9 has a higher presence in TNBC cell lines (blue lanes) than Non-TNBC cell lines (black lanes). **B.** VCP also has a higher presence in TNBC cell lines than Non-TNBC cell lines. Vinculin was included as a load control. **C.** Higher mRNA expression of SOX9 (first row) and VCP (bottom two rows) in TNBC vs. Non-TNBC cell lines. Colors are z-score normalized to depict relative values within rows. The Oncomine™ Platform (Thermo Fisher, Ann Arbor, MI) was used for analysis and visualization.

Results

VCP and SOX9 Binding

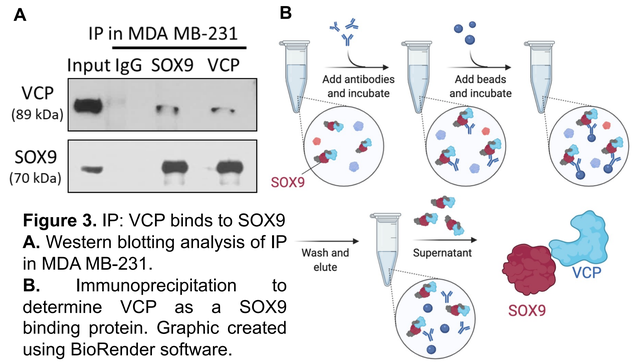

Figure 3. IP: VCP binds to SOX9
A. Western blotting analysis of IP in MDA MB-231.
B. Immunoprecipitation to determine VCP as a SOX9 binding protein. Graphic created using BioRender software.

VCP Knockdown and Cell Growth

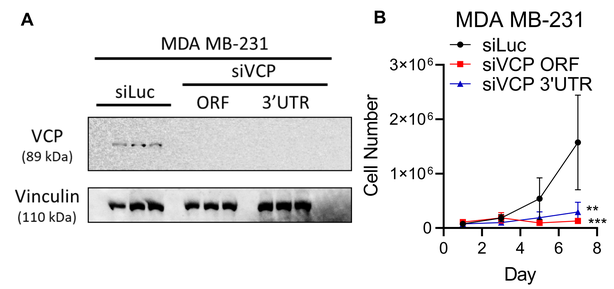

Figure 4. Knockdown of VCP in MDA MB-231 suppressed cell growth *in vitro*. Cells were treated with siLuc, siVCP ORF, and siVCP 3'UTR for 48 hours before harvest and reseeding.
A. Western blotting analysis showing successful knockdown of VCP in siVCP ORF and siVCP 3'UTR. VCP was only present in the control.
B. Cell growth curve. Cells were harvested and reseeded into 24-well plates after treatment with siRNA. Cell number was recorded on day 1, 3, 5, and 7 using the Countess Automated Cell. **: p< 0.01; ***: p< 0.001

VCP Regulates SOX9 Downstream Genes

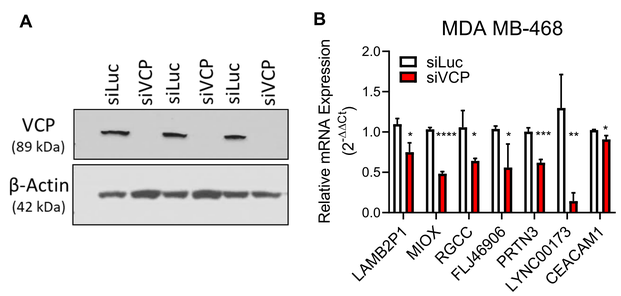

Figure 5. Knockdown of VCP reduces SOX9 downstream gene expression in MDA MB-468. **A.** Western blotting assay showing successful knockdown of VCP with no protein present in siRNA treated samples. **B.** Knockdown of VCP reduces relative mRNA expression of SOX9 downstream genes

Conclusions

- VCP expression is relatively higher in TNBC cell lines than non-TNBC cell lines.
- VCP binds with SOX9 in TNBC as shown by IP analysis.
- VCP regulates SOX9 downstream gene expression (*RGCC* and *PRTN3*) in TNBC.
- Knockdown of VCP suppresses TNBC cell growth.

Future Plans

- Perform qRT-PCR to evaluate mRNA levels of VCP in TNBC and non-TNBC cell lines.
- Further confirm the effect of VCP expression on TNBC cell growth and invasion through overexpression and knockout in TNBC cell lines
- Determine the effect of VCP inhibition on tumor growth *in vivo.*
- Investigate other SOX9 binding proteins such as CAV1 and GSK-3β as potential therapeutic targets in TNBC.

References

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