RNA splicing factors SRRM3 and SRRM4 promote cellular plasticity in castration-resistant prostate cancer

Mark P. Labrecque[1], Ilsa M. Coleman[2], Lisha G. Brown[1], Bryce Lakely[1], Lori Kollath[1], Daniel W. Lin[1], Lawrence D. True[3], Eva Corey[1], Peter S. Nelson[2,4] and **Colm Morrissey[1].**

Departments of Urology[1], Pathology[3], and Medicine[4], University of Washington School of Medicine, Seattle WA. Fred Hutchinson Cancer Research Center[2], Seattle, WA.

Background: We recently characterized five metastatic castration-resistant prostate cancer (mCRPC) phenotypes. These phenotypes include (i) AR-high adenocarcinomas with near-uniform expression of androgen receptor (AR) and prostate-specific antigen (PSA), and lack of the neuroendocrine (NE) markers synaptophysin (SYP) and chromogranin A (CHGA); (ii) AR-low tumors with weak or heterogeneous expression of AR and PSA, and negative for CHGA and SYP expression; (iii) amphicrine tumors (AMPC) composed of cells that co-express AR, PSA, CHGA and SYP; (iv) small cell or neuroendocrine tumors (SCNPC) with CHGA and SYP expression and absence of AR and PSA expression; and (v) double-negative tumors that do not express detectable levels of AR, PSA, CHGA and SYP. We show here that loss of RE1-silencing transcription factor (REST), a master regulator of neuronal differentiation, through splicing by SRRM3 and/or SRRM4 is necessary for AMPC and SCNPC conversion in CRPC.

Methods: Transcriptomic (RNASeq) and immunohistochemical/immunofluorescent analysis (IHC and IF) were conducted on amphicrine and SCNPC patient metastases, LuCaP patient-derived xenograft (PDX) models and modified CRPC cell lines. The roles of SRRM3 and SRRM4 were examined using overexpression and knockdown studies in AR-expressing and AR-null CRPC cell lines.

Results: The splicing of REST by SRRM4 and the expression of REST4 have been implicated in the loss of REST repressor activity in NE tumors. Transcriptomic analysis of AMPC patient specimens and AMPC LuCaP 77CR PDX revealed that loss of REST repressor activity occurred without SRRM4 expression in a subset of tumor specimens. BaseScope analysis using primers specific to REST4 and SRRM4 verified that AMPC LuCaP 77CR tumors were positive for REST4 expression but negative for SRRM4 expression, suggesting an alternative mechanism of REST splicing. Notably, SRRM3 and REST4 transcripts were expressed in AMPC and SCNPC patient and LuCaP PDX biospecimens that lacked SRRM4 expression, suggesting that REST is spliced and inactivated by SRRM3. Further, overexpression of SRRM3 in C4-2B and DU145 cells induced REST4 expression and loss of REST activity.

Conclusions: Our data highlights an unrecognized mechanism of adenocarcinoma to AMPC or SCNPC conversion that hinges on a SRRM3-REST regulatory axis rather than REST-loss or SRRM4-mediated REST splicing.

Conflict of Interest: None.

Funding Acknowledgements: This work was supported by a Department of Defense Idea Development Award-Partnering-PI (W81XWH-17-1-0414;W81XWH-17-1-0415), the Pacific Northwest Prostate Cancer SPORE (P50CA97186), the Department of Defense Prostate Cancer Biorepository Network (W81XWH-14-2-0183), the Prostate Cancer Foundation, the Institute for Prostate Cancer Research, and the Richard M. LUCAS Foundation.