Endocrine Signaling of Neuroendocrine Prostate Cancer Cells

Magnolia Winterlove[1,2], Tarana Arman[1], Ilsa Coleman[1], Jared Lucas[1], Pete Nelson[1]

[1]Division of Human Biology, Fred Hutchinson Cancer Center, Seattle, WA, [2]The Evergreen State College, Olympia, WA

Fred Hutch Cancer Center

Background

- Prostate Cancer (PC) is the second most diagnosed cancer among men worldwide and is driven by dysregulated androgen receptor (AR) [1].
- Initial stages of PC can be treated with androgen deprivation therapies or AR signaling inhibitors [1].
- Neuroendocrine prostate cancer (NEPC) is a subset of metastatic and therapy resistant PC characterized by lack of AR expression and high expression of canonical neuroendocrine markers (e.g., synaptophysin) [1].
- Previous studies also show a high frequency of inter- and intra-tumor heterogeneity for advanced PC [2].
- Previous studies show NE tumors express and secrete biologically active molecules that might have an advantage on the survival, progression and metastasis of AR-active prostate cancer (ARPC) cells *(Figure 1)*.

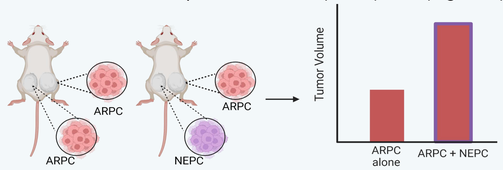


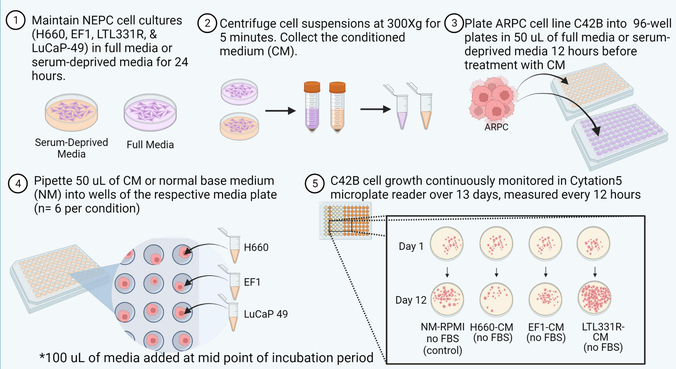
Figure 1. Schematic representation of the effect of NEPC derived factors on ARPC tumor growth, based on previous studies [3,4] .

- Preliminary mass-spec proteomics (NEPC) identified neurotrophic factors that have also been studied in the context of small cell lung cancer [5].

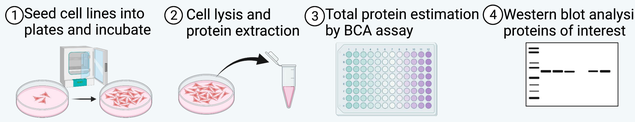
References: 1. Arman et.al., 2022 (PMID: 36440195), 2. Brady et.al., 2021 (PMID: 33658518), 3. Jin et.al., 2004 (PMID: 15289359), 4. Uchida et.al., 2006 (PMID: 16372327), 5. Kimura et.al., 2018 (PMID: 29748024).

Methods

Cell Count & Viability



Western Blot Analysis



Hypothesis & Aims

Secreted factors derived from NEPC cells influence distant ARPC cell types and promote treatment resistance.

Aim 1: Determine the endocrine influence of NEPC on ARPC growth in an in-vitro system.

Aim 2: Determine the secretory program operative in neuroendocrine prostate cancer cells.

Results

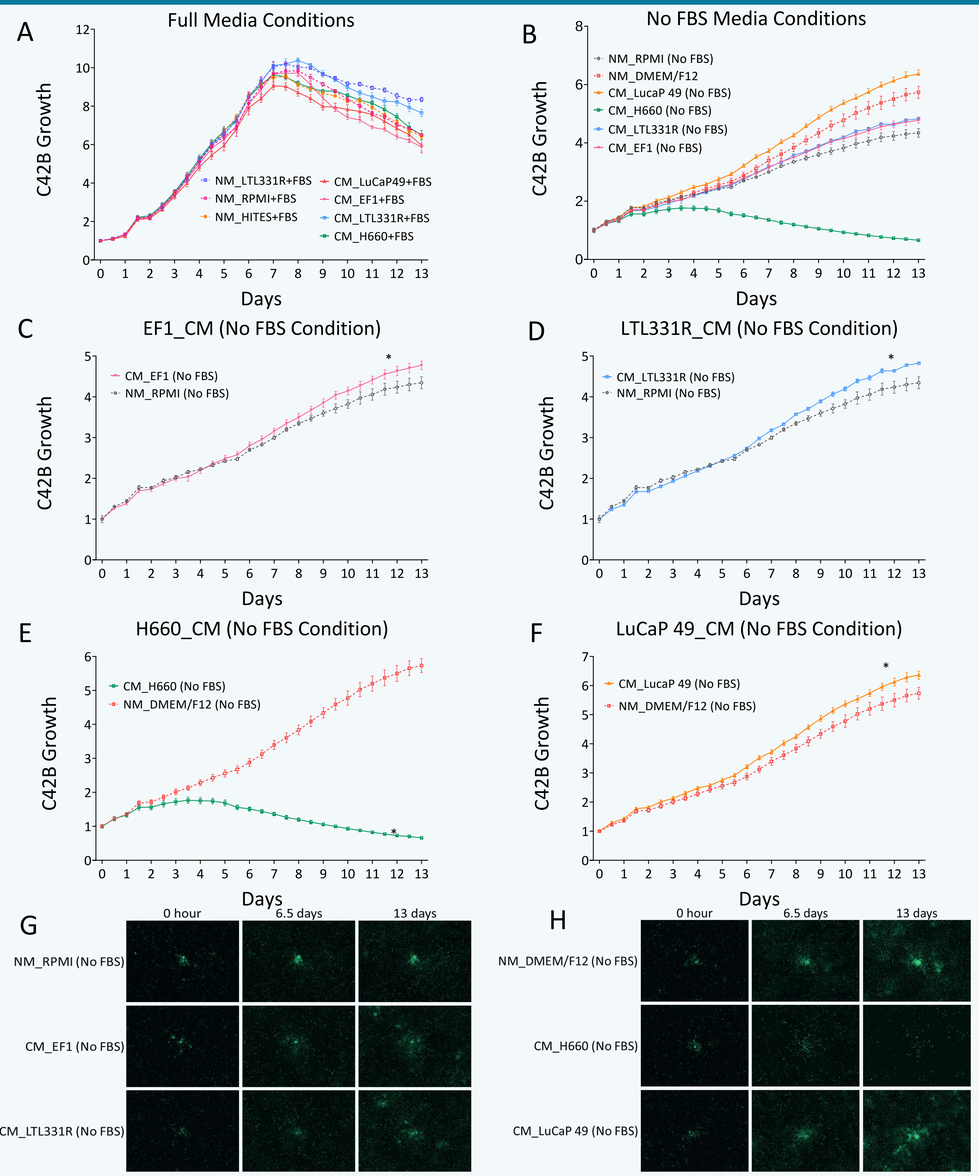


Figure 2. **Effect of NEPC conditioned media (CM) on the growth and viability of ARPC (C42B) cells**. (A) Growth of C42B cells seeded in full media after addition of full media NEPC-condition medium (NEPC-CM). (B) Growth of C42B cells seeded in serum deprived (No-FBS) condition after addition of No-FBS NEPC-CM. Media conditions: *NM*-Normal base media that are standard growth media for different NEPC cell lines. *CM*- Respective media conditioned by NEPC cells after 24 hours of growth. Effect of EF1_CM (C), LTL331R_CM (D), H660_CM (E) and LuCaP-49_CM (F) compared to their respective NM on the growth of C42B cells in a No-FBS condition over a period of 13 days. *NM_RPMI (No FBS)*:Normal base media for EF1 and LTL331R cell growth; *NM_DMEM/F12 (No FBS)*:Normal base media for H660 and LuCaP 49 cell growth. Data represent mean ± SEM. N=6 for each group. Two-way ANOVA p-values (*p-value<0.05) are shown. Panels (G-H): Representative images of C42B cell growth under different NEPC-CM conditions at 0 hr, 6.5 days, 13 days of conditioned media addition.

Results

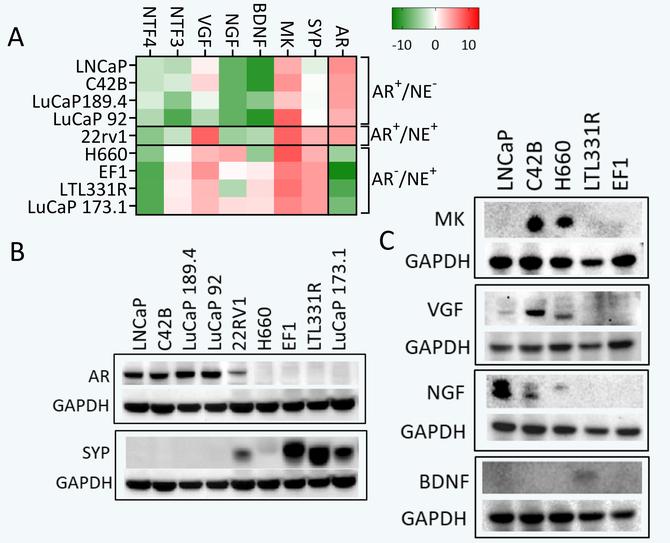


Figure 3. Expression of secreted factors in ARPC *versus* NEPC cell lines. (A) RNA-seq expression of AR-Androgen receptor, Syp- Synaptophysin, MK- Midkine, BDNF- Brain derived nerve growth factor, NGF- Nerve growth factor, VGF- VGF nerve growth factor inducible, NTF3/4- Neurotrophin 3/4 in ARPC (LnCaP and C42B) and NEPC (H660, EF1, LTL331R, LuCaP 49, LuCaP 173.1) cells. Results are expressed as log2 fragments per kilobase of transcript per million mapped reads (FPKM) and colored according to scale. Protein expression of AR, SYP (Panel B) and MK, VGF, NGF and BDNF (Panel C) in ARPC and NEPC cells.

Conclusions

- Conditioned media from three of the four NEPC cell lines (EF1, LTL331R and LuCaP 49 had significant positive effect on the growth of ARPC (C42B) cells suggesting a potential effect of NEPC secreted factors on the growth and survival of ARPC cells.
- BDNF protein uniquely expresses in NEPC (LTL331R) cells. BDNF and its receptor TrKB has previously been linked to poor prognosis and outcomes in various cancers including small cell lung cancer.

Future Directions

Expand the analysis into more ARPC cell lines.

More iterations of the immunoblotting assay required to confirm the differences in expression.

Mass-spec proteomics of secreted factors from NEPC cell lines ongoing.

Acknowledgements

This work is funded by DOD grant PC220408.

The Summer Undergraduate Research Program is supported in parts by the Fred Hutch Internship Program and individual labs/research groups.
Special thanks to Pete Nelson, Tarana Arman and the Nelson lab for mentorship and assistance.