VASOPRESSIN RECEPTORS AS THERAPEUTIC TARGETS FOR CASTRATION-RESISTANT PROSTATE CANCER

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ABSTRACT

We previously identified the G protein-coupled receptor, arginine vasopressin receptor type 1A (AVPR1A) as a potential therapeutic target in CRPC. AVPR1A mRNA levels are elevated in CRPC patients compared to primary PC and selective depletion of AVPR1A decreased growth of CRPC cells. AVPR1A antagonists were found to be safe and effective in clinical trials for conditions ranging from autism to pre-term labor. Repurposing the AVPR1A-selective antagonist, relcovaptan, inhibited CRPC tumor growth in vivo in three distinct preclinical settings: newly emergent, established and late stage growth in the bone metastatic niche (Zhao N et al. Sci Transl Med 2019). To understand more fully the mechanisms of AVPR1A action in CRPC, we examined pathways stimulated by its endogenous ligand arginine vasopressin (AVP). As it does in canonical target tissues, we found that AVP/AVPR1A signals via the G protein alpha subunit, Gq11, resulting in calcium release in CRPC. Independent of calcium signaling, AVP stimulated ERK phosphorylation leading to activation of CREB in CRPC. This pathway was blocked by an inhibitor of Gq11. We determined from analysis of human datasets that mRNA encoding pre-provasopressin (from which AVP is derived) is present at higher levels in CRPC compared to localized disease. Furthermore, we demonstrated that AVP was synthesized by CRPC cells revealing the exciting potential for autocrine signaling in advanced disease. Since AVP also serves as the endogenous ligand for another member of the AVP receptor family, AVPR2, we investigated expression of this receptor subtype in CRPC and found that AVPR2 was co-expressed with AVPR1A in select CRPC cell lines. These data suggest that AVP-mediated autocrine activation of both AVPR1A and AVPR2 may occur in CRPC. To understand the possible contribution of AVPR2 in CRPC growth, we examined the effects of sub-type selective antagonists for AVPR1A and AVPR2 on CRPC cell proliferation. We found that the combination of AVPR1A and AVPR2 antagonists inhibited CRPC cell proliferation and promoted apoptosis to a significantly greater extent than treatment with the individual antagonists. In contrast, dual use of the AVPR antagonists was not toxic to non-tumorigenic prostate epithelial cells. These findings indicate that autocrine signaling by AVP activates mitogenic and survival pathways in CRPC and further support the repurposing of drugs that target AVP receptors in CRPC.

SUMMARY + FUTURE DIRECTIONS

- Some CRPC cell lines produce AVP, which stimulates ERK phosphorylation through Gq, but not through canonical calcium effectors
- CRPC cells expressing both V1a and V2 receptors are growth inhibited through antagonism of both receptors
- We plan to examine the significance of autocrine AVP production and the impact of AVP activation of ERK and CREB in CRPC. We will evaluate the efficacy of dual antagonism of V1a and V2 receptors in preclinical in vivo models of CRPC

RESULTS

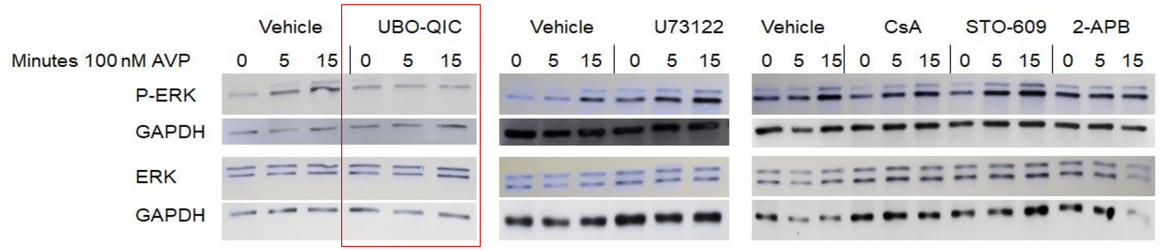

Figure 1. AVP activates ERK through the Gq subunit, but not through canonical downstream effectors of calcium signaling. 22Rv1 cells were pretreated with inhibitors of Gq (UBO-QIC 100 nM), PLCbeta (10 uM), calcineurin (cyclosporin a, CsA 10 uM), CAMKKII (STO-609 5uM), or IP3 (2-APB 10 uM) for 30 minutes. Cells were then treated with 100 nM AVP for the indicated time. Cell lysates were immunoblotted for phosphorylated ERK, total ERK, and GAPDH.

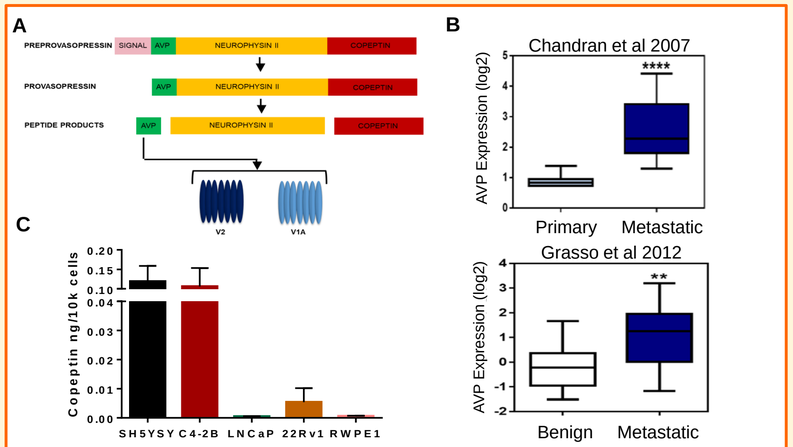

Figure 2. AVP expression is upregulated in patient samples of advanced forms of CRPC and CRPC cell lines produce AVP and copeptin. (A) Schematic for the processing of AVP from preprovasopressin. (B) AVP mRNA expression in Chandran et al., 2007 (NCBI, GSE6752) and Grasso et al 2012 (NCBI GSE35988). (C) Cells were incubated in serum free media for 48 hours. The conditioned media was then collected and analyzed for AVP or copeptin, a stable biomarker for AVP production, by ELISA. Cells excluding trypan blue were counted. SH-5YSY, a neuroblastoma cell line, is a positive control for copeptin production.

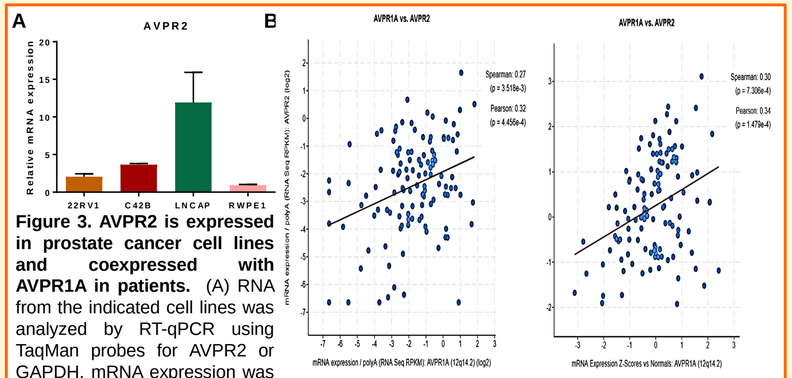

Figure 3. AVPR2 is expressed in prostate cancer cell lines and coexpressed with AVPR1A in patients. (A) RNA from the indicated cell lines was analyzed by RT-qPCR using TaqMan probes for AVPR2 or GAPDH. mRNA expression was normalized to RWPE1 levels. (B) mRNA Expression of AVPR1a and AVPR2 in two different patient sample data sets, (left) SU2C/PCF Dream Team, Cell 2015 and (right) MSKCC, Cancer Cell 2010, was evaluated for co-expression.

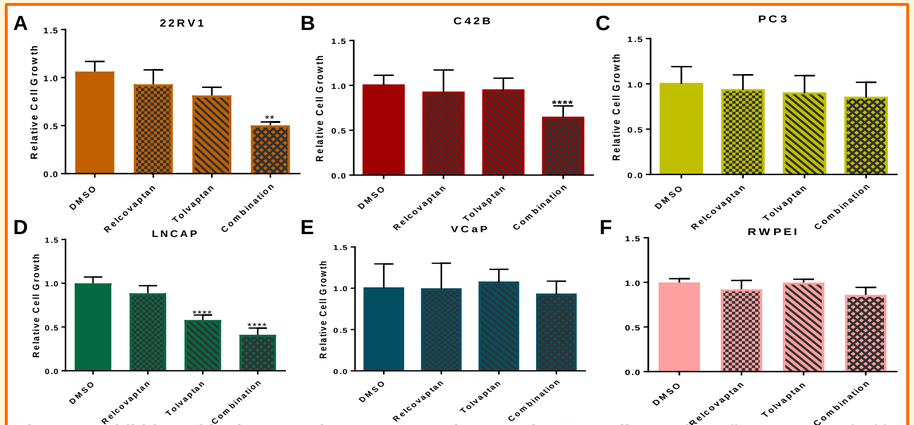

Figure 4. Inhibition of both V1a and V2 receptors decreased CRPC cell growth. Cells were treated with DMSO, 10 uM AVPR1a antagonist Relcovaptan, 5 uM Tolvaptan, or both for 7 days. (A-C) are CRPC cell lines, (D-E) is an androgen dependent cell line, and (F) is a non-tumorigenic prostate epithelial line. Cells were then counted and normalized to the DMSO treated control cells. ** = p<.01, *** = p < .001, **** = p<.0001. Errors bars = S.E.M.

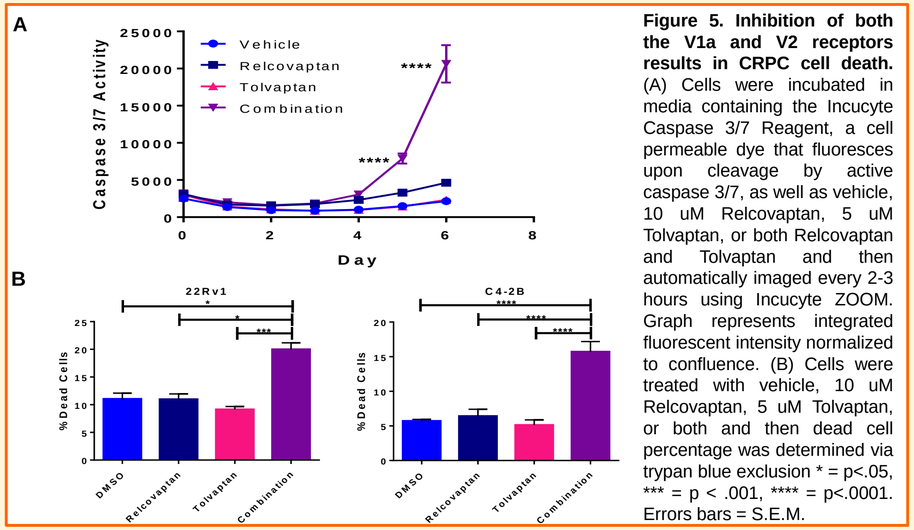

Figure 5. Inhibition of both the V1a and V2 receptors results in CRPC cell death. (A) Cells were incubated in media containing the Incucyte Caspase 3/7 Reagent, a cell permeable dye that fluoresces upon cleavage by active caspase 3/7, as well as vehicle, 10 uM Relcovaptan, 5 uM Tolvaptan, or both Relcovaptan and Tolvaptan and then automatically imaged every 2-3 hours using Incucyte ZOOM. Graph represents integrated fluorescent intensity normalized to confluence. (B) Cells were treated with vehicle, 10 uM Relcovaptan, 5 uM Tolvaptan, or both and then dead cell percentage was determined via trypan blue exclusion * = p<.05, *** = p < .001, **** = p<.0001. Errors bars = S.E.M.