

CHRM1-mediated acetylcholine signaling protects prostate cancer cells from the chemotherapy agent docetaxel

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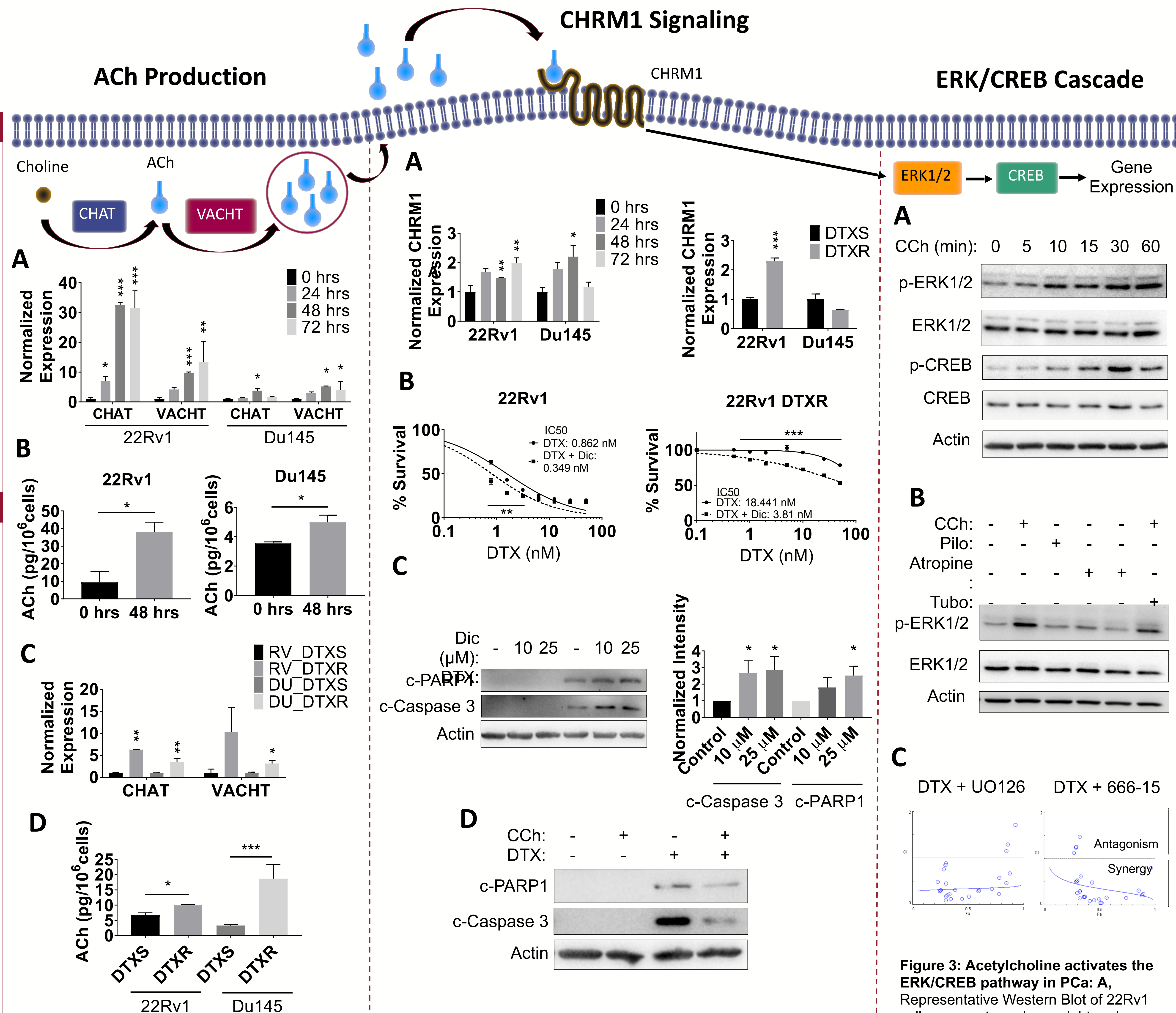
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Background

- 1 in 9 men will develop prostate cancer (PCa) sometime throughout their lifetime.
- Chemotherapy-resistant PCa remains a leading cause of cancer-related deaths in advanced PCa patients.
- Acetylcholine (ACh) and the muscarinic cholinergic receptor 1 (CHRM1) signaling has been shown to play a role in PCa growth but little is known on its role in chemotherapy resistance^{1,2}.
- The ERK/CREB cascade is known to play a role in PCa growth but it is currently unknown if ACh signaling plays a role in its activation^{3,4}.

Methods

- Cell culture:** The prostate cancer cell lines 22Rv1 and Du145 were grown in RPMI media supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Docetaxel-resistant cell lines were maintained in 3 nM DTX.
- RT-qPCR:** Total RNA was isolated using Trizol and converted to cDNA with M-MLV reverse transcriptase. All genes were amplified using PowerUp SYBR green and normalized to *GAPDH* expression.
- Acetylcholine Secretion:** Cells were grown in serum starved media supplemented with neostigmine (100 μ M) for 36 hrs before media was collected and frozen. Media was lyophilized and reconstituted with 1/5 volume water. Total ACh was measured with the QuickDetect ACh ELISA Kit.
- Crystal Violet Assay:** Cells were treated as described in the figure legends and then stained with crystal violet and absorbance was measured at 570 nm.
- Western blot:** Cells were treated as described in the figure legends. Phosphorylated proteins were normalized to the total protein intensity and non-phosphorylated proteins were normalized to actin.
- Drug synergy assay:** Cells were treated with varying concentrations of inhibitor followed by DTX. Cells were stained with crystal violet and absorbances measured at 570 nm



Conclusions

- Docetaxel treatment increases production and secretion of ACh which persists in docetaxel resistance
- Docetaxel treatment increases the expression of CHRM1 and inhibition of this receptor enhances DTX-induced cell death. This is enhanced in docetaxel-resistant cells
- ACh signaling activates the ERK/CREB cascade which is mediated by muscarinic receptor signaling.
- Inhibition of ERK or CREB enhances DTX-induced cell death in a synergistic manner
- Inhibition of CHRM1 signaling may be useful for sensitizing and even reversing chemotherapy resistance in PCa.

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