Veratridine functions as a potential anti-mTORC2-Rictor tumorigenic pathway inhibitor in human colorectal cancer

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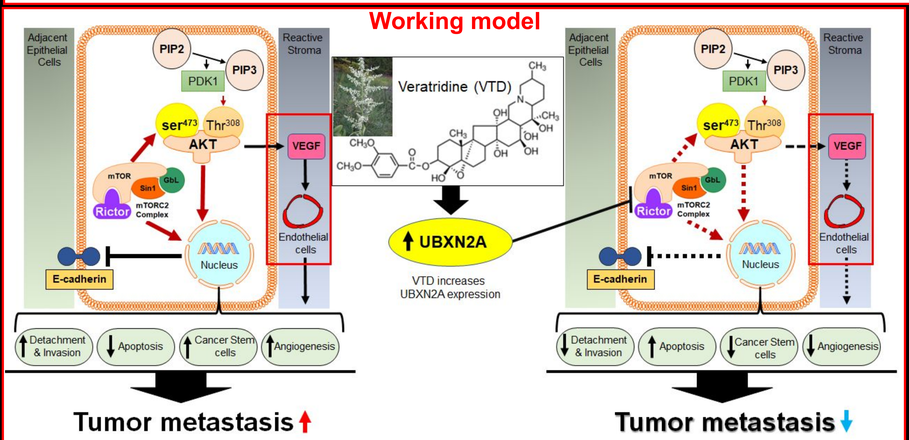
Introduction

- Colorectal cancer (CRC) is one of the most commonly diagnosed malignancies and is the second leading cause of cancer-related deaths for both men and women in the United States.
- A five-year survival rate of 91% for localized disease can be attributed to the success of surgical resection, but this rate drops to approximately 14% for patients diagnosed with the disseminated form of the disease because of the lack of effective therapies.
- There is a clear need to develop safer and more effective targeted therapies capable of significantly decreasing the high mortality rates associated with the metastatic form of human colorectal cancer.

Hypothesis: Veratridine increases UBXN2A, a novel tumor suppressor protein in CRC. UBXN2A suppresses the mTORC2-Rictor tumorigenic pathway and inhibits tumor growth, migration, and stem cell populations.



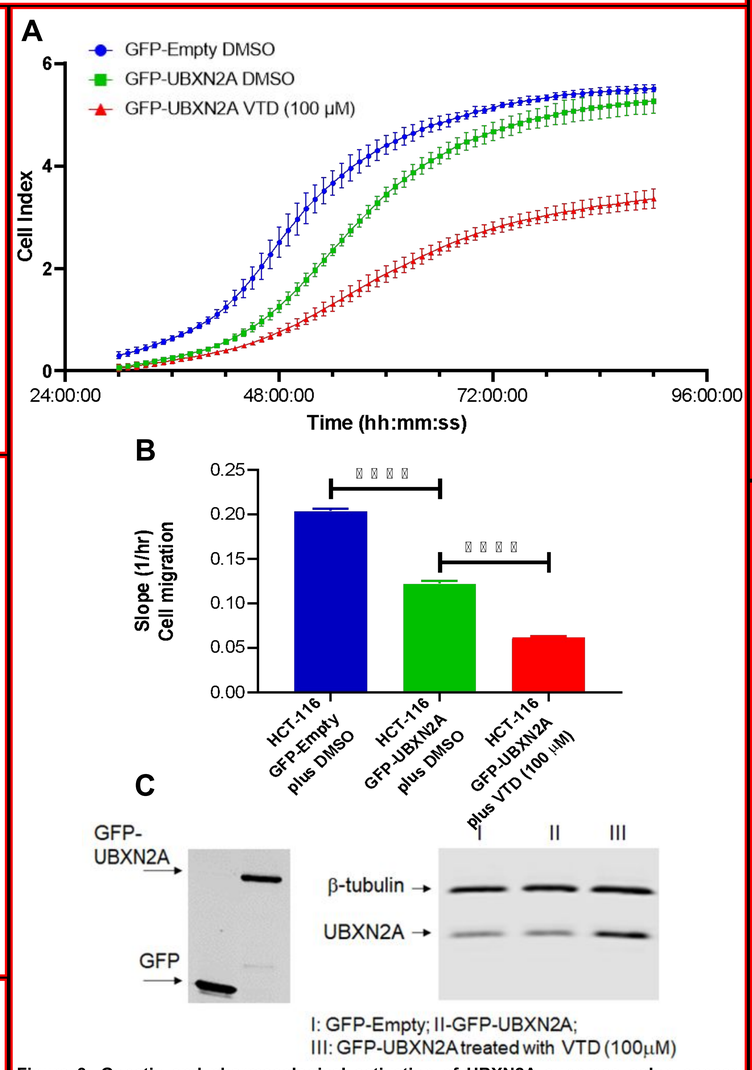


Figure 3: Genetic and pharmacological activation of UBXN2A suppress colon cancer migration. HCT-116 GFP-empty or GFP-UBXN2A cells were treated with DMSO or VTD for 72 hours and were plated in 16-well CIM plates. Cell migration was monitored using xCELLigence Real-Time technology (Panel A). Panel B shows calculated slopes for the events between the critical time points, hours 35 – 50. Interestingly, enhancement of UBXN2A endogenous protein by veratridine further decreases HCT-116 cancer cell migration (Panel B, blue versus red column). Panel C shows equal overexpression of exogenous GFP and GFP-UBXN2A in HCT-116 with the Tet-on promoter system and elevated endogenous UBXN2A in veratridine-treated cells that were subjected to xCELLigence analysis. Experiments were repeated two times with n of 4 per cell line per experiment (****P<0.0001, mean ± SD).

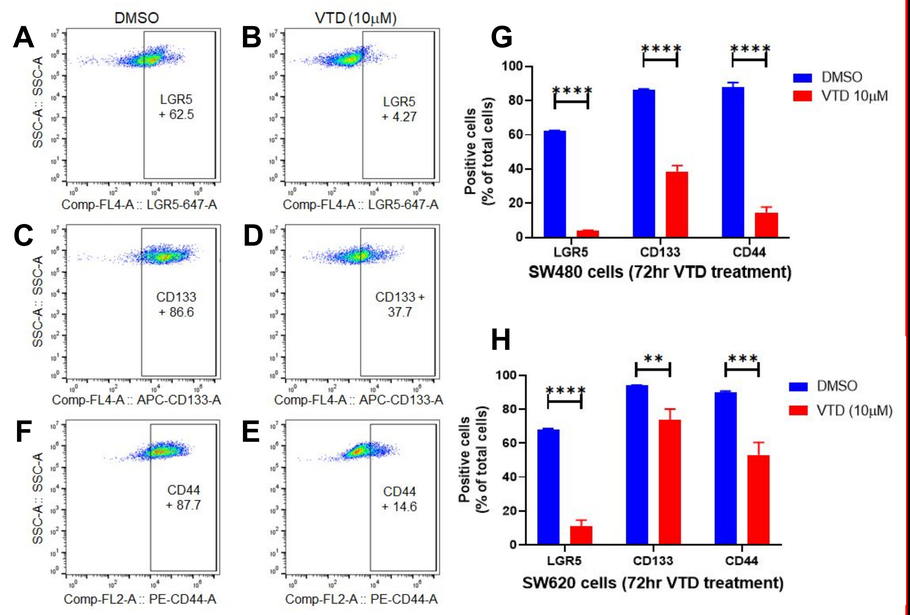


Figure 4: Veratridine (VTD) significantly decreases cancer stem cell populations in human colon cancer cells. CSCs positive for Lgr5, CD44, and CD133 are regulated by the mTORC2-hedgehog axis in cancer cells and are potentially responsible for the high recurrence rates of CRC. SW480 (primary, panels A-G) and SW620 (metastatic, panel H) cells were treated with VTD (100 µM) for 72 hours and were subjected to flow cytometry analysis using three CSC markers for Lgr5, CD44, and CD133. (n=3 per CSC marker, **P<0.01, ***P<0.001, ****P<0.0001, mean ± SD).

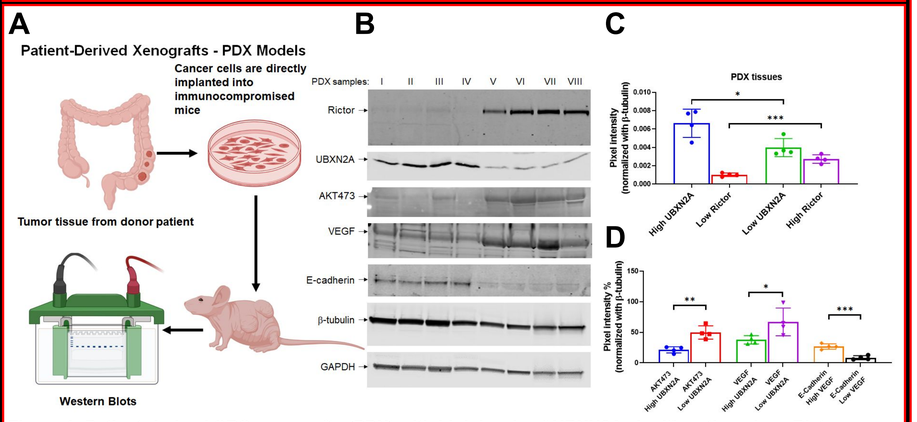


Figure 1: Patient-derived CRC xenografts (PDX) with high levels of UBXN2A significantly reduce Rictor protein and its downstream targets, including AKT-473, VEGF, and E-Cadherin. PDX tissues (n=8) were subjected to WB followed by quantitation of their corresponding bands (Panel A-B). Results revealed that the high level of UBXN2A in PDXs significantly decreases Rictor protein (Panel C), and it changes Rictor's downstream target proteins, including AKT-473, VEGF, and E-Cadherin (Panel D). (*P<0.05, **P<0.01, ***P<0.001, mean ± SD).

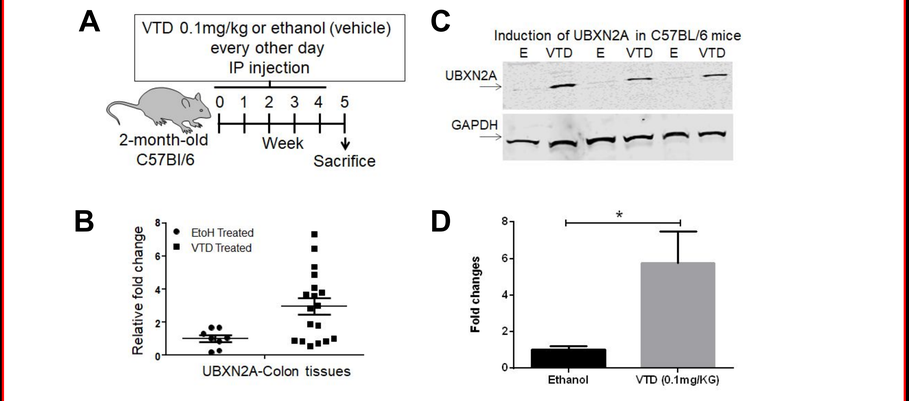


Figure 2: Treatment of mice with Veratridine transcriptionally increases UBXN2A at the RNA and protein level in mouse colon tissue. Mice were treated with VTD for 30 days (Panel A). After treatment, tissue was subject to qRT-PCR (Panel B) and Western Blot (Panels C-D). (*P<0.05, mean ± SD).

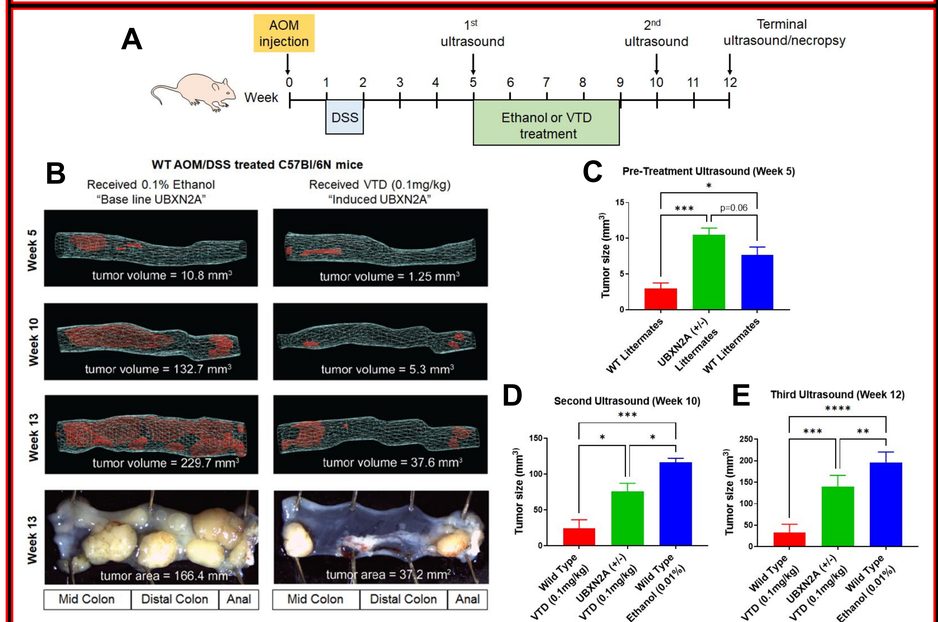


Figure 5: Veratridine (VTD) reduces tumor growth in mouse models of colorectal cancer. Mice received AOM/DSS to generate tumors in both the descending colon and rectum to mimic human CRC (Panel A). Progressing tumors were monitored by ultrasound before and during VTD treatment (0.1mg/kg) (Panel B). There was a final ultrasound before termination. Panels C, D, and E indicate that WT mice that received VTD treatment had slower tumor growth rates compared to WT mice that received the control treatment, ethanol (0.01%). The heterogenous UBXN2A (+/-) mice that received VTD treatment also had slower tumor growth rates compared to the control despite having half the expression level of UBXN2A in WT mice. Experiments were conducted under our approved IACUC animal protocol. (n=5, *P<0.05, ***P<0.001, ****P<0.0001, mean ± SD).

Acknowledgments

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Conclusion

- **Veratridine, a plant alkaloid, transcriptionally increases levels of UBXN2A, a tumor suppressor protein, resulting in suppression of the mTORC2/Rictor tumorigenic pathway.**
- **UBXN2A significantly reduces Rictor protein and its downstream targets including AKT-473, VEGF, and N-Cadherin which are involved in epithelial-mesenchymal transition (EMT).**
- **Veratridine-dependent enhancement of UBXN2A can suppress tumor growth, migration, metastasis, and cancer stem cells through the mTORC2/hedgehog signaling pathways in Colorectal cancer.**