Mechanistic studies of UBXN2A-RICTOR-mTORC2 axis in human colorectal cancer

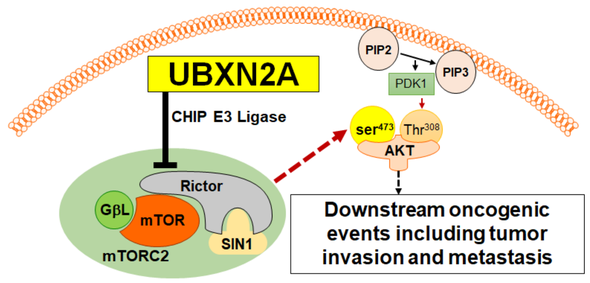
Sanam Sane,[1] Rekha Srinivasan,[1] Rashaun A. Potts,[1] Morgan Eikanger,[1] Jessica Freeling,[1] Ryan M. Antony,[1] Douglas Lynch,[2] Jonathan Bleeker,[3] Hassan Turaihi,[4] Angela Pillatzki,[5] Khosrow Rezvani [1]

1 Division of Basic Biomedical Sciences, Sanford School of Medicine, University of South Dakota, 414 E. Clark Street, Lee Medical Building, Vermillion, SD, USA. 2 Laboratory Medicine and Pathology, Sanford School of Medicine, Sioux Falls, SD, USA.
3 Sanford Cancer Center, Sioux Falls, SD, USA. 4 Sanford Surgical Associates, Sioux Falls, SD, USA. 5 Veterinary and Biomedical Sciences Department, Animal Disease Research and Diagnostic Laboratory, South Dakota State University, Brookings, SD, USA.

Introduction

There is a clear need to develop more effective targeted therapies to decrease the high mortality associated with metastatic colorectal cancer (CRC). Recent evidence points to inhibiting a signaling pathway regulated by the mTORC2 complex as a promising approach for effective targeted therapy in CRC. Suppression of mTORC2 signaling inhibits CRC cell proliferation and sensitizes CRC cells to standard-of-care therapies. The Rictor protein is a critical component of the mTORC2 complex that increases CRC and drives aberrant mTORC2 and AKT signaling in CRC cells. The current study leverages evidence that the downregulation of Rictor may be a promising approach for targeting mTORC2 signaling for therapeutic benefit in CRC patients. We found a tumor-suppressive ubiquitin-like protein, UBXN2A, induces degradation of Rictor in CRC cells, inhibiting downstream Rictor-mTORC2-regulated cancer-associated processes such as cell growth, survival, and migration. UBXN2A induction in patient tumor-derived organoids suppresses the mTORC2 pathway. These findings provide new insights into the potent anti-cancer function of a Ubiquitin-like protein in patients with CRC.

Current model



Hypothesis: UBXN2A suppresses tumor progression in colorectal cancer by suppressing the mTORC2 tumorigenic signaling pathway.

The ultimate goal: According to ClinicalTrial.gov, there are currently no active clinical trials on a selective mTORC2 inhibitor. Integration of the data achieved in this project provides a foundation for determining the druggability of UBXN2A as a selective mTORC2-Rictor inhibitor in human CRC.

Acknowledgements

- Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institute of Health under Award Numbers U54GM128729 and 1R03 CA223935-01A1 (Rezvani).

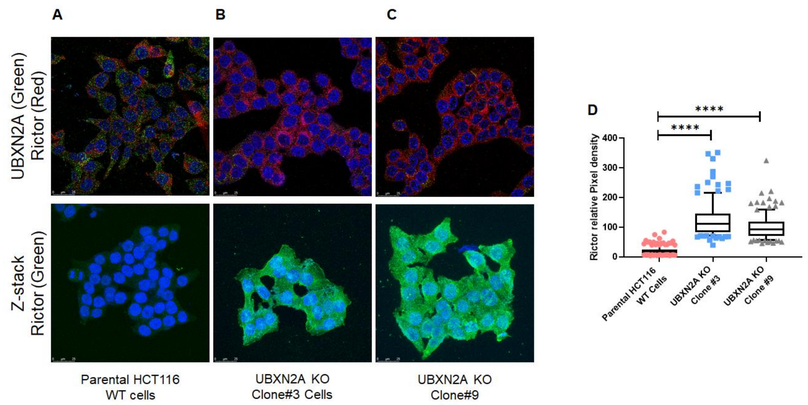


Figure 1: **Absence of UBXN2A leads to elevation of Rictor, resulting in inhibition of mTORC2's downstream protein targets**. Two stable CRISPR UBXN2A KO HCT-116 cells generated by CRISPR/Cas9 genome editing were validated by WB and flow cytometry analysis. Clones 3 and 9 UBXN2A KO were subjected to a confocal microscopy study. **Panels A-D** demonstrate that the absence of UBXN2A in HCT-116 (clones 3 and 9) significantly elevates the level of Rictor protein measured by immunocytochemistry.

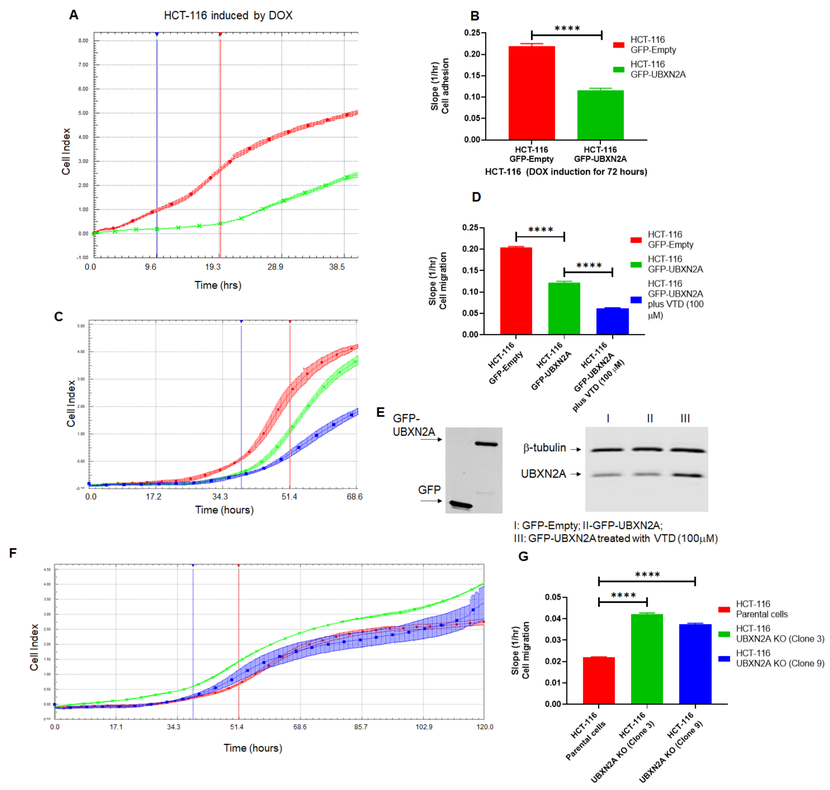


Figure 2:**Genetic and pharmacological regulation of UBXN2A suppress colon cancer migration.** HCT-116 GFP-empty or GFP-UBXN2A treated with DOX for 72 hours were plated in 16-wells E-plate or CIM-plate (xCELLigence Real-Time technology) and monitored in real-time for cell adhesion (**A-B**) and migration (**C-G**).

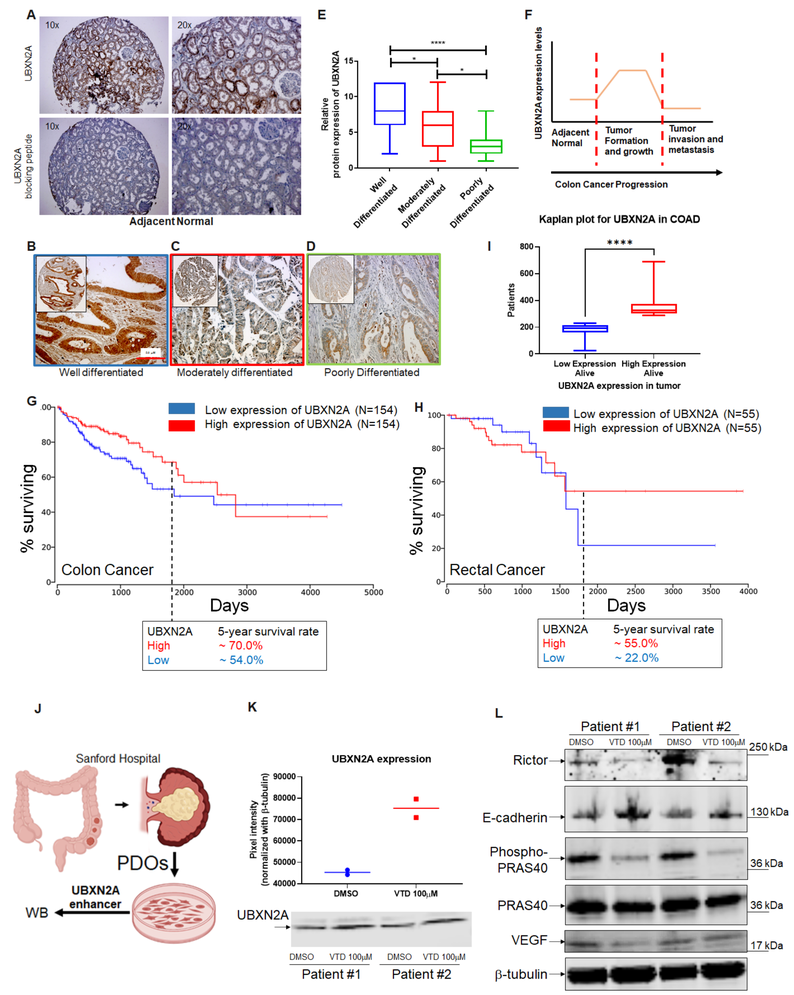


Figure 3:**UBXN2A protein levels predominantly upgrade during the early stage of tumor development and improve survival rates in colorectal cancer patients. A** shows UBXN2A's medium protein expression level in normal colon tissues. **B-F**: IHC staining was used to stain cytoplasmic and nuclear UBXN2A in well-differentiated (n=26), moderately (n=74), and poorly differentiated (n=24 tumor tissues) human colon tumor tissues. **G-I**: Kaplan–Meier's analysis of extracted survival data from TCGA shows a correlation of higher survival rate with higher UBXN2A expression in colon cancer. Further analysis shows a significantly larger portion of alive patients with COAD had higher UBXN2A expression compared to lower expression of UBXN2A. **J-K**: PDOs generated from surgically removed CRC tumors (n=2) were treated with the UBXN2A enhancer Veratridine (VTD, 100μM) for 72 hours and examined for mTORC2.

Conclusion

- **UBXN2A is a key player in intercepting the mTORC2-Rictor-AKT signaling pathway and its downstream regulators of metastasis.**
- **UBXN2A is an attractive and promising target for the treatment of CRC.**
- **Understanding the physiological and therapeutic potential of UBXN2A in human colon cancer will open a new platform for developing selective anti-mTORC2 drugs that leave the mTORC1 pathways primarily intact.**