Therapeutic development of ULK1/2 inhibitors as adjuvant therapy in bone metastatic prostate cancer

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BACKGROUND. One mechanism by which prostate cancer cells evade treatment is the upregulation of macroautophagy (ATG), a metabolic process where cells generate energy and building materials through the degradation of redundant or damaged organelles and proteins. ATG initiation due to cell stress leads to activation of the Unc-51-like autophagy-activating kinase 1 (ULK1) and ULK2 which then phosphorylate ATG effectors. ATG is upregulated in response to androgen receptor pathway inhibitor (ARPI) therapy. Using structure-activity relationship (SAR) studies, we identified ULK1/2 dual kinase inhibitors (ULKi),e.g., SBP-1750. To determine if these ULKi could be effective in CRPC, we examined the ATG pathway and efficacy of ULKi in PCa cell lines, patient-derived xenografts and organoids.

HYPOTHESIS. Small molecule inhibitors of ULK1/2 represent clinically actionable adjuvant therapies for CRPC that synergize with ARPI, chemotherapy and radiation therapy.

METHODS. PCa cells, 22RV1 or LNCaP, bmCRPC PDX models, PCSD1 and PCSD13. Assays for viability, ATG flux. Cell cycle analysis with ***Fucci2BL,*** bicistronic **F**luorescent, **U**biquitination-based **C**ell **C**ycle **I**ndicator system in live cells in 2D cultures in Incucyte real time cell cycle assay and in live cell confocal microscopy of 3D organoids. PDX tumor growth measured via calipers and IVIS. RNASeq, qRT-PCR, FACS, IFC and immunoblotting.

RESULTS. ENZA treatment in 22RV1 and LNCaP, and in intra-femoral PDX PCSD13 tumors upregulated key ATG proteins, such as ULK1, ATG13, and LC3. ENZA increased ATG flux in 22Rv1 and LNCaP cells with mCherry-LC3-GFP, a fluorescent ATG flux reporter, which could be blocked by ULKi, SBP-1750. ENZA can induce dormancy, a quiescent state where cancer cells stop dividing and ATG is upregulated. LNCaP expressing the FUCCI system showed transient S-phase arrest and cell growth inhibition when treated with ULKi, SBP1750, alone and even more so with SBP1750 + ENZA. Using live cell confocal time course analysis of FUCCI+ PCSD1 and PCSD13 organoids, we showed that SBP1750 resulted in a substantial, transient increase S-Phase followed by death of organoids, again, more acutely in SBP1750 + ENZA.

CONCLUSIONS-- Together these data support a mechanism in which PCa cells develop resistance to ARPI by upregulating ATG proteins and autophagic flux. Our novel ULKi can kill ARPI-resistant PCa cells through ATG inhibition which induced cell cycle arrest followed by cell death. Furthermore, these data indicate that our new ULKi have the potential for effective clinical application in castration resistant PCa (CRPC) and that ULKi in combination with ARPI may enhance patient outcomes.

Funding: Curebound Discovery Grant, JM Foundation
Conflict of Interest: None

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