

Implications of ketone metabolism in progression to treatment refractory bone metastasis

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Background: Prostate cancer (PCa) progression is driven by profound metabolic rewiring, yet the specific pathways supporting advanced disease remain incompletely defined. Our prior work revealed that the transition to castration-resistant PCa (CRPC) is marked by enhanced ketone body (KB) metabolism, accompanied by increased expression of the ketolytic enzymes ACAT1, OXCT1, and BDH1, with ACAT1 emerging as a clinically relevant, bone-metastasis-associated metabolic vulnerability.

Methods: We mined ACAT1 levels in the Human Protein Atlas, the MDA PCa PDX series; and its association with ketone pathways by GSCA in TCGA-PRAD and SU2C-PCF datasets. We performed spatial metabolomics in a panel of subcutaneous and intrafemoral tumors and established a pipeline to analyze metabolomics data. Castration studies were performed in both subcutaneous and intrafemoral tumor-bearing mice to evaluate androgen-dependent regulation of KB enzymes by immunohistochemistry. To improve imaging approaches of prostate cancer-driven bone remodeling, male SCID mice were intrafemorally injected with MDA PCa 183-luc, followed by surgical castration, and monitored for relapse by IVIS and X-ray.

We treated PCa cell lines and organoids from patient-derived xenografts (MDA PCa PDX), with ACAT1 inhibitor arecoline hydrobromide (AH) and assessed active phosphorylated ACAT1 through Western blot, KB content (BHB-Glo), lipid-dynamics through Bodipy-based flow cytometry (lipid droplets, lipid uptake, lipid peroxidation) and mitochondrial respiration and glycolytic flux using Seahorse XF analysis to measure real-time oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). We genetically silenced ACAT1 in 22Rv1 cells to further characterize its functions. Male SCID mice bearing MDA PCa 183, 203, and 173 PDX tumors received AH (50 mg/kg/day, i.p., 21 days) or vehicle; tumor volume, weight, and intratumoral KB levels were measured at endpoint.

Results: Spatial biology analysis reveal predominant bone site-specific tumor dependencies. ACAT1 was increased in progression samples and corresponding *in vivo* models, as well as in tumors within the bone microenvironment. Further, ACAT1 and OXCT1 levels were upregulated by castration in both subcutaneous and intrabone PCa PDXs. This was consistent with *in silico* findings, where ACAT1 was found increased in MDA PCA PDX derived from bone and exhibited enhanced association with ketone categories in castration resistant bone metastatic samples of the SU2C-PCF dataset.

AH treatment reduced phospho-ACAT1 as demonstrated by Western blot analysis, hence impairing the formation of the stabilized tetramer, essential for enzymatic activity, and induced activation of lipid metabolism and metabolic shutdown in PCa cell lines. AH reduced tumor volume and intratumoral KB levels in MDA PCA PDXs 203, 173, and 183 *in vivo*. ACAT1 genetic silencing reduced KB content, and triggered compensatory levels of several ketolytic enzymes.

Conclusions: These findings support the key role of KB metabolism in PCa progression and adaptation to the bone microenvironment proposing ACAT1 targeting as a promising therapeutic strategy for advanced PCa.

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