TARGETING KETONE BODY METABOLISM IN ADVANCED TREATMENT-RESISTANT PROSTATE CANCER

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Background: The complexity of metabolic pathways fueling prostate cancer (PCa) progression remains poorly understood. We previously identified an important metabolic shift that occurs with the onset of castrate-resistant PCa (CRPC), from elevated glycolysis to enhanced ketone body (KB) metabolism—fatty acid products that can be used as an alternative fuel source—accompanied by increased expression of key ketolytic/ketogenic enzymes, ACAT1, OXCT1, and BDH1. Among these, ACAT1 stood out as a promising druggable target whose expression is increased in bone metastases and correlates with poor outcomes. Here, we evaluated the functional role of KB metabolism in PCa bone metastasis and the potential of ACAT1 inhibition as a therapeutic strategy for advanced PCa.

Methods: We assessed cell viability in PC3, C4-2B and 22Rv1 PCa cells and MD Anderson PCa patient-derived xenografts (MDA-PCa PDX)-derived organoids treated with increasing concentrations of the ACAT1 inhibitor, arecholine hydrobromide (AH). We evaluated ACAT1 and phospho-ACAT1 levels by Western blot and immunofluorescence, lipid accumulation and lipid uptake with Bodipy 493/503 or Bodipy 500/510, respectively, KB (BHB-Glo) and ATP content (CellTiterGlo, relative to cell number) in PCa cells. Mice bearing MDA-PCa-183, -203 and -173 subcutaneous PDXs were treated with AH (50 mg/kg/day i.p., 21 days) or vehicle. Tumor volume was monitored with calipers, and tumors were weighed and KB content assesed at the end of the study. MDA-PCa-183-bearing mice (subcutaneous or intrafemoral) were subjected to surgical or sham (control) castration. RNA expression was assessed by RNA-sequencing. To assess modulation of KB enzymes under the presence of bone cells, indirect co-culture was performed between PCa cells and bone progenitors (MC3T3, pre-osteoblasts; Raw264.7 pre-osteoclasts).

Results: We observed transcriptional activation of lipid metabolism, and in particular of KB metabolic enzymes (ACAT1, OXCT1 and BDH1) in *in vitro* PCa-bone cell co-cultures. Accordingly, increased ACAT1 expression was detected in bone-localized PDXs compared with matched subcutaneous tumors. ACAT1 levels are modulated by castration in both subcutaneous and intrabone PCa PDXs.

Functionally, ACAT1 blockade with AH decreased cell viability in PCa cell lines and in PDX-derived organoids. AH also reduced phospho-ACAT1 (stabilized tetramer) and ACAT1 levels, KB levels, and increased lipid accumulation and uptake, as well as ATP levels in AH-treated cells, highlighting the link between KB and lipid dynamics. *In vivo*, AH reduced tumor volume and intratumoral KB levels in MDA-PCa-203, -173, and -183 PDXs. In the MDA-PCa-183 PDXs, AH only decreased tumor volume in sham-castrated, but not castrated mice, consistent with the observed castration-induced ACAT1 downregulation.

Conclusions: These findings underscore the pivotal role of KB metabolism in PCa progression and position ACAT1 as a compelling therapeutic target in advanced PCa.

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