Targeting metabolic adaptations in the breast cancer-liver metastatic niche nexus using dietary approaches to improve endocrine therapy efficacy

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Introduction: The liver is a common site of spread for metastatic breast tumors and patients with liver metastasis have significantly increased death risk compared to lung or bone metastases. About 70% of metastatic tumors express ERα rendering MBC responsive to endocrine-based therapies, including Fulvestrant (**Fulv**). Fulv is an ERα antagonist and the only clinically approved selective estrogen receptor degrader prescribed for MBC treatment, independent of the location of the metastatic tumors. Yet, patients with liver metastases are less responsive to Fulv, compared to patients with bone or lung metastases. There is a critical need for novel therapeutic approaches that will provide a durable therapy response or cure patients with ER^+ liver MBC. **Methods:** In this study, we focused on tumor-intrinsic metabolic mechanisms that arise specifically in the liver metastatic niche. We used tissue-specific hydrogels to partially mimic metastatic niches and liver-specific cell line xenograft (**CLX**) models to delineate ER^+ MBC metabolic adaptations in a liver metastatic tissue context. We used metabolomics, flux analysis, transcriptomics, and cistromics to associate metabolic pathway changes with ER activity. **Results:** To uncover the mechanistic basis of decreased Fulv efficacy in patients with ER^+ liver MBC, we used a preclinical cell line xenograft mouse model of MCF7 $ESR1^{Y537S}$cells. Tail vein injection of MCF7 $ESR1^{Y537S}$cells in NOD SCID gamma (**NSG**) mice resulted in liver metastasis, and Fulv treatment failed to reduce metastatic burden. RNA-Seq of liver metastatic tumors showed that the pyruvate production pathway was the top upregulated metabolic pathway and pyruvate and acetyl-CoA producing enzymes were the top regulated genes in response to Fulv. Because we observed major metabolic and gene expression changes in pyruvate metabolism and increased dependence on and utilization of glucose in models of liver metastasis, we next tested the impact of a fasting mimic diet (**FMD**) on the MCF7-$ESR1^{Y537S}$ xenograft model. Feeding animals with an

FMD synergized with Fulv treatment to reduce the metastatic burden and decrease the number of visible metastatic nodules in the liver. **Conclusions:** Using a combination of models, spatial and molecular data, and analytical methods, we illuminated the details of a key mechanism of endocrine resistance in liver MBC: niche-related metabolic plasticity in MBC cells that alter the response to ER-directed therapies. Our studies established metastatic-niche specific metabolic vulnerabilities as a novel target by uncovering the mechanistic basis of why Fulv does not improve the overall survival of patients with ER+ liver MBC, including further explorations of the metabolism-cancer nexus and response to other endocrine-based therapies.