Title

Abstract 44: Hexyl-benzyl-biguanide (HBB) potently and selectively inhibits CYP3A4 epoxygenase activity and inhibits EET stabilization of mitochondrial respiration in ER+HER2- breast cancer cells, inducing glycolysis and pyruvate biosynthesis

Journal

Cancer Research, 76(14_Supplement)

ISSN

0008-5472

Authors

Guo, Zhijun

Sevrioukova, Irina

Hanse, Eric

et al.

Publication Date

2016-07-15

DOI

10.1158/1538-7445.am2016-44

Cancer Research
Volume 76, Issue 14_Supplement
15 July 2016
Poster Presentations - Proffered Abstracts| July 15 2016

Abstract 44: Hexyl-benzyl-biguanide (HBB) potently and selectively inhibits CYP3A4 epoxygenase activity and inhibits EET stabilization of mitochondrial respiration in ER+HER2- breast cancer cells, inducing glycolysis and pyruvate biosynthesis

Zhijun Guo; Irina Sevrioukova; Eric Hanse; Xia Zhang; Ilia Denisov; Ting-Lan Chiu; Rebecca Cuellar; Christian Torres; Julia Wulfkuhle; Emanuel Petricoin; Qing Cao; Haitao Chu; Beverly Norris; Robert Schumacher; Ameeta Kelekar; Ian Blair; Jorge Capdevila; John Falck; Thomas Poulos; Steven Sligar; Gunda Georg; Elizabeth Amin; David A. Potter

Cancer Res (2016) 76 (14_Supplement): 44.
https://doi.org/10.1158/1538-7445.AM2016-44

Abstract

Cytochrome P450 3A4 (CYP3A4) promotes ER+HER2- breast cancer cell proliferation and survival, in part, by biosynthesis of epoxyeicosatrienoic acids (EETs). EETs are known to regulate mitochondrial function in non-transformed cells, but the roles of CYP3A4 and EETs in regulation of breast cancer bioenergetics are unknown. Hexyl-benzyl-biguanide (HBB) is useful probe of CYP3A4 epoxygenase activity and selectively inhibits EET biosynthesis (IC50 = 9 uM vs. IC50 = 50 uM for CYP2C8). HBB caused depolarization of mitochondria in MCF-7 cells, while (±)-14,15-EET provided partial protection. The soluble epoxide hydrolase (sEH) inhibitor t-AUCB ameliorated inhibition of oxygen consumption rates (OCR) by HBB (20 uM), while there was no effect on extracellular acidification rate (ECAR), indicating that the primary effect of HBB is on OCR. At 30 minutes, HBB added to MCF-7 cells transiently suppressed phosphorylation of pyruvate kinase muscle isozyme 2 (PKM2) on Tyr-105, which has been reported to favor enzymatically inactive dimer over active tetramer. Suppression of phosphorylated PKM2 correlated with subsequent PKM2 tetramer formation and increase of intracellular pyruvate and extracellular lactate at 1 hour. The (±)-14,15-EET regioisomer reduced the pro-glycolytic PKM2 tetramer at 1 hour, suggesting that HBB may promote PKM2 tetramer, in part, through reduction of EET. Prolonged exposure to HBB (20 uM) in cultured cells activated phosphorylation of PKM2 on Tyr-105, but there was increased cellular necrosis correlating with reduced mitochondrial respiration and reduction of ATP stores, indicating that loss of respiration was the dominant effect. HBB inhibited the ER+HER2- MCF-7 xenograft, similar to CYP3A4 silencing. HBB promoted phosphorylation of intratumoral PKM2 on Tyr-105, consistent with long-term exposure to HBB in cultured MCF-7 cells. Notably, MCF-7 tumor response to HBB did not correlate with phosphorylation of AMPK-alpha on Thr-172, a marker of AMPK activation. Metformin (5 mM) exhibited no effect on PKM2 or its phosphorylation in cultured MCF-7 cells. Together, these results indicate that part of the inhibitory effect of HBB on ER+HER2- breast cancer is mediated through inhibition of respiration.

Significance: These results establish HBB as a useful chemical probe of respiration, with indirect effects on PKM2 regulation. HBB may also be useful as a potential therapeutic candidate for ER+HER2- breast cancer.

Citation Format: Zhijun Guo, Irina Sevrioukova, Eric Hanse, Xia Zhang, Ilia Denisov, Ting-Lan Chiu, Rebecca Cuellar, Christian Torres, Julia Wulfkuhle, Emanuel Petricoin, Qing Cao, Haitao Chu, Beverly Norris, Robert Schumacher, Ameeta Kelekar, Ian Blair, Jorge Capdevila, John Falck, Thomas Poulos, Steven Sligar, Gunda Georg, Elizabeth Amin, David A. Potter. Hexyl-benzyl-biguanide (HBB) potently and selectively inhibits CYP3A4 epoxygenase activity and inhibits EET stabilization of mitochondrial respiration in ER+HER2- breast cancer cells, inducing glycolysis and pyruvate biosynthesis. [abstract]. In: Proceedings of the 107th Annual Meeting of the American Association for Cancer Research; 2016 Apr 16-20; New Orleans, LA. Philadelphia (PA): AACR; Cancer Res 2016;76(14 Suppl):Abstract nr 44.