Effect of N1-hexyl-N5-benzyl-biguanide mesylate (HBB) on Immediate Early Hormonal Signaling in MCF-7 Breast Cancer Cells

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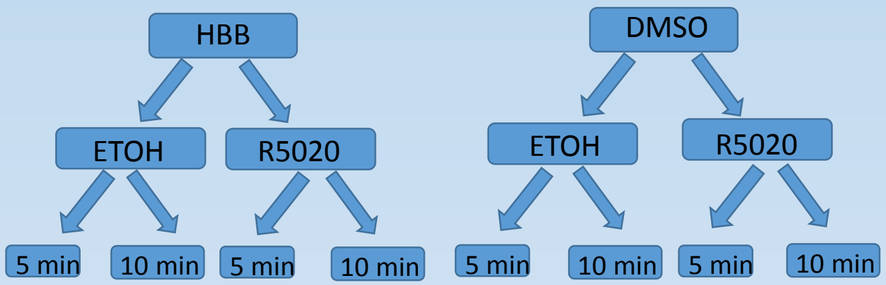
Introduction

- 70% of new breast cancers are luminal, positive for both estrogen receptors and progesterone receptors[1]
- A third of luminal breast cancers are resistant to estrogen targeted therapies[1]
- CYP3A4 is an arachidonic acid epoxygenase enzyme necessary for breast cancer cell proliferation, in part, through the formation of epoxyeicosatrienoic acids (EETS)
- Hexyl-benzyl-biguanide (HBB) induces AMPK phosphorylation promoting cancer cell catabolism and inhibits STAT3 phosphorylation, which is important for tumor cell growth[2]
- HBB binds to and inhibits CYP3A4 and can therefore serve as a chemical probe of the roles of EETs in breast cancer

Methods

- MCF7 cells from a complete media were passaged in charcoal stripped serum and put into four 6-well plates
- Cells were then starved overnight in serum free medium
- The cells were pretreated with HBB or the vehicle control (DMSO) before exposure to the progesterone receptor agonist (R5020) or its vehicle (ethanol)



- This was repeated with E2, 17-beta-estradiol
- Cells were harvested and left in -80° C overnight
- A western blot assay was used to determine the ratio of phospho-ERK (Thr202/204) (pERK) to total ERK levels.

Results

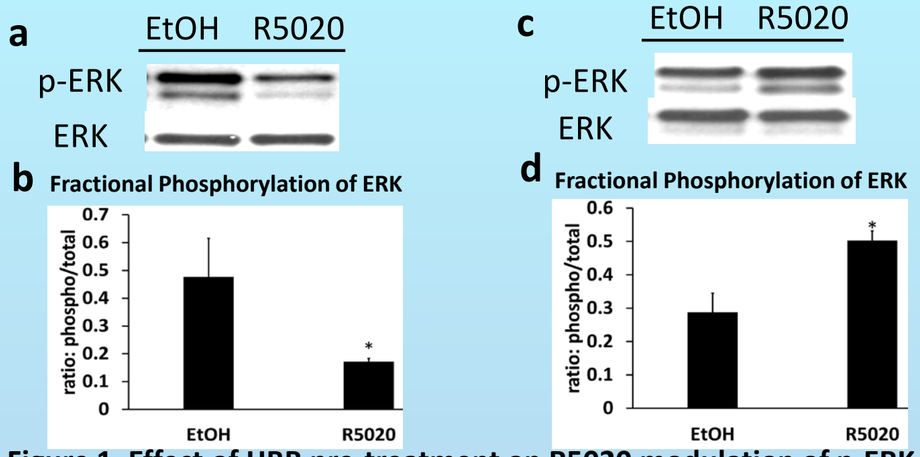


Figure 1. **Effect of HBB pre-treatment on R5020 modulation of p-ERK.** MCF7 cells were grown in phenol red free MEM with 10% charcoal stripped FBS and serum starved for 16 hours before experiment. (**a**) Western blot of 5 minute stimulation with R5020 without DMSO or HBB pretreatment. (**b**) Results are expressed as mean±S.D. (n=3, * indicates statistical significant difference from control P<0.05). (**c**) 10 minute stimulation with R5020 with 20 uM HBB pretreatment for 2 hours. (**d**) Results are expressed as mean±S.D. (n=3, * indicates statistical significant difference from control P<0.05)

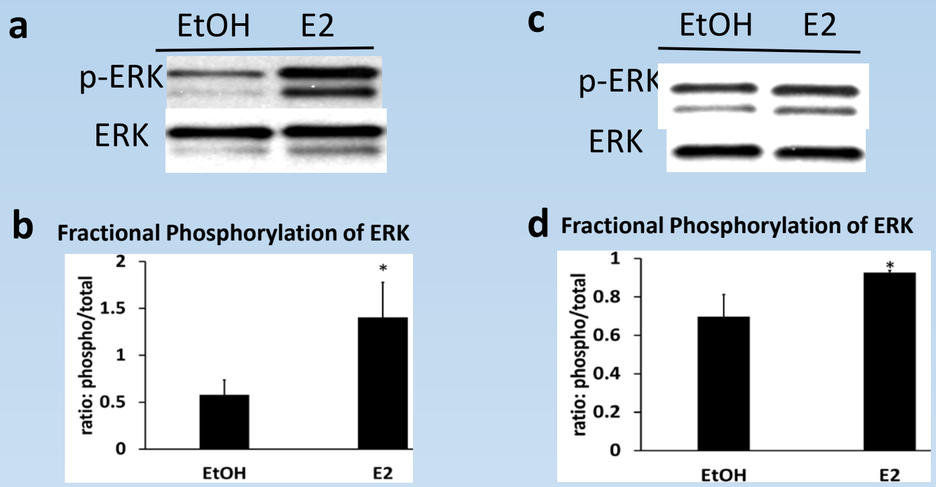


Figure 2. **Effect of HBB pre-treatment on E2 modulation of p-ERK.** MCF7 cells were grown in phenol red free MEM with 10% charcoal stripped FBS and serum starved for 16 hours before experiment. (**a**) Western blot of p-ERK and ERK after 10 minute stimulation with E2 (1 nM) after 2 hr of DMSO or HBB (20 uM) pretreatment (**b**)Results are expressed as mean±S.D. (n=3, * indicates statistical significant difference from control P<0.05) (**c**) Western blot of pERK and ERK after 10 minute stimulation with E2 1 nM with 2 hr 20 uM HBB pretreatment. (**d**)Results are expressed as mean±S.D. (n=3, * indicates statistical significant difference from control P<0.05)

Conclusions

- Estradiol and progesterone agonist R5020 increased fractional phosphorylated ERK
- HBB may potentiate E2 and R5020 signaling to ERK

Future Directions

- Test this effect in a different ER+ PR+ breast cancer cell lines
- Change the length of the pre-treatment
- Directly test the effects of EET on E2 and R5020-mediated activation of p-ERK

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Literature Cited

1. Daniel, A. R., Gaviglio, A. L., Knutson, T. P., Ostrander, J. H., D'Assoro, A. B., Ravindranathan, P., … Lange, C. A. (2015). Progesterone receptor-B enhances estrogen responsiveness of breast cancer cells via scaffolding PELP1- and estrogen receptor-containing transcription complexes. *Oncogene 34*,506–515.
2. Guo, Z., Chavez, K. J., Alvarez, J., Zhang, X., Norris, B., Maher, M., … Potter, D. A. (2014). Abstract 2689: Breast cancer inhibition by a novel and potent biguanide, N1-hexyl-N5-benzyl-biguanide. Cancer Research *74*. 2689–2689.