Eicosanoid Regulation of the Nuclear Pore Complex in Breast Cancer

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INTRODUCTION

- Cytochrome P450 (CYP) AA epoxyenase enzymes promote cancer cell proliferation, in part, through synthesis of epoxyeicosatrienoic acids (EETs)
- N1-hexyl-N5-benzyl-biguanide (HBB) binds to and inhibits CYP3A4 with high affinity, thereby serving as a chemical probe of the roles of EETs
- Mitochondrial inhibition by HBB causes activation of AMPK
- HBB causes more potent effects on depolarization of mitochondria in MCF-7 cells, inhibition of STAT3 phosphorylation, and reduction of the mitochondrial membrane potential of cancer cell lines
- Estrogen receptor (ER)-positive is known to be overexpressed in breast cancer, including in MCF-7 and T47D cell lines

METHODS

- MCF-7, T47D normal and charcoal serum cells were grown in 4-Chamber CultureSlides for 24 hr or 48 hr before treatment
- RagC and ERα staining in T47D normal and charcoal stripped cell culture medium for 6 hr
- Charcoal stripped serum removes steroid hormones, endogenous hormones, and growth factors
- 4% Paraformaldehyde for 10 min used as a fixative to ensure cell structures stay intact and steady
- 3% Normal Donkey Serum at 37 °C used as a blocking agent
- RagC and ERα primary antibodies overnight to bind to the target molecule
- Anti-Rabbit-Cy3 secondary antibody to recognize and bind to primary antibody
- DAPI staining allows nuclear viewing
- Images were captured using an Olympus FV1000 Confocal Microscope with a 120X oil immersion objective

RESULTS

- HBB restricts passive transport of fluorescent dextrans of 70 kDa through the NPC
- Restricting nuclear passage of RagC prevents it from gaining its GDP-bound state necessary to activate mTORC1
- Reducing translocation of ERα could blocks its effect on cell proliferation, thereby inhibiting tumor growth

HYPOThESIS

- HBB inhibits the nuclear pore complex (NPC) function and thereby prevents nuclear localization of macromolecules, including RagC. Whether HBB inhibits nuclear localization of ERα, which promotes breast tumor growth, remains to be determined
- EETs synthesized by peri-nuclear mitochondrial CYP3A4 open the nuclear pore complex, thereby promoting tumor growth

FUTURE RESEARCH

- Use EET agonist C22 to test nuclear localization of fluorescent dextrans of 70 kDa and ERα
- Determine whether the nuclear localization of RagC and ERα is inhibited by CYP3A4 knockdown
- Test the effects of EETs directly on 17β-estradiol (E2)
- Determine the effects of HBB on the ERα human breast cancer cell line ZR75 and the ERα mouse mammary carcinoma cell lines SSM2βd and 67NR

CONCLUSIONS

- Determine the effects of HBB on the ERα
- Determine whether the nuclear localization of RagC and ERα is inhibited by CYP3A4 knockdown
- Test the effects of EETs directly on 17β-estradiol (E2)
- Determine the effects of HBB on the ERα human breast cancer cell line ZR75 and the ERα mouse mammary carcinoma cell lines SSM2βd and 67NR

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