The relevance of xenoestrogens in breast cancer pathogenesis: Should low levels of estrogen-like compounds concern us?

Shawn Pan1, Chaoshen Yuan2, Abderrahmane Tagmount1, Ruthann Rudel3, Chris Vulpe1 and Dale Leitman1

1 Department of Nutritional Science and Toxicology, College of Natural Resources, University of California, Berkeley, California
2 Silent Spring Institute, Newton, Massachusetts

Introduction

Breast Cancer Risk Factors:
- Genetic Susceptibility: BRCA 1, BRCA 2
- Environmental Factors

Overall Risk of Breast Cancer Development

Relevance

Xenoestrogens
- Estrogen mimics (produced by the body or from working)
- Anti-androgens (inhibits androgens from working)

A closer look at environmental factors:
- Chemicals present in the environment and in our bodies

Xenoestrogens:
- 7,12-Dimethylbenz(a)anthracene
- α-Bromonaphthalene

Are these chemicals present in the environment and in our bodies at levels of concern to us?

Background:
- Target gene (e.g. c-myc) DNA → mRNA → Protein → Effect
- More binding of ERs to ERE = more gene expression
- Chromatin immunoprecipitation showing ERs recruit c-myc enhancer region

Experimental Results

C-myc mRNA levels
- DNA → mRNA → Protein → Effect
- Increased c-myc mRNA in BT-474 cells

C-myc protein levels
- DNA → mRNA → Protein → Effect
- c-myc protein encourages cell proliferation

Conclusion and Future Directions

What does this mean in terms of human health concern?
- Hergulin/HER2 activation modulates ERs response to estradiol and xenoestrogens
- In vitro, cells are exposed to hergulin and other growth factors that stimulate HER2 activation
- Most in vitro assays that are used to assess xenoestrogens test the compound of interest alone
- Our study suggests that the in vivo response to xenoestrogens, in regards to cell proliferation and breast cancer development, may be stronger than is suggested by current studies
- Performing transfection assays (overview below) is an effective way to choose xenoestrogen candidates to undergo the “HER2 activation” experiment

Overview of a Transfection Assay:
1) An electrical field is created to increase the permeability of the cell membrane. This results in the uptake of the two plasmids shown.
2) The ERE gene in the expression vector will be expressed by the cell to produce the receptor.
3) The cell is then treated with the drug of interest for 24 hours. During this time, drugs that are estrogenic will bind to the produced ERs, which will then activate the transcription of the Luciferase gene. Subsequently, Luciferase protein is made. Luciferase is an enzyme that catalyzes a light-producing reaction.
4) After 24 hours, the cell is lysed and Luciferase substrate is added. The amount of light is determined by a luminometer.
5) More light = more Luciferase produced = more ERs activated = more estrogenic the compound.