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

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Introduction

Breast Cancer Risk Factors:

Genetic Susceptibility:
- BRCA 1
- BRCA 2

Environmental Factors

→ **Overall Risk of Breast Cancer Development**

A closer look at environmental factors…

Industrialization
→ **Technological Innovation**
→ **Novel Synthetic Compounds**
+ Other Natural Compounds
=
Environmental Agents of Concern

Could these agents contribute to breast cancer development?

DNA-Damaging Agents:
- 7,12-Dimethyl-benz(a)anthracene
- *tert*-Butylhydroquinone

Xenoestrogens:
- Diethylstilbestrol (DES)
- Bisphenol A (BPA)
- Parabens
- Phthalates

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

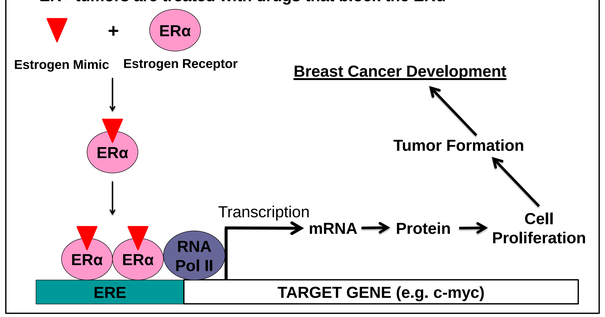
In vitro:	*In vivo:*
• Not very potent at very low levels • Assays require high doses to yield any response	• Xenoestrogen levels found in the body are extremely low

Relevance

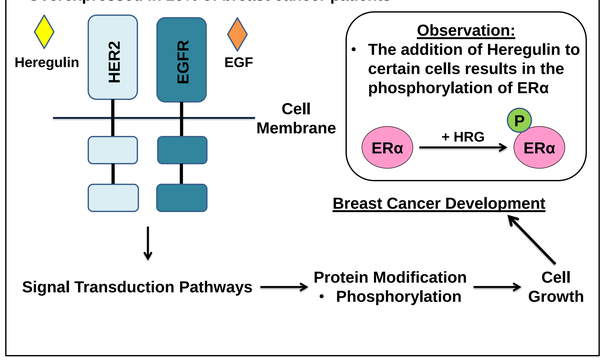
Xenoestrogens

- **Aromatase** (synthesizes estrogens)
- **Estrogen mimics** (behaves like body's estrogens)
- **Anti-androgens** (prevents androgens from working)

Estrogen mimics produce biological effects by binding to the Estrogen Receptor α and regulating gene transcription.
- **Overexpressed in 75% of breast cancer patients**
- **ER+ tumors are treated with drugs that block the ERα**



Some breast cancers are *HER2+*. These cancer cells overexpress the protein *H*uman *E*pidermal growth factor *R*eceptor 2, which is a receptor that can stimulate cell growth upon activation.
- **Overexpressed in 25% of breast cancer patients**

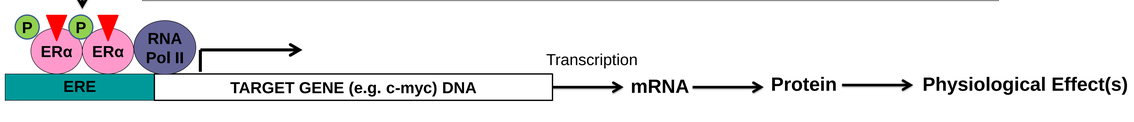


HER2 Activation

Modify? P

Do ERα and HER2 interact in response to xenoestrogens?

Hypothesis: The potency of xenoestrogens could be enhanced in the presence of HER2 activators.

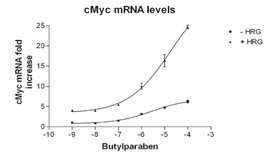


Experimental Results

Background:
- C-myc is a gene that is targeted for expression by activated ERα
- The production of c-myc protein leads to cell proliferation
- HER2 activation by Heregulin modifies ERα by phosphorylating serine 167 of ERα
- We used BT-474 cells and MCF-7 cells

Cell Type	ERα Expression	HER2 Expression
MCF-7	High	Normal
BT-474	Normal	High

C-myc mRNA levels **DNA → mRNA → Protein → Effect**

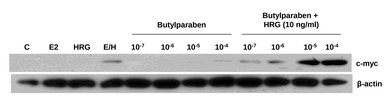


Heregulin (HRG) and butylparaben (BP) produce a synergistic stimulation of c-myc mRNA in BT-474 breast cancer cells.

C-myc protein levels **DNA → mRNA → Protein → Effect**
- **C-myc protein encourages cell proliferation**
- **In actively proliferating cells, there are high levels of c-myc**
- **C-myc is a good *indicator* of cell proliferation**

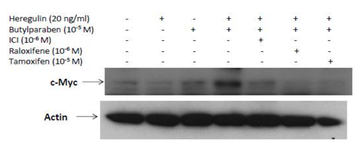
BT-474 cells (ER+)

MCF-7 cells (ER+)



SKBR3 cells (ER-)

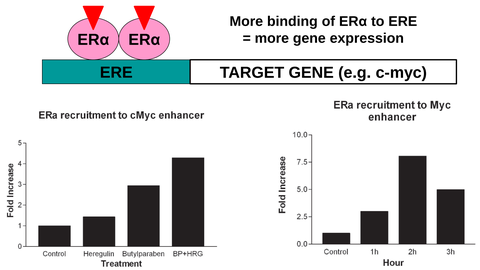
HRG and butylparaben produce a synergistic stimulation of c-myc protein in ER+, but not ER-, breast cancer cells.



The synergistic effects of HRG and BP on c-myc protein are blocked by ERα antagonists.

Chromatin Immunoprecipitation

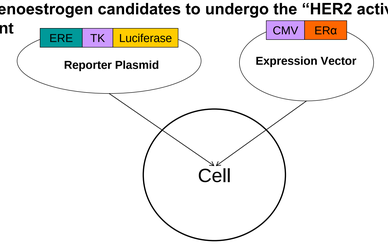
- **Is the increase in c-myc mRNA/protein due to HER2-activated ERα?**
  - **If so, we suspect that, upon HER2 activation, more ERα is bound to the c-myc promoter/enhancer region than in inactivated conditions, thus increasing c-myc gene transcription and translation**



Chromatin immunoprecipitation showing ERα recruitment to c-myc enhancer region

Conclusion and Future Directions

What does this mean in terms of human health concern?
- **Heregulin/HER2 activation modulates ERα response to estradiol and xenoestrogens**
- ***In vivo*, cells are exposed to heregulin 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, in the presence of HER2 activators, be greater than what 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 ERα 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 ERα, 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 ERα activated = the more estrogenic the compound.**