Webinar

Reducing breast cancer risk by eliminating parabens and phthalates

Featuring Dr. William Goodson

Reduction of daily-use parabens and phthalates reverses accumulation of cancer-associated phenotypes within disease-free breast tissue of study subjects

Shanaz Dairkee, Dan Moore, Gloria Luciani, Nicole Anderle, Roy Gerona, Karina Ky, Smantha Torres, Polly Marshall,

William H Goodson III

www.healthandenvironment.org Webinar

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We thank:

- Many women who participated in this and the previous studies that are the basis for this work

- The volunteers of Breast Cancer Over Time

- California Breast Cancer Research Program

- Clarence Heller Foundation

- The hundreds of *survivors* and their families who donated through the CPMC Foundation to maintain the research team (SHD, DHM, and WHG) making this and related projects possible.

Gene expression: genes are expressed when new RNA carries their message to ribosomes
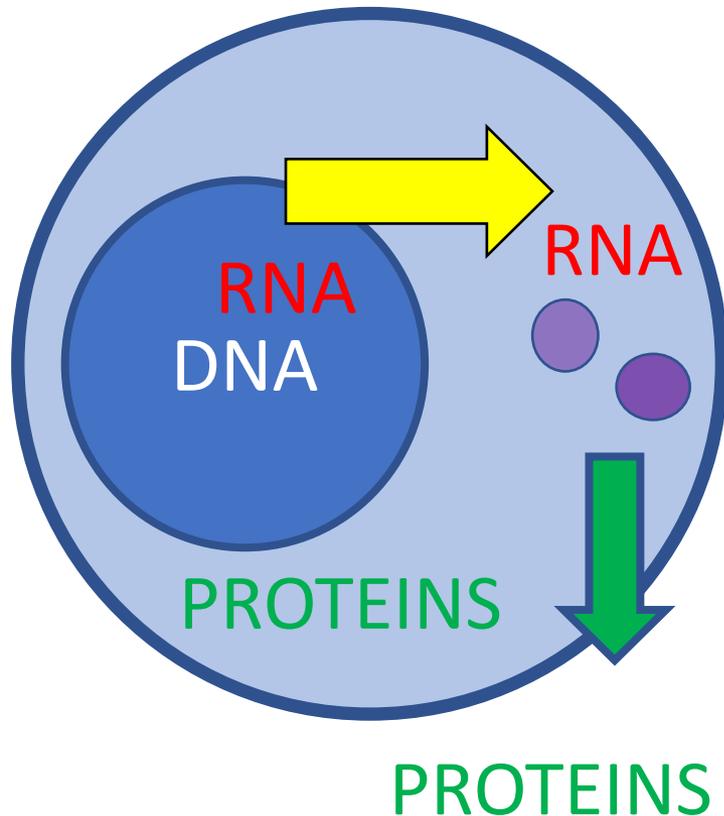
RNA is the messenger that carries the information from the DNA in the nucleus, to a ribosome ⬤ in the cytoplasm, where a new protein is made. That gene is expressed.

Measuring RNA tells us what genes are expressed. We can tell what a cell is doing by measuring its RNA.

RNA

RNA
DNA

PROTEINS

PROTEINS

Measuring RNA tells what genes are <u>expressed</u>.
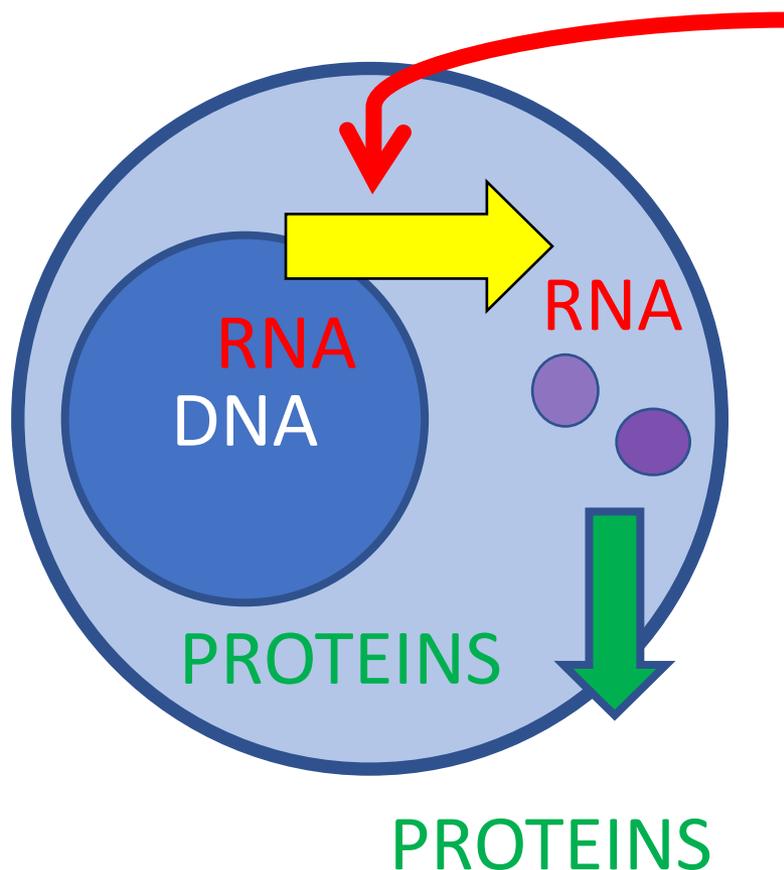
We measured RNA levels before and after withdrawal of usual personal care products (PCPs).

PCPs change gene expression, and thus change how cells function.

Takeaways from this study

Reduced exposure to phthalates and parabens from personal care products (PCPs):

- Shifted balance of gene expression away from cancer
- Redirected intact cells away from behavior that is typical of malignant cells

Note: The study is awash in double negatives:

reduced exposure x reduced risky genes = positive outcome

Why This Study is Important

- Addresses the problem of increasing breast cancer

- Challenges current assumptions about safety assessment

- Demonstrates the need for a more comprehensive experimental database

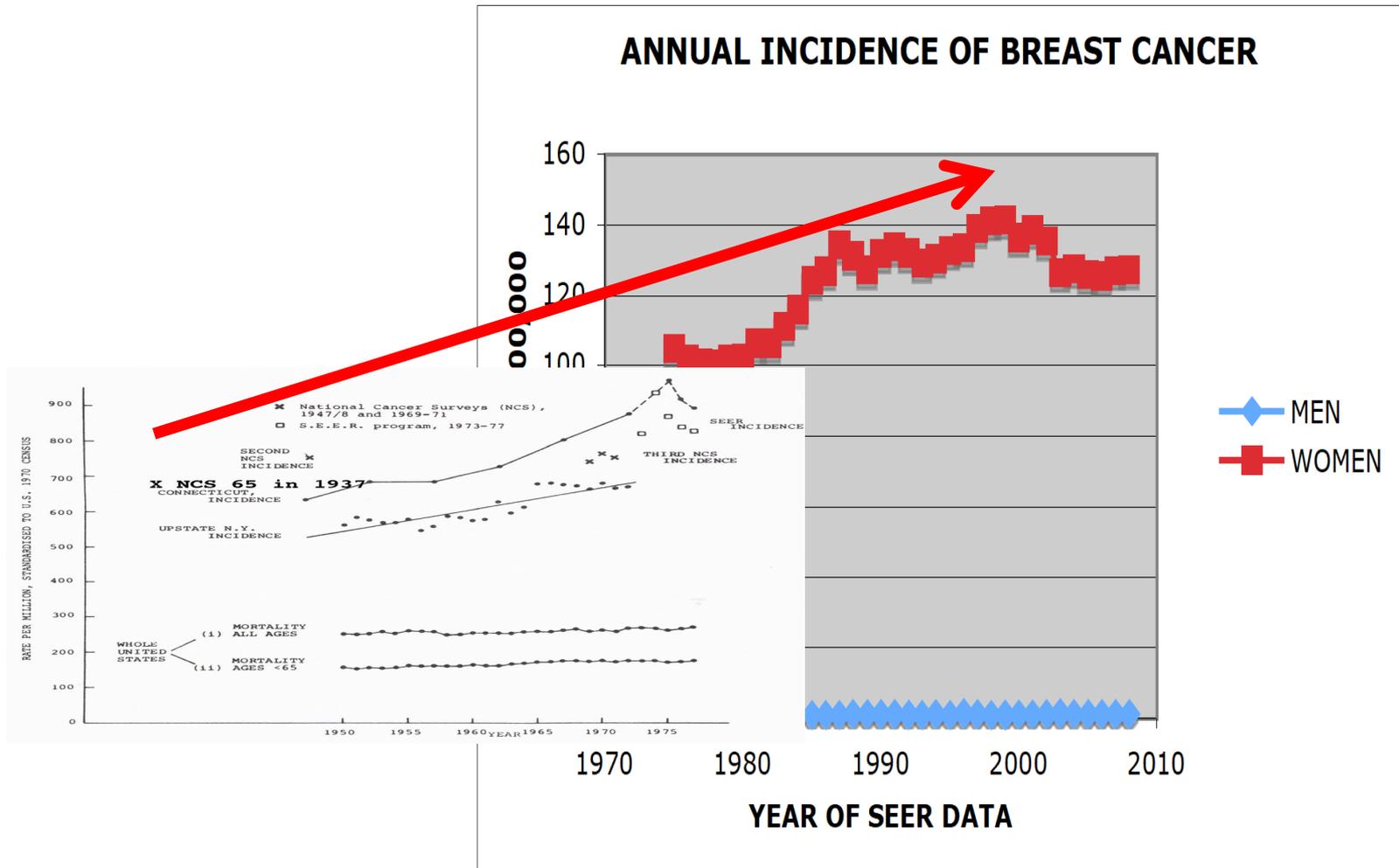- Proves feasibility of evaluating chemicals in human volunteers

  (Complements, does not replace, laboratory and animal studies)
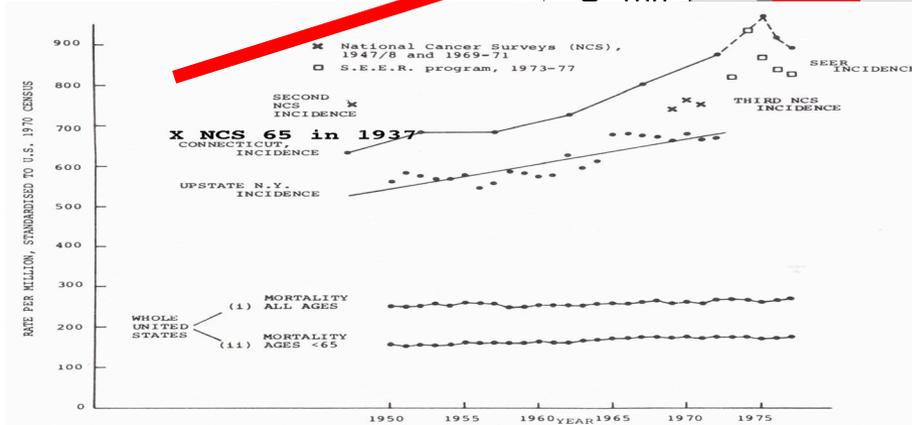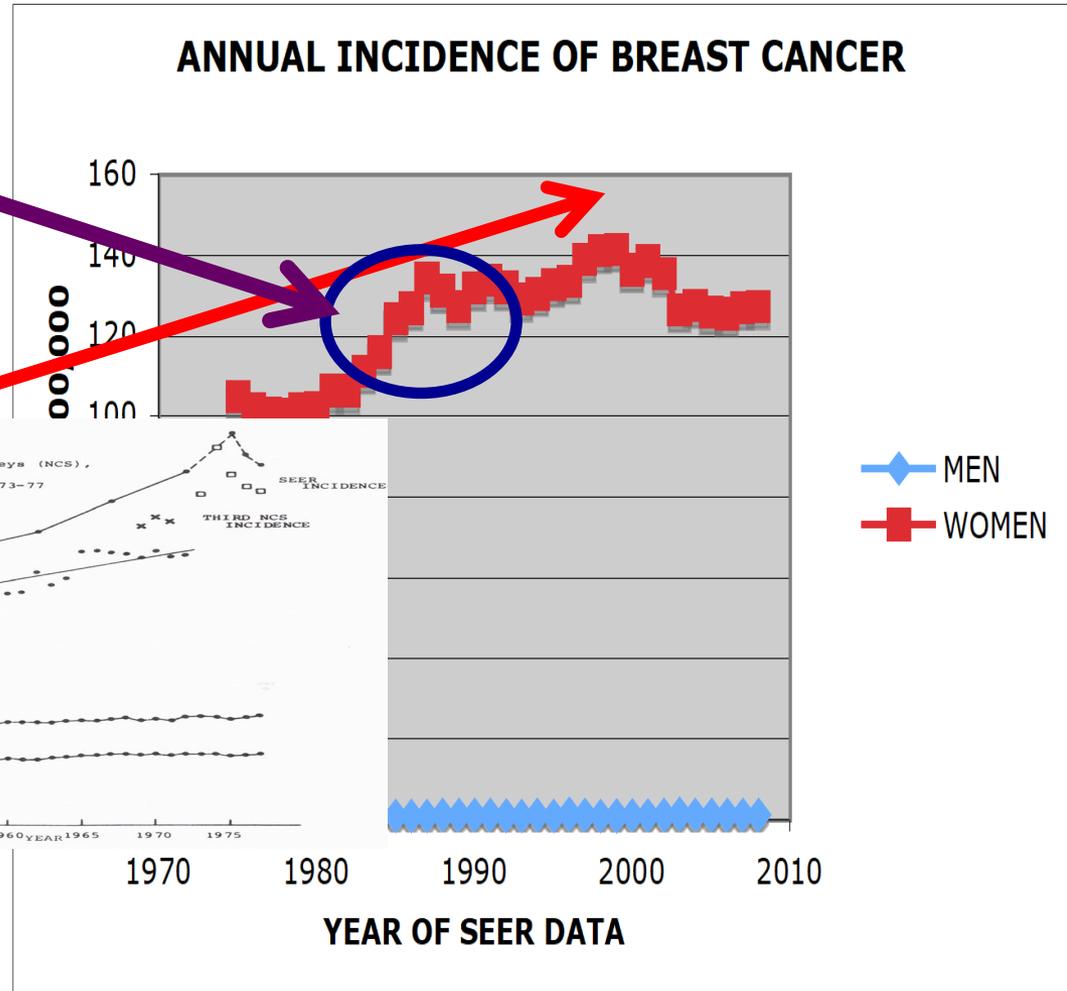
Increasing breast cancer since 1937
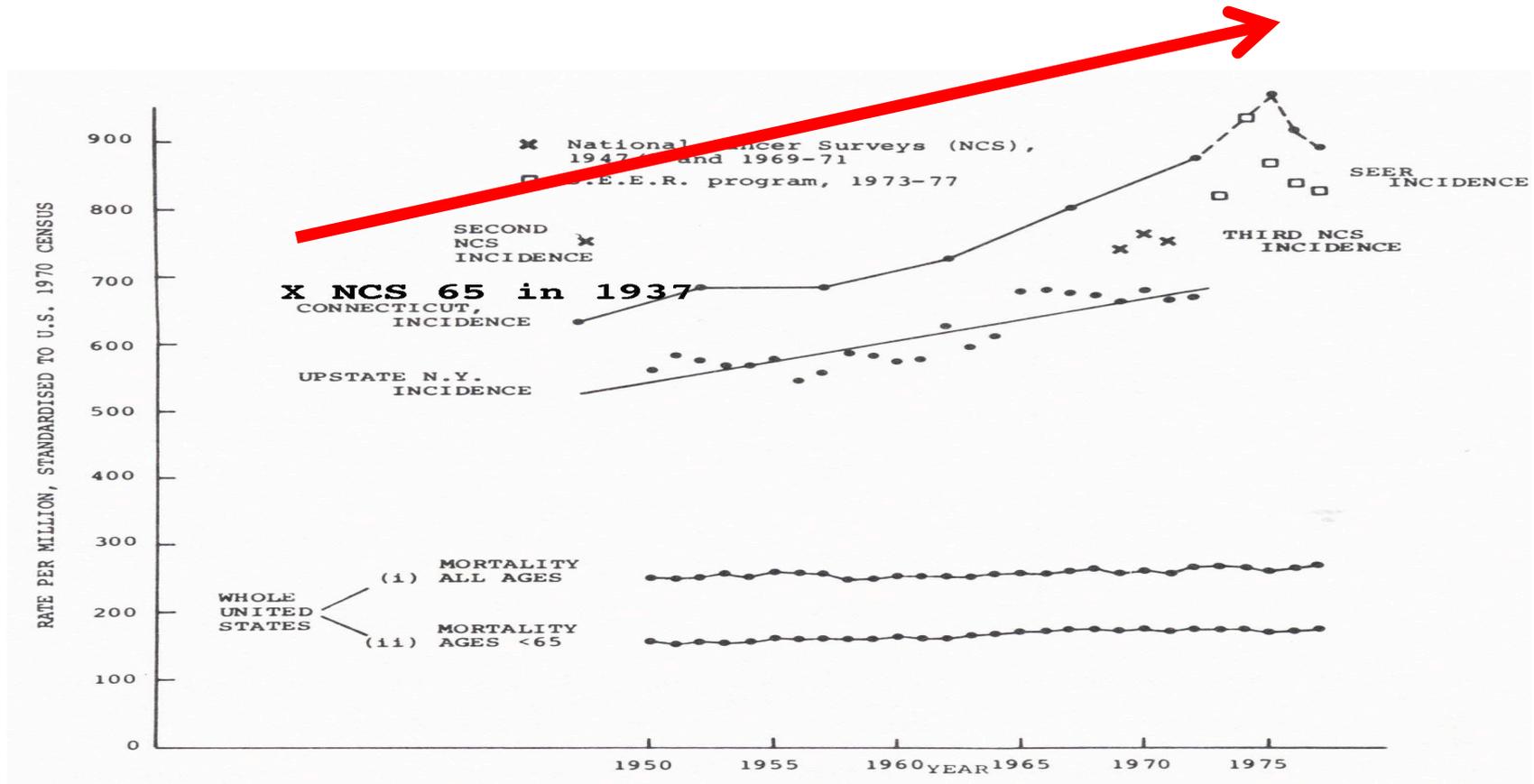
Doll & Peto, Oxford Monographs 1981

Increasing invasive breast cancer since 1937

Increasing invasive breast cancer since 1937

Increased Mammograms

ANNUAL INCIDENCE OF BREAST CANCER

160
140
120
100

MEN
WOMEN

1970   1980   1990   2000   2010

YEAR OF SEER DATA

National Cancer Surveys (NCS), 1947/8 and 1969-71
S.E.E.R. program, 1973-77

SECOND NCS INCIDENCE

THIRD NCS INCIDENCE

SEER INCIDENCE

X NCS 65 in 1937
CONNECTICUT, INCIDENCE

UPSTATE N.Y. INCIDENCE

RATE PER MILLION, STANDARDISED TO U.S. 1970 CENSUS

900
800
700
600
500
400
300
200
100

MORTALITY
(i) ALL AGES

WHOLE UNITED STATES

MORTALITY
(ii) AGES <65

1950   1955   1960   1965   1970   1975
YEAR

Increased breast CA ≤39 yo

Cathcart-Rake, et, JCO Oncology Practice, 2021

Look for other reasons for increased breast cancer because…

- Delayed childbirth and oral contraceptives around for longer than just the past decade

- Increase began 30 years before widespread mammograms

- BRCA carriers get breast cancer younger

- Increase in women under <40 years old who do not have screening mammograms

Phthalates and parabens are ubiquitous suspects

- Phthalates and parabens are xenoestrogens (XEs, chemicals that are not estrogen, but act like estrogen)

- Common in PERSONAL CARE PRODUCTS (PCPs)

- Phthalate and paraben excretion correlates with PCP use. i.e., can monitor exposure by urine testing

- There are other XE exposures from air such as food, food packaging, house dust, fireproofing, stain resistant materials, etc.

Background for this study

- Philippa Darbre (2008, UK) measured concentrations of phthalates and parabens in human breast tissue from mastectomy specimens and demonstrated that a mixture of these XEs - in the <u>concentrations measured in breast tissue</u> promoted growth of MCF7 cancer cells in culture

- Carol Fabian (2010, Kansas) and Seema Khan (2012, Northwestern) compared before and after RPFNAs to evaluate interventions

- SHD, DHM, WHG 10+ years analyzing RPFNAs

- Hermosa Study (2015, Salinas, CA) high school girls reduced urinary excretion of XEs by changing cosmetics for 3 days

Reduced Xenoestrogen Exposure (REDUXE)

- Screened volunteers to determine whether at least one daily-use PCP contained a phthalate or paraben (or fragrance as a surrogate)

- Crossover trial with IRB approval to randomize 60 subjects to REDUXE or control first 28 days with reverse for second 28 days (assuming 70% successful cell growth)

- REDUXE intervention subjects provided a 30 day supply of phthalate and paraben-free PCPs identified from the Environmental Working Group website www.ewg.org

Measured effects of REDUXE with random periareolar fine needle aspirations (RPFNA)

- RPFNA is a needle biopsy of the breast using a very small needle (less than a reverse flu shot)

- Obtained breast samples before and after REDUXE

- Local Anesthesia, <u>no attrition!</u>

- Cytology confirmed no atypical or cancerous cells

- Expanded/ grew cells to sufficient numbers to measure RNA and cell behavior

Indices of compliance

- Urine and blood testing before and after REDUXE

- Only REDUXE subjects had significant decrease 4 cosmetic-associated parabens (MP,EP,PP,BP) and 1 cosmetic-associated phthalate metabolite (DEHP metabolite MEHP)

- No decrease in common phthalates from food, so no evidence that volunteers changed other lifestyle exposures such as food

- No change estrogen, progesterone, sex hormone binding globulin

Along came COVID…

- Started accrual 2019 of planned 60 volunteer subjects
- Halted by COVID March 2020 with n=14 successful pairs, skewed toward REDUXE 12:2
- Statistical double-check
- Validate with parallel changes in whole, living cells

We measured RNA to find what genes are expressed

RNA

DNA

RNA

PROTEINS

PROTEINS

?
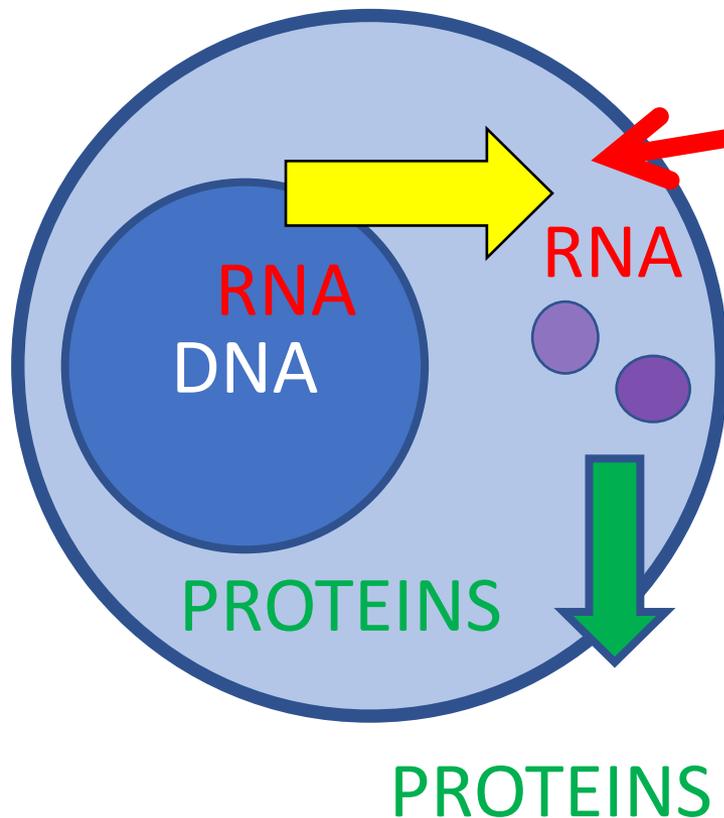
Determine which genes are differentially expressed by measuring RNA of 567 genes.

Differentially Expressed Genes (DEGs)

- For all 14 subjects measured RNA in RPFNA 1 and RPFNA 2.

- Calculated the ratio of RNA in RPFNA 2 *versus* RNA in RPFNA 1, 567 genes (log 2 of the ratio)
  - If RPFNA2 > RPFNA 1, ratio >1, Log 2 > 0, and *vice versa*

- Compare log ratios of RPFNA 2: RPFNA 1 for REDUXE *versus* control subject for each gene with t-test.

- Using $p<0.01$ as cutoff for a DEG, for 567 genes, expect 6 DEGs by random chance.

- First cut, n=64 DEGs among the 567 tested

Refining gene selection

- Used leave-out-one random deletion to see if remained significant, kept a gene only if did not lose significance with removal of one subject. n=54

- Repeatedly reassigned the 14 gene profiles randomly as 12 treated and 2 controls to determine whether genes called significant under correct assignment could have arisen by chance. Only kept genes where no random rearrangements were significant. n=26

- 18 genes were decreased relative to controls and 8 increased relative to controls

- 18 of 26 (69%) DEGs shifted toward "normal" by REDUXE; 8 shifted toward more problematic

Overall DEGs

n=18, EXPRESSION DECREASED AFTER REDUXE

LOWER is better	HIGHER would have been better	
B2M	CDKN2B	
HHEX	C190rf49 (FAAP24)	
IRS1	DKK1	
ITGB4	SPRY2	
MAP2K4		
MNAT1		
MYD88		
PIK3CA		
PLAU		
POLB		
PPP2CB		
SE3B1		
TLR4		
TNFRSF10C		

n=8, EXPRESSION INCREASED AFTER REDUXE

HIGHER is better	LOWER would have been better	
DDIT4	MCM7	UNCLEAR
MLLT4	NRAS	COL4A5
MSH2	NUPR1	
SMAD4		

Pathways affected by DEGs
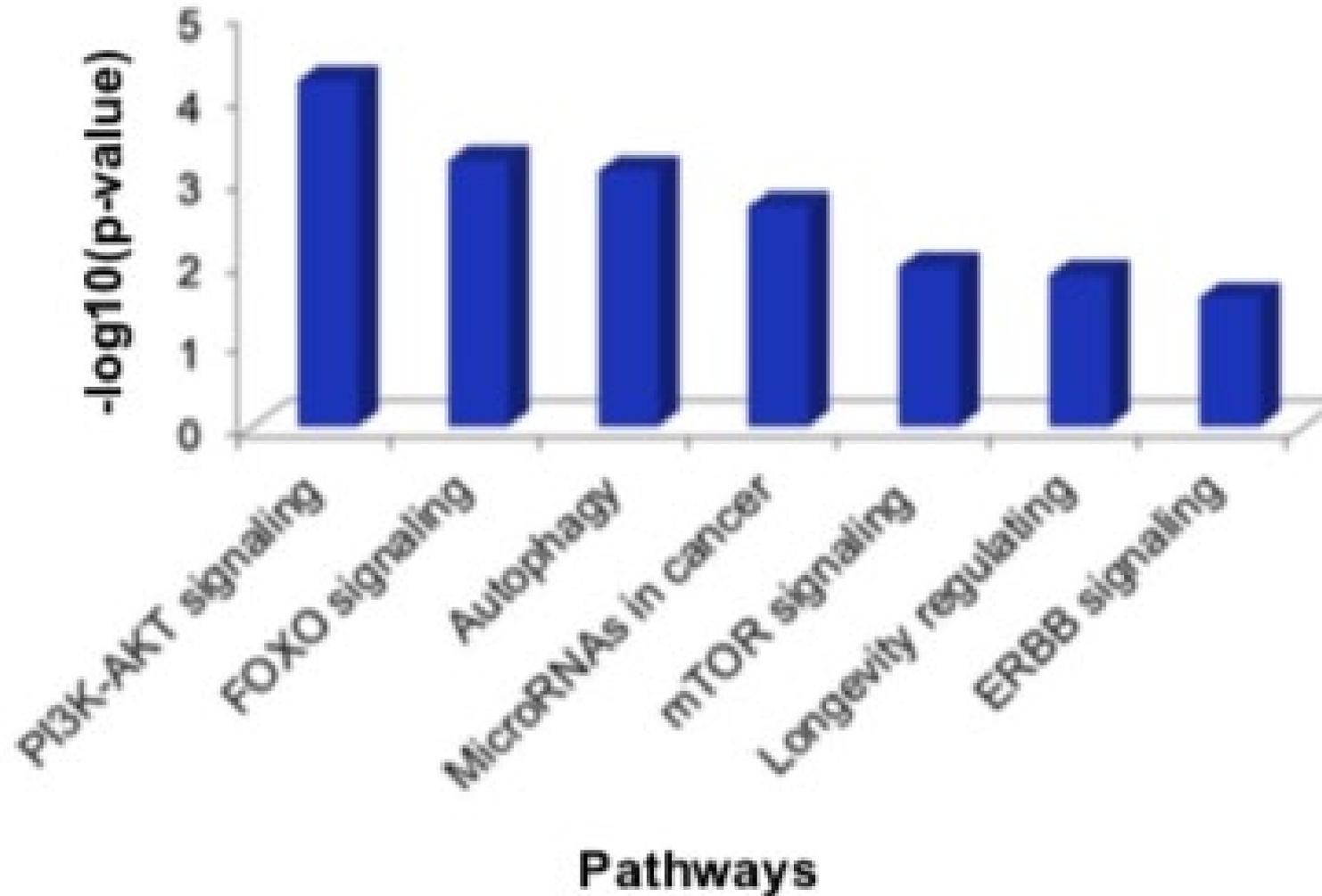
For example, 8 DEGs in PI3K-AKT pathway

- REDUCED GENE EXPRESSION
  - IRS1   Promotes cancer
  - ITGB4   Promotes cancer
  - PIK3CA   Promotes cancer
  - PPP2CB   Promotes cancer
  - TLR4   Drives BRCA when mutant P53

- INCREASED GENE EXPRESSION
  - COL4A5   MMP, may promote invasion
  - DDIT4   Suppresses cancer
  - NRAS   Drives breast cancer

How do we know overall effects of the balance of good or bad changes in gene expression?

Evaluate the behavior of intact, whole cells.

Increased Estrogen Receptor-β post REDUXE in whole cells

- There are two estrogen receptors; typically more ER-α in breast cancer

- ER-β is typically lost in transition to malignancy

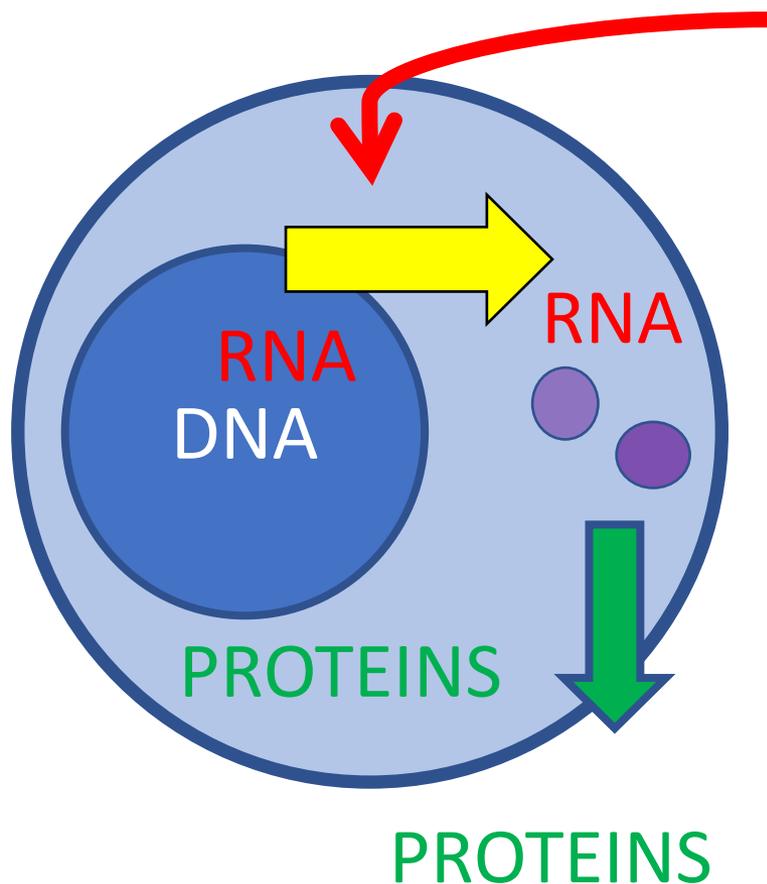- Benign cells exposed to XEs lose ER-β
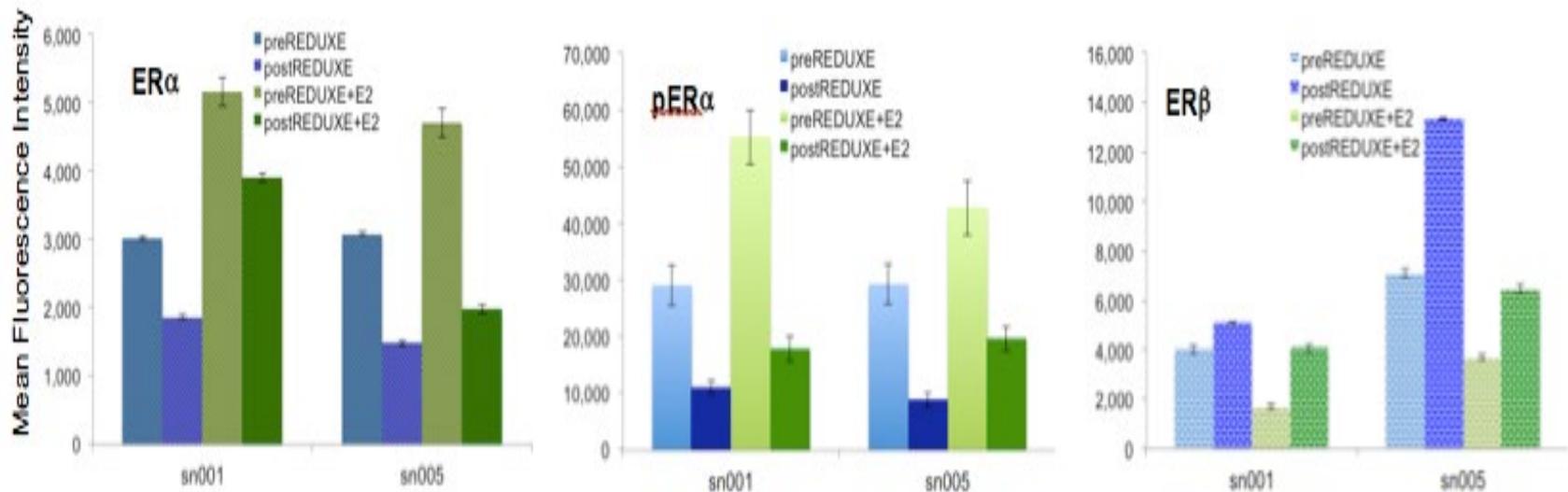
After REDUXE, increased *apoptosis* (programmed cell death)

- Normal breast cells self-destruct when trigger programmed cell death signal, e.g. with tamoxifen
- Loss of Apoptosis is a Hallmark of Cancer
- Benign cells exposed to XEs lose apoptosis response
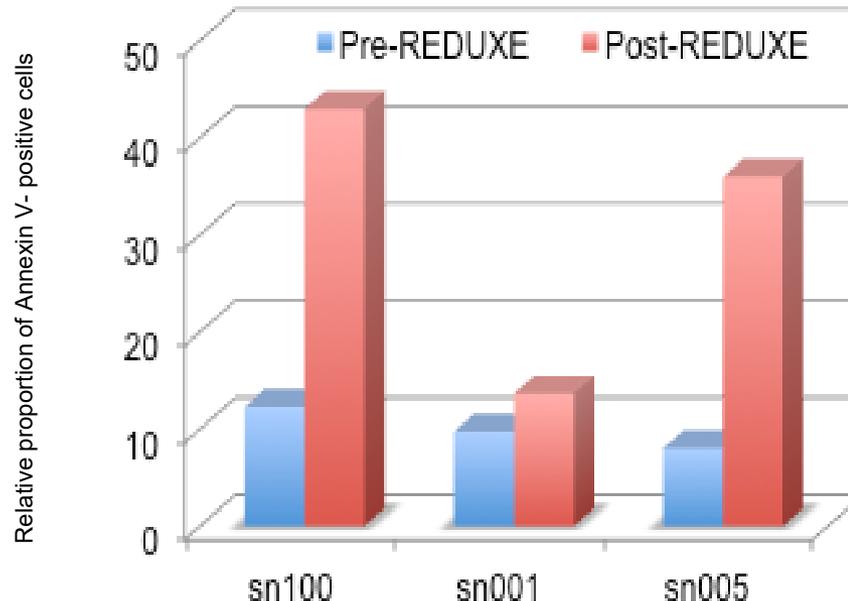
Personal care products change breast cells

Reducing exposure to chemicals absorbed from personal care products

1. Can change gene expression; and

2. Can change cell function.

Limitations of this study

- Small numbers
- Skewed randomization (however, intact, whole cell assays support conclusions)
- Needs repetition
- Like other gene studies - with the exception of a few disease-specific, deleterious mutations - we cannot predict cell behavior from changed gene expression
- We need much more data on how chemicals affect cell behavior

What this study is...

- Randomized Controlled Trial:
  - REDUXE shifted balance of gene expression away from cancer-enhancing pathways
  - Despite limitations, conclusions are supported since REDUXE also shifted behavior of whole cells toward behavior typical of non-malignant cells
- This is currently the *ONLY* human study out there, and we found that chemicals in PCPS adversely effect breast tisssue and that is not what anyone would want.

Thank you

whg3md@att.net