The impact of mixed estrogenic chemicals on non-malignant cell function

Shanaz H. Dairkee, PhD
California Pacific Medical Center Research Institute, San Francisco, CA
dairkes@cpmcri.org

May 20, 2020
Population-based Alternatives to Routine In Vitro Models

Random Periareolar Fine Needle Aspirates (RPFNA)

HRBECs  High-Risk donor derived Breast Epithelial Cells
ARBECs  Average-Risk donor derived Breast Epithelial Cells

Study approved by CPMC Institutional Review Board
Prior written consent obtained from donors
Overexposure to natural estrogens is strongly associated with breast cancer. Exposure to persistent synthetic estrogen mimics, also known as xenoestrogens (XEs) is thus potentially carcinogenic.

A causal role for XE exposure in breast cancer progression will be revealed by employing test systems representative of carcinogen-targeted healthy epithelial cells in the human breast - the cells it is hoped will not become malignant.

A finite in vitro life span of such healthy human cells is not a barrier for experimentation. Instead, by sampling a wide spectrum of individuals, the limitations of data collection from rare immortalized cancer cell lines will be surmounted.

Improvements in key parameters of breast carcinogen screening assays will advance breast cancer prevention.
RPFNA-derived non-malignant breast epithelial cells

Cytopathology  3D phenotype in vitro
Expansion *in vitro*

Fresh sample

7-d *in vitro* colony
Functional heterogeneity

[Graph showing % Apoptosis for HRBECs and ARBECs]
Maintenance of estrogen receptor (ER) expression

<table>
<thead>
<tr>
<th></th>
<th>T47D</th>
<th>SKBR3</th>
<th>MCF7</th>
<th>PA115</th>
<th>PA024</th>
<th>PA025</th>
<th>PA134</th>
<th>PA135</th>
<th>PA136</th>
<th>PA138</th>
<th>PA139</th>
<th>PA140</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERα</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERβ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cancer - Non-malignant

Spontaneous immortalization - Primary finite-life
RPFNA Data Acquisition Flow Chart

1. Expansion of minimal numbers of live breast cells in samples collected from consented donors for *in vitro* chemical exposure.

2. Treatment with environmental chemicals of human relevance at a concentration range detected in body fluids and tissues.


4. Validation of exposure effects, singly and as mixtures, across additional population-based samples.

**Further applications**
- Surrogate biomarkers of functional perturbations
- Reversal of chemically-induced perturbations
Bisphenol A Induces a Profile of Tumor Aggressiveness in High-Risk Cells from Breast Cancer Patients

Shanaz H. Dairkee,1 Junhee Seok,2 Stacey Champion,1 Aejaz Sayeed,1 Michael Mindrinos,2 Wenzhong Xiao,2 Ronald W. Davis,2 and William H. Goodson1

BPA signature and clinical outcome
BPA deregulates mTOR pathway of cell survival in non-malignant breast cells

Dairkee et al., Cancer Research 68 (7):2076, 2008
Goodson et al., Carcinogenesis 32 (11):1724, 2011
BPA obliterates normal limit of cellular lifespan in non-malignant breast cells

Dairkee et al., *Carcinogenesis* 34 (3):703, 2013
Mixture treatment of non-malignant breast epithelial cells

• Three high volume estrogenic chemicals: bisphenol A (BPA), methyl paraben (MP), and perfluorooctanoic acid (PFOA), were selected within the environmentally relevant concentration range of 1-10nM. Additionally, 10-fold higher exposure levels were also studied.

• We measured treatment effects upon total ERα, and ERβ based on their contrasting roles in cell cycle regulation. Additionally, we measured activated ERα phosphorylated at serine-118.

• Direct downstream consequences of effects on ER isoforms were assayed as the S-phase fraction of the cell cycle, and the proportion of cells that evaded experimentally induced apoptosis.
Mixture vs. single components
Modulation of ER isoforms

Dairkee et al., *Toxicological Sciences* 165:131-144, 2018
Mixture vs. single components

S-phase induction

Anti-BrdU, FITC

DNA content, PI

Control 16.03±0.69

E2-I 34.1±0.62

XE Mix-I 52.38±0.02

BPA-I 30.28±0.91

MP-I 30.78±1.86

PFOA-I 44.07±0.07
Mixture vs. single components

Increased rate of cell proliferation

Viable cells (% of control)

Hours of exposure

Low

- MIX
- BPA (p=0.01)
- MP (p=0.02)
- PFOA (p=0.04)
- E2

Intermediate

- MIX
- BPA (p=0.02)
- MP (p=0.03)
- PFOA (p=0.28)
- E2

High

- MIX
- BPA (p=0.01)
- MP (p=0.05)
- PFOA (p=0.3)
- E2
Mixture vs. single components

Programmed cell death evasion

Increase in Annexin-FITC post 24-h tamoxifen
Differential mixture effects on non-malignant vs. breast cancer cells

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Apoptosis</th>
<th>ERβ</th>
<th>ERα</th>
<th>pERαS118</th>
<th>S-phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>T47D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCF7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA231</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Concentration**

**Fold-change relative to untreated control**
Conclusions

• The RPFNA-derived non-malignant breast cell model is as close as is ethically possible to carcinogen-targeted cells within human breast tissue.

• Some functional endpoints are more readily perturbed in chemically-exposed benign cells than in malignant cells. Testing cancer cell lines alone can miss important dysfunctional events.

• Unlike generalized one-size-fits-all screening schemes, RPFNA samples allow a direct test of population variability in regard to complex issues, such as perturbations induced by chemical mixtures.
THANKS!

Colleagues
Bill Goodson, M.D.
Ian Jaffee, M.D.
Dan Moore, Ph.D.
Gloria Luciani, Ph.D.
Aejaz Sayeed, Ph.D.
Tri Lu, B.S.

Funders
CBCRP Award # 12IB-0115, 17UB-8702
Clarence Heller Charitable Foundation
CPMC Foundation