Cellular Signaling Mechanisms by which Xenoestrogen Pollutants Disrupt Normal Estrogenic Signaling

utmb Health

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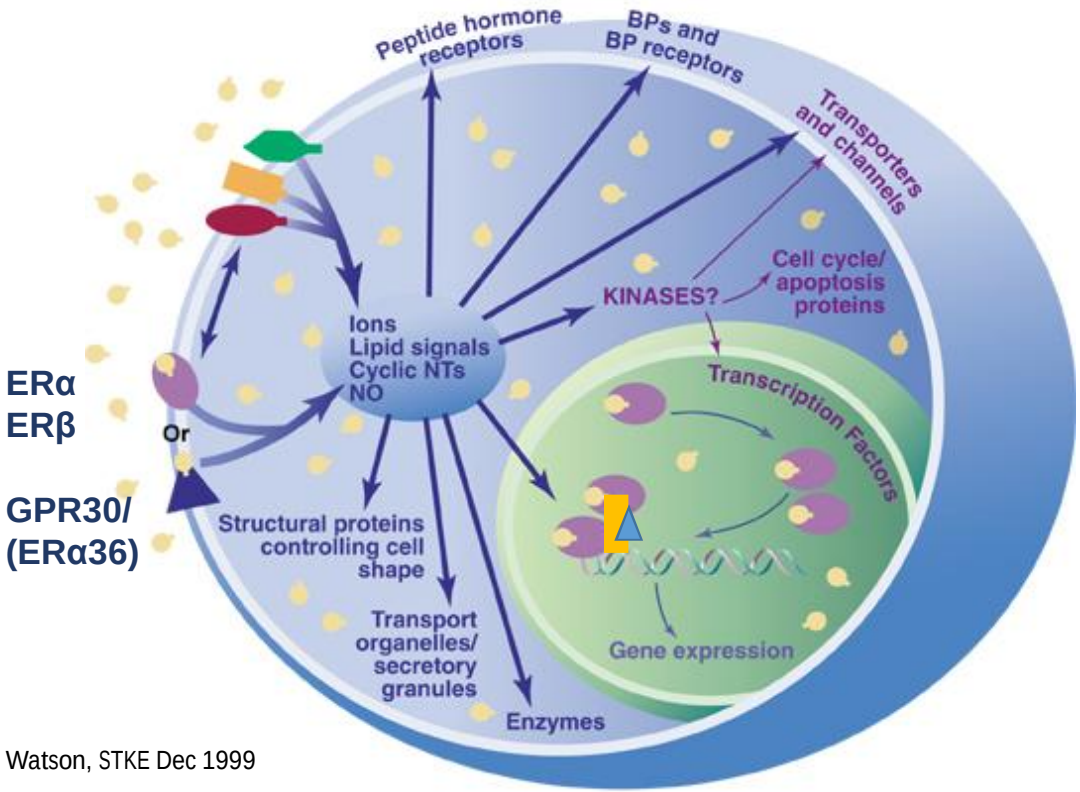
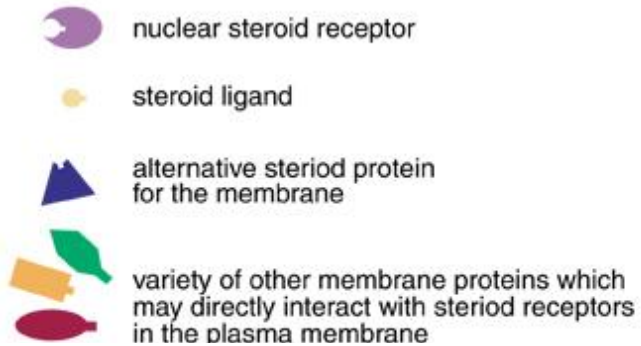
How do estrogens of all types signal at the cell?



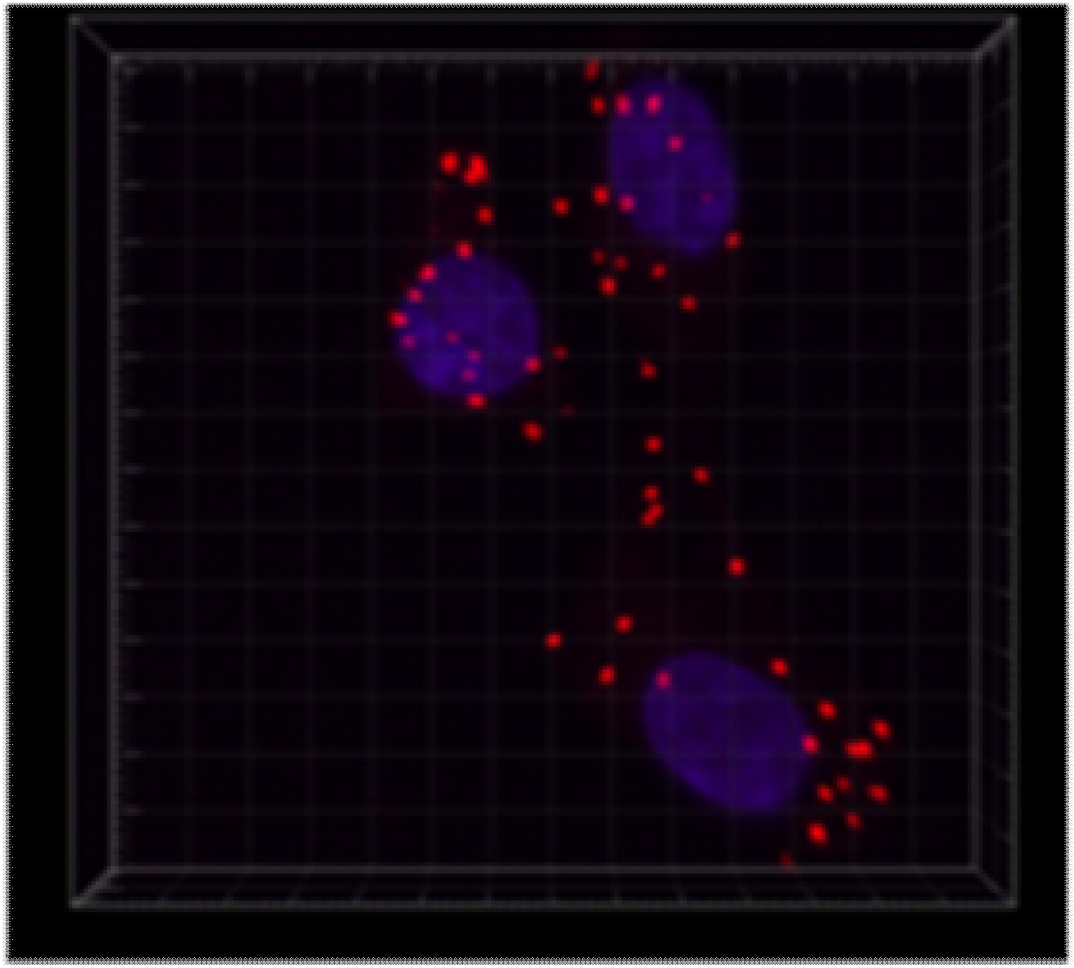
Watson, STKE Dec 1999

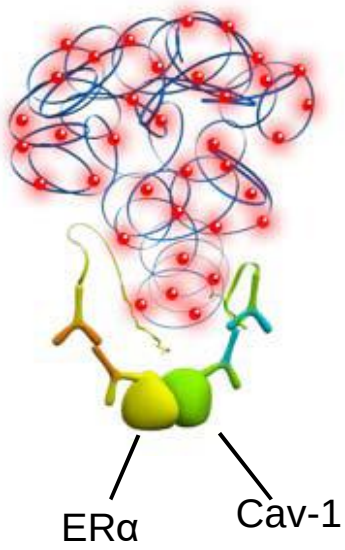


- From the **nucleus** (classical genomic mechanism)
- From the **membrane** (novel, rapid, nonclassical, nongenomic mechanism)

Proteins "moonlight" (do different jobs in different locations in the cell and in different tissues), and therefore partner with a lot of different proteins in those alternative locations

We work on membrane estrogen receptors. What do they look like? Do they partner with other known membrane proteins?





...also with Gαi

Guangzhen Hu - pituitary

Cells/Tissues studied in the Watson lab:

mERα in a pituitary tumor cell line (GH3/B6) mediates rapid (1 min) PRL release and control of cell proliferation

mERα in breast cancer cell lines – mediates control of cell proliferation/growth inhibition

mERs in a pheochromocytoma neuronal cell line (PC-12) mediates rapid inhibition of dopamine uptake and stimulation of dopamine efflux

mERs in prostate cancer cells mediating cell killing or slower growth

Collaborative projects:

mGRs in human and rodent T lymphoma cell lines mediate glucocorticoid-induced cell death (Bahiru Gametchu)

mERα in human and rodent mast cells and lymphocytes mediating histamine and leucotriene release and epigenomic Δs in IFNγ synthesis (Terumi Midoro-Horiuti and Randy Goldblum)

mERα in hippocampal and mERß in medullary raphe cells mediates rapid inhibition of serotonin transport (Mary Thomas)

mERs in responding to xenoestrogens affecting amniotic membranes and pre-term birth (Ram Menon)

What are the health issues for estrogens (Es)

You can't live without 'em (as individuals or as a species) as they prevent disease in both ♀**s and** ♂**s**:

Reproductive failure
Bone loss
Vasomotor disturbances (hot flashes)
Some cardiovascular system vulnerabilities
Some cognitive declines, mood disorders
Skin, immune system, and metabolic aging

You can't live with 'em (too long, too much, or at inappropriate developmental windows):

Cancer (breast, uterus, colon, pituitary)
Blood clots
Nausea and eating disorders
Asthma
Obesity and other metabolic disorders

So you need a balanced and highly regulated amount of exposure……

If you have the **wrong** ones (xenoestrogens, XEs), you can have endocrine disruption of many types

So many estrogens…..so little time

We have examined >40 estrogens or antagonists so far, in at least one of our experimental systems:

Physiological: E_1, E_2, E_3, $17\alpha E_2$, and metabolites

Pharmaceutical: agonists - EE_2, DES; antagonists - ICI182700, tamoxifen, and many newer selective agonists and antagonists – which can become pollutants

Plant: coumesterol, daizein, genistein, R-equol (all from soy), trans-resveratrol (grapes), 8-prenylnaringenin (hops)

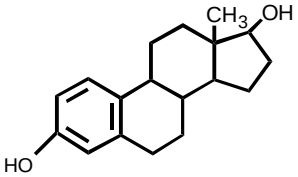
Environmental:
--surfactants, plastics and their additives and metabolites: EP, PP, OP, NP, BPA (+ chlorinated, sulfated, glucuronidated); BPS, phthalates, PCB153
--pesticides/fungicides: dieldrin, endosulfan, DDE, heptachor, tributyltin

…but there are so many more…

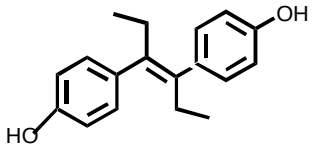
Do XEs look like physiological Es?

Small molecules ~270-400 MW

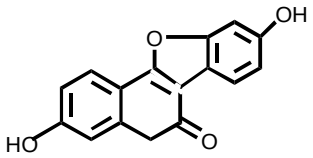
physiological estrogen
pharmaceutical estrogen
phytoestrogen
environmental estrogen



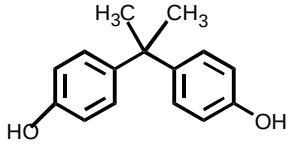
17ß estradiol (E_2)



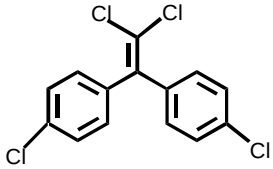
diethylstilbestrol (DES)



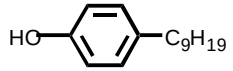
coumestrol



bisphenol A (BPA)



DDE (DDT metabolite)



Nonylphenol (NP)

…these molecules can also “twist” into similar shapes

From our collective studies thus far we know that…..

Xenoestrogens can act very potently via rapid nongenomic (non-nuclear) mechanisms and membrane ERs……unlike slower genomic actions via nuclear ERs where sensitivity to xenoestrogens appears to be very low FAST POTENT

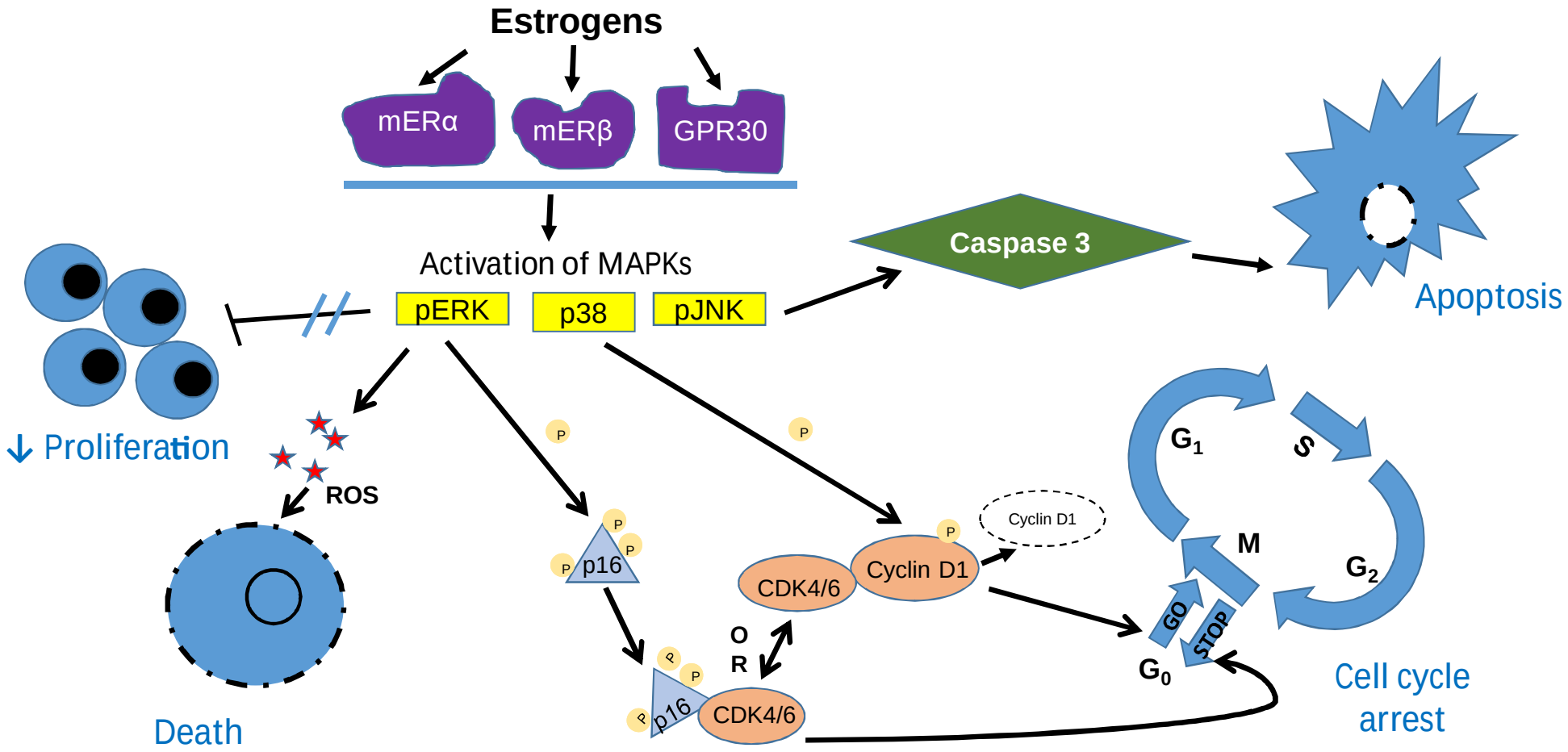
Xenoestrogens are imperfect estrogens – they do not exactly mimic endogenous estrogen actions… the signaling and functional patterns are different IMPERFECT

Xenoestrogens, like the physiological estrogens they mimic, cause non-monotonic concentration dependence (lower doses are often more effective than higher doses) UNEXPECTED DOSE PATTERN

Xenoestrogens present in mixtures (like they are in our environment) can cause much greater disruption – even completely negating or reversing the actions of a physiological estrogen MIXTURES DISRUPT

I'll show you examples of these…….

Concerns for cancer cells -- Which E signaling pathways affect cell growth, halt proliferation, or cause cell death?? ---------- here are shown the signaling pathways to ↓ cell number



Signals – these are FAST (seconds to minutes) and POTENT (down to 10^{-15}M)
These are the ones we have demonstrated.

- **2nd messengers: Ca^{++}, cAMP, ROS**
- **mitogen-activated protein kinases (MAPKs)**
- **other kinases/phosphatases-PKA; AKT; others tested -- selective by tissue) – perhaps leading to pEZH2 and methylated histones involved in epigenetic changes**
- **G protein activations via GTP binding**
- **p'ation leading to rapid degradation of cell cycle proteins**
- **caspases (programmed cell killing)**
- **downstream transcription factor post-translational modifications (P), leading to genomic effects**

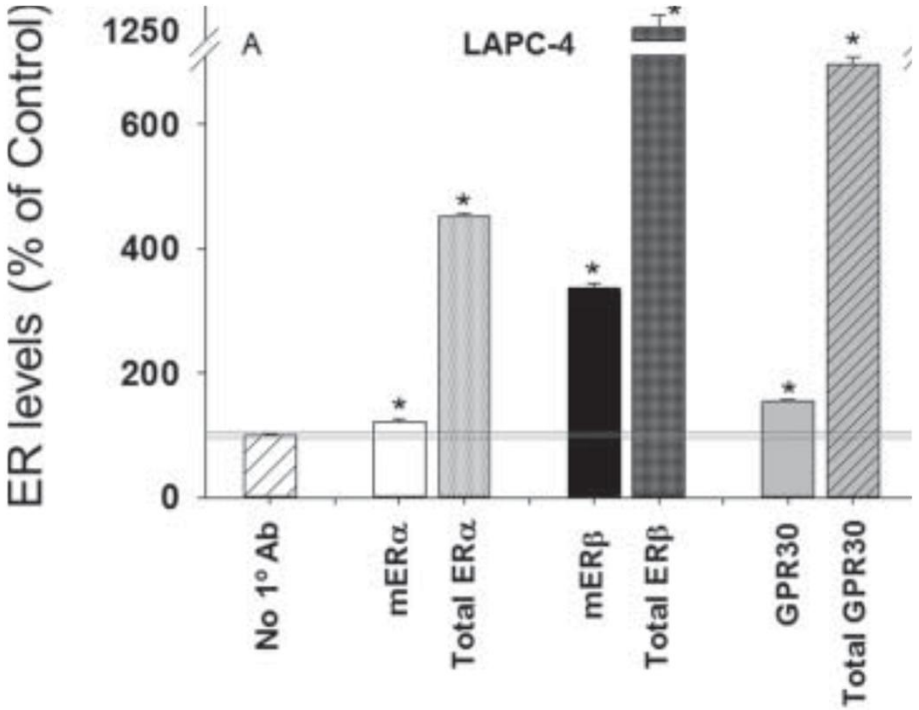
Each has been related to functional endpoints and presence of mERs

These data collectively generated by Jennifer Jeng, Nataliya Bulayeva, Gaga Zivadinovic, Ann Wozniak, Guangzhen Hu, Mikail Kochukov, Celeste Finnerty, Andrea Norfleet, Manish Saraf, Luke Koong, Rene Vinas -- Acknowledgements: These and names of other contributers listed on each slide of their data

Some examples of the principles of nongenomic actions by XEs that we have learned:

What ER subtypes could be involved?

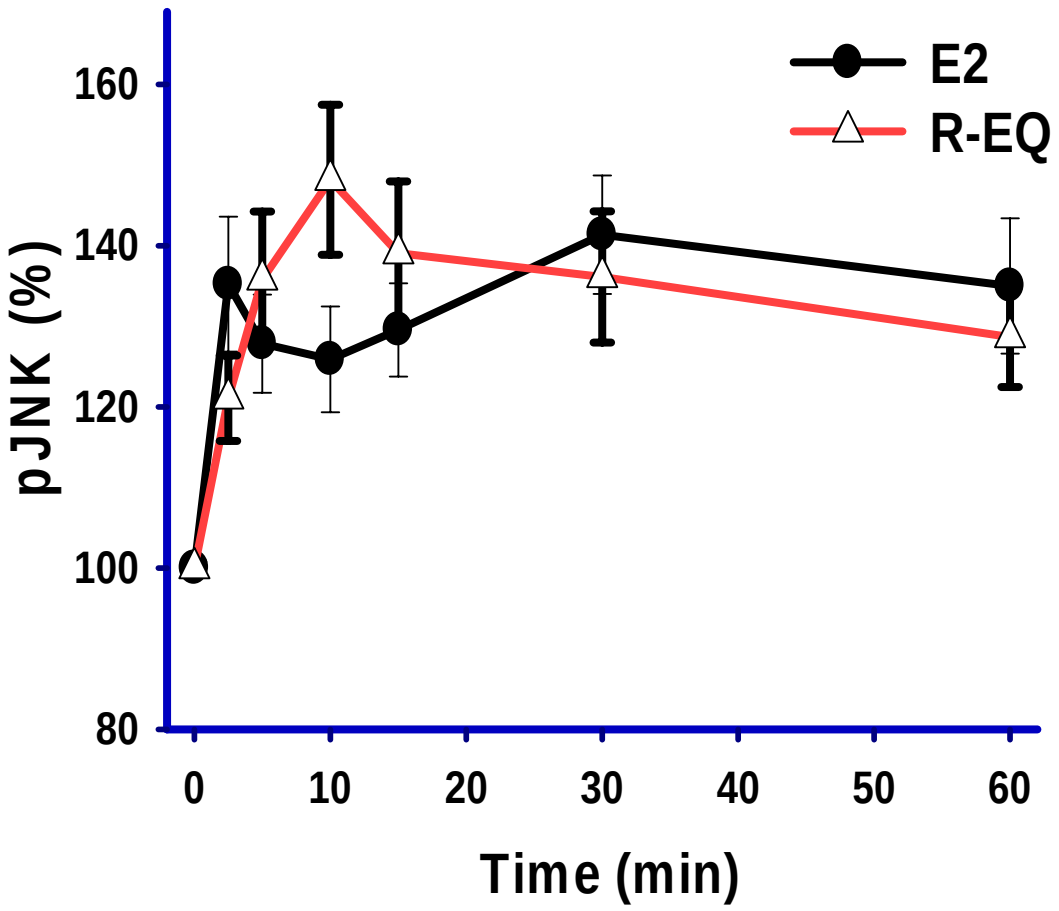
–→ <u>all three</u> (**α**, **β**, and GPR30) in both the membrane and the nucleus



Luke Koong - prostate

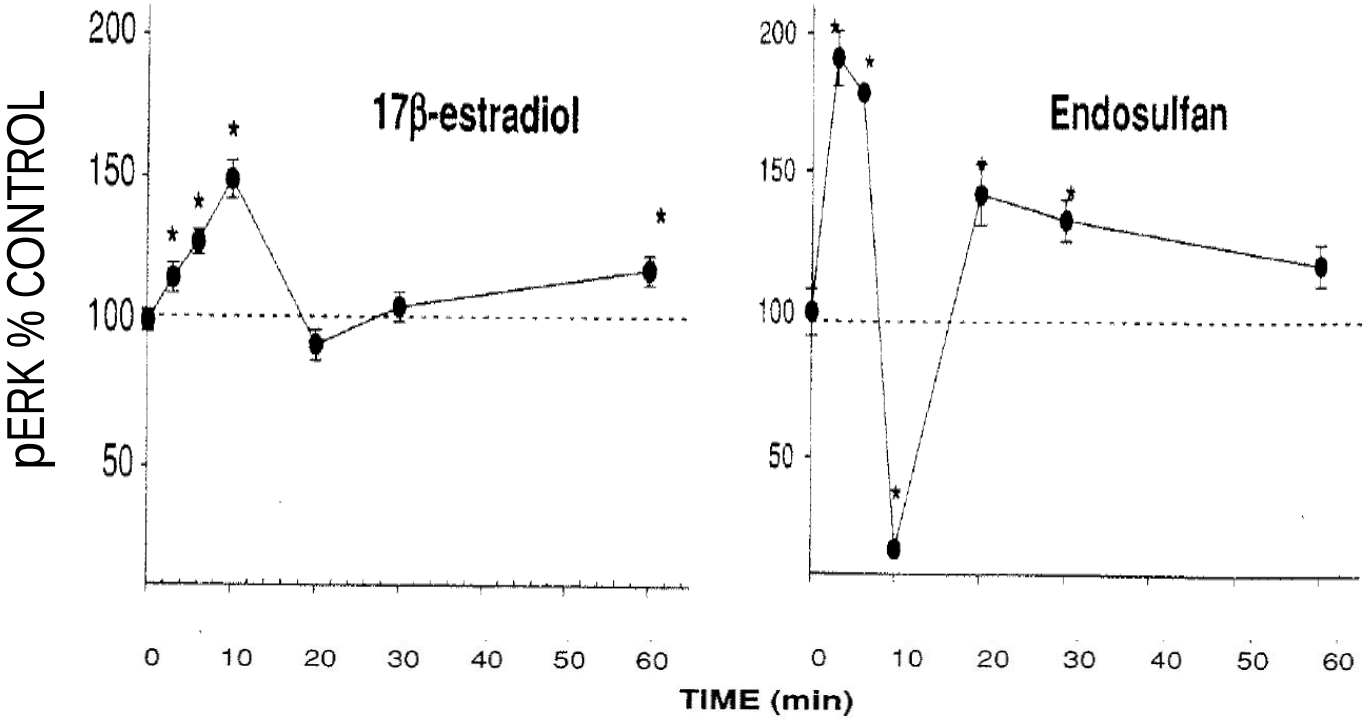
FAST

IMPERFECT



FAST

IMPERFECT



Gaga Zivadinovic – breast cancer cells

Summary table of many mechanisms expected to control cell proliferation in prostate cancer cells

Red mechanisms ↓ cell #;

Green mechanisms ↑ cell #;

Black mechanisms were activated but equivalent in all cases;

Gray, not mechanistically involved

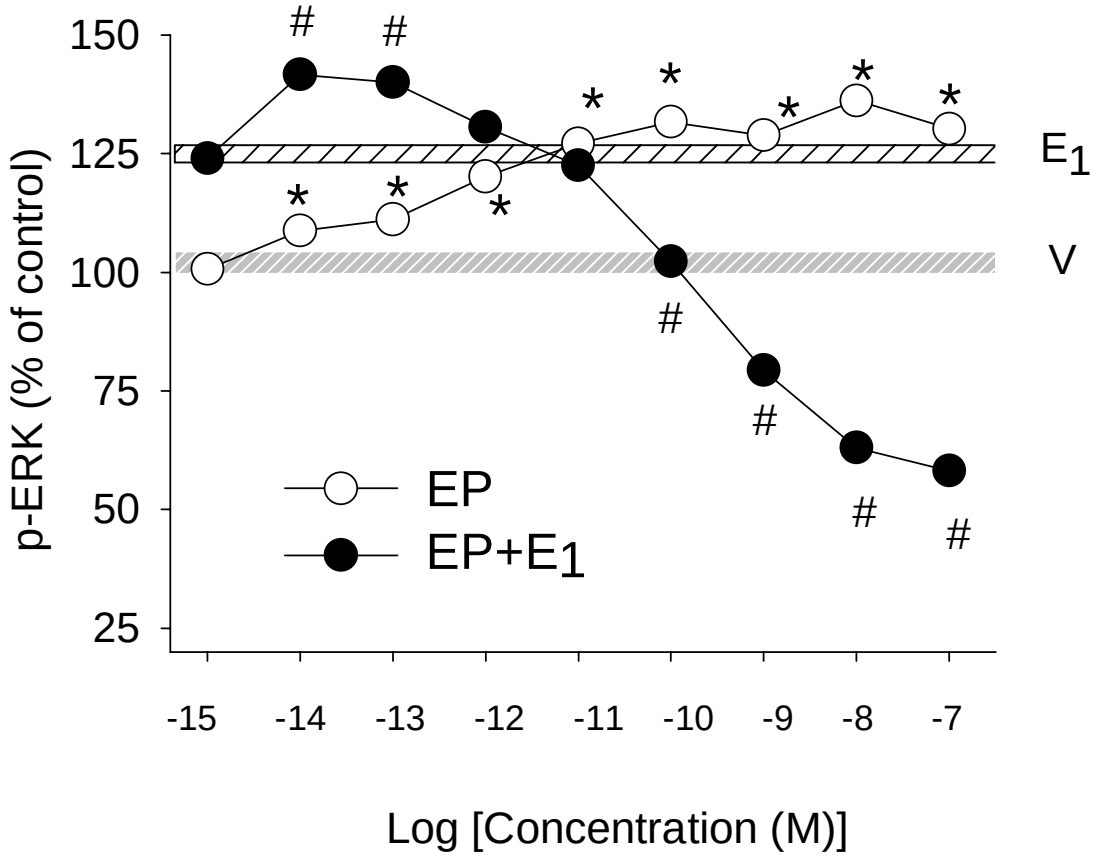
	Estradiol	Diethylstilbestrol
LAPC-4 (Early)	**↓↓ Cell Viability** **↑↑ pERK→↑ ROS via ERα, ERβ** **↓ pJNK** **↑ p-p38** **↑ Caspase 3** **6** Necroptosis **1** **↑ p-p16^{INK4A}** **↑ p-cyclin D1, via ERα, ERβ** **↓ Total cyclin D1**	**↓ Cell Viability: 10^{-14}-$10^{-11}$and 10^{-6} M** **↓ pERK** →ROS **↓ pJNK** **↑ p-p38** **↑ Caspase 3** **5** Necroptosis **1** **↑ p-p16^{INK4A}** **↑ p-cyclin D1, via ERβ, GPR30** **↓ Total cyclin D1**
PC-3 (Late)	**↓ Cell Viability: 10^{-10}-10^{-8} M** **↑↑ pERK→↑ ROS via ERβ, GPR30** **↑ pJNK** **↑ p-p38** Caspase 3 **6** Necroptosis **0** **↑ p-p16^{INK4A}** **↑ p-cyclin D1, via ERβ, GPR30** **↓ Total cyclin D1**	**↔ Cell Viability** **↓ pERK**→ ROS **↓ pJNK** **↑ p-p38** Caspase 3 **2** Necroptosis **2** **↑ p-p16^{INK4A}** **↓ p-cyclin D1, via no ERs** Total cyclin D1

Surprising outcome -- DES R_x to late stage tumors wouldn't be expected to work very well, yet this is the most common estrogenic therapy. E_2 R_x should be the most effective.
***Can xenoestrogens interfere in the prostate cancer killing effects of estradiol?????

What about combinations of XEs that challenge physiological Es ??? – which is the way we experience them as environmental contaminants

enhance a physiologic estrogenic response; inhibit at more effective concentrations – this is the most typical effect of combinations

POTENT UNEXPECTED DOSE PATTERN MIXTURES DISRUPT



Have assessed this for 15 cases (3 physiologic estrogens – E_1, E_2, and E_3 - challenged by 5 different XEs)

(physiologic estrogens all at 1 nM for 5 min responses)

Jennifer Jeng – pituitary

Are we making progress in getting rid of any xenoestrogens in the environment? – we are just playing “whack-a-EDC”

What can we do about this in the future????

The Future of Green Chemical Design: A unique alliance between biologists and chemists to test and then design much *safer* chemicals for the marketplace

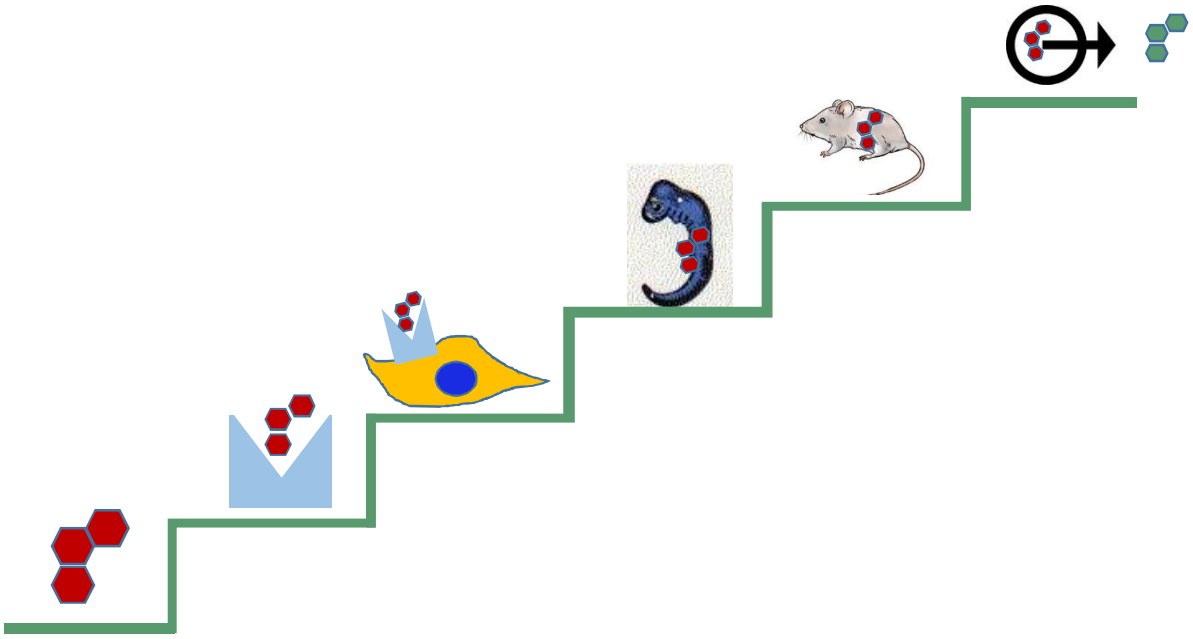
TiPED (Tiered Protocols for Endocrine Disruptors) http://www.tipedinfo.com/

Outlined in this publication:

Green Chemistry (DOI 10.1039/c2gc35055f; www.rsc.org/greenchem PAPER)

Designing endocrine disruption out of the next generation of chemicals

T. T. Schug, R. Abagyan, B. Blumberg, T. J. Collins, D. Crews, P. L. DeFur, S. M. Dickerson, T. M. Edwards, A. C. Gorei L. J. Guillette, T. Hayes, J. J. Heindel, A. Moores, H. B. Patisaul, T. L. Tal, K. A. Thayer, L. N. Vandenberg, J. C. Warner, C. S. Watson, F. S. vom Saal, R. T. Zoeller, K. P. O'Brien and J. P. Myers



…and we are organizing and focusing our ideas, responses, and commentaries into talks and written forums (journals, blogs, FB pages, and our web site) so everyone can share and debate them….

- Signaling mechanisms
- Endocrine disruption-based disease
- High throughput, structure-based, and modeling methodologies
- Experimental models
- Perinatal origins of adult disease
- Natural endocrine disruptors
- Cross-training for chemists and biologists
- Public policy
- Education and Outreach

…..the journal

waivers for articles submitted through September 30, 2016

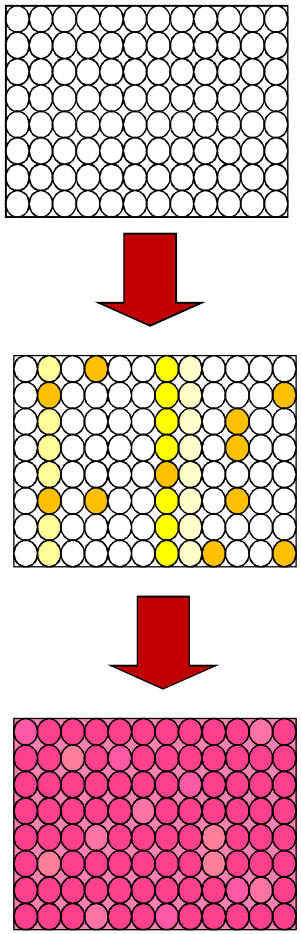
Taylor & Francis – open access

Because we compare multiple classes and structurally related XEs at multiple concentrations, times, signaling mechanisms, and combinations.....we need to do a lot of samples at one time for comparisons.

We developed medium through-put assays more accurate and sensitive than Westerns or commercial "in-cell Westerns" -- a fixed-cell 96-well plate immunoassay that:

- is optimized for each task -- cell type, epitope-Ab pair, and cell compartment (based on permeabilization)
- makes use of the many Abs now available to detect activation (usu. p'ation, also GTP-charging, methylation) of proteins, or the subcellular location of proteins (membrane vs. intracellular)
- comparing different Es vs. XEs and their mixtures over long time courses, at wide ranges of concentrations brackets real life exposures
- with adaptations for each molecule assessed, one can do these assays in parallel
- has recently been automated using a BioMek robot

We use an assay of receptors and signaling responses on fixed cells growing in small wells of a plastic culture plate -- specialized immuno-assays for quantitation of membrane vs. intracellular proteins, their trafficking, and their activation state.



Fix cells: various methods to optimize for epitope & cell type
-- unpermeabilized measures membrane proteins
-- permeabilized (with detergent) measures intracellular proteins

Incubate with 1°Ab (have used it for various ERs, DAT, pMAPKs & other kinases, p-cell cycle proteins, and p-transcription factors, GTP-G proteins, and methyl-enzymes in many different cell types)

Incubate with biotinylated 2° Ab; avidin-conjugated alkaline phosphatase

Incubate with pNpp →→ pNp at 37° in dark, & read at 405 nm → Ag quantitation

Wash off reagents

Stain with 0.1% crystal violet, wash, extract, read at 562 nM → cell number (normalization for each well)

Robots like this help make tests faster and cheaper
http://www.youtube.com/watch?v=6Jb7xBjWTtA