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Synthetic chemicals found in the environment have the capacity to disrupt the development and function of the endocrine system in both wildlife and humans. This has drawn public concern since many of these chemicals may bind to estrogen receptors (ERs) and evoke estrogenic effects. Early evidence that exposure to estrogenic chemicals during development could pose a threat to human health came from studies of a synthetic hormone, diethylstilbestrol (DES), which was used to prevent premature birth and spontaneous abortion. Laboratory experiments showed that exposure of animals to sex hormones during critical windows of perinatal life caused irreversible alterations to the endocrine and reproductive systems of both sexes. In the immune and nervous systems, bone, muscle, and the liver were also affected. Although many of these chemicals can bind to ERs in wildlife and humans, the molecular basis for the action of environmental estrogens remains poorly understood. Thus, understanding the molecular mechanisms through which environmental estrogens and sex hormones act during critical developmental windows is essential.

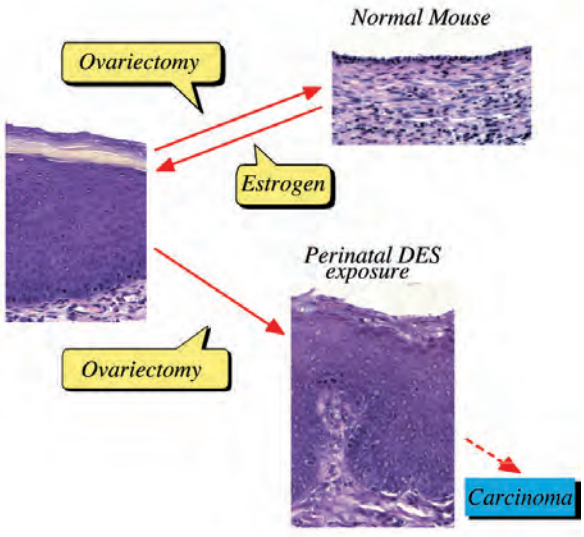

Figure 1. Scheme of estrogen-dependent and -independent vaginal epithelial cell proliferation in mice induced by perinatal estrogen exposure.

Ⅰ. Perinatal estrogen exposure induces persistent changes in reproductive tracts

The emerging paradigm of the "embryonic/fetal origins of adult disease" provides a powerful new framework for considering the effects of endocrine disrupters on human and animal health. In 1971, prenatal diethylstilbestrol (DES) exposure was found to result in various abnormalities of the reproductive tract in women and this syndrome was named the DES syndrome. Similar abnormalities have been demonstrated in experimental animals exposed perinatally to estrogens: for example, developmental estrogen exposure in mice induces persistent proliferation of vaginal epithelial cells. We found that the persistent changes in the vagina in mice exposed neonatally to estrogens result from the persistent phosphorylation of erbB2 and estrogen receptor α (ERα), sustained expression of EGF-like growth factors and phosphorylation of JNK1, IGF-I receptor and Akt. Recently, we found that Wnt 4, notch1, notch 3, and other genes also show persistent expression changes in neonatally DES-exposed mouse vaginae. Currently, we are analyzing the methylation status of genes showing altered expression in the mouse vagina. The number of DES-induced genes in the mouse vagina during the critical developmental exposure window was smaller than after the critical period.

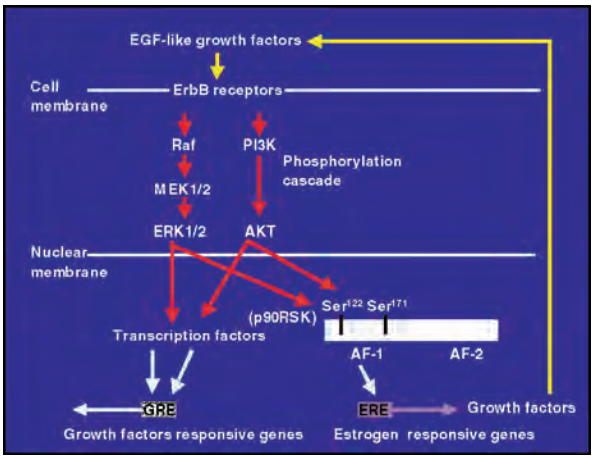

Figure 2. A hypothetical model for the estrogen-independent ER activation pathway in mouse vaginae.

Ⅱ. Microarray analysis of estrogen responsive genes

To understand the mechanisms through which estrogenic chemicals act on mouse reproductive organs, data documenting the temporal and spatial expression patterns of estrogen-responsive genes is essential. A large number of genes affected by estrogen treatment were identified in tissues of wild-type mice using a microarray approach. For most of the identified genes, expression was induced by 17 β-estradiol (E_2) in a dose-dependent manner. Subsequently, several environmental (xeno)estrogens were tested and

characteristic gene expression patterns were observed for each compound tested; these patterns were distinct from that obtained following E_2 exposure. We also found that xenoestrogenic chemicals and dioxin have distinct effects on the liver as well. Therefore, possible tissue-specific effects should be considered when elucidating the distinct effects of various EDCs.

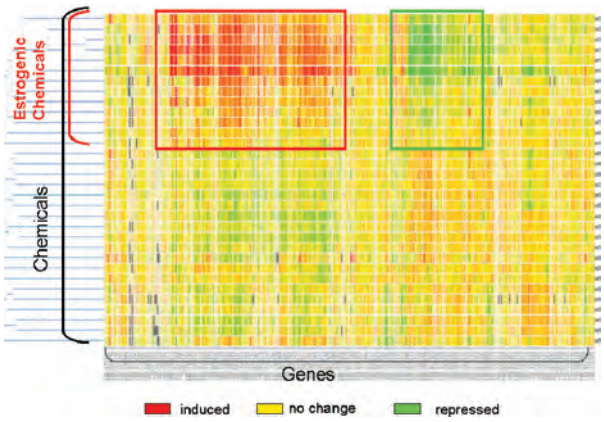

Figure 3. Scatter plot of average expression levels in control and chemical-treated uterus

Ⅲ. Steroid hormone receptors of reptiles, amphibians and fishes

Steroid and xenobiotic receptors (SXR) have been cloned from various animal species (fish, amphibian, reptiles, birds and mammals) by our group and we have demonstrated species-specific differences in their responses to various environmental and endogenous chemicals (receptor gene zoo). Thus, simple predictions of chemical effects based on data from a few established model species are not sufficient to develop real world risk assessments. ER and ER-like genes have been cloned from various animal species including rockshell, *Amphioxus*, lamprey, lungfish, sturgeon, roach, stickleback, mosquitofish, mangrove *Rivulus*, Japanese giant salamander, newt, *Silurana tropicalis*, American alligator, Nile crocodile, freshwater turtle, various snakes and vultures. Functional studies showed that the rockshell ER-like sequence does not bind estrogen but exhibits ligand-independent transactivation, whereas lamprey ER exhibited ligand-dependent transactivation, proving that primitive vertebrates, such as the Agnatha, have a functional ER.

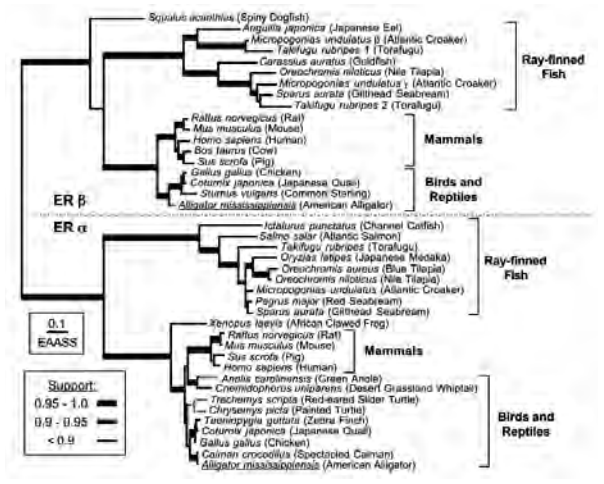

Figure. 4 Evolutionary relationships of estrogen receptor sequences

Ⅳ. Male production in Daphnids by juvenile hormones

Daphnia magna has been used extensively to evaluate the organism- and population-based responses of invertebrates to pollutants by their inclusion in acute toxicity or reproductive toxicity tests. These tests, however, provide no information about the mode of action of the tested compounds. Therefore, we applied an ecotoxicogenomic assessment of *D. magna*. We established a *Daphnia* EST database and developed an oligonucleotide-based DNA microarray with high reproducibility. Exposure of *D. magna* to several chemicals resulted in characteristic gene expression patterns that are chemical-specific, indicating that the established DNA microarray can be used for the classification of toxic chemicals as well as for the development of a mechanistic understanding of chemical toxicity in a common freshwater organism. *D. magna* reproduce asexually (parthenogenesis) when they are in an optimal environment for food, photoperiod and population density. Once environmental conditions become sub-optimal, they alter their reproductive strategy from asexual to sexual reproduction. Chemicals are able to affect sex determination of *D. magna* and we observed that juvenile hormone agonists (pesticides), for example, induce the production of male offspring. The molecular basis of environmental sex determination is largely unknown in these organisms. Therefore, we isolated sex determination-related genes to understand the molecular mechanisms of this phenomenon in *Daphnia*. DM-domain genes are well known as sex-related genes. We identified four DM-domain genes: DMRT11E, DMRT93B, DMRT99B and DSX. Quantitative gene expression analysis in daphnid gonads revealed that DMRT93B and DSX were expressed only in the testis. Recently, we have developed a method to inject silencing genes into *D. magna* embryos which will allow us to study gene function in more detail in this species.

Ⅴ. Gene zoo

We are establishing cDNA library banks of animal species in collaboration with the University of Pretoria, South Africa, the University of Florida, USA, San Diego Zoo, USA, and the Asa Zoo in Hiroshima.

Ⅵ. Molecular target search

We found that the persistent and ubiquitous environmental contaminant, tributyltin chloride (TBT), induces the differentiation of adipocytes *in vitro* and increased adipose mass *in vivo*. TBT is a dual noanomolar affinity ligand for both the retinoid 'X' receptor (RXR) and the peroxisome proliferators activated receptor γ (PPARγ). TBT promotes adipogenesis in the murine 3T3-L1 cell model and perturbs

key regulators of adipogenesis and lipogenic pathways *in vivo*. Moreover, *in utero* exposure to TBT leads to strikingly elevated lipid accumulation in the adipose depots, liver, and testis of neonate mice and results in increased epididymal adipose mass in adults. In the amphibian *Xenopus laevis*, ectopic adipocytes form in and around gonadal tissues following organotin, RXR or PPARγ ligand exposure. TBT represents the first example of an environmental EDC that promotes adipogenesis through RXR and PPARγ activation. Developmental or chronic lifetime exposure to orgaotins may therefore act as a chemical stressor for obesity and related disorders.

Publication List

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