CENTER FOR INTEGRATIVE BIOSCIENCE

Interim Head: Hideo Mohri

The center is jointly managed by NIBB and two other institutes in Okazaki, IMS and NIPS. The following projects will be the main focus of the center: 1) Development, Differentiation and Regeneration. 2) Strategic Methodology. 3) Bio-environmental Science.

DEPARTMENT OF BIOENVIRONMENTAL RESEARCH I

Professor:	*Taisen Iguchi*
Associate Professor:	*Hajime Watanabe*
Research Associate:	*Yoshinao Katsu*
Technical Staffs:	*Takeshi Mizutani*
Institute Research Fellow:	*Hiroki Okumura*
Post doctoral fellow:	*David Buchanan*
Graduate Students:	*Hiroshi Urushitani* [1)]
	Satomi Khono[1)]

[1)] *Graduate School of Yokohama City University*

Synthetic chemicals found in the environment have the capacity to disrupt endocrine system development and function in both wildlife and humans. This has drawn public concern since many of these chemicals may bind to estrogen receptors and evoke estrogenic effects. Early evidence that estrogenic chemicals could pose a threat to human health during development came from studies of diethylstilbestrol (DES), which was used to prevent premature birth and spontaneous abortion. Laboratory experiments have demonstrated that exposure of animals to sex hormones during perinatal life can cause permanent and irreversible alterations of the endocrine and reproductive systems as well as the immune system, nervous system, bone, muscle, and liver in both sexes. Although many of these chemicals may bind to estrogen receptors and evoke estrogenic effects in wildlife and humans, the effects of estrogen are not well understood even now. Thus, understanding the effects of sex hormones at the molecular level, especially during development, is very important to resolve these problems.

I Estrogen-induced irreversible changes

Perinatal sex-hormone exposure has been found to induce lesions in reproductive tracts in female mice. The possible relevance of the mouse findings to the development of cancer in humans has been emphasized. In the early seventies, a close correlation between occurrence of vaginal clear cell adenocarcinoma in young women and early intrauterine exposure to diethylstilbestrol (DES) was demonstrated. Many chemicals released into the environment have the potential to disrupt endocrine function in wildlife and humans. Some of these chemicals induce estrogenic activity by binding to the estrogen receptor (ER). The neonatal mouse model has been utilized especially to demonstrate the long-term effects of early sex hormone exposure on the female reproductive tract. Neonatal treatment of female mice with estrogens induces various abnormalities of the reproductive tract: ovary-independent cervicovaginal keratinization, adenosis, uterine hypoplasia, epithelial metaplasia, oviductal tumors, polyovular follicles and polyfollicular ovaries. Female reproductive tracts in mice exposed prenatally to estrogen show altered expression of Hoxa genes and Wnt genes and the analysis of knockout mice lacking Hoxa-10 or Wnt7a show uterine hypoplasia. The growth response of neonatally DES-exposed reproductive organs to estrogen is reduced, as are ER levels and EGF receptor levels, in addition to other hormone receptor levels.

Estrogenic compounds such as bisphenol A (BPA) and nonylphenol as well as dioxins and PCBs were found in the human umbilical cord. BPA can easily cross the placenta and enter the fetus in Japanese monkey and mice. Bisphenol-A (BPA) can be found in fetal brain, testis and uterus when given to pregnant mice. Neonatal exposure to a high BPA dose induced ovary-independent vaginal changes, polyovular follicles and infertility lacking corpora lutera. Thus, the developing mammal is sensitive to exposure to estrogenic agents as evident by the induction of long-term changes in female reproductive organs.

In order to clarify the molecular mechanisms of these effects, we are studying changes in gene expression patterns induced by perinatal exposure to chemicals or estrogen using differential display and DNA microarry techiniques. We have found genes possibly related to the ovary-independent changes by differential display. We also have clustered groups of genes that are respon-

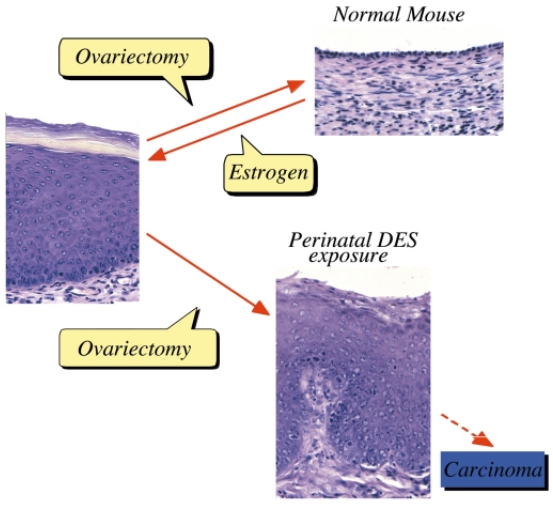

FIG. 1

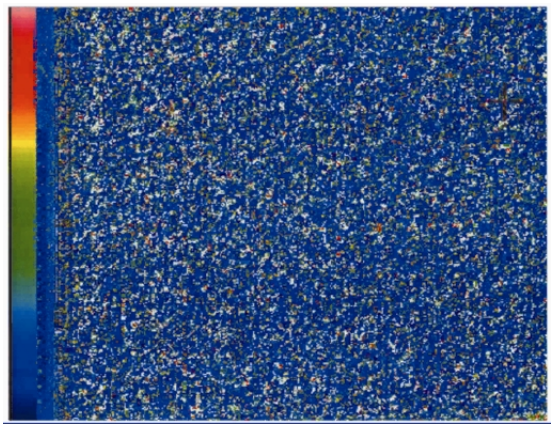

FIG. 2 Fluorescence image of an array

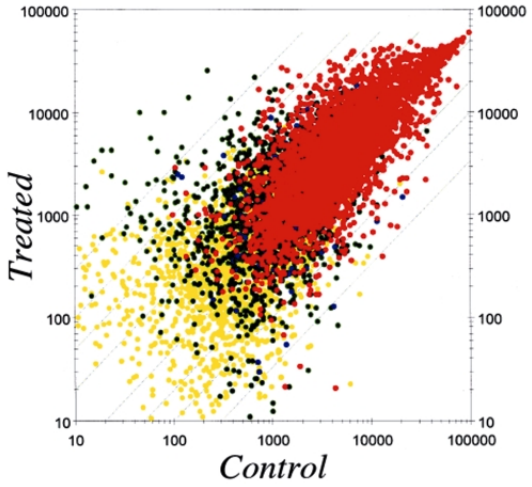

FIG.3 Scatter plot of average expression levels in control and chemical-treated uterus

sive to estrogenic stimuli in uterus by using the DNA microarray system. We need to understand the molecular background of the critical period during development, the low dose effect of estrogenic chemicals and the molecular metabolism of hormones and hormone-like agents in animals including humans.

II Effect of estrogen on amphibian and fishes.

During embryogenesis, exogenous estrogen exposure induces abnormal sex differentiation and the abnormal bone formation in African clawed frog, *Xenopus laevis* and the cyprinodont fish, mummichog (*Fundulus heteroclitus*). In these animals estrogen receptor is present in the embryo. To analyze the function of estrogen, we have isolated cDNA clones of estrogen receptor α and β. The estrogen-responsive genes must play important roles. So, we try to isolate the estrogen-responsive genes to understand the molecular physiology of estrogen action. Vitellogenin has been well characterized in avian, amphibian, and fish as a precursor of egg yolk. As the vitellogenin gene is responsive to estrogen, we can examine the effect of endocrine disruptors in the environment using a vitellogenin-specific and sensitive enzyme-linked immunosorbent assay (ELISA). Japanese tree frog (*hyla japonica*) takes water through ventral skin. We found that sex steroids and endocrine disruptors interfere with water absorption through ventral skin in frogs. Further, using the amphibian and fish as model animals we aim to analyze the effects of numerous chemicals released into the environment on endocrine system function in wildlife.

III Molecular Target Search

Abnormalities caused by endocrine disrupting chemicals are reported but the molecular mechanisms of the effects are not well studied. Although estrogen receptor is one of the strongest candidates possibly responsible for the endocrine disrupting function of many chemicals, it alone cannot explain the variety of phenomena induced by endocrine disrupting chemicals. Thus, we are also looking for new target molecules that may be responsible for endocrine disruption. In parallel, we also are studying the ligand-binding mechanisms of nuclear receptors to hormones and other chemicals using Surface Plasmon Resonance technology.

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