Title of Research:

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Development of new in vivo screening method to validate the low-dose effects induced by estrogenic chemicals using estrogen reporter mice

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Summary of Research:

Objective: The uterotrophic bioassay has been proposed as a short-term *in vivo* screening test to detect the estrogenic properties of potentially endocrine disrupting chemicals by the Organisation for Economic Co-operation and Development (OECD). Although the test is already standardized as a OECD test guideline (Test No.440), there is a possibility that the test might overlook a certain estrogenic properties which has no effect to the uterus. On the other hand, we recently generated a new transgenic mouse carrying “E-Rep” reporter gene which consisted of the luciferase (Luc) with c-Myc tag under the control of estrogen-responsive elements coupled to a minimal promoter. E-Rep mouse is a potential powerful tool for assessing the kinetics of gene activation by estrogenic properties *in vivo*. We here established a new *in vivo* screening assay using E-Rep mice to detect the tissue-specific estrogenic effects of xenobiotics and validated the assay to compare with the uterotrophic bioassay.

Methods: The experimental protocols of the new assay using E-Rep mice are based on the uterotrophic bioassay with some modifications. Briefly, E-Rep female mice (8 weeks of age) were ovariectomized and then fed phytoestrogen-free purified diet for 7 days. And then, mice were fed phytoestrogen-free purified diet containing estradiol (0, 0.01, 0.1, 1, 10 ppm) for another 7 days. Mice were dissected and then the estrogenic effects were evaluated by measurement of uterine weight, detection of Luc activity, or detection of c-Myc tag production by using immunohistochemical analysis.

Results: Luc activity in the uterus increased significantly when estradiol was administered at 0.1 ppm or greater. In contrast, the uterine weight rose significantly only when estradiol was administered at 1 ppm or greater, suggesting that the E-Rep mice provide a more sensitive assay of estrogenic activity than the uterotrophic bioassay. In addition, estradiol at 10 ppm caused a significant increase in Luc activity of the all tested organs. Furthermore, estradiol at above 1 ppm made clearly detection of estrogen-dependent production of Luc with c-Myc in the mammary gland by immunohistochemical staining.

Conclusion: E-Rep mice provide a potential new assay system to measure estrogenic activity of endocrine disrupting chemicals in multiple organs with high sensitivity.

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