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ES 666

Development of Biomarkers of Endocrine Disrupting Activity in Emerging Amphibian Model, *Silurana (Xenopus) tropicalis*

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Because amphibians show peculiar ecological features and interesting responses to some hormones, it is conceivable that amphibians are very useful animals for assessing the toxic effects of environmental contaminants, including endocrine disrupters. To develop methods of detecting endocrine toxicity of environmental chemicals in amphibians, we have started to assemble a biomarker tool kit for an emerging amphibian model, *Silurana* (*Xenopus*) *tropicalis*. We isolated full-length cDNAs encoding estrogen receptor α (ERα), ERβ, thyroid hormone receptor α (TRα), and TRβ of *S.* (*X.*) *tropicalis* to develop a reporter gene assay system, as an estimation tool for environmental chemicals. The amino acid sequences inferred from the four full-length cDNAs were highly homologous to those of ERα, TRα and TRβ of *X. laevis*, and ERβ of the Japanese quail. In particular, the *S.* (*X.*) *tropicalis* ERα shared a higher similarity of amino acid sequence with *X. laevis* ERα than the previously reported *S.* (*X.*) *tropicalis* ERα, as determined by Wu *et al.*[(1)] RT-PCR analysis showed that the two *ERα* and *ERβ* transcripts were expressed relatively abundantly in the brain, liver, and gonad/kidney complex of the *S.* (*X.*) *tropicalis* tadpole after gonadal sex differentiation occurring at developmental stages 54–59, suggesting that they are susceptible to estrogenic substances. A similar result was obtained in the two *TR* transcripts, although their expression levels were lower in the gonad/kidney complex than in the other tissues. Moreover, we identified *vitellogenin A* (*Vtg A*) and *Vtg B* as estrogen-responsive genes expressed in the female *S.* (*X.*) *tropicalis* liver using macroarray analysis and RT-PCR. In addition, *Rana japonica* Vtg was purified from serum using anion-exchange chromatography to produce anti-Vtg antibody as a protein marker. In the future, we are going to construct reporter gene assay systems using the full-length *ER* and *TR* cDNAs, analyze histologically the differentiation of gonads and thyroid glands in the *S.* (*X.*) *tropicalis* tadpole exposed to estrogenic chemicals, and produce sex-reversed male *S.* (*X.*) *tropicalis* to obtain all-male tadpoles. Using these tools, we hope to be able to identify endocrine disrupting compounds in laboratory experiments for hazard assessment purposes, and also detect endocrine toxicity in environmental samples as part of an integrated approach to assessing the impact of environmental contaminants on wild amphibian populations in Japan and the UK.