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A Study of Tissue-Specific Expression and Analysis of Structure and DNA Sequences of Porcine Cytochrome P450 Aromatase Genes

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By use of RT-PCR coupled with DHPLC technique (WAVE analysis), pattern of isoforms of porcine cytochrome P450 aromatase gene was investigated. Relatively higher expression of aromatase mRNA was observed in testis than in ovary and this result accounted for the previous findings of higher blood estrogen level in male compared with female in this species. The result from the DHPLC demonstrated that PCR amplified DNA fragments of ovary and testis tissues, using unique PCR primers for all three types of aromatase genes, were different from those of type II and III genes. Further nucleotide sequence analyses of the plasmid clones containing the PCR products revealed that nucleotide sequences of all clones were identical to type I aromatase gene (ovary type). In addition, as an initial step towards understanding regulatory mechanism of each aromatase gene and its physiological role in this species, PCR-based screening of the BAC library of the Korean native pig with the PCR primers designed from exon 10 of the gene gave birth to ten positive clones. Among the ten clones, two BAC clones showing distinct hybridization pattern by Southern blot analysis were selected and generated about 1,500~2,000 plasmid clones from each BAC clone by shot-gun library construction approach. Random sequencing of all the plasmid clones with universal primers and assembling the sequences using bioinformatic tools allowed us to obtain the full-length nucleotide sequences of the two BAC clones. Computer analysis revealed that the two clones overlapped each other and spanned the whole area of the three aromatase genes. Moreover, comparison of nucleotide sequences and gene structures showed that all three genes are homologous in their nucleotide sequences including exon, intron, and 5' untranslated region. In conclusion, the result from the present study indicates that the ovary and testis express the same type of aromatase gene and three isoforms of porcine aromatase genes are neighboring each other on the same chromosome and arose through gene duplication mechanism in this species.

Key Words : *Cytochrome P450 aromatase, 19-norandrogens, Gene, DHPLC, Pig*