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Zhou et al., Supplemental Methods

Hormone Measurements

The minimum detectible level of LH in most assays was 0.013 ng/ml, because 75-µl samples, instead of 25 µl as previously used, were assayed (1). The cross-reactivities of rat FSH and human recombinant FSH in the rat LH assay were 0.26% and <0.03% respectively.

The levels of human FSH (hFSH) in rat serum were directly measured with an assay specific for hFSH, which showed no detectable cross-reaction with rat FSH (rFSH). However, there was cross-reactivity of the hFSH in the rat assay (2), which was quantified by running samples of freshly prepared recombinant hFSH in both a rat FSH assay (using rFSH as the standard) and a human FSH assay (using hFSH as the standard). The cross-reactivity ratio for recombinant hFSH, 0.49 ng/ml (in the rat FSH assay) per 1 U/L (in the human FSH assay), was multiplied by the measured units of hFSH from the human FSH assay in each rat serum sample to calculate the cross-reaction contribution of hFSH, which was then subtracted from the total reading in the rat FSH assay to estimate the actual concentration of rFSH in the serum sample.

Data Analysis

The following criteria were added for eliminating small changes in gene expression with hormonal treatment that, although statistically significant, were unlikely to be biologically significant. Since the largest changes in hormone levels occurred between the irradiated rats (X) and the irradiated rats treated with GnRH-ant + flutamide (XAF), the criterion for the difference (X-XAF, where X and XAF are the log base 2 transformed values of the expression levels) being significant was $|X-XAF|\geq 0.5$, which corresponds to a 1.41-fold change in expression, in addition to $P<0.05$ (Student's *t*-test). Since the changes in hormone levels due to T replacement were less than that of the GnRH-ant + flutamide treatment, the criterion for the difference between the expression levels in GnRH-ant + flutamide-treated rats and the GnRH-ant + T-treated rats (XAF-XAT) being significant was less stringent. The difference was considered potentially biologically significant if $|XAF-XAT|\geq 0.333$, which corresponds to only a 1.26-fold change in expression and $P<0.05$ was still used for statistical significance. The 1.26-fold change in expression was also used as the criterion for changes in expression levels with addition of FSH (XAF-XAFF) to the regimen. These criteria were mainly used to count the genes in different categories and identify potentially regulated genes.

Identified genes were subjected to further analysis or stricter criteria to ensure that they were not falsely discovered. False Discovery Rate (FDR) was calculated using software SAM (3) with all the probes that were present in at least one of the comparing groups.

References used in Supplemental Data

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