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Global Analysis of Gene Expression in the Estrogen Induced Pituitary Tumor of the F344 Rat.

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Abstract

The F344 rat rapidly forms large prolactinomas in response to chronic estrogen treatment. To identify genes expressed in the course of this estrogen induced pituitary tumor growth, we performed microarray analysis on the F344 rat pituitary after chronic estrogen treatment and on untreated controls. At a significance level set to minimize type I error, some 72 genes were found to be differentially expressed between estrogen treated and untreated. Of those genes, 70 have not been reported previously as being affected by estrogen in the F344 rat pituitary. Since many other investigators have studied the effect of estrogen on specific gene expression in rat pituitary, we also examined the mRNA expression of the 36 genes that have been previously reported as having their expression affected by estrogen in the rat pituitary. Of these, 13 were found to have their expression affected by estrogen treatment in the same direction as had been reported by others.

Keywords

estrogen; pituitary; gene expression; Fischer 344; prolactinoma

1. Introduction

The rat pituitary has been an important experimental system for the study estrogen action and many laboratories have used this model system to study the effects of estrogen on specific gene expression. Such work has been carried out over many years with each group focusing on one or a few genes. Categories of genes whose expression have been studied are those encoding hormones and hormone receptors [1-5], paracrine regulators of cell proliferation and their receptors [6-12], tumor suppressors [13,14], intracellular signaling proteins [5,15,16], proteases [5,17,18], and angiogenic factors and their receptors [9,19, 20].

Many of the studies on the effect of estrogen on specific gene expression in the rat pituitary have been performed in the estrogen induced prolactinoma of rats of the F344 strain. Chronic estrogen treatment can induce tumor growth in the anterior pituitary of rats of several strains, but it is greatest in the F344 [21-24]. The tumors that are formed are not of clonal origin. Rather, estrogen treatment induces uncontrolled proliferation of the entire lactotroph population [25-27,31]. The resulting tumors develop rapidly. There really is no latency because proliferation initiates as soon as estrogen treatment begins [28] and the tumors are highly

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uniform consisting almost entirely of lactotrophs [29,30]. In addition to uncontrolled lactotroph proliferation, there is increased angiogenic activity in the anterior pituitary of estrogen treated F344 rat [19,25, 29,32-35]. After prolonged treatment, the F344 rat pituitary tumor exhibits neoplastic transformation and invades surrounding tissue [26,30,36].

Here we report the first global analysis of the effect of estrogen on gene expression in the F344 rat pituitary. Our results confirm some of the results published by others, but the majority of genes have not been previously reported in this system.

2. Materials and Methods

2.1. Animals and estrogen treatment

21 day old ovary-intact female rats were given subcutaneous Silastic tubing implants containing 5 mg of the synthetic estrogen diethylstilbestrol (Sigma Chemical Company, St. Louis, MO, USA) as described by Wiklund *et al* [37]. Implants were left in place until the animal was sacrificed 70 days later. Untreated rats were animals of the same age which did not receive implants [24]. All procedures performed on live animals were approved by the Oakland University Institutional Animal Care and Use Committee.

2.2. Microarray Analysis of mRNA

Total RNA was obtained from anterior pituitary of six estrogen treated and six untreated F344 rats. During dissection, as soon as the pituitary was exposed it was bathed in RNALater preservative solution (Ambion, Inc., Austin, TX, USA). The intermediate lobe was removed while the pituitary was still in place in the cranium. The anterior pituitary was collected and stored in RNALater solution at -80 C until use. RNA was extracted from anterior pituitary tissue using Trizol reagent (Invitrogen Corp., Carlsbad, CA, USA) and total RNA was purified using an RNAeasy Mini Kit (Qiagen Inc., Valencia, CA, USA). RNA quality was verified using an Agilent Technologies 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA).

Total RNA was used to probe the Affymetrix GeneChip Rat Genome 230 2.0 array. Probing and analysis was done in collaboration with the Wayne State University Applied Genomics Technology Center. Within each treatment group, the six RNA samples were combined into pools of two (with equal amounts of RNA from each sample). Each pool was then used to probe a separate microarray chip. Thus, the degree of within-group variability in the expression data is based on three separate chips, while the mean expression values for a treatment group are the average of 6 animals. Affymetrix arrays were imaged and cel files of probe intensity produced using the Affymetrix GCOS 1.0 software (www.affymetrix.com). These were then analyzed using the DNA-Chip Analyzer (dChip) program of Li and Wong (www.dchip.org), initially to normalize the probe intensity to an invariant set across the chips. Probeset intensities were then assessed using Cheng Li's model-based expression algorithm [38] after selecting the PM-only option, based on our experience of the reduction in variance this produces between probeset repeats. All arrays passed the Affymetrix 3'/5' ratio tests, p-call percentage and dChip MBEI outlier criterion.

2.3. Statistical analysis of microarray data

For each treatment group, the mean gene expression from the three chips probed was taken as the representative expression value for the gene and dChip was used to derive a fold change of estrogen treated relative to untreated and p-value based upon student's t-test of this mean and repeat variance. Any probeset with a majority of absence calls made in both repeat sets (both estrogen treated and untreated) was discarded from analysis. Initial results from dChip indicated that with a p-value threshold of 0.05 and a 2 fold absolute cutoff, around 1,500 genes would be discovered significantly changed between treatments out of a potential set of 31,099

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probesets. However this would necessarily include a significant type I error. In order to correct for this potential error in a distribution-specific manner and without excessively impacting type II error, Storey's Q value [39] approach was employed to correct the false discovery rate. Taking the maximum desired false discovery rate amongst the genes showing significant change to be 0.01, the q-value tool (http://faculty.washington.edu/~jstorey/qvalue/index.html) was used in command line mode within the R package to examine the p-value distribution and suggest an appropriate p value threshold to correct the q-value to 0.01. On this basis a p-value threshold of 0.0015 was selected and validated by trivially permuting signal intensities between control and experimental datasets. This p-value of 0.0015 was thus used as the significance threshold for comparing mRNA level between estrogen treated and untreated groups based solely on the microarray data.

In this study those genes that have been reported elsewhere as having their expression affected by estrogen treatment in the rat pituitary was also evaluated. In this case, incorporating prior knowledge mitigates to an extent the need for a p-value adjustment. Therefore, a p-value of 0.05 was applied as the criterion for significant fold change between estrogen treated and untreated, given that it was of the same direction as reported previously.

2.4. Quantitative rtPCR

The same preparations of total RNA used for the microarray analysis were also used for real-time PCR to validate expression results of selected genes. For each RNA sample, cDNA was synthesized from 1.5 μg of total RNA and oligo-dT primers using SuperScript II Reverse Transcriptase (Invitrogen Corporation, Carlsbad, CA).

Real-time PCR was performed with an iCycler Thermal Cycler (BioRad Laboratories, Hercules, CA) and products detected in real time by intercalation of SYBR Green I (Molecular Probes, Eugene, OR). For real-time PCR, equal amounts of cDNA were pooled in the same manner as described above for microarray analysis. In addition to cDNA template, reactions contained 0.03 U/μl Amplitaq Gold DNA Polymerase, 2.5 mM $MgCl_2$, 0.2 mM of each nucleosidetriphosphate, 300 nM forward primer, 300 nM reverse primer, 8% glycerol, 0.2X SYBR Green I, 10 nM fluorescine, and 1X AmpliTaq Gold Buffer. PCR was carried out by 3 min at 95 degrees followed by 45 cycles of 15 seconds at 94 degrees, 45 seconds at 55 degrees, and 30 seconds at 72 degrees. Threshold cycle (Ct) values were collected and the ?? Ct method was used to calculate the fold difference in transcript level of the estrogen treated relative to untreated. Beta actin was used as the reference gene for each gene validated.

PCR primers were designed using the program Primer3[40] and their nucleotide sequences are given in Table 1. All primer pairs were designed to span at least one large intron in order to eliminate the possibility of amplification of genomic DNA. Information on exon structure was obtained from the Ensembl Genome Browser (version 37, February 2006) [41].

3. Results

3.1. Genes differentially expressed between DES treated and untreated F344 pituitary

At a significance level of $p < 0.0015$, a total of 72 genes were differentially expressed between the estrogen treated group and untreated control in the F344 rat anterior pituitary (Table 1). 46 of the genes were higher in the estrogen treated compared to untreated, while 26 were lower in the estrogen treated compared to untreated. Of the 72 genes that we found to be differentially expressed with high significance, only two, *Vip* [6] and *Dusp1* (a.k.a. *Mkp-1*) [5] have been previously reported as having their expression affected by estrogen in the rat pituitary. Thus, the other 70 genes are novel findings. Fujimoto *et al* [5] recently reported 33 gene products whose expression was affected by estrogen in GH3 rat somatolactotroph cell line. Of these,

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only one, *Dusp1* (aka *Mkp-1*) is among those genes detected as differentially expressed with $p < 0.0015$ in our study.

3.2. Genes previously reported as affected by estrogen in the rat pituitary

The rat pituitary, and the F344 rat pituitary in particular, has been used as an experimental system for research on estrogen action by many different laboratories over the years. Thus, there is a large list of genes for which the effect of estrogen on their expression is documented (Table 3). Out of 36 gene products previously reported, we found that 14 of them had their expression significantly affected by the estrogen treatment in our study. In the case of all but one, *Myc*, the direction of change was the same as previously reported (Table 3). In the case of *Myc* the difference between estrogen treated and untreated was statistically significant, but very small. We detected another 14 genes as expressed (called present in the Genechip analysis) but without a statistically significant change in expression. The remaining genes were simply not detected (called absent in the Genechip analysis).

Because several genes which have been reported as regulated by estrogen in the rat pituitary were not so found in our study, we validated the expression data of a selected group of genes by real-time PCR (Table 4). Of the eight such genes tested, we found only one, *Pttg1*, to give a different result by real-time PCR than by microarray. Our real-time PCR assay found *Pttg1* to be elevated in the estrogen treated relative to untreated which is consistent with what has been reported previously for this gene[13]. For the other seven, our real-time PCR was consistent with the microarray data. In the microarray analysis, *Tgfb1* and *Fgf2* were not detected (called absent). We do detect them by real-time PCR but do not find evidence for any difference between estrogen treated and untreated. We also tested the genes *Gal* and *Vip*, which we had found by microarray analysis to be upregulated by estrogen treatment, and confirmed that realtime PCR assay also detected them as highly upregulated.

4. Discussion

We report here the first global analysis of gene expression used to study the effect of estrogen treatment on the F344 rat pituitary. The vast majority of genes that we have identified have not been reported before in this system, even though many laboratories have studied the effect of estrogen on specific gene expression in this tumor model. Of the genes with a known function which we find to be differentially expressed with high statistical significance, slightly more than one-half (18 out of 30) have been reported to play a role in cell signaling and/or growth control (Table 2). For the all but three genes identified which are known to play a role in cell signaling and/or growth control, the direction of change of expression is consistent with expected affect on tumor growth, *i.e.* growth promoters are upregulated and *vice versa.* Three genes which would be expected to function as negative regulators of growth, *Cgref1*, *Gch*, and *Dusp1*, are actually higher in the estrogen treated relative to untreated. This may reflect an effort of the cells to control growth in the face of mitogenic stimulus, but such effort is insufficient due to other growth promoting factors being upregulated. Much remains to be learned about the significance of the transcripts that we have identified since only 30 out of the 72 are genes of known function.

We find that *Litaf* is upregulated by estrogen treatment in the F344 rat pituitary consistent with what has been reported in the female reproductive tract. Litaf is a transcription factor that is needed for the induction of expression of TNF-alpha and other cytokines[42]. Everett *et al* [43] first identified *Litaf* (then named *EET-1*) as a novel transcript that was upregulated by estrogen in the female reproductive tract (uterus, vagina, and cervix) and in kidney, but not in brain, heart, liver and spleen, but its expression in pituitary was not tested.

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Surprisingly, we do not find an effect of estrogen treatment on the expression of genes encoding TGF-β3 or FGF-2. Through a series of several papers, the Sarkar laboratory has clearly established an important paracrine pathway for the estrogenic stimulation of lactotroph proliferation involving TGF-β1, TGF-β3, FGF-2, and TGFβR2 [44,45]. They showed that the release of TGF-β3 by lactotrophs is increased by estrogen treatment [9]. This in turn stimulates the folliculostellate cells to release FGF-2 which in combination with estrogen stimulates lactotroph cell proliferation [9]. In our study, we do not detect any difference in expression of mRNA between estrogen treated and untreated for either the *Tgfb3* or the *Fgf2* gene (Tables 2 and 3). Likewise, *Tgfb1* mRNA was not found to change, even though the level of TGF-β1 protein is known to decrease in the F344 pituitary upon estrogen treatment [44]. When comparing the results we report here with reports by others, it should be noted that we are measuring a different endpoint. While we report here on mRNA, the Sarkar group mainly measured the release of the proteins, so the important regulation could be acting as some stage post-mRNA, perhaps even on the storage and release of the factors. Supporting such an idea are the observations by Gonzalez *et al* who reported that in the rat pituitary, FGF-2 protein was abundant, while the level of its message was extremely low [46].

Our microarray data do show evidence of estrogen treatment affecting the TGF-β pathway through other routes. We find that *Bambi*, which encodes a TGF-βI pseudoreceptor that inhibits TGF-β signaling [47], is down regulated in the estrogen treated group, relative to untreated.

In comparing our results to those reported by the Sarkar group, one should also consider the difference in timing of estrogen treatment and tissue. While their work used cells in primary culture or in rats after a 4-week estrogen treatment [9,48], our data come from rats after 10 weeks of estrogen treatment. It is possible that after long-term treatment, other paracrine factors become more significant. For example we do detect a large effect of estrogen treatment on the expression of the *Vip* and *Gal* genes which encode paracrine factors known to promote lactotroph cell proliferation [49].

Although we detect prolactin mRNA, we find no difference in its level between treated and untreated (Table 3). This may be unexpected given that *Prl* has been a model for the study of estrogen-induced transcription [50]. Estrogen treatment, either for a few days [51], or for longer periods such as 30 days[52], has been shown to significantly increase the level of *Prl* mRNA in the pituitary of female F344 rats, compared to untreated ovectomized controls. However, our present findings on *Prl* mRNA expression are consistent with lactotroph density data that we have previously reported for F344 rat pituitary. We consistently find no difference in lactotroph density in F344 rat pituitary with both DES-treated and age-matched ovary-intact controls, and both consist of more than 90% PRL-positive pituicytes [29]. The discrepancy may reflect a difference in treatment protocols. We have used ovary-intact rats and a 10-week DES treatment. Also, the message level from both the microarray and real-time PCR analysis for *Prl* is very high in the untreated samples (not shown). Thus, it is not that *Prl* mRNA is not being expressed in the tumors, but that both treated and untreated have high levels.

Fujimoto *et al* reported that in estrogen treated F344 rats the level of mRNA for *Myc*, *Calb3*, and *Pvalb* are much greater (18, 90, and 75 -fold, respectively) in the pituitary of estrogen treated F344 female rats compared to untreated [5]. Our results are quite different from theirs with neither microarray nor real time PCR showing any increase. One possible explanation is a difference in experimental protocol. The pituitary RNA collected by Fujimoto *et al* was from rats after 30 days estrogen treatment, while we used a 70 day treatment. Another possibility is that, the discrepancy could be due to different levels in the untreated controls; the data from microarray and rtPCR gene expression are relative differences of estrogen treated to untreated, not absolutes. Fujimoto *et al* used ovectomized females while ours were ovary-intact females. Ovectomy is a control used to reduce circulating estrogen levels in the untreated controls, but

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has not been found to affect the development of pituitary tumors in estrogen treated rats [24, 53]. However, ovary-produced estrogen could raise the baseline level of expression of specific genes in our study.

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Table 1

Nucleotide sequence of primers used for real-time PCR

Gene	Ensembl Transcript	Forward Primer	Reverse Primer
Calb3	ENSRNOP00000005622	TGACTCTGGCAGCACTCACT	CTTGGACAGCTGGTTTGGAT
Fgf2	ENSRNOP00000023388	GAACCGGTACCTGGCTATGA	CCGTTTTGGATCCGAGTTTA
Gal	ENSRNOP00000020425	ATGCCATTGACAACCACAGA	GTGGGTGTGGTCTCAGGACT
Myc	ENSRNOP00000006188	ACGGCCTTCTCTTCTTCCTC	GTTTGCTGTGGCCTCTTGAT
Prl	ENSRNOP00000023412	ATCAATGACTGCCCCACTTC	TCATTTCCTTTGGCTTCAGG
Pttg1	ENSRNOP00000005070	GTAAACCCCTGCAATCGAAA	CCATTCAAGGGGAGAAGTGA
Pvalb	ENSRNOP00000009062	GCGGATGATGTGAAGAAGGT	GTCAGCGCCACTTAGCTTTC
Tgfb1	ENSRNOP00000028051	GCGTCTCAAGAAGCAGAAGG	TAGGTTCGTGGACCCATTTC
Tgfb3	ENSRNOP00000013516	TGGAGGAGAACTGCTGTGTG	GTCAGAGGCTCCAGGTCTTG
Vip	ENSRNOP00000025477	TGATGAGAAGGGTCCTCTGG	GCCTTTCACGAGCTAAAGATG

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Table 2

Genes with greater than 2-fold change in expression between estrogen-treated and untreated and $p < 0.0015$

Accession	Gene	Description	fold change[a]	Role in cell signaling or growth control[b]
AI412212	*Vip*	vasoactive intestinal polypeptide	+182.24	growth factor
AI009059	*Spink4*	Kazal type serine protease inhibitor 4	+33.4	
AW526160	*Mef2d*	myocyte enhancer factor 2D	+28.5	
BM384311	*Pdgfrl (p)*	platelet-derived growth factor receptor-like (predicted)	+14.98	growth factor receptor
NM_031721	*Prss11*	protease, serine, 11 (Igf binding)	+13.79	growth factor binding
AI044556	*Arhgap15*	Rho GTPase activating protein 15	+11.06	activation of RHO GTPase
AW530436		Transcribed sequences	+8.99	
NM_130748	*Slc38a4*	amino acid transport system A3	+6.91	
BE108969		Similar to Insulin-like growth factor binding protein 4 precursor (LOC360622)	+6.75	
BM387419		moderately similar to matrilin-2 precursor (M.musculus)	+6.73	
BF398091		Transcribed sequence with moderate similarity to protein pdb:1LBG (E. coli) B Chain B, Lactose Operon Repressor	+6.67	
U66470	*Cgref1*	cell growth regulator with EF hand domain 1	+6.63	inhibits cell growth
BI296368		Similar to 2010004A03Rik protein (LOC361406)	+6.55	
BG665530		Similar to RIKEN cDNA 1700001E04 (LOC301204)	+6.47	
BI296600		Transcribed sequences	+6.02	
M88469	*Sponf*	f-spondin	+6.01	
AA850290		Similar to type IV putative aminophospholipid transporting ATPase (LOC360932)	+5.53	
AI233288		Similar to D11Ertd498e protein (LOC303630)	+5.48	
AI227769		Transcribed sequences	+5.24	
BI285321		Similar to ATP sulfurylase/APS kinase 2 (LOC294103)	+4.83	
AW433978		Similar to glycoprotein (LOC361875)	+4.63	
AI763990		Similar to UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase T10; UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase T14 (LOC313878)	+4.39	
AA963863		Similar to chondroitin beta1,4 N-acetylgalactosaminyltransferase (LOC306375)	+4.02	
BI278482		Similar to aortic carboxypeptidase-like protein ACLP (LOC305494)	+3.81	
BF284235		Similar to retinoblastoma-binding protein mRbAp48 (LOC313048)	+3.7	
AA801107	*Ehd4*	pincher	+3.66	endocytosis of NGF receptor for cytoplasmic signaling
AI012419	*Gch*	GTP cyclohydrolase 1	+3.55	may mediate cell death
BF415436		Transcribed sequences	+3.49	
AI101330		Similar to neurocalcin delta (LOC366916)	+3.41	
BI284739	*Litaf*	LPS-induced TNF-alpha factor	+3.38	transcription factor, upregulated by estrogen
BI275795		Highly similar to sorting nexin 9 (H.sapiens)	+3.33	
BF550033		EST	+3.3	
NM_053827	*Plod*	procollagen-lysine, 2-oxoglutarate 5-dioxygenase (lysine hydroxylase, Ehlers-Danlos syndrome type VI)	+3.22	
AI029492		Transcribed sequences	+3.08	
BF397529	*MGC94555*	Hypothetical LOC306165 (LOC306165)	+3.03	
U72660	*Ninj1*	ninjurin 1	+2.97	
U02553	*Dusp1*	dual specificity phosphatase/MAP kinase phosphatase 1	+2.82	inactivates MAP kinase
BI280304		Similar to Bcl2-associated athanogene 1 (LOC297994)	+2.82	
NM_053607	*Acsl5*	fatty acid Coenzyme A ligase, long chain 5	+2.82	synthesis of signaling molecules
BE099470		Transcribed sequences	+2.79	
NM_031357	*Cln2*	ceroid-lipofuscinosis, neuronal 2	+2.76	
X04440	*Prkcb1*	protein kinase C, beta 1	+2.69	intracellular signaling
AI235294		Similar to RIKEN cDNA 1110014L17 (LOC305502)	+2.62	
BG668421	*Sdc2*	syndecan 2	+2.42	
AA891634		Transcribed sequences	+2.16	
AI232217		Transcribed sequences	+2.15	

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Accession	Gene	Description	fold change[a]	Role in cell signaling or growth control[b]
NM_031070	*Nell2*	nel-like 2 homolog (G. gallus)	-2.22	may regulate intracellular signaling
BG670246		Transcribed sequences	-2.24	
AF078779	*Vgcnl1*	voltage gated channel like 1	-2.33	
BI299169		Transcribed sequences	-2.75	
NM_022209	*Ppp2r2b*	protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), beta isoform	-3.08	
AI070438		Similar to 1700060H10Rik protein (LOC309790), mRNA	-3.33	
AF387513	*Bambi*	BMP and activin membrane-bound inhibitor, homolog (X. laevis)	-3.48	negative regulator of TGF-ß signaling
BF417335		Transcribed sequences	-3.53	
AI171799		Similar to mammalian ependymin related protein-2 (LOC291180)	-3.58	
BG664461		Transcribed sequences	-3.69	
M94043	*Rab38*	Rab38, member of RAS oncogene family	-3.72	small GTPase-mediated signal transduction
AI709768		Transcribed sequences	-3.84	
BE107978	*Mapt*	microtubule-associated protein tau	-3.86	apoptosis
AF385402	*Kcnk2*	potassium channel, subfamily K, member 2	-4.37	G-protein coupled receptor protein signaling pathway
AI112199		EST	-4.42	
AA858564		Transcribed sequences	-4.51	
BM382847		Moderately similarity to protein sp:P00722 (E. coli) BGAL_ECOLI Beta-galactosidase	-4.74	
AI716676		LOC362136 (LOC362136)	-4.91	
AI412750	*Hap1*	huntingtin-associated protein 1	-6.07	
BI296915		Transcribed sequences	-6.19	
BF394545	*Nfl*	neurofilament, light polypeptide	-6.37	
BG671865	*Ndn*	necdin	-8.15	negative regulator of cell proliferation
BI281230		Similar to death effector filament-forming Ced-4-likeapoptosis protein isoform 2; caspaserecruitment domain protein 7; NAC-alpha/beta/gamma/delta (LOC360556)	-9.17	
BE121330		Similar to Brain-specific angiogenesis inhibitor 3 precursor (LOC301309)	-10.41	
NM_019169	*Snca*	synuclein, alpha	-12.65	anti-apoptosis
BI289110		Similar to DNA polymerase delta subunit 3	-16.44	

[a] estrogen treated relative to untreated.

[b] Annotations for roles in cell signaling and/or growth control are from the Rat Genome Database (http://rgd.mcw.edu/).

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Table 3

Genes reported previously to be regulated by estrogen in anterior pituitary of F344 rats.

Gene	Gene Product	Published Effect of Estrogen in F344 RNA	Protein	Ref	10 week DES-treated F344 vs. untreated, Rat 230 ver. 2 Chip Genbank Accession	fold chnge.	p-value
Vegf	vascular endothelial growth factor A	up	up	[19]	AI175732	+ 2.45	0.0032
Vip	vasoactive intestinal peptide precursor	up	up	[6]	AI412212	+182.2	0.0005
Pttg1	pituitary tumor transforming gene	up	up	[13]	NM_022391	P	n.s.
Esr1	truncated estrogen receptor products	up	up	[1]	NM_012689	A	--
Gal	galanin precursor	up	up	[7]	NM_033237	+ 8.05	0.0052
Fos	subunit of AP-1 transcription factor	up	n.d.	[15]	BF415939	+ 2.37	0.0043
Nrp	neuropilin	up	n.d.	[20]	AF016296	P	n.s.
Pace4	PACE4 or Subtilisin - like endoprotease	up	n.d.	[5]	NM_012999	+ 1.40	0.0163
Dusp1	dual specificity phosphatase / MAP kinase phosphatase 1	up	n.d.	[5]	U02553	+ 2.82	0.0004
Stat5a	regulator of transcription 5a1	up	n.d.	[5]	NM_017064	P	n.s.
Myc	c-myc protein	up	n.d.	[5]	NM_012603	- 1.42	0.0256
Calb3	Calbindin-D9k	up	n.d.	[5]	NM_012521	P	n.s.
Kcnj1	K+ channel ROMK2.1 isoform	up	n.d.	[5]	L29403	A	--
Pvalb	Parvalbumin	up	n.d.	[5]	AI175539	P	n.s.
Pgr	progesterone receptor	up	n.d.	[5]	NM_022847	+ 1.40	0.0306
Scl25a30	“solute carrier family 25, member 30”	up	n.d.	[5]	H35736	+ 1.77	0.0114
Prl	prolactin	up	n.d.	[51]	NM_012629	P	n.s.
Cpe	carboxypeptidase E	down	down	[17]	NM_013128	P	n.s.
Tgfbr2	transforming growth factor beta receptor II	down	down	[8]	L09653	A	--
Tgfb1	transforming growth factor beta I	down	down	[8]	NM_021578	A	-
Calca	Calcitonin	down	n.d.	[3]	M11597	A	--
Kdr	VEGF receptor 2	n.d.	up	[19]	AW918207	+ 2.49	0.0122
Ar	androgen receptor	n.d.	up	[4]	NM_012502	P	n.s.
Tgfb3	transforming growth factor beta III	n.d.	up	[9]	NM_013174	P	n.s.
Fgf2	fibroblast growth factor 2	n.d.	up	[9]	NM_019305	A	--
Npy1r	neuropeptide Y receptor	down[a]	up	[10,11]	BI395810	A	--
Ngfg	glandular kallikrein or true kallikrein	n.d.	up	[12]	NM_031523	+ 6.52	0.0023
Mmp9	matrix metalloproteinase 9	n.d.	up	[18]	NM_031055	A	--
Rb1	retinoblastoma susceptibility protein	n.d.	down	[14]	AI178012	P	n.s.
Rab3a	Ras-related small GTP binding protein 3A	n.d.	down	[16]	NM_013018	P	n.s.
Vamp2	synaptobrevin 2	n.d.	down	[16]	NM_012663	P	n.s.
Syt1	synaptotagmin 1	n.d.	down	[16]	AI413003	A	--
Snap25	synaptosomal-associated	n.d. protein 25	down	[16]	NM_030991	P	n.s.
Stx1a	syntaxin 1 (Syntaxin 1A?)	n.d.	down	[16]	NM_053788	- 2.19	0.0110
Vamp3	“cellubrevin, a.k.a. synaptobrevin 3”	n.d.	down	[16]	NM_057097	- 1.41	0.0312
Penk-rs	Met5- and Leu5-enkephalins	n.d.	down	[54]	NM_017139	- 6.19	0.0087

n.s. = p >0.05

A = called absent

P = all called present, but no statistically significant change

[a] NPY mRNA is down in pitutiary as a whole but is up in gonadotropes [10,11].

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Table 4

Confirmation of Selected Genes by Real-Time PCR

Gene	Fold Change[a]
Calb3	- 2.0
Fgf2	-2.5
Gal	+126.0
myc	-1.4
Prl	+1.7
Pttg1	+4.9
Pvalb	-1.3
Tgfb1	+1.1
Tgfb3	1.0
Vip	+535.0

[a] estrogen treated relative to untreated.