Expressions of hepatic genes, especially *IGF-binding protein-1*, correlating with serum corticosterone in microarray analysis

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Abstract

Microarray technology was evaluated for usefulness in assessing relationships between serum corticosterone and hepatic gene expression. Nine pairs of female Swiss mice were chosen to provide a wide range of serum corticosterone ratios; cDNA microarray analysis (~8000 genes) was performed on their livers. A statistical method based on calculation of 99% confidence intervals discovered 32 genes which varied significantly among the livers. Five of these ratios correlated significantly with serum corticosterone ratio, including *tyrosine aminotransferase*, *stress-induced protein*, *pleiotropic regulator 1* and *insulin-like growth factor-binding protein-1*; the latter has a potential role in cancer development. Secondly, linear regression of gene expression vs corticosterone ratios was screened for those with $r \geq 0.8$ ($P < 0.01$), yielding 141 genes, including some known to be corticosterone regulated and others of interest as possible glucocorticoid targets. Half of these significant correlations involved data sets where no microarray ratio exceeded ± 1.5 . These results showed that microarray may be used to survey tissues for changes in gene expression related to serum hormones, and that even small changes in expression can be of statistical significance in a study with adequate numbers of replicate samples.

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Introduction

Glucocorticoids are well known to regulate the expression of numerous genes in liver for control of energy metabolism, in response to stress, and as part of other physiological processes. In the past, these regulatory events have been revealed by experimental manipulation *in vivo* through ablation or hormonal supplementation studies, and by hormone treatment of hepatic cells in culture. In general, such investigations have involved large changes in hormone levels and hence pertain particularly to gene responses under extreme conditions. Fewer studies have been carried out to find changes in gene expression that occur in liver during the hourly fluctuations in circulating glucocorticoids that accompany diurnal rhythms, varying physical activity levels, and feeding. Also,

there have been no comprehensive assessments of the total number and nature of the hepatic genes that may be influenced by glucocorticoids.

As a first step in gathering information on these topics, we have undertaken microarray analysis of normal mouse liver genes with a set of approximately 8000 cDNAs, and have correlated gene expression ratios with ratios of levels of serum corticosterone. Mice were analyzed in pairs, chosen on the basis of differing serum corticosterone; microarray analysis of gene expression was carried out for each liver. The ratio of serum corticosterone for the pair was then matched with the ratio of expression of each hepatic gene for that pair, for xy plotting of corticosterone ratio vs hepatic gene expression ratio. With nine pairs of livers, we found significant ($P < 0.01$) correlations between serum corticosterone and microarray ratios for 141 genes.

Some of these genes are known to be glucocorticoid responsive, while others are revealed as potential glucocorticoid targets for the first time. Of particular interest was *insulin-like growth factor-binding protein-1* (*Igfbp1*), which, of all the genes correlated with serum corticosterone, showed the widest range of expression ratios. This protein plays a key role in regulating the actions of insulin-like growth factors (IGF)-I and -II, important contributors to growth control and cancer development (Wetterau *et al.* 1999). The results with *Igfbp1* were confirmed by both reverse transcription (RT)-PCR and real-time PCR. These studies have established, for the first time, the utility of microarray for relating serum parameters to gene expression in organs and establish a possible mechanism by which altered serum corticosterone could influence neoplasia development in multiple tissues.

Materials and methods

Animals and corticosterone analyses

Animals were maintained and handled in a facility fully accredited by the American Association for the Accreditation of Laboratory Animal Care, in accordance with the policies established by that organization and by National Institutes of Health (NIH) guidelines (Guide for the Care and Use of Laboratory Animals, NIH Publication No. 86-23 1985). Female Swiss Cr:NIH(S) mice were bred from parents obtained from the Animal Production Area at the National Cancer Institute at Frederick. They were maintained on hardwood shavings as bedding, given NIH open formula autoclavable mouse chow (PMI, Inc., Richmond, IN, USA) and acidified water, under conditions of $72 \pm 7^\circ\text{C}$, $50 \pm 20\%$ humidity, and a fluorescent light cycle of 12 h light:12 h darkness. The mice were killed by decapitation between 0930 and 1200 h. Preliminary studies showed that stress-related changes in serum corticosterone could be reduced by acclimatizing the mice to the laboratory and to brief handling before they were killed (not shown). Sera and livers were immediately frozen in liquid nitrogen and stored at -70°C until use. Total serum corticosterone was assayed by AniLytics, Inc. (Gaithersburg, MD, USA), utilizing a ^{125}I radioimmunoassay kit from ICN Biomedicals, Inc. (Irvine, CA, USA).

Liver samples were chosen so as to represent mice with a range of serum corticosterone values. In experiment 1, these values ranged from 25 to 740 ng/ml. Pairs for microarray analysis were selected so as to construct a wide range of serum corticosterone ratios, including eight that were uniformly distributed plus a lower extreme of 0.034 (pair 9 in Table 1). For the second confirmatory experiment assayed with real-time PCR, sera and livers were from an independent group of female mice of the same strain.

Microarray analysis

Total RNA was extracted from a sample of each liver by homogenization in the presence of Trizol (Life Technologies, Inc.). mRNA was isolated with oligo dT (Oligotex; Qiagen Co., Valencia, CA, USA). One microgram mRNA of each sample was submitted to Incyte Genomics, Inc. (Fremont, CA, USA) for the RT, microarray hybridization, and quantification process. ScanArray (Perkin Elmer Co., Willsley, MA, USA) and GenePix Software (Union City, CA, USA) were used to acquire microarray image analysis and for quantification. Both endogenous and exogenous internal controls were included to monitor the quality and quantity of each array. Further information regarding Incyte Genomics may be found on the web site (www.incyte.com). Identifications of the Integrated Molecular Analysis of Genes and their Expression (I.M.A.G.E.) clones, used for preparation of the Incyte Genomics chips, were by reference of chip identification numbers to GenBank and dbEST, as of 15 April 2003. The reader is advised that definitive identification of some I.M.A.G.E. clones is still an evolving process.

Microarray data were downloaded from Incyte Genomics with a file transfer protocol to a Microsoft Windows NT 4.0 workstation equipped with GEMTools, a proprietary microarray analysis software from Incyte Genomics. Tab-delimited format microarray data files and pseudocolor plate-view PDF files were also downloaded from the company website. The data were prefiltered such that spots that were not at least 40% covered or that had a signal/background ratio less than 2.5 were omitted. Tab-delimited data files were imported to Microsoft Excel 97/2000 and to various mathematical and statistical packages for analysis.

The distribution of the data for each microarray pair was evaluated by the method of Chen *et al.* (1997). Briefly, this method requires the calculation of a maximum likelihood estimate for the common coefficient of variation of the entire cDNA set. This method is used to specify the probability density function for the ratio of the set. Knowledge of this, in turn, enables one to calculate the lower and upper limits of a confidence interval (CI) for the array (Chen *et al.* 1997, Powell *et al.* 2002). As noted previously, by chance alone, only one cDNA of the ~8000 assessed is expected to be found outside of the 99% CI in two array sets. It is highly unlikely that any given cDNA will occur outside of the 99% CI of three or more array sets. This strategy enabled us to identify genes whose apparent changes in expression were not due to chance alone.

As an additional analytical strategy, correlations of microarray ratios were sought with serum corticosterone ratios and with the logs of these ratios. In addition, corticosterone ratios less than one were converted to negative reciprocals, to parallel the representation of downregulated genes as negative values in the microarray. This format is referred to in this report as a reciprocal ratio. All three presentations of the corticosterone ratio data were tested for significant correlation with the expression of each of the ~8000 genes in nine arrays. All genes with a correlation value of $r=0.80$ or better for any of the three corticosterone ratio data sets were selected. This corresponded to a significance of $P=0.01$ or better. There were 141 genes that met this criterion. Each of these genes was then examined separately for significance of the correlations by linear regression analysis. Each correlation was also tested for significance when the lower extreme value was omitted.

RT-PCR for *Igfbp1*

Specific IGF-binding protein-1 (*Igfbp-1*) primers (forward: ctctgcatgccctcacc and reverse: ccagcagctgttcctct) were designed with Primer3 (www.genome.wi.mit.edu). The 18s RNA, amplified in parallel, served as an internal and amplification control. Synthesis of the first strand of cDNA was performed by the use of RETROScript (Ambion Corp.). A 20 µl reaction mixture, containing 2 µg total RNA, 100 U M-MLV RT, 1.25 µM random hexamers, 10 U placental RNase inhibitor, 0.5 mM

dNTPs, 1.5 mM MgCl₂, 50 mM KCl, and 10 mM Tris-HCl, pH 8.3, was incubated at 42 °C for 1 h and then heated to 92 °C for 10 min to denature the RT. An aliquot of 2 µl cDNA mixture was then amplified for the *Igfbp* and Classic 18S RNA internal amplification control (Ambion Quantum-RNA 18s RNA). The amplification reaction mixture consisted of 0.125 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 5 µM each forward and reverse primers, and 2.5 U Taq DNA polymerase (Ambion SuperTaq Plus) in a final volume of 50 µl. An RT-free reaction was run as a negative control. Optimum linearity conditions for the multiplex RT-PCR amplification along with 18s RNA were established (57 °C, 25 cycles). PCR products were electrophoresed through a 10% polyacrylamide gel in Tris-borate-EDTA buffer. Bands were quantified with Sybr Green II (Molecular Probes, Inc., Eugene, OR, USA) and luminescence was measured in a digital image system (Alpha Innotech, IS-1000, San Leandro, CA, USA). The intensity of each band was normalized to the intensity of the corresponding 18s RNA control bands.

Real-time PCR for *Igfbp1*

Specific primers for real-time PCR quantification of *Igfbp1* mRNA were designed using Primer Express software (Applied Biosystems, Foster City, CA, USA) and optimized for amplification and minimal primer dimer formation. Accurate quantification of *Igfbp1* mRNA was achieved using a plasmid vector containing the mouse *Igfbp1* cDNA obtained from the American Type Culture Collection (Manassas, VA, USA; I.M.A.G.E. no. 4161889). DNA sequencing from both the 5' and 3' ends verified identity. The real-time PCR assays for *Igfbp1* plasmid DNA or cDNA were carried out in 20 µl reactions using primers (forward primer CTC TGC ATG CCC TCACC; reverse primer CCA GCA GCT GTT CCT CT) and SYBR Green master mix (Qiagen Inc.) and run on an ABI 8900 (Applied Biosystems, Inc., Foster City, CA, USA) for 40 cycles (95 °C for 15 min, 95 °C for 15 s, 60 °C for 30 s; then a dissociation curve: 95 °C for 15 s, 60 °C for 15 s and then a 2% ramp rate to 95 °C for 15 s). The standard, mouse *glyceraldehyde-3-phosphate dehydrogenase* (*Gapdh*) plasmid DNA was purchased from Serologicals (Gaithersburg, MD, USA). The real-time PCR

Table 1 Pairs of female mouse serum and liver utilized

Mouse pair	Serum corticosterone			
	ng/ml	Ratio	Log ratio	Reciprocal ratio
R19	284	2.20	0.34	2.20
R34	129			
R20	502	1.22	0.09	1.22
R24	411			
R38	587	1.12	0.05	1.12
R26	522			
R18	350	1.08	0.03	1.08
R33	324			
R16	405	0.66	−0.18	−1.51
R29	611			
R15	256	0.60	−0.22	−1.67
R28	427			
R17	96	0.27	−0.57	−3.75
R30	360			
R27	72	0.15	−0.83	−6.79
R7	489			
R3	25	0.03	−1.47	−29.6
R4	740			

assays for *Gapdh* plasmid DNA was carried out in 20 µl reactions using the mouse GAPDH control kit (Applied Biosystems) and run on an ABI 7900 for 40 cycles (95 °C for 15 min, 95 °C for 15 s, 60 °C for 30 s, then a dissociation curve: 95 °C for 15 s, 60 °C for 15 s and then a 2% ramp rate to 95 °C for 15 s. Sensitivity and linear dynamic range were checked on the serial dilutions (10 to 10⁶ copies/reaction) of *Igfbp1* and *Gapdh* plasmid DNA and found to be >98% efficient with a slope of 3.6. The *Igfbp1* mRNA expression was normalized to the *Gapdh* expression in multiplex and quantified with its own standard curve, >97% efficiency with a slope of −3.8.

Results

Genes with significant among-pairs variation

The features of the nine pairs of mice used are given in Table 1, including the absolute concentration of corticosterone in the serum and the serum corticosterone ratios for each pair. The last pair listed, with a corticosterone ratio of 0.03, is referred to as the 'lower extreme value' in the analyses which follow.

cDNA at 35 locations on the microarray chips showed a significant range in values among pairs, after application of the winnowing procedure based on the 99% confidence limits (see Materials and methods). Of these, 23 represented known genes. Two were present at two or more chip locations, providing confirmation of the results. Thus, expressions of 21 known genes showed significant variability among these normal livers, by this conservative statistical procedure.

Since the liver pairs had been selected on the basis of serum corticosterone, we next tested each of the 35 array ratios for significance of correlation with serum corticosterone ratios. Six were significant at $P < 0.05$: one EST, one hypothetical protein, and *Igfbp1*, *stress-induced protein*, *pleiotropic regulator 1*, and *tyrosine aminotransferase* (Table 2). Microarray ratios for all of these genes were positively associated with serum corticosterone ratios, except for *pleiotropic regulator 1*, for which the correlation was negative.

Genes which showed significant expression variation among livers, but did not correlate with serum corticosterone, included *metallothionein 1*, *lipin 1*, *presenilin 2*, *cytochrome P450 4a14*, *cytochrome P450 4a10*, *fatty acid synthase*, *aquaporin 1*, *macrophage activation protein 2*, *N-methyl-D-aspartate receptor-regulated gene 1*, *chloride intracellular channel 1 (mitochondrial)*, *deleted in azoospermia-like*, *glutamate oxaloacetate transaminase 1*, *pancreatic colipase*, *aminolevulinic acid synthase 1*, *long chain fatty acyl elongase*, and *enolase 1*, *alpha non-neuron*. Many of these showed wide ranges in array ratios among the nine liver pairs, from −5 to +5.8.

Gene expression ratios correlating with serum corticosterone

Additional correlations of gene expression with serum glucocorticoid were sought by linear regression analysis and selection of cDNAs with $r \geq 0.8$ for the nine pairs, as related to corticosterone ratio, log of this ratio, or reciprocal ratios (see Materials and methods). A total of 141 genes met this criterion (Table 3); of these, 112 correlated positively with serum corticosterone, and 29 correlated negatively. Ratios of 83 genes, listed in Table 4, maintained their significant correlation with corticosterone ratio when the lower extreme value was omitted; thus these showed correlation over a wide range of corticosterone values and

Table 2 Genes showing significant expression variation among the nine pairs of livers, based on CI calculations, and also significant correlation with serum corticosterone ratios

Clone location number	Clone name	I.M.A.G.E. clone identification	Range of microarray values	Direction and significance of correlation with serum corticosterone (<i>P</i>)
376	Insulin-like-growth factor-binding protein 1 (Igfbp1)	404595	-6.2 to 1.3	↑0.0093
1310	Stress-induced protein (Stip)	619418	-2.7 to 2.0	↑0.034
2559	Hypothetical protein 1190009B22	355442	-2.3 to -1.0	↑0.0061
6573	Pleiotropic regulator 1, PRL1 homolog (Plrg1)	776426	-2.4 to 3.0	↓0.030
7768	RIKEN cDNA 1110030N17	480706	-3.0 to 1.3	↑0.0043
8478	Tyrosine aminotransferase (Tat)	680259	-3.6 to 1.5	↑0.014

The clone location number indicates the position on the Incyte Genomics mouse cDNA microarray chip. The distribution of the data for each of the nine microarray pairs was evaluated by the method of Chen *et al.* (1997), leading to calculation of the lower and upper limits of a 99% CI for the array. A total of 32 cDNAs at 35 chip locations presented array ratios which occurred outside the 99% CI for three or more data sets, a degree of variation with a very low probability of occurring by chance. The array ratios for each cDNA were examined by linear regression analysis for correlation with serum corticosterone ratios for the same animals. Of the 32 genes, five gene expression ratios correlated with serum corticosterone ratios; these results are shown above.

Table 3 Hepatic genes showing a significant correlation with serum corticosterone over nine pairs of livers

Category	No.	Correlation		Ratio expression giving most significant correlation		
		Positive	Negative	Direct	Log	Reciprocal
(1) Significant without lower extreme value	83	76	7	15	64	4
(2) Significant only with inclusion of the lower extreme value	58	36	22	0	2	57
Total	141	112	29	15	66	61

The nine serum corticosterone ratios included eight that were spaced as a continuum over regular intervals, plus a ninth that was markedly lower; the latter is referred to as the lower extreme value. Gene array ratios were examined for correlation with the serum corticosterone ratios from the same animals, with a cut-off of $r=0.8$, $\sim P=0.01$. The set was queried with the corticosterone ratios calculated in three different ways: as a direct ratio, as a log of the direct ratio, and as a format where ratios <1.0 were utilized as reciprocals, to mimic the format of the array ratios. These processes yielded 141 significantly correlated genes. These were further subdivided into those that maintained a significant correlation even when the lower extreme value was omitted (category 1) and those that were significantly correlated only with inclusion of the lower extreme value (category 2).

ratios. These are designated category 1. The remaining 58 gene ratios (category 2; Table 5) correlated with corticosterone ratios only when the lower extreme value was included and so were most

likely related to more marked differences in corticosterone. In both Tables 4 and 5, the genes are presented in approximate rank order according to the magnitude of the array ratios.

Table 4 Hepatic genes correlating with serum corticosterone, still with significance after omission of lower extreme value

Clone location number	Clone name	I.M.A.G.E. clone identification	Function	Correlation with corticosterone	Gene ratios	
					#≥1.5	#≥2.0
376	Insulin-like growth factor-binding protein-1 (Igfbp1)	404595	Regulates <i>in vivo</i> activity of IGF-I, usually negatively	↑	6	5
8478	Tyrosine aminotransferase (Tat)	680259	Metabolism	↑	5	3
7768	Hypothetical protein MGC36388	480706	Unknown	↑	3	2
4163	Carnitine palmitoyl transferase 1, liver (Cpt1a)	737898	Phospholipid metabolism	↑	3	1
4649	Translocator of inner mitochondrial membrane 17 kDa, a (Timm17a)	318134	Preprotein import machinery component	↓	3	1
2435	RIKEN cDNA 1110033E03 gene	333669	Unknown	↑	3	0
8505	Forminotransferase cyclodeaminase (Ftcd)	680146	Has both transferase and deaminase activity; links histidine with folate metabolism	↑	3	0
2559	EST	355442	Unknown	↓	2	1
2022	RIKEN cDNA 1200014D15 gene	748958	Unknown	↑	2	0
2999	RIKEN cDNA 1110036H21	459538	Unknown	↑	2	0
3060	EST	466788	Unknown	↑	2	0
3506	Zinc finger protein 103 (Zfp103)	596277	Presumed transcription factor	↑	2	0
3953	Proline dehydrogenase (oxidase) 2 (Prodh2)	676559	Proline metabolism	↑	2	0
6673	Williams-Beuren syndrome chromosome region 14 homolog (human) (Wbscr14)	521951	Transcriptional repressor	↑	2	0
5854	Eph receptor A3 (Epha3)	635020	Lymphoid function	↓	1	1
6237	Peroxisomal trans-2-enoyl-CoA reductase (Pecr)	680935	Metabolism	↑	1	1
8796	Butyryl coenzyme A synthetase 1 (Bucs1)	748351	Mitochondria metabolism	↑	1	1
189	DNA segment, Chr 2, ERATO Doi 120, expressed	651903	Unknown	↑	1	0
2990	EST weakly similar to T lymphoma oncogene protein	463157	Unknown	↓	1	0
3580	RIKEN cDNA 0710008A13 gene	634469	Unknown	↑	1	0
3929	Aconitase 1 (Aco1)	677092	Metabolism	↑	1	0
4171	Cerebellar postnatal development protein 1 (Cpd1)	736660	Associated with cerebellum development	↑	1	0
6961	Heterogeneous nuclear ribonucleoprotein A2/B1 (Hnrpa2b1)	315740	Pre-mRNA processing, telomere regulation	↑	1	0

Continued

Table 4 Continued

Clone location number	Clone name	I.M.A.G.E. clone identification	Function	Correlation with corticosterone	Gene ratios	
					#≥1.5	#≥2.0
7039	RIKEN cDNA 1300001I01 gene	373505	Unknown	↑	1	0
7120	Phytanoyl-CoA hydroxylase (Phyh)	403728	Peroxisomal metabolism of phytanic acid	↑	1	0
7614	Pescadillo homolog 1, containing breast cancer gene 1 carboxyterminal (BRCT) domain (zebrafish) (Pes1)	477900	Cell cycle, DNA repair; may be necessary for oncogenic transformation and tumor progression	↑	1	0
7790	Protein distantly related to the calcium channel gamma subunit family (Pr1)	571643	Stabilization of inactive calcium channels	↑	1	0
8202	Hypothetical protein MGC38585	638204	Unknown	↑	1	0
8560	Calcium-binding protein, intestinal (Cai)	695696	Protein disulfide isomerase, endoplasmic reticulum	↑	1	0
8564	Ribosomal protein S5 (Rps5)	696769	Protein synthesis	↑	1	0
8676	Cis-retinol/3alpha hydroxysterol short-chain dehydrogenase-like (Crad-L)	747207	Regulates production of retinol and testosterone metabolite; gene expression	↑	1	0
8936	Histidine triad nucleotide bonding protein (Hint)	533117	Adenosine hydrolase?	↑	1	0
176	Expressed sequence AL022671	335302	Unknown	↑	0	0
235	Peroxisome biogenesis factor 16 (Pex16)	350336	Peroxisome biosynthesis and integrity	↑	0	0
470	RIKEN cDNA 1110018G07 gene	422206	Unknown	↑	0	0
749	Peroxisomal biogenesis Factor 3 (Pex3)	463255	Biogenesis of peroxisomes	↑	0	0
1060	RIKEN cDNA 0610038K03 gene	492407	Unknown	↑	0	0
1192	Inositol hexaphosphate kinase 1 (Ihpk1)	580257	Releases calcium from intracellular stores	↑	0	0
1529	Nuclear factor of activated T cells, cytoplasmic 2 (Nfatc2)	850163	Proinflammatory transcription factor	↑	0	0
1582	RIKEN cDNA 1700021K19	890852	Unknown	↓	0	0
1717	EST, moderately similar to rat amine oxidase (flavin-containing) B (monoamine oxidase)	680958	Metabolism, neurotransmission	↑	0	0
1935	Scavenger receptor class B, member 2 (Scarb2)	747497	Mediates cholesterol uptake	↓	0	0
1941	secretory protein 14 (SEC14)-like 2 (<i>S. cerevisiae</i>) (Sec14I2)	747543	Carrier protein, may have transcriptional activity	↑	0	0

Continued

Table 4 Continued

Clone location number	Clone name	I.M.A.G.E. clone identification	Function	Correlation with corticosterone	Gene ratios	
					#≥1.5	#≥2.0
2064	Glutathione S transferase alpha 3 (GSTa3)	766582	Anti-oxidant, catabolism	↑	0	0
2294	Serine/threonine kinase 11 (Stk11)	933770	Signaling; putative tumor suppressor	↑	0	0
2825	Transmembrane protein 4 (Tmem4)	438550	Unknown	↑	0	0
2850	RIKEN cDNA 0710001K01 gene	424968	Unknown	↑	0	0
3045	Mitochondrial ribosomal protein L15 (Mrpl15)	464084	Protein synthesis	↑	0	0
3143	Phosphatidylinositol transfer protein, beta (Pitpnb)	476642	Catalyzes the transfer of phosphatidylinositol and phosphatidylchloride between membranes	↑	0	0
3229	cDNA sequence BC002318	480204	Unknown	↑	0	0
3239	LIM and Sh3 protein 1 (Lasp1)	482313	Involved in cAMP signaling; ion transport; regulation of cytoskeleton; amplified in breast cancers	↑	0	0
3305	NADH dehydrogenase (ubiquinone) Fe-S protein 2 (Ndusf2)	572585	Mitochondrial electron transport; metabolism	↑	0	0
3312	Isocitrate dehydrogenase 3 (NAD+)-beta (Idh3b)	570685	Metabolism	↑	0	0
3325	CDC-like kinase 2 (Clk2)	492518	Autophosphorylating kinase; possibly involved in mRNA splicing	↑	0	0
3382	Regenerating islet-derived 3 gamma (Reg3g)	575773	Possible role in islet β-cells	↑	0	0
3455	Acid phosphatase 6, lysophosphatidic (Acp6)	582668	Catabolism	↑	0	0
3480	EST, weakly similar to beta-tubulin cofactor E (<i>H. sapiens</i>)	583526	Tubulin folding	↑	0	0
3520	cDNA sequence BC003281	596556	Unknown	↑	0	0
3579	Expressed sequence AI662535	636324	Unknown	↑	0	0
3626	Alcohol dehydrogenase 5 (Adh5)	636207	Metabolism, catabolism	↑	0	0
3640	Syntaxin 4A (placental) (Stx4a)	634982	Membrane protein involved in exocytosis	↑	0	0
3702	Expressed sequence AI465155	639991	Unknown	↑	0	0
3851	Eukaryotic translation initiation factor 3 (Eif3)	889816	Translation initiation	↑	0	0

Continued

Table 4 Continued

Clone location number	Clone name	I.M.A.G.E. clone identification	Function	Correlation with corticosterone	Gene ratios	
					≥ 1.5	≥ 2.0
4108	Beta-1,3-glucuronyltransferase 3 (glucuronosyltransferase I) (B3gat3)	734282	Catabolism	↑	0	0
4158	Ankyrin repeat domain-containing SOCS box protein 13 (Acb13)	735500	Couples suppressor of cytokine signalling (SOCS) proteins and their binding partners with the elongin B and C complex, possibly targeting them for degradation	↑	0	0
4224	Expressed sequence R74866	749778	Unknown	↑	0	0
4374	Ubiquitin 1 (Ubn1)	820195	Interacts with the basic domains of transcription factors EB1 and c-Jun and competes with their binding to the AP1 consensus site	↑	0	0
4766	RIKEN cDNA 5430429M05 gene	353294	Unknown	↓	0	0
4813	Solute carrier family 25 (mitochondrial carrier; oxoglutarate carrier), member 11 (Slc25a11)	367801	Mitochondrial function	↑	0	0
5560	Tripartite motif protein 24 (Trim24)	571091	Transcriptional intermediary factor	↑	0	0
5638	Expressed sequence AU042952	574974	Unknown	↑	0	0
6231	Glucosidase 1 (Gcs1)	680150	First enzyme in the N-linked oligosaccharide processing pathway	↑	0	0
6464	DNA segment, Chr 16, human D22S680E, expressed	750147	Unknown	↑	0	0
7055	SH3-binding kinase (Sbk)	389034	Signaling	↑	0	0
7136	Transmembrane 9 superfamily member 1 (Tm9sf1)	404535	Vesicle transport?	↑	0	0
7475	RIKEN cDNA 2610003B19	456543	Unknown	↑	0	0
7656	Translin-associated factor X (Tsnax)	476185	A DNA-binding protein that binds consensus sequences at breakpoint junctions of chromosomal translocations	↑	0	0
7842	WD repeat domain 1 (War1)	493109	Actin-binding protein, putatively involved in protein interaction	↑	0	0
7846	EST	484315	Unknown	↑	0	0

Continued

Table 4 Continued

Clone location number	Clone name	I.M.A.G.E. clone identification	Function	Correlation with corticosterone	Gene ratios	
					# ≥ 1.5	# ≥ 2.0
8397	Degenerating spermatocyte homologue (Dega)	676578	Lipid desaturase	↑	0	0
8771	Ribosomal protein S5 (Rps5)	775645	Protein synthesis	↑	0	0
8966	RIKEN cDNA 2010012D11 gene	524682	Unknown	↑	0	0
8969	RIKEN cDNA 2510008H07 gene	532770	Unknown	↑	0	0

The clone location number refers to the position on the Incyte Genomics mouse cDNA microarray chip. For each cDNA, the array ratios for nine pairs of female mouse livers were plotted against the ratios of serum corticosterone levels for the same animals. Those showing correlation at $P < 0.01$ or better by linear regression analysis are listed, along with the direction of the correlation. The number of microarray ratios equal to or exceeding 1.5 and 2.0 are also shown.

The majority of the genes in category 1 showed strongest correlation with absolute or, more frequently, log corticosterone ratios; only four correlated best with the reciprocal value (Table 3). Most correlated positively, with seven showing a negative association (Table 4). A convention in microarray analysis is to regard ratios of ± 1.5 as potentially significant. In category 1, 32 genes included at least one ratio ± 1.5 ; some of these genes are illustrated in Fig. 1. The first two genes on the list, *Igfbp1* and *tyrosine amino transferase*, had multiple gene ratios ± 2.0 , and had accordingly also appeared on the list of significant changes based on the analysis presented in Table 2. However, even genes with only one ratio value ± 1.5 showed highly significant correlations with serum corticosterone ratios, for example, *pescadillo homologue 1* ($P = 0.0055$), *heterogeneous nuclear ribonucleoprotein A2/B1* ($P = 0.0019$), *cis retinol-androgen dehydrogenase* ($P = 0.0043$), *proline oxidase 2* ($P = 0.0045$), and *histidine triad nucleotide-binding protein* ($P = 0.0033$) (Fig. 1). Of these, proline oxidase is known to be regulated by corticosterone (Kowaloff *et al.* 1977). The heterogeneous nuclear ribonucleoprotein A2/B1 belongs to a class of molecules known to interact with the glucocorticoid receptor (Eggert *et al.* 2001). Thus, correlations of expression of these genes with serum corticosterone are plausible.

Fifty-one genes in category 1 had no microarray ratio ± 1.5 (Table 4). In spite of presentation

exclusively of low gene expression ratios, many of the correlations showed very highly significant correlations between cDNA ratios and serum corticosterone, as illustrated in Fig. 2. Several of these are known to be regulated by glucocorticoids: *nuclear factor of activated T cells* ($P = 0.0028$, not shown) (Refojo *et al.* 2001), *glutathione S transferase $\alpha 3$* ($P = 0.0019$) (Falkner *et al.* 2001), *alcohol dehydrogenase 5* ($P = 0.0030$) (Edenberg 2000), and *glucosidase 1* ($P = 0.0037$, not shown) (Kalamidas & Kotoulas 2000). The protein product of *scavenger receptor class B* ($P = 0.0063$) has an important role in regulating plasma levels of cholesterol for use in glucocorticoid synthesis (Galman *et al.* 2002, Malerod *et al.* 2002).

Category 2 genes included many correlating negatively with corticosterone ratios, and almost all showed strongest correlation when the reciprocal corticosterone ratio values were used (Table 3). Fourteen genes in this class had more than one ratio ± 1.5 and/or one or more ≥ 2.0 (Table 5). Nevertheless, some genes with only one ratio ± 1.5 had very highly significant correlations between gene and corticosterone ratios (Fig. 3), for example, *interleukin 15 receptor* ($P = 0.0001$), *fibroblast growth factor receptor* ($P < 0.0001$), *nuclear factor, erythroid derived* ($P = 0.0001$), and *programmed cell death 8* ($P < 0.0001$). Genes in this group known to be influenced by glucocorticoids include *fibroblast growth factor receptor* (Riva *et al.* 1998) and the myeloblastosis oncogene, *myb* (Eastman-Reks & Vedeckis 1986).

Table 5 Hepatic genes correlating with serum corticosterone, significant only with inclusion of the lower extreme value

Clone location number	Clone name	I.M.A.G.E. clone identification	Function	Correlation with corticosterone	Gene ratios	
					#≥1.5	#≥2.0
5604	BTB (POZ) domain containing 1 (Btbd1)	576401	Interacts with topoisomerase 1	↑	4	1
7214	EST highly similar to DEAD-box protein p72	418920	DNA helicase	↑	4	0
4127	RIKEN cDNA 2210403K04	722566	Unknown	↑	3	2
7154	RIKEN cDNA 1110003O22	404057	Unknown	↑	3	1
8236	Moderately similar to putative integral membrane transport protein (USTlr)	643257	Solute transport?	↑	3	1
5676	RIKEN cDNA 2610007K22	573857	Unknown	↑	3	0
8479	RIKEN cDNA 1810018P12	679244	Unknown	↑	3	0
5178	par-3 (partitioning defective 3) homolog (<i>C. elegans</i>) (Pard3)	443916	Essential for asymmetric cell division and polarized growth	↓	2	1
5699	Hypothetical protein MGC25977	578525	Unknown	↑	2	0
2714	RIKEN cDNA 1110006I15	422123	Unknown	↓	1	1
6313	Myeloblastosis oncogene (Myb)	721803	Transcription factor	↓	1	1
6389	Pellino 1 (Peli 1)	718768	Signaling; toll receptor-related protein	↓	1	1
7066	f-box and leucine-rich repeat protein 11 (Fbxl11)	373036	Protein targeting?	↓	1	1
7545	RIKEN cDNA 1200003O06 gene	464280	Unknown	↑	1	1
1071	Similar to hypothetical protein CG2803	493256	Unknown	↑	1	0
1103	RIKEN cDNA 2610002D18 gene	573595	Unknown	↓	1	0
1245	Expressed sequence C78339	599069	Unknown	↑	1	0
1578	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6 (14 kDa, B14) (Ndufa6)	871020	Mitochondrial electron transport	↑	1	0
1810	Fibroblast growth factor receptor 1 (Fgfr1)	734032	Receptor; cell growth control	↓	1	0
1851	Nuclear factor, erythroid derived, 45 kDa (Nfe2)	733953	Presumed transcription factor regulates hemoglobin production	↓	1	0
1860	Programmed cell death 8 (Pcd8)	719457	Involved in apoptosis	↑	1	0
2032	Ubiquitin-conjugating enzyme E2, J1 (Ube2j1)	751477	Protein regulation	↓	1	0
3938	EST highly similar to chloride intracellular channel protein p64	670375	Presumed chloride transport nuclear membrane	↑	1	0

Continued

Table 5 Continued

Clone location number	Clone name	I.M.A.G.E. clone identification	Function	Correlation with corticosterone	Gene ratios	
					#≥1.5	#≥2.0
4220	Inositol polyphosphate-5-phosphatase, 75 kDa (Inpp5b)	749425	Signaling	↑	1	0
4236	Nitrogen fixation gene 1 (<i>S. cerevisiae</i>) (Nfs1)	750126	Mitochondrial iron transport; iron-sulfur cluster	↑	1	0
4260	Interleukin 15 receptor, alpha chain (Il15ra)	777103	IL15 action Interleukin	↑	1	0
4561	<i>M. musculus</i> , clone I.M.A.G.E.: 5102170, mRNA, partial	761319	Unknown	↓	1	0
566	Pregnancy upregulated non-ubiquitously expressed CaM kinase (Pnck)	834617	Signaling	↓	1	0
715	Expressed sequence AI194968	457071	Unknown	↓	1	0
7356	ATP-binding cassette, sub-family G (WHITE), member 4 (Abcg4)	427248	Expressed predominantly in liver tissue; function may involve cholesterol transport	↓	1	0
7461	Cyclin D-type binding protein 1 (CCNDBP1) interactor (Cdpin)	445520	Protein interaction; cell cycle control	↓	1	0
7834	EST	571219	Unknown	↑	1	0
87	RIKEN cDNA 9030401P18	574725	Unknown	↑	1	0
8894	Peroxisomal sarcosine oxidase (Pso)	790721	Peroxisomal metabolism	↑	0	0
539	Transactivating region (TAR) DNA binding protein (Tardbp)	427346	A transcriptional repressor that binds to chromosomally integrated TAR DNA and represses HIV-1 transcription	↑	0	0
852	EST	476972	Unknown	↓	0	0
917	Expressed sequence C86142	479186	Unknown	↓	0	0
1040	RIKEN cDNA 2310004N11	573093	Unknown	↓	0	0
1507	Cadherin 11 (Cdh11)	658334	Adhesion protein	↑	0	0
1759	RIKEN cDNA 1110027L01	693341	Unknown	↓	0	0
1896	Protein kinase, cAMP dependent, regulatory, type 1 beta (Prkar1b)	736724	Signal transduction	↓	0	0
2057	Interferon regulatory factor 1 (Irf1)	777393	Transcription factor; anti-oncogenic actions	↑	0	0
3522	RIKEN cDNA 1700056O17 gene	596719	Unknown	↑	0	0
4201	EST AI426782	737425	Unknown	↑	0	0
4240	Carcinoembryonic antigen (CEA)-related cell adhesion molecule 2 (Ceacam2)	762299	Cell adhesion; immunoglobulin family; anti-oncogenic actions	↑	0	0

Continued

Table 5 Continued

Clone location number	Clone name	I.M.A.G.E. clone identification	Function	Correlation with corticosterone	Gene ratios	
					#≥1.5	#≥2.0
4452	EST	535794	Unknown	↑	0	0
4904	Protein tyrosine kinase 9 (Ptk9)	402345	Cell regulation	↓	0	0
5561	Small protein effector 1 of Cdc42 (Spec1)	573520	May act as a scaffold to co-ordinate and/or mediate Cdc42 regulation of kinase signaling and cell shape changes	↓	0	0
6214	<i>M. musculus</i> , clone I.M.A.G.E.: 3597827, mRNA, partial	676898	Unknown	↑	0	0
6811	<i>M. musculus</i> , similar to tripartite motif-containing 6, clone	805046	Signaling?	↑	0	0
7307	B-cell CLL/lymphoma 11A (zinc finger protein) (Bcl11a)	425021	May be a leukemia/lymphoma-associated disease gene; translocations associated with B-cell malignancies deregulate its expression	↓	0	0
7362	RIKEN cDNA 2700081N06	426076	Unknown	↑	0	0
7835	SH3-domain binding protein 5 (protein tyrosine kinase (BTK)-associated) (Sh3bp5)	571398	Inhibits the auto- and transphosphorylation activity of Bruton tyrosine kinase	↑	0	0
8160	Protein tyrosine phosphatase 4a2 (Ptp4a2)	634539	Signal control	↑	0	0
8312	A disintegrin and metalloproteinase domain 9 (meltrin gamma) (Adam9)	644907	Transmembrane metalloprotein; secretase?	↑	0	0
8460	Golgi autoantigen, golgin subfamily a, 5 (Golga5)	659182	Golgi interactions	↑	0	0
8489	RIKEN cDNA 2510006C20	693767	Unknown	↑	0	0
8901	DNA segment, Chr 4, Brigham & Women's Genetics 0593 expressed	831951	Unknown	↑	0	0

The clone location number refers to the position on the Incyte Genomics mouse cDNA microarray chip. For each cDNA, the array ratios for nine pairs of female mouse livers were plotted against the ratios of serum corticosterone levels for the same animals. Those showing correlation at $P<0.01$ or better by linear regression analysis are listed, along with the direction of the correlation. The number of microarray ratios equal to or exceeding 1.5 and 2.0 are also shown.

There were 24 genes in category 2 that showed no ratio ± 1.5 . Several of these (Fig. 4) showed highly significant correlations, and are from families with known glucocorticoid regulation:

protein kinase A, regulatory, type 1 β ($P=0.0002$) (Dwivedi & Pandey 2000), *interferon regulatory factor* ($P<0.0001$) (Franchimont *et al.* 2000), and *cadherin 11* ($P<0.0001$) (Foty *et al.* 1998).

Figure 1 Representative genes showing significant correlation between microarray ratios and serum corticosterone (cort) ratios (or log corticosterone ratios), for category 1 genes with one or more gene pairs showing an array ratio of ± 1.5 or greater. The correlation for category 1 genes was still significant when the lower extreme value was omitted.

Figure 2 Representative genes showing significant correlation between microarray ratios and serum corticosterone ratios (or log corticosterone ratios), for category 1 genes with no gene pairs showing an array ratio of ± 1.5 or greater. The correlation for category 1 genes was still significant when the lower extreme value was omitted.

Figure 3 Representative genes showing significant correlation between microarray ratios and serum corticosterone reciprocal ratios, for category 2 genes with one or more gene pairs showing an array ratio of ± 1.5 or greater. The correlation for category 2 genes was not significant when the lower extreme value was omitted.

Figure 4 Representative genes showing significant correlation between microarray ratios and serum corticosterone reciprocal ratios, for category 2 genes with no gene pairs showing an array ratio of ± 1.5 or greater. The correlation for category 2 genes was not significant when the lower extreme value was omitted.

Figure 5 Confirmation of the *Igfbp1* microarray results by RT-PCR. The normalized RT ratio correlated well with the microarray ratios (left panel) and with the corticosterone ratios (right panel).

Figure 6 Confirmation of the correlation between *Igfbp1* expression in liver and serum corticosterone, in a separate set of control livers, using real-time PCR, with raw *Igfbp1* values (left panel) and *Igfbp1* values normalized to *Gapdh* expression (right panel).

Confirmation of correlation of hepatic *Igfbp1* expression with serum corticosterone levels

In order to confirm the striking correlation between gene expression ratios for *Igfbp1* in liver with corticosterone ratios in serum, *Igfbp1* mRNA levels were determined by semi-quantitative RT-PCR in the 18 liver samples. The ratios of these results correlated well with both microarray results (Fig. 5, left panel) and with serum corticosterone ratios (Fig. 5, right panel).

In addition, to confirm the result with *Igfbp1* further, livers and sera were analyzed from ten female mice of the same strain, but obtained at a different time. For this experiment, *Igfbp1* message was quantified by real-time PCR. As illustrated in Fig. 6 (left panel), absolute levels of *Igfbp1* message in liver correlated very strongly with serum corticosterone. Significant correlation was also observed when the values were normalized to *Gapdh*, though the scatter was greater (Fig. 6, right panel).

Discussion

The results of this investigation reveal several novel applications of microarray technology. The first is the discovery of genes showing marked, significant variation in expression in the livers of identically handled mice. There were 30 such genes, as listed in Table 2. Six of these, including four known genes, correlated with serum corticosterone ratios for the same animals. *Igfbp1* is known to be regulated by glucocorticoids (Lee *et al.* 1997, Wetterau *et al.* 1999), and *tyrosine aminotransferase* is so strongly glucocorticoid-responsive in liver that it is often used as a marker for the effects of these hormones (Granner & Hargrove 1983, Ramakrishnan *et al.* 2002). The *stress-induced protein* gene might be expected to be regulated by corticosterone, a stress hormone. Pleiotropic regulator 1 is a part of nuclear spliceosomes (Ajuh *et al.* 2001) and in *Arabidopsis* exercises pleiotropic control of glucose and steroid hormone responses (Nemeth

et al. 1998, Salchert *et al.* 1998); its regulation by glucocorticoids in mammals may not have been described.

A total of 23 genes, including 16 known genes, had significantly variable expression among the liver pairs for reasons not clearly related to serum corticosterone. Many of these code for enzymes involved in intermediary metabolism/catabolism. There were several others of possible interest in the context of individual variation in disease susceptibility. *Lipin* codes for a nuclear protein that plays a key role in nutrient sensing and adipocyte development (Peterfy *et al.* 2001, Huffman *et al.* 2002). Presenilin 2 contributes to Alzheimer's disease risk, and has been implicated in the processing of amyloid precursors (Edbauer *et al.* 2002), control of apoptosis (Alves da Costa *et al.* 2002), and protein synthesis (Gamliel *et al.* 2002). Although presenilin is expressed in liver (Benkovic *et al.* 1997), its function there has not been studied. The protein product of *Clic4*, a mitochondrial chloride channel protein, has been implicated in apoptotic response to DNA damage (Fernandez-Salas *et al.* 2002). The water channel protein aquaporin 2 apparently is not expressed in rat liver (Umenishi *et al.* 1996, Huebert *et al.* 2002), but has not been studied in mice; it may have a role in glucose-driven water transport in liver (Masyuk *et al.* 2002).

The reasons for the variability in the expression of these genes among this homogeneous group of mice will require more study. The contribution of genetics cannot be ruled out, since NIH Swiss mice are not inbred. However, inter-individual variation in physiological and biochemical hepatic parameters is commonly observed even among inbred animals. A practical conclusion from this aspect of the study is that the common practice of pooling two or three livers to obtain a common reference control sample for microarray studies could lead to erroneous conclusions for some genes.

The second new use for microarray was the survey of the ~8000 genes for correlation between hepatic gene expression and serum corticosterone. Corticosterone regulation of gene expression has long been known and studied in cells, animals, and humans, often with molecular constructs and/or extreme variations in concentration. Our results show that it is possible to survey comprehensively changes in tissue gene expression as related to normal variations in serum corticosterone in

untreated animals. Of particular interest here was the finding that, in most of the significant correlations, the majority of the microarray values did not exceed ± 1.5 . Indeed, a total of 75 correlations, listed in Tables 3 and 4, had no microarray ratio values greater than ± 1.5 . Some of these correlations were very highly significant, and involved genes known to be regulated by corticosterone (see above). Heretofore, microarray ratios of $< \pm 1.5$ have been regarded as noise by most investigators. Our results suggest that these small changes are in fact meaningful. A recent study of the *familial adenomatous polyposis* gene revealed that a 50% reduction in expression at the RNA level was sufficient to lead to development of polyposis (Yan *et al.* 2002).

As noted above, many of the genes showing significant correlation with serum corticosterone in this study are already known to be glucocorticoid responsive, or at least from gene families with this characteristic. These results collectively confirm the validity of the experimental approach, and point to these genes as ones that are responsive to normal physiological variations in serum corticosterone. The other genes that correlated significantly with serum corticosterone ratios could be directly or indirectly associated. Many of these gene products are involved in metabolism and a relationship with corticosterone levels is not surprising. Also of interest are the numerous genes involved in cell growth control, that may have been revealed as glucocorticoid related for the first time. These are summarized in Table 6. In all, significant correlations with serum corticosterone were found for four growth factor/receptor genes, 18 genes for extracellular, membrane and/or signaling proteins, 16 genes for products involved in transcription or gene expression control, and five genes related to cell cycle, DNA repair, or apoptosis.

The gene whose expression correlated most strikingly with serum corticosterone was *Igfbp1*. This gene is well known to be inducible through glucocorticoid response elements in the promoter in both human and rat liver cells (Suh *et al.* 1994, 1995, 1996, Goswami *et al.* 1994). It is reasonable to conclude that the *Igfbp1* genes in the livers in our study were directly controlled by serum corticosterone, over a wide range of titers. However, insulin strongly suppresses *Igfbp1* expression through a response element that interacts with the glucocorticoid control sequences (Robertson *et al.* 1994,

Table 6 Summary of hepatic cell/growth control genes correlating with serum corticosterone

Functional category	Gene and direction of correlation with serum corticosterone		References for known association with glucocorticoids
Growth factor/receptor	<i>Igfbp1</i>	↑	Wetterau <i>et al.</i> (1999)
	<i>Fgfr1</i>	↓	Lee <i>et al.</i> (1997)
	<i>Epha3</i>	↓	n/f
Extracellular/membrane/signaling	<i>Il15ra</i>	↑	n/f
	<i>Cdh11</i>	↑	Foty <i>et al.</i> (1998)
	<i>Prkar1b</i>	↓	Dwivedi & Pandey (2000)
	<i>Ihpk1</i>	↑	n/f
	<i>Stk11</i>	↑	n/f
	<i>Tmem4</i>	↑	n/f
	<i>Pitpnb</i>	↑	n/f
	<i>Lasp1</i>	↑	n/f
	<i>Sbk</i>	↑	n/f
	<i>Peli1</i>	↓	n/f
	<i>Inpp5b</i>	↑	n/f
	<i>Pnck</i>	↓	n/f
	<i>Ceacam2</i>	↑	n/f
	<i>Ptk9</i>	↓	n/f
	<i>Spec1</i>	↓	n/f
	<i>Sh3bp5</i>	↑	n/f
	<i>Ptp4a2</i>	↑	n/f
	<i>Adam9</i>	↑	n/f
Transcription/gene expression	<i>Hnrpa2b1</i>	↑	Eggert <i>et al.</i> (2001)
	<i>Nfatc2</i>	↑	Refojo <i>et al.</i> (2001)
	<i>Myb</i>	↓	Eastman-Reks & Vedeckis (1986)
	<i>Irf1</i>	↑	Franchimont <i>et al.</i> (2000)
	<i>Plrg1</i>	↓	Ajah <i>et al.</i> (2001), Salchert <i>et al.</i> (1998), Nemeth <i>et al.</i> (1998)
	<i>Zfp103</i>	↑	n/f
	<i>Eif3</i>	↑	n/f
	<i>Wbscr14</i>	↑	n/f
	<i>Crad-L</i>	↑	n/f
	<i>Sec14l2</i>	↑	n/f
Cell cycle/DNA repair/apoptosis	<i>Clk2</i>	↑	n/f
	<i>Ubn1</i>	↑	n/f
	<i>Trim24</i>	↑	n/f
	<i>Nfe2</i>	↑	n/f
	<i>Tardbp</i>	↑	n/f
	<i>Bcl11a</i>	↓	n/f
	<i>Pes1</i>	↑	n/f
	<i>Tsnax</i>	↑	n/f
	<i>Btbd1</i>	↑	n/f
	<i>Pard3</i>	↓	n/f
	<i>Pdcd8</i>	↑	n/f
	<i>Cbpin</i>	↓	n/f

The known genes represented by the cDNAs in Tables 4 and 5 were searched using MedLine and LocusLink NCBI databases, for functions and for published reports indicating regulation by, or other relationship with, glucocorticoids. Genes with a likely relationship to cell growth control are listed.

n/f, no references found.

Suwanichkul *et al.* 1994), and can override the inductive effects of glucocorticoids. It has become generally accepted that insulin is the major

regulator of *Igfbp1* expression, with glucocorticoids and other factors playing modulatory roles (Lee *et al.* 1993). Our results may be somewhat

surprising, therefore, in suggesting that corticosterone had a predominate role in the regulation of *Igfbp1* expression. The suppressive effect of insulin has a concentration threshold (Lee *et al.* 1993) and under conditions of hypoinsulinemia in humans cortisol causes significant increase in plasma IGFBP-1 (Conover *et al.* 1993). Our mice were killed in the morning, when diurnal and meal-related levels of insulin are expected to be low (Gagliardino & Hernandez 1971, Petersen 1978). Under these circumstances, corticosterone may provide the main control of *Igfbp1* expression. IGFBP-1 is known to be upregulated by fasting, exercise, and various forms of stress (Lee *et al.* 1993), all situations involving marked elevations in serum corticosteroids.

It is clear that regulation of *Igfbp1* is complex and involves numerous signaling pathways and transcription factors (Neau *et al.* 1995, Suh *et al.* 1995, Lewitt *et al.* 1996). Nevertheless, it may be that glucocorticoids often play a more primary role in the regulation of *Igfbp1* expression than hitherto suspected, and in this way may influence cancer risk. Circulating levels of IGFBP-1 regulate the bioavailability of IGF-I and IGF-II, which can be growth factors for neoplastic cells; this axis is under study as a target for cancer prevention and treatment efforts (McCarty 1997, Kari *et al.* 1999, Yee 2002). In addition, direct effects of IGFBP-1 on tumors have been reported (Perks *et al.* 1999, Zhang & Yee 2002).

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