Studies of the Effects of Vilon and Epithalon on Gene Expression in Mouse Heart using DNA-Microarray Technology

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Expression of 15,247 clones from a cDNA library in the heart of mice receiving Vilon and Epithalon was studied by DNA-microarray technology. We revealed 300 clones (1.94% of the total count), whose expression changed more than by 2 times. Vilon changed expression of 36 clones, while Epithalon modulated expression of 98 clones. Combined treatment with Vilon and Epithalon changed expression of 144 clones. Vilon alone or in combination with Epithalon activated expression of 157 clones (maximally by 6.13 times) and inhibited expression of 23 clones (maximally by 2.79 times). Epithalon alone or in combination with Vilon activated expression of 194 clones (maximally by 6.61 times) and inhibited expression of 48 clones (maximally by 2.71 times). Our results demonstrate the specific effects of Epithalon and Vilon on gene expression.

Key Words: *gene expression; DNA-microarray; peptides; Vilon; Epithalon*

Recent studies showed that the pineal gland plays an important role in aging [1,14]. Nocturnal production of pineal hormone melatonin decreases, and circadian rhythms of its secretion are impaired during aging [1,14]. Pinealectomy is accompanied by a decrease in animal life span, while treatment with melatonin increases it [1,13,14]. Transplantation of the pineal gland from young to old mice and administration of Epithalamin peptide preparation from the pineal gland to mice, rats, and flies increase their life span [1,2,4,11,13]. Tetrapeptide Epithalon (Ala-Glu-Asp-Gly) was constructed and synthesized on the basis of amino acid analysis of Epithalamin by the method of V. Kh. Khavinson [10]. Long-term treatment with Epithalon increased life span of CBA mice [3]. The molecular mechanism of Epithalon-produced changes and its effects on gene expression are of particular interest. Here we studied the effects of Epithalon in geroprotective doses on expression of 15,247 genes in the heart in CBA mice. Dipeptide Vilon (Lys-Glu) possessing geroprotective activity was used as a reference preparation [3].

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MATERIALS AND METHODS

Experiments were performed on 30 female CBA mice obtained from the Rappolovo nursery (Russian Academy of Medical Sciences) and receiving *ad libitum* food and water. Six-month-old mice were randomly divided into 3 groups (10 animals in each group). Experimental mice received subcutaneous injections of Vilon and Epithalon (1 μg) in 0.1 ml 0.9% NaCl for 5 days. These peptide preparations were synthesized at the St. Petersburg Institute of Bioregulation and Gerontology. Control mice were injected with 0.1 ml 0.9% NaCl. The animals were decapitated on day 6. The hearts were removed, immediately frozen in liquid nitrogen, and stored at -80°C for isolation of total

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RNA. We used 15,247 clones from a cDNA library (National Institute on Aging, USA, NIA mouse 15K cDNA clone set) [9]. Clones in the pSPORT-1 vector were grown in *E. coli* using the nutrient broth LB/ampicillin. Plasmid DMA was isolated using Edge Biosystems kits. cDNA inserts were amplified on a PCT-225 Peltier amplifier (MJ Research) using the direct primer 5'-CCAGTCACGACGTTGTAAAACG AG-3' and reverse primer 5'-GTGTGGAATTGTGAG CGGATAACAACAA-3'. Reactions were performed in a mixture (100 ml) containing 10 ng plasmid DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.5 mM direct primer, 0.5 mM reverse primer, 0.8 mM dNTPs, and 5 U Taq polymerase (PE Biosystems). Polymerase chain reaction (PCR) products were precipitated with ethanol, resuspended in TE buffer, and placed on 2% agarose gel to control PCR and confirm the absence of cross-contamination. PCR products of the set containing 15 247 clones were placed on 7 individual nylon membranes (2.5×7.5 cm, Schleicher and Scuell). The distance between centers of the points (diameter 300 m, indexes A-G) was 665 m. DNA samples were placed on membranes using a GMS417 microarrayer robot (Genetic Microsystems). Total RNA was isolated by the guanidine isothiocyanate method [6] and used as the substrate (20 mg) to introduce a radioactive labels phosphorus α-^{33}P dCTP (Amersham) during cDNA synthesis with Super-Script II reverse transcriptase (Life Technologies) on oligo $(dT)_{12-18}$ primer (Amersham). Samples were purified on ProbeQuant G-50 microcolumns (Amersham). Emission of samples (1 ml) was measured on a LS5801 liquid scintigraph (Beckman). Prehybridization and hybridization of microchips with independently synthesized probes (3 for each group) were performed in hybridization cylinders that included 7 membranes of the 15K set (more than 15,000 clones). Membranes were hydrolyzed in 2× SSC buffer (sodium chloride/sodium citrate) at room temperature for 3 min and prehybridized with heat-denatured mouse Cot1 DNA (1 mg/ml, Life Technologies) and Poly(A) RNA (50 mg/ml, Amersham) in 10 ml MicroHyb solution (Research Genetics) at 65°C for 6 h. Hybridization was performed in a freshly prepared mixture of MicroHyb, Cot1, and Poly(A). Volumes of the hybridization mixture and samples were normalized to a sample concentration of 80,000 dpm; total radioactivity did not surpass 63 million decays. Hybridization was performed at 42°C for 18 h. Membranes were rinsed 4 times with a small volume of 2×SSC/0.1% sodium dodecyl sulfate (SDS) at room temperature. Then these membranes were washed with 25 ml 2×SSC/0.1% SDS (2 times, 50°C, 15 min), 25 ml 1×SSC/0.01% SDS (2 times, 50°C, 15 min), and 25 ml 0.8×SSC/0.1% SDS (2 times, 50°C, 15 min).

Washed membranes were exposed with a Storage Phosphor Screen photographic screen (Molecular Dynamics) at room temperature for 5 days. Screens were scanned using a Storm 860 automatic radiation detector (Amersham, resolution 50 m/pixels). The image was formatted using ImageTools 2.1 software and analyzed by means of ImageQuant 5.1 software (Molecular Dynamics). Coordinate grids were imposed on each image to estimate the intensity of signals from individual clones. In each hybridized membrane we recorded background signals from 18 regions not containing fixed DNA. The intensity of signals from individual clones was expressed in relative units. The mean signal intensity was subtracted form the background signal intensity. The relative expression of individual clones was estimated by Z transformation of signals (ratio of the difference between a common logarithm of the signal intensity and common logarithm of the mean signal intensity to the mean deviation of a common logarithm of the signal). Independent hybridization was performed 3 times for each sample using individual specimens.

The results of experimental hybridizations were compared to the control by calculating Z ratios (ratio of the difference between Z transformations in experimental and control samples to the mean deviation of this difference). Z ratios for experimental and control samples were averaged. The variation coefficient was calculated for each membrane as the percent ratio between the mean deviation of a common logarithm of the signal and mean common logarithm of the signal. The correlation coefficient was calculated for each complex of clones using MS Excel 97 software (statistical function Correl).

RESULTS

Variability of the results in control mice was lower than in experimental animals. After 3 hybridizations the variation coefficients in control animals and mice receiving Vilon and Epithalon were 14.9-16.5, 16.1-16.6, and 18.9-19.3%, respectively. The mean correlation coefficients in these animals were 0.935, 0.976, and 0.969, respectively, which that the efficiency of hybridization of independently synthesized radioactive samples with microarrays was highly reproducible. We compared the results of hybridization of microarrays with 15,247 cDNA clones in heart samples from control animals and mice receiving Vilon and Epithalon for 5 days. Expression of 300 clones (1.94% of the total count) in heart samples from mice receiving Vilon and Epithalon changed more than by 2 times compared to the control. Administration of Vilon changed expression of 36 clones, while Epithalon modulated expression of 98 clones. Combined treatment

with Vilon and Epithalon changed expression of 144 clones. These results show that Vilon and Epithalon changed expression of 180 and 242 clones, respectively. Vilon alone or in combination with Epithalon activated expression of 157 clones (maximally by 6.13 times), but inhibited expression of 23 clones (maximally by 2.79 times). Epithalon activated expression of 194 clones (maximally by 6.61 times), but inhibited expression of 48 clones (maximally by 2.71 times).

In 7 measurements, 2-15 of 300 clones corresponded to the same genes. Overall, 300 clones were associated with 278 genes. Some clones were similar to mitochondrial (n=5, 1.8%) and nuclear mouse genes (n=83, 29.9%). Other clones showed strong similarity to nucleotide sequences of genes in humans (n=51, 18.3%) and other organisms (n=5, 1.8%). We revealed no similarity between 134 clones (48.2%) and genes in humans and other organisms.

Nucleotide sequences of 144 clones were similar to genes in mice and other organisms, including *Homo sapiens*, *Rattus norvegicus*, *Macaca fascicularis*, and *Bos taurus*. Vilon and Epithalon changed expression in 5 of 13 genes of the mitochondrial genome. Expression of 4 mitochondrial genes (16S, Vilon and Epithalon; NADH dehydrogenases 4 and 5 and cytochrome b, Epithalon) increased by 2.03-6.61 times. However, Vilon decreased the intensity of expression for ATPase 6 genes by 2.25 times. According to functional classification of genes in the cardiovascular system [8] 139 nuclear genes were divided into several functional categories. Some genes (n=44) did not belong to any category or encoded hypothetical proteins. Other genes (n=95) represented 6 main functional categories, including genes for cell division (n=14), cell signaling systems and communication (n=15), cell structure and motility (n=7), protective systems of cells and organisms (n=16), expression of genes and proteins (n=24), and metabolism (n=19, Table 1).

Genes, whose expression changed under effects of Vilon and Epithalon, functionally belong to various cell systems. It should be emphasized that the ratio of genes belonging to various functional categories and subcategories in the heart did not correspond to normal [8]. The ratio of genes for expression of genes and proteins, including posttranscriptional factors (10 genes), and metabolism was highest. Categories of genes for cell signaling systems, communication, and cell structure and motility did not differ from the control. However, categories of genes for cell division (10.07 *vs*. 5.68% in the control) and protective systems of cells and organisms (11.51 *vs*. 6.71% in the control) included a higher number of genes with modified expression. The data suggest that genes of these subclasses are most potent effectors for biological effect of Vilon and Epithalon. Detailed studies of functional subcategories showed that changes in the ratio of genes for cell cycle regulation (subcategory 1d, 5 genes, 3.6 *vs*. 1.59% in the control) and protective systems of cells and organisms, including membrane transport, transport proteins (subcategory 4b3, 6 genes, 4.32 *vs*. 0.9% in the control), and immune system (subcategory 4c, 5 genes, 3.6 *vs*. 1.75% in the control), are of considerable importance. We compared the effects of Vilon and Epithalon on genes in heart samples. Epithalon selectively modulated expression of genes associated with ribosomal proteins (subcategory 5b3). Vilon activated expression of only 1 gene belonging to this subcategory (ribosomal protein S6 kinase 1, p70/p85 s6 kinase). Epithalon activated expression of not only s6 kinase gene, but also genes encoding ribosomal proteins L8, L27, S6, and S10 (by 2.02-2.46 times, Table 1). The gene for ribosomal protein S10 was presented by 2 clones similar by nucleotide sequences to rat and human genes (Table 1 shows only human gene). Expression of these genes increased by 2.02 and 2.09 times, respectively.

It is important that Vilon and Epithalon modulated expression of several genes associated with oncogenesis. Vilon and Epithalon inhibited expression of myeloblastic oncogene-like gene 1 and protooncogene Bcl-3, respectively. These peptides activated expression of genes for protein kinase C-ζ (Epithalon), LIM/PDZ-domain enigma proteins (Epithalon), and homologue 2 (both peptides), which are probably involved in oncogenesis and suppress expression of functionally related genes for PDZ-domain Cipp protein (Vilon) [5,12,15]. It should be emphasized that these peptides affect genes that are related to calcium exchange. Both peptides increase expression of culline-5 and genes for Kcnn4 and Dcamkl1. Epithalon activates expression of calmodulin, but inhibits expression of genes for Ca^{2+}-binding protein calbindin and Kcnn2. Vilon and Epithalon increase expression of genes for serine/threonine kinases Pctk3, FUSED, and Stk11 that belong to the same functional category (cell signaling systems and communication). At least one of these kinases (Stk11) with unknown functions possesses anticarcinogenic activity. Mutations in its gene lead to the development of Peits-Gingers syndrome, which increases the risk of tumors [7]. These data are consistent with the inhibitory effects of Epithalon and Vilon on the development of spontaneous tumors [3].

Our results show for the first time the specific effects of peptide bioregulators Epithalon and Vilon on gene expression.

This work was performed in accordance with the agreement between National Institute on Aging (USA) and St. Petersburg Institute of Bioregulation and Gerontology. Our joint study resulted in the realization of a large-scale project, which is unique in modern

TABLE 1. Effects of Vilon and Epithalon on Gene Expression in Female CBA Mice

ΔE		Clone	Type	Gene	Category
Vilon	Epithalon				
				Cell division	
3.28	3.14	H3056F10	Hs	Epidermal growth factor receptor pathway substrate 15 (EPS15)	1a
-2.03		H3064C09	Mm	Myeloblastic oncogene-like gene 1 (Mybl1)	1a
	2.50	H3108D12	Mm	Gene deleted in polyposis 1 (Dp1)	1a
	-2.21	H3082H10	Hs	Protooncogene Bcl-3 (BCL3)	1a
2.12	2.31	H3051A09	Hs	MCM10 homologue	1b
	-2.11	H3067E06	Mm	DNA primase, p58 subunit (Prim2)	1b
3.35	3.08	H3052B05	Hs	Culline 5	1d
2.34	2.60	H3025B08	Hs	Proliferating cell nucleolar antigen P120 (proliferation-associated nucleolar antigen P120, NOL1)	1d
2.08	2.24	H3054F09	Mm	Cyclin gene ania-6b	1d
	2.09	H3149G05	Mm	Cyclin I (Ccni)	1d
	-2.06	H3061H06	Mm	Activator of S phase kinase (Ask-pending)	1d
3.09	2.78	H3052C04	Hs	APG5L, *S. cerevisiae* APG5 homologue (autophagy 5)	1e
2.03	2.06	H3054D02	Mm	Histone H3, family 3B (H3f3b)	1e
	-2.14	H3103D04	Mm	Gene 1b homologue for *S. cerevisiae* budding uninhibited by benzimidazoles (Bublb)	1e
				Cell signaling systems and communications	
-2.79		H3093A07	Mm	Platelet ligand selectin (p-selectin, Selpl)	2a
2.76	2.66	H3052F06	Mm	Platelet/endothelial cell adhesion molecule (Pecam)	2a
2.31	2.11	H3054E05	Mm	Thrombospodin 3 (Thbs3) and mucin 1 (Muc1)	2a
2.52	2.46	H3054H04	Mm	Ca-activated medium/minor K-conducting channel, member 4 of subfamily N (Kcnn4)	2b
	-2.71	H3097C12	Mm	Intracellular mitochondrial Cl channel 4 (Clic4)	2b
	-2.32	H3100C10	Rn	Ca-activated medium/minor K-conducting channel, member 2 of subfamily N (Kcnn2)	2b
	-2.19	H3098H10	Mm	Calbindin (PCD-29)	2c
	2.15	H3019D01	Mm	Calmodulin (Cam I)	2c
2.90		H3001A02	Mm	Secretin (Sct)	2d
	-2.01	H3101E12	Mm	Small GTPase gene (Rab11a)	2e
3.49	3.27	H3052B11	Mm	Protein kinase 3 with PCTAIRE motive (Pctk3)	2g
2.40	2.35	H3056E03	Hs	Serine/threonine kinase FUSED	2g
2.38	2.41	H3052D01	Mm	Doublecortin and Ca/calmodulin protein kinase-like 1 (Dcamkl1)	2g
2.38	2.25	H3052C12	Mm	Serine/threonine kinase 11 (Stk11)	2g
	2.57	H3047A06	Mm	Protein kinase C-z (Pkcz)	2g
				Cell structure and motility	
3.28	3.02	H3056C01	Mm	Formin 2 (Fmn2)	3c
2.69	2.66	H3056C12	Mm	Dystonin (Bpag1-n)	3c
	2.32	H3154A09	Mm	Filamin	3c
	-2.21	H3102A05	Mm	Centromeric autoantigen H (Cenph)	3c
2.81	2.08	H3121F01	Mm	Wingless-related MMTV integration site 4 (Wnt4)	3d
	2.13	H3010B10	Mm	Procollagen, type V, a3 (Col5a3)	3d

	2.26	H3133C10	Hs	Golgi complex autoantigen (golgin, subfamily a, 1; GOLGA1)	3e
				Protective systems of cells and organism	
3.27	3.09	H3052D11	Mm	Adenylate kinase 2 (Ak2)	4b1
	2.10	H3023E12	Mm	Topoisomerase (DNA) IIIb (Top3b)	4b2
3.35	2.94	H3055D08	Mm	Protein COP1 (Cop1)	4b3
2.37	2.47	H3056G12	Mm	Homologue 2 of *R. norvegicus* enigma gene (Enh2-pending)	4b3
	2.76	H3082E06	Hs	Enigma (LIM-domain protein, ENIGMA)	4b3
	-2.43	H3032G08	Mm	Corticosteroid-binding globulin (Cbg)	4b3
	2.09	H3023H11	Mm	Ferritin L-subunit gene	4b3
	2.09	H3145H02	Hs	Translocation protein 1 (TLOC1)	4b3
2.49		H3042G07	Mm	Heat shock protein 84 (HSP84)	4b4
	2.42	H3139E01	Mm	70-kDa heat shock protein (HSC70 and HSP73)	4b4
	2.33	H3054D05	Rn	Protein associated with small stress protein PASS1 (Pass1)	4b4
3.05	2.96	H3054E04	Mm	Class II major histocompatibility protein, chains a and b	4c
2.38		H3147A04	Mm	Butyrophilin precursor (BT, BUTY)	4c
2.23	2.26	H3054E02	Hs	Chromosome 1 mRNA similar to BAT2 genes	4c
2.16	2.15	H3055B08	Mm	Class III major histocompatibility protein	4c
	-2.35	H3103C12	Mm	Signal lymphocyte activation molecule (Slam)	4c
				Expression of genes and proteins	
-2.54		H3058C11	Hs	RNA-binding motive protein 9 (RBM9)	5a2
	-2.26	H3015G02	Mm	RNA-binding motive protein 6 (Rbm6)	5a2
3.69	3.27	H3056E09	Hs	Zinc finger protein ZNF01 and HUMORFKG1B	5a3
3.65	3.19	H3056G09	Mm	Scm-similar gene with 4 mbt-domains (Sfmbt)	5a3
3.04		H3133B04	Mm	Transcriptional factor 20 (Tcf20)	5a3
2.71	2.69	H3054G09	Mm	SMAR1	5a3
2.49	3.37	H3133A12	Mm	Zinc finger protein 61 (Zfp61)	5a3
2.33	3.30	H3116H10	Mm	ets-Related transcriptional factor (Etv5)	5a3
2.04	2.38	H3139D10	Hs	Thyroid hormone receptor-interacting protein 12 (TRIP12)	5a3
2.01		H3098G05	Mm	DNA-binding protein inhibitor ID-2	5a3
	2.46	H3131F02	Hs	Transcription-enhancing MADS-box factor 2, polypeptide B (myocyte-enhancing factor 2B, MEF2B)	5a3
	-2.05	H3074C11	Hs	TFIIB-related factor 2	5a3
3.08	2.99	H3047B05	Hs	FLJ12848 fis highly homologous to Hs mRNA for nuclear transport receptor	5b1
	3.03	H3080D01	Mm	Polyubiquitin C (Ubc)	5b1
	-2.13	H3103H06	Mm	Neighbor of A kinase-anchoring protein 95 (Nakap95-pending)	5b1
	-2.07	H3068G02	Mm	Ubiquitin-conjugating enzyme 2e (Ubc2e)	5b1
-2.47	-2.16	H3049A01	Hs	SUMO-1-specific protease FKSG6	5b2
-2.06		H3042F12	Mm	Serine protease inhibitor 4 (Spi4)	5b2
2.06	2.75	H3047B07	Mm	Tripeptidyl peptidase II (Tpp2)	5b2
2.94	2.88	H3054F04	Mm	p70/p85 s6 kinase	5b3
	2.46	H3011E01	Mm	Ribosomal protein L8 (Rpl8)	5b3
	2.09	H3133B09	Mm	Ribosomal protein S6 (Rps6)	5b3

	2.09	H3126B04	Hs	Ribosomal protein S10	5b3
	2.07	H3126D05	Mm	Ribosomal protein L27 (Rpl27)	5b3
				Metabolism	
	2.12	H3148E08	Mm	Ornithine decarboxylase antizyme	6b
	2.26	H3055F07	Mm	d-Aminolevulinate dehydratase (Lv, ALAD)	6c
-2.31	-2.26	H3061H04	Mm	Homologue of ATP synthetase b-chain	6d
-2.09		H3065E03	Mm	Soluble isocitrate dehydrogenase 1 $NADP^+$ (Idh1)	6d
	2.95	H3005E04	Hs	ATP synthetase b-subunit (ATPSB)	6d
	2.57	H3001A07	Bt	15-kDa subunit of NADH ubiquinone oxidoreductase, 15 kDa (NIPM)	6d
	2.50	H3009D02	Rn	ATPase F1F0 d-subunit	6d
	2.06	H3020D10	Hs	Isocitrate dehydrogenase 3 (NAD^+) a (IDH3A)	6d
2.91	2.70	H3055C05	Hs	Inositol-1,3,4-triphosphate 5/6 kinase (ITPK1)	6e
2.17	2.39	H3047D05	Hs	Enoyl-CoA hydratase	6e
	2.15	H3095C08	Mm	Dodecenoyl-CoA d-isomerase	6e
3.18	2.90	H3056E08	Mm	H-type AMP deaminase	6f
	5.95	H3147A06	Mm	APEX nuclease	6f
	2.47	H3147B06	Mm	Glyceraldehyde-3-phosphate dehydrogenase (Gapd)	6h
	2.04	H3114C12	Mm	Class 3 cytoplasmic aldehyde dehydrogenase (Adh4)	6h
	2.04	H3145A07	Mm	Aldehyde reductase (Akr1A4)	6h
2.95	2.73	H3053H06	Mm	Citrine (Slc25a13)	6i
2.78	2.72	H3052B06	Mm	ATP-binding cassette, subfamily B (MDR/TAP), member 1 (Abcb1)	6i
2.42	2.54	H3033B04	Hs	Family of soluble carriers 7 (cationic amino acid transporter, system y+), member 6 (SLC7A6)	6i
				Unclassified genes	
5.34	5.64	H3047A02	Mm	Serine/threonine protein kinase of protooncogene A-Raf (KRAA)	7a
4.91	5.18	H3047H01	Mm	Nulp1	7a
3.38	3.07	H3056D02	Hs	FLJ22439 fis	7a
3.20	2.85	H3056G05	Hs	Protein KIAA0029	7a
3.19	3.06	H3052B09	Mm	Nuclear antigen Sp100	7a
3.12	2.97	H3055D05	Hs	Protein KIAA0970	7a
3.05	2.91	H3056E07	Hs	FLJ13697 fis	7a
2.93	2.78	H3052C02	Hs	KIAA0308 gene	7a
-2.77		H3095C07	Mm	Channel-interacting PDZ-domain protein (Cipp)	7a
2.67	2.60	H3053G06	Mm	mg53d08.r1	7a
2.61	2.75	H3039H10	Mm	Epithelial protein lost in neoplasm (Eplin)	7a
2.60	2.29	H3056E11	Hs	Brefeldin A-inhibited guanine nucleotide-exchange protein 2 (BIG2)	7a
2.59	2.81	H3054E11	Hs	Phosphoinositol-3-phosphate-binding protein 2 (PEPP2)	7a
2.55	2.53	H3052C05	Hs	Putative sialoglycoprotease type 2 (LOC64172)	7a
-2.29		H3015A03	Hs	Protein CGI-63 (LOC51102)	7a
2.28	3.07	H3047F05	Hs	Gene 3 for translocational myeloid/lymphoid or mixed-lineage leukemia, trithorax (*Drosophila*) homologue, MLLT3	7a
2.28	2.41	H3056H01	Hs	MSTP028	7a
2.25	2.48	H3052G11	Mm	Ganglioside-induced differentiation-associated protein 1 (Gdap1)	7a

2.21	2.20	H3054H11	Hs	FLJ10977 fis	7a
2.14		H3033B01	Hs	FLJ22386 fis	7a
2.13	3.64	H3047C01	Hs	Hypothetical protein FLJ10914	7a
2.13	2.14	H3053G04	Hs	Hypothetical protein FLJ20476	7a
-2.12		H3002A11	Hs	Hypothetical protein FLJ10252	7a
2.11	2.50	H3002D05	Hs	FLJ12166 fis	7a
2.07	2.28	H3054C10	Hs	Protein KIAA0699	7a
	2.94	H3074A01	Hs	KIAA0182	7a
	2.83	H3021G11	Mm	Calreticulin (Calr)	7a
	-2.62	H3142H11	Hs	FLJ22230 fis	7a
	-2.56	H3097C06	Mm	Transforming growth factor-β 1-induced transcript 4 (Tgfb1i4)	7a
	-2.49	H3102C10	Mm	ERIC1 (Eric1)	7a
	2.48	H3158F09	Hs	FLJ22903 fis	7a
	2.37	H3084E12	Hs	FLJ21480 fis	7a
	2.30	H3139C11	Mm	MS4A11	7a
	2.19	H3022D10	Hs	Brain acid-soluble protein 1 (BASP1)	7a
	2.18	H3144C06	Mm	24,6-kDa protein	7a
	2.16	H3007C12	Hs	Hypothetical protein HSA011916	7a
	2.15	H3095C05	Mm	Shfdg1	7a
	-2.14	H3060A02	Mm	Cysteine-rich repeat-including protein CRIM1 (Crim1)	7a
	-2.12	H3061F04	Hs	Protein HYA22	7a
	2.08	H3018F08	Hs	Hypothetical protein FLJ20419	7a
	-2.07	H3063C10	Mf	Brain clone QnpA-21065	7a
	-2.07	H3097G04	Mm	Protein MLN 64 (protein ES 64, ML64)	7a
	-2.06	H3097G02	Hs	Protein KIAA1157	7a
	2.03	H3136B04	Mm	Uterine protein LOC55978	7a
Mitochondrial genes					
6.13	6.61	H3139C10	Mm	Mitochondrial gene 16S	
-2.25		H3049A03	Mm	ATPase 6 mitochondrial gene	
	2.32	H3024A09	Mm	NADH dehydrogenase 5 mitochondrial gene (ND5)	
	2.19	H3023G11	Mm	Cytochrome b mitochondrial gene (CYT b)	
	2.03	H3011D05	Mm	NADH dehydrogenase 4 mitochondrial gene (ND4)	

Note. Table includes only identified genes. Δ*E*: changes in gene expression; *Hs*: *Homo sapiens*; *Mm*: *Mus musculus*; *Rn*: *Rattus norvegicus*; *Mf*: *Macaca fascicularis*; *Bt*: *Bos taurus*.

biomedical science. The count of more than 15 000 clones reaches an all-time high.

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