한국한의학연구원논문집 12권1호(통권16호), 2006
Korean Journal of Oriental Medicine Vol. 12 No. 1 2006

藁本의 초산으로 유발된 생쥐 통증모델에 대한 마이크로어레이 유전자 발현 양상

김명규, 김창주[*], 홍미숙[*], 정주호[*], 부영민, 김윤경[**], 홍승헌[**], 임강현[***]
우석대학교 한약학과
* 경희대학교 의과대학
** 원광대학교 한약학과
*** 세명대학교 한의과대학

Abstract

Microarray Profiles of Ligustici Rhizoma on the Pain Model of Mouse Induced by Acetic Acid

Kim Myunggyou, Kim Changju[*], Hong Meesuk[*], Chung Jooho[*],
Boo Yungmin, Kim Yunkyung[**], Hong Seungheon[**], Leem Kanghyun[***]
College of Pharmacy, Woosuk University
* College of Medicine, Kyung Hee University
** College of Pharmacy, Wonkwang University
*** College of Oriental Medicine, Semyung University

The present study was designed to investigate the effects of Ligustici Rhizoma on the expression of genes in the pain model induced by acetic acid. cDNA microarray (GenePlorer TwinChipTM Mouse 7.4K) was used to evaluate the gene expressions. The expressions of 32 genes were up-regulated in the Ligustici Rhizoma-treated group; they include the genes coding Casp6, Hrh3, Basp1, Sprr2h, Zfp131, Copz2, LOC432436, Itpr5, etc. The expressions of 16 genes were down-regulated in the Ligustici Rhizoma-treated group; they include the genes coding Il16, Zfpm1, Cacna2d1, Xpo7, Smpdl3b, Dscr1, Harp, etc. The conclusion is that the expressions of 32 genes were up-regulated and the expressions of 16 genes were down-regulated in Ligustici Rhizoma-treated group.

Keywords : Ligustici Rhizoma, cDNA microarray, gene expression

* **접수** : 2006년 3월 19일 **수정** : 2006년 4월 6일 **채택** : 2006년 4월 17일
교신저자 : 임강현, 충북 제천시 신월동 세명대학교 한의과대학.
Tel: 043-649-1341, Fax: 043-649-1349, E-mail: lkh@semyung.ac.kr

Ⅰ. Introduction

Ligustici Rhizoma is a natural drug widely known in Korean Oriental medicine where it is called Ko-Bon (KB). The first appearance of KB is the *Shin-Nong-Bon- Cho-Gyoung (The Divine Husbandman's Classic of the Materia Medica)*, a classic Chinese herbal medicine text written over 2,000 years ago[1].

In traditional Oriental medicine, KB is described as acrid and warm and distributed through bladder channel and governing vessel with the kidneys. KB is used clinically to expels wind and alleviates pain. It used for externally contracted wind cold patterns, especially for headache. And it also used in any wind pattern that presents with pain at the vertex or pain that travels from the vertex down to the cheeks and teeth. It is also used for acute lower back pain from invasion of wind cold, as it treats both ends of the governing channel[2,3].

Visceral pain is one of the most common forms of disease-induced pain. It occurring in both pathologic conditions such as ureteral colic or myocardial infarction, and in nonpathologic conditions such as bowel or bladder distention. An early description of a muscle or somatic component of pain that both resulted from visceral organ injury and mimicked the pain from such an injury were left pectoral muscle trigger points that occurred with acute myocardial infarction, and which were relieved by procaine injection into the trigger areas reported by Travell and Rinzler in 19524).

The objective of the present study is to find out differences that expression of genes between KB treated mice and control.

Ⅱ. Materials and Methods

1. Materials

KB was purchased from Semyung University Oriental Medicine Hospital. Dried Ligustici Rhizoma (100.0 g) extracted with distilled water at 100ºC. The filtrates were united and evaporated under vacuum to give the extract. The extract was then powdered and stored at -20℃ till use. The sample was dissolved in distilled water prior to use.

The microarray chip (GenePlorer Twin ChipTM Mouse 7.4K) was purchased from DigitalGenomics Co. (Seoul, Korea). A complete list of the genes is available on the DigitalGenomics web site (http://www.digital-genomics.co.kr). Each of the cDNAs printed on the array has been sequenced and verified by the company.

2. KB treatment and RNA isolation

We prepared two groups of adult male ICR mice. Control group was administrated with distilled water and other KB treated-group was administrated with KB (200 mg/kg, p.o.). After 1 hour, all mice were received an i.p. injection of 0.3 ml of 1% acetic acid to evoke writhing reflex and observed for 30 min. Then, two mice were selected in either control group and KB treated-group and cerebral

hemispheres were isolated to obtain total ribonucleic acid (RNA).

RNA was isolated from the tissue with the TRIzol reagent (GibcoBRL, Grand Island, USA) as the manufacturer's instructions.

3. Synthesis of fluorescence probe

100 μg of total RNA in a volume of 22 μl and 2 μl of oligo dT at a concentration of 500 μg/ml were mixed together and incubated at 70℃ for 10 min. The mixture was cooled immediately after incubation on ice. 10 μl of 5 x reaction buffer, 0.1M DTT, 5 μl of 10 x dNTP (5mM d(ACG)TP and 2mM TTP), 2 μl of fluorescent nucleotide (1mM Cy3-dUTP for the control group and Cy5-dUTP for the KB-treated group), 1.5 μl of RNAsin, and 2.5 μl of superscript Ⅱ reverse transcriptase (200 U/ μl) were added to the mixture, which was then incubated at 37℃ for 2 hours. Afterwards, 6 μl of 3N NaOH was added to the reaction mixture to eliminate the RNA. 20 μl of 1M Tris HCl (pH 7.5) and 12 μl of 1N HCl were added to neutralize the NaOH. The reaction mixture was loaded into Biospin 6 columns, centrifuged at 1,000 x g for 4 min, loaded into Microcon 30 tubes, and centrifuged at 12,000 x g for another 3 min.

4. Prehybridization of slide

For preparation of hybridization, prehybridization buffer (6 x SSC, 0.2% SDS, 5 x Denhardt's solution, 1 mg/ml salmon sperm solution) was sprayed on the array under a 22 mm x 22 mm glass coverslip, sealed with glue, and left for 2 hours at room temperature. Slides were then unsealed, washed by 2 x SSC, 0.2 x SSC, and spun dry by centrifugation for 2 min in a 50 ml tube at 300 G.

5. Microarray hybridization and scanning

28.2 μl of the fluorescence probe, 1 μl of poly(dA) (4 mg/ml in TE), 1 μl of yeast tRNA (4 mg/ml), 1 μl of human Cot I DNA (Amersham Pharmacia Biotech Inc. Piscataway, USA) (10 mg/ml), 0.8 μl of 10% SDS, and 8 μl of 20 x SSC were added together and mixed. The mixture was incubated at 99℃ for 5 min. After staying for 5 min. at room temperature, the mixture was centrifuged at 12,000 X g for 2 min. The mixture was then sprayed on the microarray, and the microarray was incubated at 62℃ overnight. The hybridized microarray was washed with 1 x SSC, 0.1% SDS solution. The microarray was washed with a 0.1 x SSC/0.1% SDS solution at 50℃ for 10 min, and then in a 0.1 x SSC solution for another 10 min.

6. Array quantification and data processing

Following hybridization, arrays were scanned using a confocal laser-scanning microscope (Scan Array 5000, Affymetrix, Santa Clara, CA, USA) at 543 nm (Cy3, GHeNe laser) and 632 nm (Cy5, RHeNe

laser). Each spot was defined by manual positioning of a grid of circle over the array image. For each fluorescent image, the average pixel intensity within each circle was determined, and the local background beyond a 3-pixel buffer range from the circle was computed for each spot. Net signal was determined by subtraction of this local background from the average intensity of each spot. Signal intensities between the two fluorescent images were normalized by the intensities of the house-keeping genes provided on the arrays.

7. Data Analysis

Signal intensities of the fluorescent images for Cy3 and Cy5 were considered significant when they were more than twice the average intensity of the negative control signals. Genes with consistently low signals in subsequent repetition of the experiment were omitted from the analysis.

Ⅲ. Results

The following results show that KB regulates the expression of genes in mouse brain. Two experiments were performed.

1. Cyanine dye-labelled cDNA was hybridized to the microarray

A two-color image of a cDNA microarray is shown in Fig. 1. Red dots represent Cy3, and green dots represent Cy5. After scanning for each fluorescent dye false colour images were superimposed. Red dots or green dots represent genes that show altered expression, and yellow dots represent genes that show no change in expression (Fig. 1).

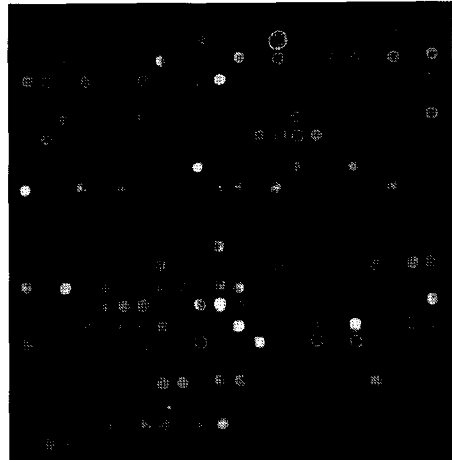

Fig. 1. Two-color image of a cDNA microarray. The array consists of PCR-amplified cDNA clones printed on glass; the diameter of each spot is 500 μm.

A sample mixture consisting of Cy3-labelled mRNA from untreated group and Cy5-labelled mRNA from KB-treated group was added. After scanning for each fluorescent dye false colour images (red for Cy3, green for Cy5) were superimposed. Yellow dots represent genes that show no change in expression. The hybridized microarray was scanned with the confocal laser microscope Scan Array 5000 (General Scanning) at 543 nm (Cy3, GHeNe laser) and 632 nm (Cy5, RHeNe laser). Two independent experiments were performed.

2. Genes up-regulated in the KB-treated group

The expression of 32 genes, Casp6, Hrh3, Basp1, Sprr2h, Zfp131, Copz2, LOC432436, Itpr5, 1300012G16Rik, Sec61a1, Mpg, Ubqln2, Pom121, Cklfsf8, Tmeff2, Mdm2, Cyp4b1, Ecgf1, Enpp1, Kif3a, Mtf2, Ubtf, Myom2, Tcea3, Pcsk1n, C1qb, 9530008L14Rik, 1110001A05Rik, 2610040E16Rik,

and three unnamed genes, were up-regulated in KB-treated group (Table Ⅰ).

Table Ⅰ. Genes upregulated in KB-treated group

Title	Gene.Symbol	GenBank.ACC	global.M
Caspase 6	Casp6	AI326797	1.01
Histamine receptor H 3	Hrh3	AI452091	1.02
--	--	AI835427	1.02
Brain abundant, membrane attached signal protein 1	Basp1	AI894132	1.03
Transcribed locus, strongly similar to NP_031604.1 calcium channel, voltage-dependent, P/Q type, alpha 1A subunit [Mus musculus]	--	AI839543	1.03
Small proline-rich protein 2H	Sprr2h	AI661967	1.05
Zinc finger protein 131	Zfp131	AI429320	1.05
--	--	AI840210	1.05
Coatomer protein complex, subunit zeta 2	Copz2	AI327160	1.06
Hypothetical gene supported by AK090213	LOC432436	AA590694	1.06
Inositol 1,4,5-triphosphate receptor 5	Itpr5	AA980905	1.06
RIKEN cDNA 1300012G16 gene	1300012G16Rik	AI839592	1.07
Sec61 alpha 1 subunit (S. cerevisiae)	Sec61a1	AI327023	1.08
N-methylpurine-DNA glycosylase	Mpg	AI851936	1.08
Ubiquilin 2	Ubqln2	AI854423	1.08
Nuclear pore membrane protein 121	Pom121	AI430911	1.08
Chemokine-like factor super family 8	Cklfsf8	AI327124	1.09
Transmembrane protein with EGF-like and two follistatin-like domains 2	Tmeff2	AI427489	1.10
Transformed mouse 3T3 cell double minute 2	Mdm2	AI448103	1.18
Cytochrome P450, family 4, subfamily b, polypeptide 1	Cyp4b1	AI648959	1.18
Endothelial cell growth factor 1 (platelet-derived)	Ecgf1	AI452183	1.20
Ectonucleotide pyrophosphatase/phosphodiesterase 1	Enpp1	AI851683	1.23
Kinesin family member 3A	Kif3a	AI429298	1.28
Metal response element binding transcription factor 2	Mtf2	AA208917	1.29
Upstream binding transcription factor, RNA polymerase I	Ubtf	AI893975	1.30
Myomesin 2	Myom2	AI594057	1.38
Transcription elongation factor A (SII), 3	Tcea3	AI323893	1.42
Proprotein convertase subtilisin/kexin type 1 inhibitor	Pcsk1n	AI604765	1.60
Complement component 1, q subcomponent, beta polypeptide	C1qb	AI854126	1.69
RIKEN cDNA 9530008L14 gene	9530008L14Rik	AW319682	1.86
RIKEN cDNA 1110001A05 gene	1110001A05Rik	AW228771	2.60
RIKEN cDNA 2610040E16 gene	2610040E16Rik	AI837674	3.97

Normalized ratios of medians were calculated as Cy5 dividing Cy3 and the ratios more than 2.0 (Global. M is more than 1.0) are shown in this table.

3. Genes down-regulated in the KB-treated group

The expression of 16 genes, 2700038L12Rik, Il16, Zfpm1, Cacna2d1, Xpo7, Smpdl3b, Dscr1, Harp, 4930532J02Rik, Enah, Elmo3, Agpat2, Polr2a, Fgfr4 and two unnamed genes, were down-regulated in KB-treated group (Table Ⅱ).

Table Ⅱ. Genes downregulated in KB-treated group

Title	Gene.Symbol	GenBank.ACC	global.M
RIKEN cDNA 2700038L12 gene	2700038L12Rik	AI449714	-2.57
Interleukin 16	Il16	AI851902	-1.96
--	--	AI836484	-1.72
Similar to eukaryotic translation initiation factor 4A, isoform 1	--	AW411795	-1.69
Zinc finger protein, multitype 1	Zfpm1	AI849004	-1.65
Calcium channel, voltage-dependent, alpha2/delta subunit 1	Cacna2d1	AI428484	-1.61
Exportin 7	Xpo7	AI429475	-1.55
Sphingomyelin phosphodiesterase, acid-like 3B	Smpdl3b	AI666692	-1.53
Down syndrome critical region homolog 1 (human)	Dscr1	AI429645	-1.46
Harmonin interacting ankyrin repeat containing protein	Harp	AI452087	-1.45
RIKEN cDNA 4930532J02 gene	4930532J02Rik	AI465833	-1.43
Enabled homolog (Drosophila)	Enah	AI853020	-1.29
Engulfment and cell motility 3, ced-12 homolog (C. elegans)	Elmo3	AI117357	-1.13
1-acylglycerol-3-phosphate O-acyltransferase 2 (lysophosphatidic acid acyltransferase, beta)	Agpat2	AI835177	-1.09
Polymerase (RNA) II (DNA directed) polypeptide A	Polr2a	AI853301	-1.06
Fibroblast growth factor receptor 4	Fgfr4	AI385693	-1.05

Normalized ratios of medians were calculated as Cy5 dividing Cy3 and the ratios less than 0.5 (Global. M is less than -1.0) are shown in this table.

Ⅳ. Discussion

In this paper, we studied the gene expression profiles in KB-treated pain-alleviated mice. The microarray technology was adapted for this purpose. Genes up-regulated in KB-treated group were Casp6, Hrh3, Basp1, Sprr2h, Zfp131, Copz2, LOC432436, Itpr5, 1300012G16Rik, Sec61a1, Mpg, Ubqln2, Pom121, Cklfsf8, Tmeff2, Mdm2, Cyp4b1, Ecgf1, Enpp1, Kif3a, Mtf2, Ubtf, Myom2, Tcea3, Pcsk1n, C1qb, 9530008L14Rik, 1110001A05Rik, 2610040E16Rik, and three unnamed genes.

Of the above genes, SEC61 complex is an essential translocation component that can associate with either ribosomes or the SEC62/SEC63 complex to perform cotranslational

or posttranslational transport, respectively[5]. It was originally thought to have a role only in translocation of proteins from the cytosol into the ER. However, Wiertz et al., Bebok et al., Chen et al., and Petaja-Repo et al. presented evidence suggesting that the human SEC61 complex can also function in retrograde transport of multidomain integral membrane proteins from the ER to the cytosol for proteasomal degradation[5-8].

Lin et al. provided insight into the role of KIF3A gene in the cellular mechanisms that trigger cyst formation. Their studies followed previous work in which Cre-loxP conditional mutagenesis was used to inactivate the KIF3A subunit of photoreceptor cells. They showed that kidney-specific Cre-loxP inactivation of the gene for the KIF3A subunit of kinesin II, an anterograde (outward moving) ciliary motor protein, causes PKD, thus directly implicating cilia in the cyst-forming mechanism. The work raised intriguing questions about the possible basis of the disorder combining polycystic kidney and congenital blindness[9,10].

UBTF is a complex of proteins that could modulate the activity of polymerase. From experiments with mouse embryonic fibroblasts, Drakas et al. presented evidence that a nuclear complex forms between IRS1, UBTF, and PI3K, leading to the serine phosphorylation of UBF1 and regulation of rRNA synthesis[11].

Overexpression of Pcsk1n led to reduced processing of proopiomelanocortin (POMC). Using the recombinant protein in vitro assays, they found that Pcsk1n could inhibit PC1 but not PC2, and kinetic analysis indicated that Pcsk1n is either a noncompetitive inhibitor of PC1 or a tight-binding competitive inhibitor with a slow off rate[12]. Fortenberry et al. confirmed that mouse Pcsk1n inhibits PC1. Using a truncation mutant, they localized the inhibitory domain to the first 24 residues of the C-terminal peptide[13].

Genes down-regulated in KB-treated group were 2700038L12Rik, Il16, Zfpm1, Cacna2d1, Xpo7, Smpdl3b, Dscr1, Harp, 4930532J02Rik, Enah, Elmo3, Agpat2, Polr2a, Fgfr4 and two unnamed genes.

Of the above genes, IL16 is well known to be involved in a proinflammatory cytokine signals via CD4, inducing chemotactic and immunomodulatory responses of CD4+ lymphocytes, monocytes, and eosinophils14). Bandeira-Melo et al. showed that, in the absence of CD4 blockade, IL16 signaled through CD4 on eosinophils in a dose-dependent manner and induced the release of leukotriene C4 (LTC4), as well as eotaxin (CCL11) and RANTES (CCL5). In an autocrine manner, RANTES and eotaxin signaled through their membrane receptor, CCR3, and enhanced eosinophil secretion of LTC4 and IL4, but not IL12[15].

DSCR1 protein is reported to be overexpressed in the brain of Down syndrome fetuses, and interacts physically and functionally with calcineurin A, the catalytic subunit of the Ca(2+)/calmodulin-dependent protein phosphatase PPP3CA. The DSCR1-binding region in calcineurin A is located in the linker region between the calcineurin A catalytic domain and the calcineurin B-binding domain, outside

of other functional domains previously defined in calcineurin A. DSCR1 belongs to a family of evolutionarily conserved proteins with 3 members in humans: DSCR1, ZAKI4, and DSCR1L2. Overexpression of DSCR1 and ZAKI4 inhibited calcineurin-dependent gene transcription through the inhibition of NFAT translocation to the nucleus. The authors hypothesized that members of this family of human proteins are endogenous regulators of calcineurin-mediated signaling pathways and may be involved in many physiologic processes[16]. Kingsbury and Cunningham referred to the proteins encoded by the MCIP genes as calcipressins. Functional analysis showed that when expressed in yeast, DSCR1 and ZAKI4 inhibited calcineurin function. The authors proposed that increased expression of DSCR1 in trisomy-21 individuals may contribute to the neurologic, cardiac, or immunologic defects of Down syndrome[17]. Ermak et al. showed significant expression of DSCR1 in brain, spinal cord, kidney, liver, mammary gland, skeletal muscle, and heart. Within the brain, DSCR1 was predominantly expressed in neurons of the cerebral cortex, hippocampus, substantia nigra, thalamus, and medulla oblongata without regard to age in humans or rats. Postmortem studies of 8 patients with Alzheimer disease (AD) and 8 controls showed that DSCR1 expression in the cerebral cortex and hippocampus of AD patients was approximately double that of controls, and moreover, that DSCR1 levels in brains with extensive neurofibrillary tangles were 3 times higher than in controls[18]. Previous studies had shown that decreased calcineurin phosphatase activity allowed accumulation of hyperphosphorylated tau protein and cytoskeletal changes in the brain similar to those observed in AD[19]. Since DSCR1 inhibits calcineurin activity, they suggested that increased DSCR1 levels may cause accumulation of hyperphosphorylated tau protein and production of neurofibrillary tangles, thereby promoting the development of AD. Cell studies showed that toxic levels of aggregated amyloid beta 1-42 peptide directly stimulated DSCR1 expression, perhaps as a protective mechanism against calcineurin- induced apoptosis. The authors suggested that while DSCR1 overexpression may initially be protective, chronic overexpression may eventually lead to the formation of neurofibrillary tangles associated with AD18). Yao and Duh demonstrated that DSCR1 was induced in human endothelial cells in response to VEGF, TNFA, and calcium mobilization, and this up-regulation was inhibited by inhibitors of the calcineurin-NFAT signaling pathway, as well as by PKC inhibition and a calcium chelator. Yao and Duh hypothesized that up-regulation of DSCR1 in endothelial cells may act as an endogenous feedback inhibitor of angiogenesis by regulating the calcineurin-NFAT signaling pathway[20].

HARP has been suggested to play a role in facilitating signal transduction in epithelia[21].

Using a variety of RNA-binding assays, Kaneko and Manley showed that the CTD of mammalian POLR2A interacted with RNA in a sequence-specific manner in vitro and in vivo. The CTD-binding consensus sequence

downstream of a polyadenylation signal suppressed mRNA 3-prime end formation and transcription termination. In vitro assays indicated that the inhibition of processing is CTD dependent[22].

Ⅴ. Acknowledgment

This study was supported by a grant of the Oriental Medicine R&D Project, Ministry of Health & Welfare, Republic of Korea(B05-0049-AM0815-05N1-00030B).

Reference

1. Kang BS, Ko UC, Kim KY, Kim SH, Kim IR, Kim HC, No SH, Park YK, Seo BI, Seo YB, Song HJ, Sin MK, Ahn DK, Lee SY, Lee YJ, Lee TH, Cho SY, Ju YS, Choi HY. Bonch-Hak. Seoul: Young-Lim press 1991: 132-133.
2. Dan B, Andrew G. Chinese Herbal Medicine: Materia Medica (8th edn). Seattle: Eastland Press, Inc. 1992: 39-40.
3. Dan B, Steven C, Erich S, Andrew G. Materia Medica (3th edn). Seattle: Eastland Press, Inc. 2004: 22-23.
4. Travell J, Rinzler S. The myofascial genesis of pain. Postgrad. Med. 11: 452-434, 1952.
5. Wiertz EJ, Tortorella D, Bogyo M, Yu J, Mothes W, Jones TR, Rapoport TA, Ploegh HL. Sec61-mediated transfer of a membrane protein from the endoplasmic reticulum to the proteasome for destruction. Nature 384: 432-438, 1996.
6. Bebok Z, Mazzochi C, King SA, Hong JS, Sorscher EJ. The mechanism underlying cystic fibrosis transmembrane conductance regulator transport from the endoplasmic reticulum to the proteasome includes Sec61-beta and a cytosolic, deglycosylated intermediary. J. Biol. Chem. 273: 29873-29878, 1998.
7. Chen Y, Le Caherec F, Chuck SL. Calnexin and other factors that alter translocation affect the rapid binding of ubiquitin to apoB in the Sec61 complex. J. Biol. Chem. 273: 11887-11894, 1998.
8. Petaja-Repo UE, Hogue M, Laperriere A, Bhalla S, Walker P, Bouvier M. Newly synthesized human delta opioid receptors retained in the endoplasmic reticulum are retrotranslocated to the cytosol, deglycosylated, ubiquitinated, and degraded by the proteasome. J. Biol. Chem. 276: 4416-4423, 2001.
9. Lin F, Hiesberger T, Cordes K, Sinclair AM, Goldstein LSB, Somlo S, Igarashi P. Kidney-specific inactivation of the KIF3A subunit of kinesin-II inhibits renal ciliogenesis and produces polycystic kidney disease. Proc. Nat. Acad. Sci. 100: 5286-5291, 2003.
10. Marszalek JR, Liu X, Roberts EA, Chui D, Marth JD, Williams DS, Goldstein LSB. Genetic evidence for selective transport of opsin and arrestin by kinesin-II in mammalian photoreceptors. Cell 102: 175-187, 2000.
11. Drakas R, Tu X, Baserga R. Control of cell size through phosphorylation of upstream binding factor 1 by nuclear phosphatidylinositol 3-kinase. Proc. Nat. Acad. Sci. 101: 9272-9276, 2004.
12. Fricker LD, McKinzie AA, Sun J, Curran E, Qian Y, Yan L, Patterson SD, Courchesne PL, Richards B, Levin N, Mzhavia N, Devi LA, Douglass J. Identification and characterization of proSAAS, a granin-like neuroendocrine

peptide precursor that inhibits prohormone processing. J. Neurosci. 20: 639-648, 2000.

13. Fortenberry Y, Hwang JR, Apletalina EV, Lindberg I. Functional characterization of proSAAS: similarities and differences with 7B2. J. Biol. Chem. 277: 5175-5186, 2002.
14. Cruikshank WW, Center DM, Nisar N, Wu M, Natke B, Theodore AC, Kornfeld H. Molecular and functional analysis of a lymphocyte chemoattractant factor: association of biologic function with CD4 expression. Proc. Nat. Acad. Sci. 91: 5109-5113, 1994.
15. Bandeira-Melo C, Sugiyama K, Woods LJ, Phoofolo M, Center DM, Cruikshank WW, Weller PF. IL-16 promotes leukotriene C4 and IL-4 release from human eosinophils via CD4- and autocrine CCR3-chemokine-mediated signaling. J. Immun. 168: 4756-4763, 2002.
16. Fuentes JJ, Genesca L, Kingsbury TJ, Cunningham KW, Perez-Riba M, Estivill X, de la Luna S. DSCR1, overexpressed in Down syndrome, is an inhibitor of calcineurin-mediated signaling pathways. Hum. Molec. Genet. 9: 1681-1690, 2000.
17. Kingsbury TJ, Cunningham KW. A conserved family of calcineurin regulators. Genes Dev. 14: 1595-1604, 2000.
18. Ermak G, Morgan TE, Davies KJA. Chronic overexpression of the calcineurin inhibitory gene DSCR1 (Adapt78) is associated with Alzheimer's disease. J. Biol. Chem. 276: 38787-38794, 2001.
19. Kayyali US, Zhang W, Yee AG, Seidman JG, Potter H. Cytoskeletal changes in the brains of mice lacking calcineurin A-alpha. J. Neurochem. 68: 1668-1678, 1997.
20. Yao YG, Duh EJ. VEGF selectively induces Down syndrome critical region 1 gene expression in endothelial cells: a mechanism for feedback regulation of angiogenesis. Biochem. Biophys. Res. Commun. 321: 648-656, 2004.
21. Johnston AM, Naselli G, Niwa H, Brodnicki T, Harrison LC, Gonez LJ. Harp (harmonin-interacting, ankyrin repeat-containing protein), a novel protein that interacts with harmonin in epithelial tissues. Genes Cells 9: 967-982, 2004.
22. Kaneko S, Manley JL. The mammalian RNA polymerase II C-terminal domain interacts with RNA to suppress transcription-coupled 3-prime end formation. Molec. Cell 20: 91-103, 2005.