Fluctuations in the gene expression of peripheral blood mononuclear cells between the active and inactive phases of systemic lupus erythematosus

M. Kawasaki[1], M. Fujishiro[1], A. Yamaguchi[1,2], K. Nozawa[1,3], H. Kaneko[3], Y. Takasaki[2], K. Takamori[1], H. Ogawa[1], I. Sekigawa[1,3*]

[1]Institute for Environment and Gender Specific Medicine, Juntendo University Graduate School of Medicine, Chiba, Japan; [2]Department of Internal Medicine and Rheumatology, Juntendo University School of Medicine, Tokyo, Japan; [3]Department of Internal Medicine, Juntendo University Urayasu Hospital, Chiba, Japan.

Abstract

Objective

The changes in the gene expression in peripheral blood mononuclear cells (PBMC) associated with disease progression in systemic lupus erythematosus (SLE) patients with their diseases activities were examined and genes related to the pathogenesis and/or disease activities of SLE were investigated.

Methods

Analyses of gene expression were performed by both DNA microarray and real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) methods.

Results

Nine known genes showing either significantly increased or decreased expression were detected between patients with active and inactive disease phases of SLE or normal volunteers.

Conclusion

Among these nine genes, three genes were related to interferon (IFN) regulatory factor and four genes associated with ribosomal proteins (RPs), and two genes were associated with genetic translation factor (GTF), respectively. These three gene groups appear to contribute to the pathogenesis and/or disease progression of SLE.

Key words

Systemic lupus erythematosus, DNA microarrays, Interferons, ribosomal proteins, genetic translation

Mikiko Kawasaki, PhD
Maki Fujishiro, BS
Ayako Yamaguchi, MD
Kazuhisa Nozawa, MD
Hiroshi Kaneko, MD
Yoshinari Takasaki, MD
Kenji Takamori, MD
Hideoki Ogawa, MD
Iwao Sekigawa, MD

This work was supported by the grants from The Institute for Environment and Gender-specific Medicine, Juntendo University Graduate School of Medicine.

Please address correspondence and reprint requests to:
Iwao Sekigawa, MD,
Department of Internal Medicine,
Juntendo University Urayasu Hospital,
2-1-1 Tomioka, Urayasu-shi,
Chiba 271-0021, Japan.
E-mail: sekigawa@juntendo-urayasu.jp

Received on July 7, 2009; accepted in revised form on December 4, 2009.

Competing interests: none declared.

Introduction

The pathogenesis of SLE, which is a representative autoimmune disease, remains still unclear although numerous studies have addressed this issue in humans and mouse models. Several complicated factors such as genetic and environmental factors including viral infection, hormone, and ultraviolet rays have been reported to mediate to the onset and exacerbation of SLE (1-3). Several kinds of genes have been suggested to contribute to the development of SLE. Therefore, the pathogenesis of SLE may not be associated with a single factor. Recently developed technology for comprehensive gene analyses such as DNA microarrays appear to be useful for pathogenic studies of diseases induced by complicated factors such as SLE (4, 5). In fact, these methods have been used in certain studies for pathogenic analyses of SLE (6-11). However, the number of genes loaded on DNA microarray slides in many of these previous studies was limited and the sensitivity of gene detection was also lower in comparison to recently developed microarray systems. Furthermore, there are few reports about the fluctuations of gene expression between the active and inactive phases of SLE in the same patients.

This study examined the change of the gene expression in PBMC from SLE patients at different stages in comparison with those of normal controls, using a DNA microarray carrying numerous human genes (4, 5). The results obtained here may elucidate the genes important to the development and/or onset of SLE.

Materials and methods

Patients and controls

Peripheral blood samples were obtained from 10 healthy individuals (8 females and 2 males; age 25-45 years old) and 10 SLE patients (8 females and 2 males; age 14–46 years old). The patients were diagnosed as having SLE according to the 1982 revised criteria of the American College of Rheumatology (12). The disease activity was assessed using the SLE Disease Activity Index (SLEDAI) (13). All patients were hospitalised for the onset or exacerbation of their SLE and treated with steroids. Their PBMC were collected before (active phase) and after (inactive phase) either the initiation of steroid therapy or increasing their dosages. The profiles of SLE patients, their disease duration, main clinical symptoms, dosages of steroid (prednisolone), and SLEDAI score during the active and inactive phase are summarised in Table I. All patients were treated with steroid and some patients (SLE 2, 4, 9) were also treated with immunosuppressant at the time of sampling of RNA. Furthermore, increases of serum anti-DNA antibodies and decreases of serum compliments (total compliment activity; CH50) levels were observed in all of the patients, and serum autoantibodies detected in each patient were summarised in Table I.

All of the patients and healthy individuals gave written informed consent to the study, and this study was approved by the local ethics committee.

Peripheral blood mononuclear cell (PBMC) isolation and total RNA extraction

PBMC were separated using Ficoll-Paque Plus (GE Healthcare, UK Ltd, Buckinghamshire, UK). Briefly, total RNA was isolated from PBMC of healthy persons and patients using an RNeasy Mini Kit (Qiagen Inc., Valencia, CA). RNA quality was checked using the Agilent 2100 BioAnalyzer RNA 6000 Nano LabChip assay (Agilent Technologies, Palo Alto, CA).

Oligo DNA microarray hybridisation

Fluorescence-labelled complementary antisense RNA targets were synthesised using an amino-allyl RNA amplification Kit (Sigma-Aldrich Co., St. Louis, MO). RNA targets synthesised from human PBMC were coupled with Cy5. RNA targets were also synthesised from Universal Human Reference RNA (Stratagene, La Jolla, CA) and coupled with Cy3 as reference samples for the two-colour microarray method. These labelled targets were competitively hybridised to an AceGene Human 30K 1 Oligo Chip Version (DNA Chip Research Inc., Kanagawa, Japan) and incubated overnight at 50° C. The hybridised images were scanned using a ScanArrray Lite (PerkinElmer, Inc.,

Table I. Profiles of the SLE patients.

Patients	Age	Sex	Disease duration (year)	Main symptoms	Autoantibodies	Therapy		SLEDAI core[2]	
						Prednisolon[1] (mg/day)	Immuno-suppressant	Active Phase	Inactive Phase
SLE 1	14	F	0	Nephropathy, rash, fever	anti-DNA	40	(-)	21	4
SLE 2	46	F	6	Central nervous symptom, rash, fever	anti-DNA, anti-Sm, anti-RNP	60	Cyclophosphamide	15	8
SLE 3	21	F	6	Nephropathy	anti-DNA	50	(-)	17	0
SLE 4	45	F	6	Central nervous symptom, rash, nephropathy	anti-DNA	60	Cyclophosphamide	20	1
SLE 5	40	F	18	Nephropathy	anti-DNA	30	(-)	18	4
SLE 6	38	F	11	Nephropathy	anti-DNA, anti-RNP	50	(-)	13	0
SLE 7	39	F	10	Pancytopenia	anti-DNA, anti-cardiolipin	20	(-)	14	6
SLE 8	27	F	4	Nephropatathy, rash, fever	anti-DNA, anti-RNP	50	(-)	16	4
SLE 9	40	M	15	Pancytopenia, fever	anti-DNA, anti-cardiolipin	20	Cyclosporine	13	2
SLE 10	23	M	2	Pancytopenia, rash	anti-DNA, anti-cardiolipin, anti-Sm, anti-SSA	20	(-)	12	4

[1]Dosages of prednisolone at the active phase of SLE in each patient.
[2]SLEDAI score before (active phase) and after (inactive phase) initiation of steroids therapies or increasing their dosages.

Wellesley, MA) and 570 and 670 nm were used to quantify the amplified RNA (aRNA) probes labelled separately with Cy3 and Cy5 separately.

Microarray data analyses

The signal intensity data were quantified from hybridisation images using ScanArray Express Software (PerkinElmer, Inc.). First, the genes showing signal intensities equal to or less than 100 were omitted from data list. Subsequently, the obtained numerical data were normalised using the global median normalisation per slide and per channel. Furthermore, the normalised Cy5 and Cy3 signal intensities were converted by taking logarithms of base two, and the Cy5/Cy3 ratio was calculated for each slide. The values in the given dataset explain normalised logarithmic gene expression profiles for each of the thirty PBMC samples. All of the gene expression values were divided by control average values in every gene, and the obtained data were described as an expression index (E.I.).

Whole analyses were carried out using the Microsoft Excel (Microsoft, Redmond, WA) and Avadis software package (Strand Life Sciences, Bangalore, India). The gene functions were obtained from the database at the National Center for Biotechnology Information.

mRNA amplification and real time qRT-PCR

As templates for real time qRT-PCR, the sense messenger RNA (mRNA) were amplified by SMART mRNA Amplification Kit (Takara Bio Inc., JAPAN), using the total RNA extracted from PBMC. The amplified mRNA were reverse transcribed and diluted using the PrimeScript® RT reagent Kit (Takara Bio Inc.), then the SYBR Green method qRT-PCR was performed using SYBR® Premix Ex Taq™ (Takara Bio Inc.) and ABI Prism 7900HT Sequence Detection System (Perkin Elmer Applied Biosystems, Foster City, CA). Those methods were carried out according to the manufacturer's instructions. Adequate primers sets were designed and employed to detect the twenty nine genes described in Table II (Texas Genomics Japan Co., Ltd., Japan). The β-actin gene was also detected using the HA067803 primer set (Takara Bio Inc.) as an internal control. The results were described as an E.I., which was calculated as the mean amount of mRNA obtained by three separate experiments divided by the control averages, and β-actin expression, respectively.

Statistical analyses

Statistical analyses were made using Microsoft Excel and GraphPad InStat Software package (GraphPad Software Inc., San Diego, CA) for microarray and qRT-PCR data, respectively. Welch's *t*-test was applied for comparison of healthy control versus active or inactive SLE patients, and paired *t*-test was applied for comparison of active *vs*. inactive SLE patients. In every analysis, $p<0.05$ criterion was used for significance.

Results

Process of gene selection in this experiment was summarised in Table III. Among 1,947 genes showing signal intensities equal to or more than 100 in healthy controls and active or inactive

Table II. Genes detected by qRT-PCR.

GenBank ID	Gene Symbol	Gene Name	qRT-PCR primers
NM_002038	IFI6	Interferon, alpha-inducible protein 6	(F) TACGCCACCCACAAGTATCTC (R) GCCAAGAAGGAAGAAGAGGTTC
NM_003641	IFITM1	Interferon induced transmembrane protein 1 (9-27)	(F) CAACATCCACAGCGAGACC (R) CCAGACAGCACCAGTTCAAG
NM_001572	IRF7	Interferon regulatory factor 7	(F) TACCTGTCACCCTCCCCAAG (R) TGTCCCACCACCTTCTGC
NM_005101	ISG15	ISG15 ubiquitin-like modifier	(F) AGGCAGCGAACTCATCTTTG (R) CCAGCATCTTCACCGTCAG
NM_004335	BST2	Bone marrow stromal cell antigen 2	(F) GCTTCTGCTGGGGATAGG (R) ATGGGTGACATTGCGACAC
NM_030978	ARPC5L	Actin related protein 2/3 complex, subunit 5-like	(F) TCCCGTCAACACCAAGAATC (R) GCCTGCTCAATCTCACTGC
NM_033632	FBXW7	F-box and WD-40 domain protein 7 (archipelago homolog, Drosophila)	(F) AAGGTCCCAACAAGCATCAG (R) CCCGTTTTCAAGTCCCATAG
NM_002201	ISG20	Interferon stimulated exonuclease gene 20kDa	(F) GGAAGATGCGAGGGCAAC (R) CTGTCCCAAAAAGCCGAAAG
NM_016459	MGC29506	Hypothetical protein MGC29506	(F) CTCCTATCCTCCCCACCTTC (R) AGTTTGGTCTCTGCCTTTGC
NM_013439	PILRA	Paired immunoglobin-like type 2 receptor alpha	(F) CAACCAAAACACCTCTCAGC (R) TAGAAGGACTGCCTGTGGAAG
NM_000942	PPIB	Peptidylprolyl isomerase B (cyclophilin B)	(F) GATGGCACAGGAGGAAAGAG (R) GAAGAACTGGGAGCCGTTG
NM_014814	PSMD6	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 6	(F) TCCAGCAGTTCGGCAGTATC (R) TCATTTCCTGTTCCACAACC
NM_033280	SEC11C	SEC11 homolog C (S. cerevisiae)	(F) TGGAAAAGAAGGACGTGGTG (R) CCCATTACAGCCAAAAGAGC
NM_003254	TIMP1	TIMP metallopeptidase inhibitor 1	(F) CTTCTGGCATCCTGTTGTTG (R) TCTGGTTGACTTCTGGTGTCC
NM_001002926	TWISTNB	TWIST neighbour	(F) GAAAATGGCACTGAGGAAGC (R) TCCATTGGAGTGTCATCTGC
NM_001959	EEF1B2	Eukaryotic translation elongation factor 1 beta 2	(F) TATGGTCCTGCCGATGTG (R) TCCCTTAGCCTCTTTGCTTC
NM_016091	EIF3S6IP	Eukaryotic translation initiation factor 3, subunit 6 interacting protein	(F) GGCAATGATGCTGTCTTCC (R) TTCAAACCTCTGCTCCAAGG
NM_000182	HADHA	Hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), alpha subunit	(F) CCGCTTTTCTGCCTTCAG (R) AACTGCCACATCCCCTTTG
NM_000978	RPL23	Ribosomal protein L23	(F) ACATCCAGCAGTGGTCATTC (R) TGGCAGAACCTTTCATCTCG
NM_001015	RPS11	Ribosomal protein S11	(F) AGCAGCCGACCATCTTTC (R) TAGCCTCCTTGGGTGTCTTG
NM_002954	RPS27A	Ribosomal protein S27a	(F) ACACCACTCCCAAGAAGAATAAG (R) CATAAACACCCCAGCACCAC
NM_001009	RPS5	Ribosomal protein S5	(F) GCTCAGTGTCCCATTGTGG (R) AGAGGGTTCTCGCCTGTG
NM_152862	ARPC2	Actin related protein 2/3 complex, subunit 2, 34kDa	(F) TGGGAATAAGAGGAGGAAGC (R) GGAACCAAAACGGAGAATCC
NM_001568	EIF3S6	Eukaryotic translation initiation factor 3, subunit 6 48kDa	(F) TTGACTTTGATGGGGCTCAG (R) ATGAAGAGACGGGCATTTTC
NM_001688	ATP5F1	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit B1	(F) GCGTCGAAAGGAACAAGAAC (R) TTTCCTGCTGTGTGGAGATG
NM_003773	HYAL2	Hyaluronoglucosaminidase 2	(F) TGAGTTTGAGTTCGCAGCAC (R) AAAGAGGTAGAAGCCCCAGAG
NM_030905	OR2J2	Olfactory receptor, family 2, subfamily J, member 2	(F) GGAAAAGACCATCTCGTATGC (R) TCACCACCAGTAGGACACACTC
NM_199334	THRA	Thyroid hormone receptor, alpha (erythroblastic leukemia viral (v-erb-a) oncogene homolog, avian)	(F) AGGATTGAGAAGGGACAAGC (R) TGTGTGTAGGGGGTGAGTTG
NM_033468	ZNF257	Zinc finger protein 257	(F) TCTTCCTGGGTATTGCTGTCTC (R) ACACATAACTGGGGGTTTGG

Among the 47 genes described in Fig. 1C, the 29 known genes detected successfully by qRT-PCR. The effective primer sets were also described in the right end column; (F) and (R) indicate forward and reverse primers, respectively. The expressions of nine genes in the active , inactive SLE patients, and normal controls described by boldface type are shown in Fig. 1. (See Fig. 1)

Table III. Process of gene selection.

Category	Numbers of detected genes
Detected genes showing signal intensities equal to or more than 100 in SLE patients and normal controls.	1.949
↓	
Genes showing statistically significant different expression between healthy controls and active phase of SLE patients.	534
↓	
Genes showing statistically significant increased or decreased expression in active SLE patients in compared to either inactive SLE patients or healthy controls.	47
↓	
qRT-PCR successfully amplified genes among these 47 genes.	27

phase SLE patients in our DNA microarray system, forty-seven genes showed statistically significant increased or decreased expression intensities in SLE patients with an active phase in compared to either the SLE patients with an inactive phase or healthy controls. There were no statistical significantly differences between the SLE patients with inactive phase and healthy controls among these forty-seven genes. qRT-PCR successfully amplified twenty nine genes of the forty-seven genes and this information is described in Table II. As shown in Figure 1, the expression of three and six genes significantly increased or decreased in the active phase of SLE, respectively, in compared to the inactive phase of SLE or normal controls. No statistically significant differences in the quantitative expressions of these nine genes were observed between the inactive phase of SLE and normal controls. The data regarding these genes, including gene names, symbols, and GenBank accession number are described in Table II.

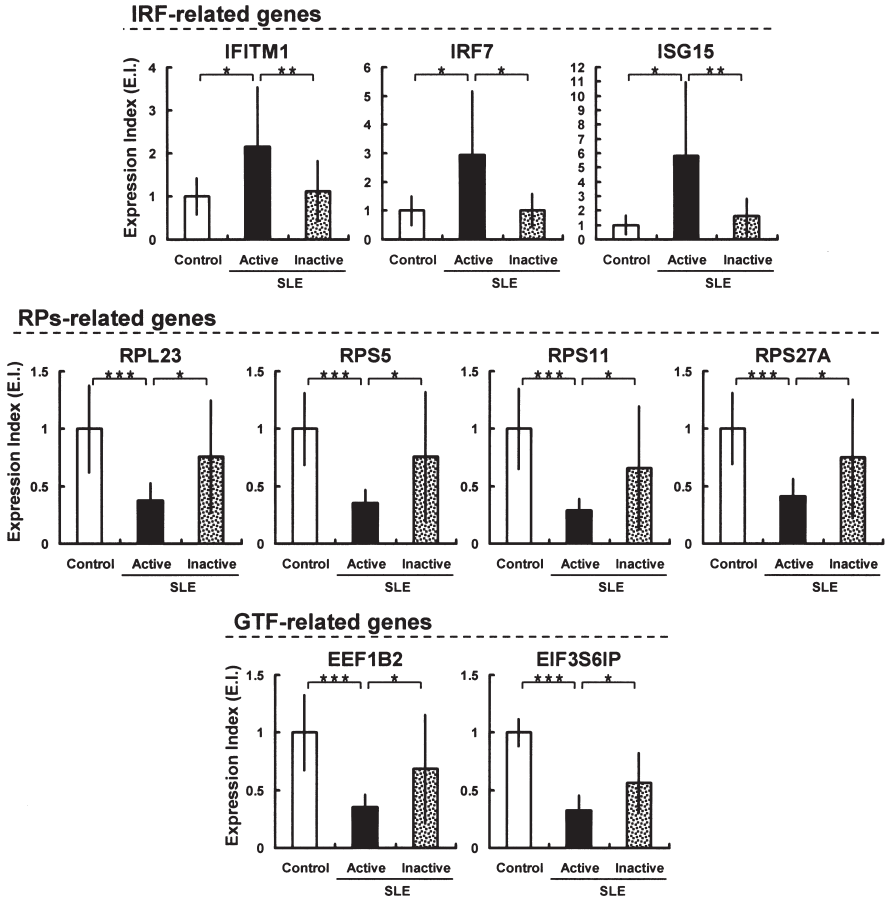

Fig. 1. Comparison of the quantitative gene expression detected by qRT-PCR between patients with active phase and inactive phase SLE or healthy controls. Among the genes described in Table II, genes showing statistically significant differences between active SLE and inactive SLE or normal patients are described in this figure. No statistically significant differences were observed between the inactive phase of SLE and normal controls in each gene described here. Information regarding the genes in this figure are summarised in Table II. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

Discussion

Fluctuations in the gene expression in PBMC were examined in SLE patients in different phases in comparison to the gene expression of normal controls. A recently developed DNA microarray, loaded with approximately 30,000 target genes was used to perform more a precise analysis of the gene expression in comparison to previous studies (4, 5). Therefore, the accuracy of the data obtained in this study seems to be higher than that of previously performed analyses of gene expression in patients with SLE using DNA microarray methods.

As described in Figure 1 and Table II, three genes demonstrated an increased quantitative expression and six genes showed a decreased expression in active SLE phase in compared to those in inactive SLE phase or normal controls. The former three genes (IRF7, ISG15, and IFITM1) belong to the interferon regulatory factor (IRF) family. Four genes (RPS5, RPS11, RPL23, and RPS27A) in the latter group are members of RPs and other two genes (EEF1B2 and EIF3S61P) are GTF-related genes (Table II).

Certain families of IRF induce proinflammatory cytokines such as tumour necrosis factor (TNF)-alpha, interleukin (IL)-6, and IL-12 in the association with toll like receptor (TLR) (14). In fact, IRF7 is reported to play an important role in the production of IFN-alpha (which is thought to contribute to the development of SLE) through an association with TLR 4 (15). Furthermore, sera or IgG from SLE patients can induce IFN-alpha/-beta production from mouse dendritic cells and this requires the presence of IRF7 and TLRs (16). In addition, the ISG15 genes detected by

qRT-PCR are highly expressed in SLE patients with active disease, in comparison to normal controls and patients with rheumatoid arthritis (17). IFITM1 is an IFN target gene and its related genes such as IFIT1 are induced by IFN-alpha in SLE plasma and are related to the production of autoantibodies to RNA binding proteins in SLE patients (18, 19). Therefore, a higher expression of IRF7, ISG15, and IFITM1 genes in active SLE phase as detected by the DNA microarray seems to be associated with the pathogenesis of SLE. Five independent studies using DNA microarray have found that IFN-regulated and/or IFN-inducible genes are highly expressed in PBMC from SLE patients in compared to normal controls, supporting a crucial role for IFN in SLE (6, 11). The current study also revealed the expression of IRF-related genes to significantly increase in the active phase of SLE, while decreasing to normal control levels in the inactive phase of SLE in each patient (Fig. 1). Therefore, these IRF-related genes appear to play an important role in the disease activity and the pathogenesis of SLE.

Antibodies to RPs are present in sera from SLE patients with active disease and these are associated with certain symptoms in SLE patients such as central nerve disorders and hepatic injury (20-22). Although the precise roles of these antibodies in the pathogenesis of SLE are still unclear, some reports indicate the penetration of anti-RPs antibodies into living cells to lead to cell dysfunction such as apoptosis and this may be related to autoimmune phenomena in SLE (22). The roles of RPs as an autoantigen are unknown in the pathogenesis of SLE. However, a decreased expression of RPs-related genes with SLE disease activities seems to play important roles in the development of SLE, because four different kinds of RPs-related genes (RPS5, RPS11, RPL23, and RPS27A) were detected coincidently in the current system (Table II). The EEF1B2 gene encodes a translation elongation factor and the protein is a guanine nucleotide exchange factor involved in the transfer of aminoacylated transfer RNA (tRNA) to the ribosome (23) and the EIF3S61P gene is also related to a mammalian translation initiation factor and the protein plays an important role in the control of cell growth (24). There have so far been no reports addressing the relationship between this EEF1B2 or EIF3S61P and SLE although EEF1B2 has been reported to be associated with certain disorders (25, 26). Further investigations are required to elucidate the biological/clinical meaning and the mechanism of the decreased expression of RPs-related genes (RPS5, RPS11, RPL23, and RPS27A) or GTF-related genes (EEF1B2 and EIF3S61P) in the active phase of SLE.

Some genes (related to immune responses such as IRF-related genes but not shown in our results) showed tendencies of quantitative gene expression similar to the genes described in Fig. 1, although no statistical significance was observed between the active and inactive phase of SLE or normal controls. Further improvements in the disease conditions with treatments may induce quantitative differences in the expression of these genes and demonstrating a statistically significant difference between the active phase and inactive phase of SLE or normal controls. More precise investigations about IRF- and RPs-, and GTF-related genes are expected to clarify the pathogenesis of SLE and open new possibilities of the treatments and diagnoses of SLE.

References

1. SEKIGAWA I, KAWASAKI M, OGASAWARA H *et al*.: DNA methylation: its contribution to systemic lupus erythematosus. *Clin Exp Med* 2006; 6: 99-106.
2. SEKIGAWA I, NAITO T, HIRA K *et al*.: Possible mechanisms of gender bias in SLE: new hypothesis involving a comparison of SLE with atopy. *Lupus* 2004; 13: 217-22.
3. SEKIGAWA I, OGASAWARA H, KANEKO H, HISHIKAWA T, HASHIMOTO H: Retroviruses and autoimmunity. *Intern Med* 2001; 40: 80-6.
4. KAWASAKI M, SEKIGAWA I, NOZAWA K *et al*.: Changes in the gene expression of peripheral blood mononuclear cells during the menstrual cycle of females is associated with a gender bias in the incidence of systemic lupus erythematosus. *Clin Exp Rheumatol* 2009; 27: 260-6.
5. KAWASAKI M, KANEDA K, KANEKO H, SEKIGAWA I, TAKASAKI Y, OGAWA H: Two cases of dermatomyositis associated with interstitial pneumonia: a comprehensive study of gene expression. *Clin Exp Rheumatol* 2008; 26: 379-80.
6. LEE HM, MIMA T, SUGINO H *et al*.: Interactions among type I and type II interferon, tumor necrosis factor, and beta-estradiol in the regulation of immune response-related gene expressions in systemic lupus erythematosus. *Arthritis Res Ther* 2009; 11: R1.
7. NAKOU M, KNOWLTON N, FRANK MB *et al*.: Gene expression in systemic lupus erythematosus: bone marrow analysis differentiates active from inactive disease and reveals apoptosis and granulopoiesis signatures *Arthritis Rheum* 2008; 58: 3541-9.
8. NIKPOUR M, DEMPSEY AA, UROWITZ MB, GLADMAN DD, BARNES DA: Association of a gene expression profile from whole blood with disease activity in systemic lupus erythaematosus. *Ann Rheum Dis* 2008; 67: 1069-75
9. DAI Y, HUANG YS, TANG M *et al*.: Microarray analysis of microRNA expression in peripheral blood cells of systemic lupus erythematosus patients. *Lupus* 2007; 16: 939-46.
10. GLADKEVICH A, NELEMANS SA, KAUFFMAN HF, KORF J: Microarray profiling of lymphocytes in internal diseases with an altered immune response: potential and methodology. *Mediators Inflamm* 2005; 2005: 317-30.
11. QING X, PUTTERMAN C: Gene expression profiling in the study of the pathogenesis of systemic lupus erythematosus. *Autoimmun Rev* 2004; 3: 505-9.
12. TAN EN, COHEN AS, FRIES JF *et al*.: The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982; 25: 1271-7.
13. BOMBARDIER C, GLADMAN DD, UROWITZ MB, CARON D, CHANG CH: Derivation of the SLEDAI. A disease activity index for lupus patients. The committee on prognosis studies in SLE. *Arthritis Rheum* 1992; 35: 630-40.
14. TAKAOKA A, YANAI H, KONDO S *et al*.: Integral role of IRF-5 in the gene induction programme activated by Toll-like receptors. *Nature* 2005; 434: 243-9.
15. RICHEZ C, YASUDA K, WATKINS AA *et al*.: TLR4 ligands induce IFN-alpha production by mouse conventional dendritic cells and human monocytes after IFN-beta priming. *J Immunol* 2009; 182: 820-8.
16. YASUDA K, RICHEZ C, MACIASZEK JW *et al*.: Murine dendritic cell type I IFN production induced by human IgG-RNA immune complexes is IFN regulatory factor (IRF)5 and IRF7 dependent and is required for IL-6 production. *J Immunol* 2007; 178: 6876-85.
17. FENG X, WU H, GROSSMAN JM *et al*.: Association of increased interferon-inducible gene expression with disease activity and lupus nephritis in patients with systemic lupus erythematosus. *Arthritis Rheum* 2006; 54: 2951-62.
18. HUA J, KIROU K, LEE C, CROW MK: Functional assay of type I interferon in systemic lupus erythematosus plasma and association with anti-RNA binding protein autoantibodies. *Arthritis Rheum* 2006; 54: 1906-16.
19. SCHILD-HAY LJ, LEIL TA, DIVI RL, OLIVERO OA, WESTON A, POIRIER MC: Tamoxifen induces expression of immune response-related genes in cultured normal human mammary epithelial cells. *Cancer Res* 2009; 69: 1150-5.

20. ABDEL-NASSER AM, GHALEB RM, MAHMOUD JA, KHAIRY W, MAHMOUD RM: Association of anti-ribosomal P protein antibodies with neuropsychiatric and other manifestations of systemic lupus erythematosus. *Clin Rheumatol* 2008; 27: 1377-85.
21. HANLY JG, UROWITZ MB, SIANNIS F *et al*.: Autoantibodies and neuropsychiatric events at the time of systemic lupus erythematosus diagnosis: results from an international inception cohort study. *Arthritis Rheum* 2008; 58: 843-53.
22. TOUBI E, SHOENFELD Y: Clinical and biological aspects of anti-P-ribosomal protein autoantibodies. *Autoimmun Rev* 2007; 6: 119-25.
23. CHAMBERS DM, ROULEAU GA, ABBOTT CM: Comparative genomic analysis of genes encoding translation elongation factor 1B(alpha) in human and mouse shows EEF1B1 to be a recent retrotransposition event. *Genomics* 2001; 77: 145-8.
24. MORRIS-DESBOIS C, RÉTY S, FERRO M, GARIN J, JALINOT P: The human protein HSPC021 interacts with Int-6 and is associated with eukaryotic translation initiation factor 3. *J Biol Chem* 2001; 276: 45988-95.
25. PILTTI J, HÄYRINEN J, KARJALAINEN HM, LAMMI MJ: Proteomics of chondrocytes with special reference to phosphorylation changes of proteins in stretched human chondrosarcoma cells. *Biorheology* 2008; 45: 323-35.
26. SORDET C, GOETZ J, SIBILIA J: Contribution of autoantibodies to the diagnosis and nosology of inflammatory muscle disease. *Joint Bone Spine* 2006; 73: 646-54.