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Short communication

Gene profiling involved in immature CD4+ T lymphocyte responsible for systemic lupus erythematosus

Y.J. Deng [a,b,1], Z.X. Huang [b,1], C.J. Zhou [a], J.W. Wang [a], Y. You [a], Z.Q. Song [a], M.M. Xiang [a], B.Y. Zhong [a], F. Hao [a,*]

[a] *Department of Dermatology, Southwest Hospital, The Third Military Medical University, ChongQing 400038, PR China*
[b] *Department of Pathology, Southern Medical University, Guangzhou 510515, PR China*

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Abstract

We attempted to characterize the genes expression of CD4+ T lymphocytes for the pathogenesis of systemic lupus erythematosus (SLE). Genomewide gene expression profiles of CD4+ T cells, which were isolated from the disease severe activity (T4-1s) and nonactivity (T4-2s) with an SLE patient by using long serial analysis of gene expression (LongSAGE). We picked out 289 genes matching to Unigene cluster with different expression more than four copies between T4-1s and T4-2s libraries and analyzed their roles from the collectedly published articles of PubMed by genes functional clustering. The genes functions were related to a diverse cellular process including: (1) most of these genes were associated with CD4+ T cells functions, particularly related to cellular developments; (2) Ras pathway genes as RANBP10, GMIP, RASGRP2 and ARL5 might be responsible for the abnormal development of CD4+ T cells of SLE; (3) HIG2, TCF7, KHSRP, WWP1, SMAD3, TLK2, AES, CCNI and PIM2 belong to Wnt/beta-catenin way, they could play roles in modulating proliferation and differentiation of T lymphocytes; (4) uncertain viral infections may initiate autoimmunity because high levels expression genes were detected in T4-1s such as TRIM22, IER2, ABCE1, DUT, G1P2, G1P3, HNRPUL1, EVER2, IFNAR1, TNFSF14, TMP21 and PVRL2; and (5) apoptosis relating genes as EIF3S8, SH3BGRL3, GPX4, TOSO, PFDN5, BIN1, XIAPAF1, TEGT and CUGBP2 may contribute to over uploading of selfantigens in SLE cells. Abnormalities findings of multiple genes expression involving with a variety of CD4+ T cells process might be meaningful to understanding the pathogenesis of SLE, and immature CD4+ T cells may be responsible for SLE.

Keywords: Systemic lupus erythematosus; CD4+ T lymphocytes; Gene expression profiling; Cell differentiation; Growth and development; Expressed sequence tags

1. Introduction

Our understandings in animal models and in human patients have found that the pathogenesis of systemic lupus erythematosus (SLE) is associated with both genetic predispositions and environmental influences. This combination of various factors, which may differ among individuals, results in dysfunction of the adaptive immune system (Qing and 3 Putterman, 2004). The production of autoantibodies immune complexes, along with autoreactive T lymphocytes mediate this altered immune response at the cellular level in SLE together cause pathological changes in several target organs, including skin, blood vessels, lung and kidney (Shlomchik et al., 2001). As SLE is a multigenic and age-dependent disease, the final disease phenotype is probably the result of many interactions arising from an initial loss of peripheral tolerance followed by the amplification of specific autoimmune responses. CD4+ T lymphocytes are considered to play a pivotal role in the generation of losing self-tolerance (Bouzahzah et al., 2003; Chang et al., 2004; Kolowos et al., 2001; Soltesz et al., 2002; Tsai et al., 2004).

* Corresponding author. Fax: +86 23 68754416.
E-mail address: haofei@mail.tmmu.com.cn (F. Hao).
[1] These authors equally contributed to this work.

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A tremendous amount of work has been done to investigate the mechanisms underlying the pathogenesis of SLE. However, traditional molecular biology methods that focus on a single likely molecule or pathway may be limited in their ability to identify potential disease-related candidates from a broad spectrum of multiple interacting factors. Genomewide gene profiling using serial analysis of gene expression (SAGE) technology emerged in 1995 (Velculescu et al., 1995), and long serial analysis of gene expression (LongSAGE) were subsequently promoted in 2002 (Saha et al., 2002). LongSAGE derives 21 bp tags for a given transcript while SAGE generates 14 bp tags. A LongSAGE/SAGE library can produce adequate tags of transcripts which allow for the unbiased quantitative analysis of transcriptomes with given tissues or cells, but the LongSAGE tags can be used to rapidly identify novel genes and exons rather than SAGE tags. Although many aspects of T lymphocytes with SLE have been studied in detail, the molecular mechanisms underlying the pathogenesis of SLE for T cells remain elusive. This work is in an attempt to identify genes of T cells with SLE that determine commitment to the disease active state or nonactive state, the gene expression profiles of CD4+ T lymphocytes isolated from a SLE patient in the disease active state (T4-1s) and nonactive state (T4-2s) were analyzed by LongSAGE, which can detect novel genes and known genes that have not been implicated in CD4+ T cell with SLE so far.

2. Materials and methods

2.1. Patient's selection and management

A 26-year-old woman who gave birth to a daughter four years ago was selected. The patient complained erythema eruptions on her hands, feet, back and face (see Supplementary data, Fig. s1) for five months along with arthropathy involving her shoulders, knees and coxae joints. The disease has not been identified and she did not take any medications until she was referred to our department. Laboratory examinations found that the patient was not with impaired renal, hepatic and cardiac function except with mild pericardial effusion. The clinical manifestations and laboratory findings revealed that the patient suffered from SLE according to the American College of Rheumatology revised criteria for the classification of SLE (Hochberg, 1997) with a 29-score of systemic lupus erythematosus disease activity 2000 (SLEDAI-2K) (Gladman et al., 2002). The patient's disease was at a severe active state. Twenty millilitres peripheral blood was taken for isolation of CD4+ T cells before chemotherapy. An intravenous pulse regimen with cyclophosphamide (600 mg for two consecutive days, monthly) and methylprednisolone (1000 mg, for three consecutive days, monthly) plus orally prednisolone and hydrochloroquine was established. After five months of treatment, the abnormalities of laboratory findings returned to normal levels. The patient had not any complaints, the skin lesions faded away and SLEDAI-2K score gradually decreased from 29 to 0 point. As disease remission achieved successfully, prednisolone and hydrochloroquine were gradually reduced and all of the orally pills were discontinued with trial on the sixth month. The patient only received monthly course of cyclophosphamide and methylprednisolone without orally medicine. In this time, the disease got through to a nonactive state and kept for two months. On the seventh month and before the administration of chemotherapy, 20 ml peripheral blood was extracted again for purification of CD4+ T cells.

2.2. Isolation of CD4+ T cells

Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll–Hypaque (Pharmacia, Sweden) gradient cell density centrifugation. Cells were stained with anti-CD4-FITC (Dyclone, France) antibodies, and then combined to anti-CD4 immunomagnetic beads (Miltenyi Biotec, Germany). CD4+ T cells were extracted from MS Mini-MACS column (Miltenyi Biotec, Germany). All samples were obtained according to the principle of manufactures' protocol.

2.3. Verification of CD4+ T cells subset purification

The identity of the purified CD4+ T cell subsets was verified genotypically and phenotypically. The purified cells were examined by flow cytometry for verification of CD4+ T cells phenotype. The genotype of purified CD4+ T cells was assessed by PCR amplification by using primers of CD4, CD8 and CD19 at gene transcriptional level.

2.4. LongSAGE analysis

RNA isolation was obtained by using Tripure isolation kit (Roche, USA). cDNA synthesis and LongSAGE library construction were carried out by I-SAGE Long Kit (Invitrogen, USA) according to LongSAGE manual. RNA was equal to 10^6 isolated CD4+ T cells for the construction of T4-1s and T4-2s LongSAGE library, respectively, and the superfluous RNAs were used to further verification experiments. The LongSAGE tags were extracted by using SAGE2000 analysis software v4.5 downloaded from http://www.invitrogen.com/sage/. The Unigene reference database (April 2004) was obtained at ftp://ftp.ncbi.nlm.nih.gov/pub/sage/. T4-1s LongSAGE library produced a total of 14,130 tags, and 16,147 tags were obtained from T4-2s LongSAGE library. All LongSAGE tags were compacted in a unique data set with quantitative information and matched to the LongSAGE reference database for gene identification.

2.5. Verification of quantitative accuracy of LongSAGE data

To validate quantitative differences in gene expression between T4-1s and T4-2s as attained from LongSAGE, semi-quantitative RT-PCR analysis was performed for a set of 30 selected genes by using cDNAs from T4-1s and T4-2s purified from the SLE patient as template. RT-PCR was done for 20, 26 and 32 cycles and normalized for the beta-actin gene. In the RT-PCR process, a LongSAGE tag sequence was used as the sense primer, and a universal antisense primer located at the 3′ end of cDNA was used as the antisense primer to amplify the original cDNA template from which the LongSAGE tag was derived.

2.6. Functional clustering of LongSAGE data

LongSAGE tags derived from known transcripts were arranged in functional clusters and sorted based on the ratio of their frequency in T4-1s and T4-2s. Clustering analysis is derived from the genes with known functions and comments in all published articles downloaded from www.ncbi.nlm.nih.gov. Ribosome and mitochondrion genes are omitted. CLUSTER and TREEVIEW software were used (downloaded from http://rana.lbl.gov/, kindly provided by Michael B. Eisen, Lawrence Berkeley National Laboratory) for transformation and graphic representation of LongSAGE tag counts.

3. Results and discussion

3.1. Chemotherapy results and follow-up observations

Skin lesions showed on the patient's face, back, hands and feet at her first hospitalization (see Supplementary data, Fig. s1). The erythemas shrank along with chemotherapy and disappeared in the third hospitalization without relapse (see Supplementary data, Fig. s2). Red blood cells (RBC) count, white blood cells (WBC) count, erythrocyte sedimentation rate (ESR), 24 h protein of urine, C3, IgG, and antinuclear–antibodies (ANA) titer returned to normal levels (see Supplementary data, Figs. s3–s9). SLEDAI-2K score was also from 29 point to 0 (Table 1) with the laboratory findings. The disease turned to a nonactive state. On the first hospitalization, the patient suffered from SLE with a severe disease activity and did not take any pills. T4-1s could represent the naive set of SLE and the genes expression of T4-1s could unroll molecular portrait of pathogenesis. In the period of disease nonactivity on the sixth chemotherapy, the patient's oral medicine had been discontinued with trial and the disease had been still on the nonactivity for two months. In fact, the patient had a good health in the subsequent follow-up of eight months except received a monthly course of intravenous pulse therapy without took any other orals. For illustration of the molecular characteristics of CD4+ T cells in the nonactive state of SLE, T4-2s were extracted from the checkpoint on the seventh chemotherapy. At the collection of peripheral blood, it was one month away from the last dose of cyclophosphamide and methylprednisolone. Therefore, cyclophosphamide could have been sufficiently cleared (Busse et al., 1999; Chen et al., 1997; Haubitz et al., 2002; Petros et al., 2002), and methylprednisolone pharmacokinetics (Booker et al., 2002; Lew et al., 1993; Tornatore et al., 2004) could be presumed that the clearance of methylprednisolone in the patient was enough not to have effect on T helper cell. By this time, the genes expression profile of T4-2s could represent the totality of CD4+ T cell's transcripts of nonactive state with SLE.

Table 1
SLEDAI-2K scores of the patient at the time of chemotherapy

SLEDAI-2K weight	The time at chemotherapy						
	1	2	3	4	5	6	7
Haemorrhagic eruption (8)	√						
Urine cast (4)	√	√	√	√			
Hematuria (4)	√	√	√	√			
Proteinuria (4)	√	√	√	√	√		
Pyuria (4)	√	√					
Eruption relapse (2)	√						
Pleuritis (2)	√	√	√				
Leucopenia (1)	√						
Total score	29	18	14	12	4	0	0

3.2. Isolation and verification of CD4+ T lymphocytes

The isolated CD4+ T cell from PBMC with anti-CD4 immunomagnetic beads showed a higher positive percent by flow cytometry examination (Figs. 1 and 2). It is recommended that the purity of isolated cells is more than 96% should be transferred to downstream experiments (Zhou et al., 2001). In this research, the purity of CD4+ cells for construction of LongSAGE libraries was above 95%. RT-PCR amplification of the purified CD4+ T cells only

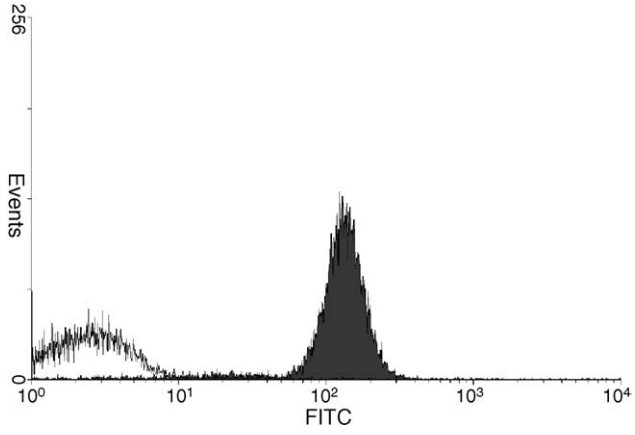

Fig. 1. CD4+ T cells isolated from the patient of SLE at severe disease activity state were positive for 95.8%.

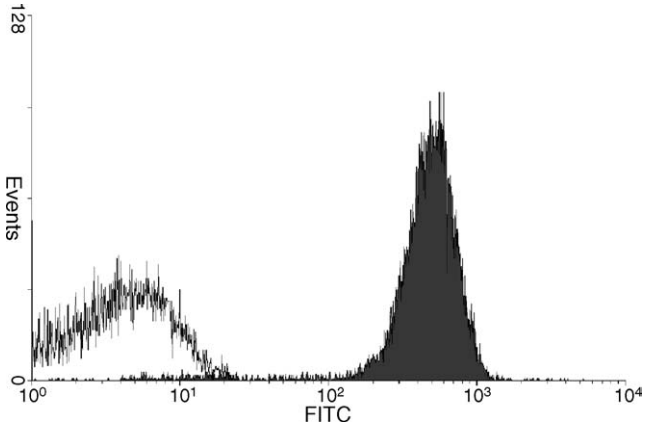

Fig. 2. CD4+ T cells isolated from the patient of SLE at nonactive state were positive for 96.2%.

produced CD4 bands, and did not produce CD19 and CD8 bands (Fig. 3). The verification of isolated CD4+ T cells with genotype and phenotype guaranteed that the constructed libraries could be available for genomewide genes profiling analysis.

3.3. Assessment of LongSAGE libraries

For both T4-1s and T4-2s, more than 14,000 tags were collected and matched to Unigene. Detailed features of T4-1s and T4-2s LongSAGE libraries are described in Table 2. T4-1s and T4-2s longSAGE libraries contained 6162 and 6960 unique tags, respectively, but more unique tags were identified in the T4-1s library. This finding indicates that the repertoire of gene expression at mRNA level is far less complex in the CD4+ T cells and suggests that CD4+ T lymphocytes express many fewer genes in the active state than in the nonactive state of SLE. Except for the fact that more than 40% of the genes expressed in T4-2s were silenced in T4-1s, the quantitative distribution of LongSAGE tags matched to Unigene database is also different between T4-1s and T4-2s LongSAGE profiles, but the abundance classes are similar in both LongSAGE libraries (Table 2). Although there are 15%

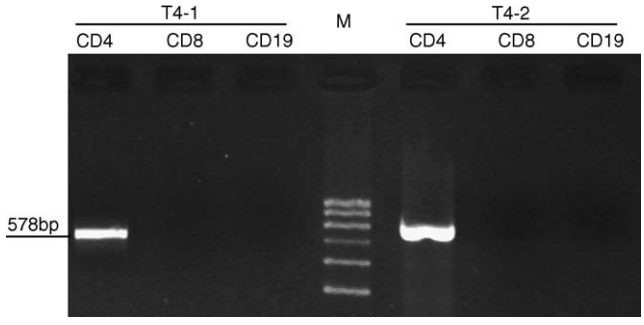

Fig. 3. 578 bp CD4 bands were amplified successfully in T4-1s and T4-2s RT-PCR products, respectively, while CD8 and CD19 were without any amplification. Marker is 100 bp DNA ladder from 300 to 800 bp.

Table 2
Distribution of LongSAGE tags in T4-1s and T4-2s

	CD4+ T cells	
	T4-1s	T4-2s
Total tags	14130	16147
Unique tags	6162	6960
Tags matching multiple genes[a]	897 (15)	670 (10)
Tags matching single gene	3267 (53)	2701 (39)
Novel tags	1998 (32)	3589 (51)
Abundance classes of LongSAGE tags[b]		
1	3660 (59)	4000 (57)
2–4	2164 (35)	2537 (36)
5–9	199 (3)	261 (4)
10–99	133 (2)	157 (2)
≥100	6 (<1)	5 (<1)

[a] Percentages are given in parentheses.
[b] Abundance classes refer to tag counts in the two LongSAGE libraries.

and 10% unique LongSAGE tags match to more than one Unigene cluster in T4-1s and T4-2s libraries, the matching range is between two to seven genes, which is far less than SAGE tags matching to multiple genes (ranging between two and more than 99 matches (Lee et al., 2001; Muschen et al., 2002; Zhou et al., 2001)). A 21 bp LongSAGE tag compared with 14 bp SAGE tag tended to be more specific for representing a gene resulting in presence of fewer tags matching multiple genes and lower matching ranges. Fifty three percent and 39% matched to one single Unigene cluster, 32% and 51% of tags do not have a match in Unigene and are likely to be derived from novel genes in T4-1s and T4-2s unique tags (Table 2). Novel SAGE tags can be further investigated by GLGI (Chen et al., 2000) and 5′ rapid amplification of cDNA ends to identify full-length cDNAs from novel candidate genes. The proportions of abundance classes are comparable in T4-1s and T4-2s (Table 2). Multiple LongSAGE tags matching to one Unigene cluster (i.e., the presence of splice variants in the 3′ region of a given gene) show a more frequent tendency in T4-2s as compared with T4-1s and point to another level, at which the pattern of gene expression is more complex in the CD4+ T lymphocytes of the nonactive state than in the active state for SLE.

Semiquantitative RT-PCR was performed to corroborate quantitative differences of gene expression for 30 selected tags that matched to single gene (Fig. 4). RT-PCRs were used cDNAs from the SLE patient of T4-1s and T4-2s (i.e., RNA that was not subjected to LongSAGE analysis) as templates, and the selected LongSAGE tags sequence and universal primers were added to the cocktail mixtures as primers. Among 30 genes tested, amplified fragments lengths of the expected size were ranged from 80 to 500 bp. The intensity of the RT-PCR bands reflected the quantitative differences between T4-1s and T4-2s as tag counts in the LongSAGE analysis for all 30 informative cases. This indicated that the quantitative differences observed in the comparison of LongSAGE profiles for T4-1s and T4-2s are consistent with semiquantitative RT-PCR results.

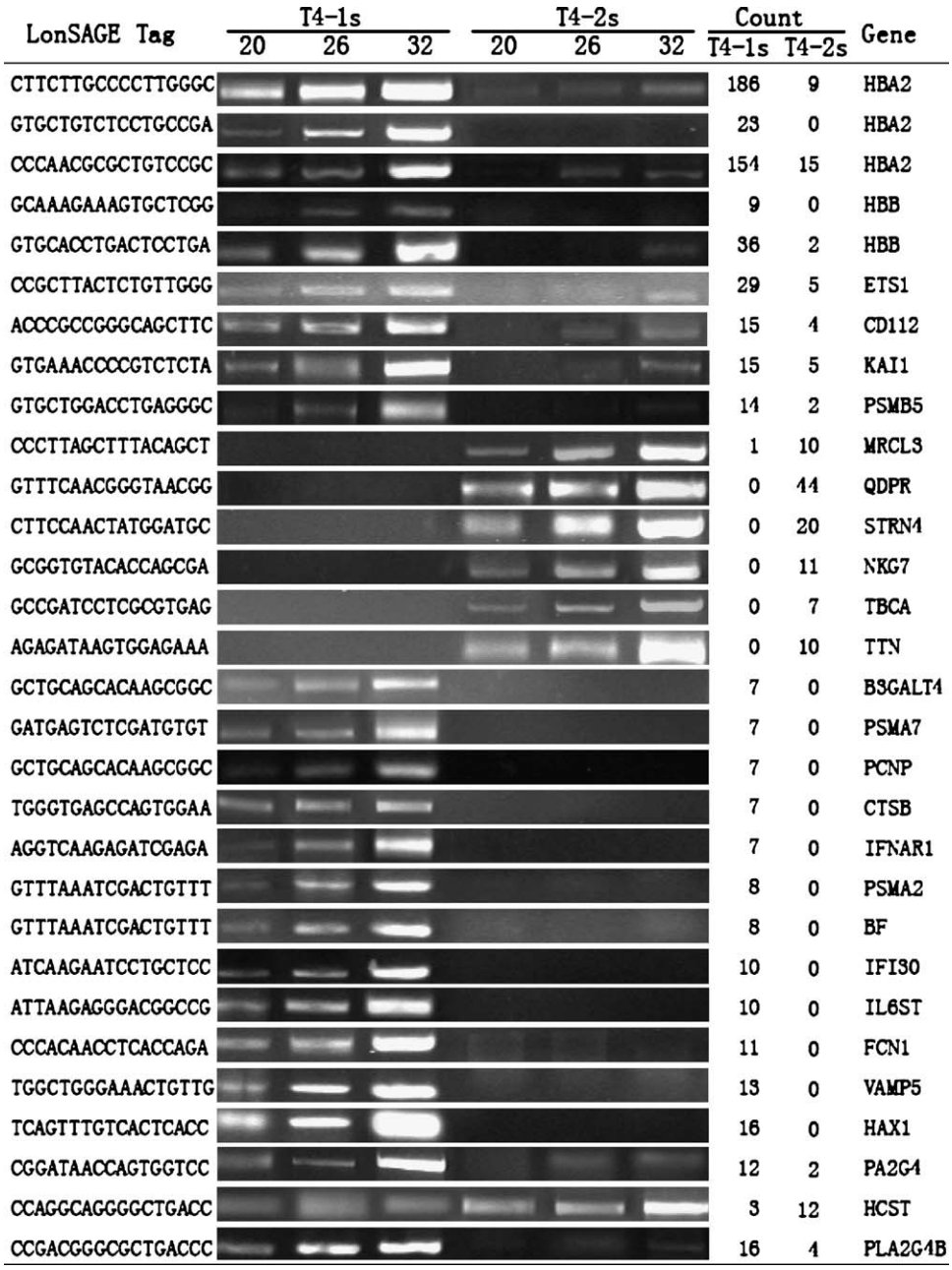

Fig. 4. Assessment of quantitative accuracy of LongSAGE data. To corroborate different gene expression between T4-1s and T4-2s as assessed by LongSAGE, semiquantitative RT-PCR was performed for 30 selected genes. The RT-PCR was normalized for 20, 26, and 32 cycles by using a cDNA fragment of the beta-actin gene as a standard. Amplified fragments lengths of the expected size were ranged from 80 to 500 bp (data not shown).

3.4. Functional clustering of Unigenes

We have analyzed the matched genes amounting to 289 genes only with the expression difference more than four copies between T4-1s and T4-2s; ribosome and mitochondrion genes are excluded. The published articles for a given gene related to corresponding cells and possible functions comments were collected from PubMed (http://www.ncbi.nih.gov/). We compared the documents related to CD4+ T cells with other blood cells, and non-associated cells (e.g. endothelium, cancer cells, osteoblast) as well. Gene's function is involved with apoptosis, lupus, immune response and regulation, antigen presentation, development, cell cycle control, hypoxia, and Ras and Wnt/beta-catenin signal transduction pathways (Fig. 5A–D).

In the collected articles about these genes, most of them are related to thymocytes functions including lupus, antigen presentation, infection, cytotoxicity, CD8 lymphocytes, CD4 lymphocytes, dendritic cells, immune responses, immune regulation, B lymphocytes, stem cells, bone marrow cells, cell cycle control, apoptosis, development, proliferation, cancer, Ras and Wnt/beta-catenin signal transduction pathways. Few

of them are associated with eosinophil, basophil, hepatocyte, endothelium, muscle cell, fibroblast, chondrocyte, osteoblast, erythrocyte, hypoxia and mesenchymal (Fig. 5A–D). It can be concluded that the screened genes with different expression in T4-1s and T4-2s by LongSAGE can exhibit the functions of CD4+ lymphocytes.

The remarkable column in the maps is referred to development, as if most of the genes have a role with it (Fig. 5A–D). Many of these genes have a variety of roles except for development, such as TCF7, CLEC2D, PDCD4, DC2, GZMB, CD7, SELL, SMAD3 and TLK2. It can be understood that some genes related to malignant tumors are also the effectors of developmental process (Fig. 5C). Although the relatively high levels of expression of these genes in T4-1s or T4-2s are not well known, they must be potentially meaningful and their function in the mechanism of pathogenesis for SLE remains to be clarified. We presume that one or more genes handicap in cellular signal transduction pathways should be in charge of the abnormal development of CD4+ T cells with SLE.

In the 289 genes with different expression between T4-1s and T4-2s, only a few of them have been involved in the published articles related to CD4+ T cells (Fig. 5A). Among these genes, TCF7 (TCF-1), BID, IER2, DUT, G1P2, IFNAR1, DC2, ACTN4, GANAB, UBE1L, PSMA7, IFI30, TNFSF14, TMP21, ITGB7, CD6, IL10RA, PVRL2, LTBP2, NKTR, LILRB1, IL7R and CD7 expressed at high levels in T4-1s, while the genes at low levels expression in T4-1s are PDCD4, GIMAP7, TES, CTSS, MED28, ITGB4BP, NKG7, GZMH, HCST, GZMB and SELL. TCF7 expresses in immature thymocytes (Staal et al., 2004), and IL7R signals can

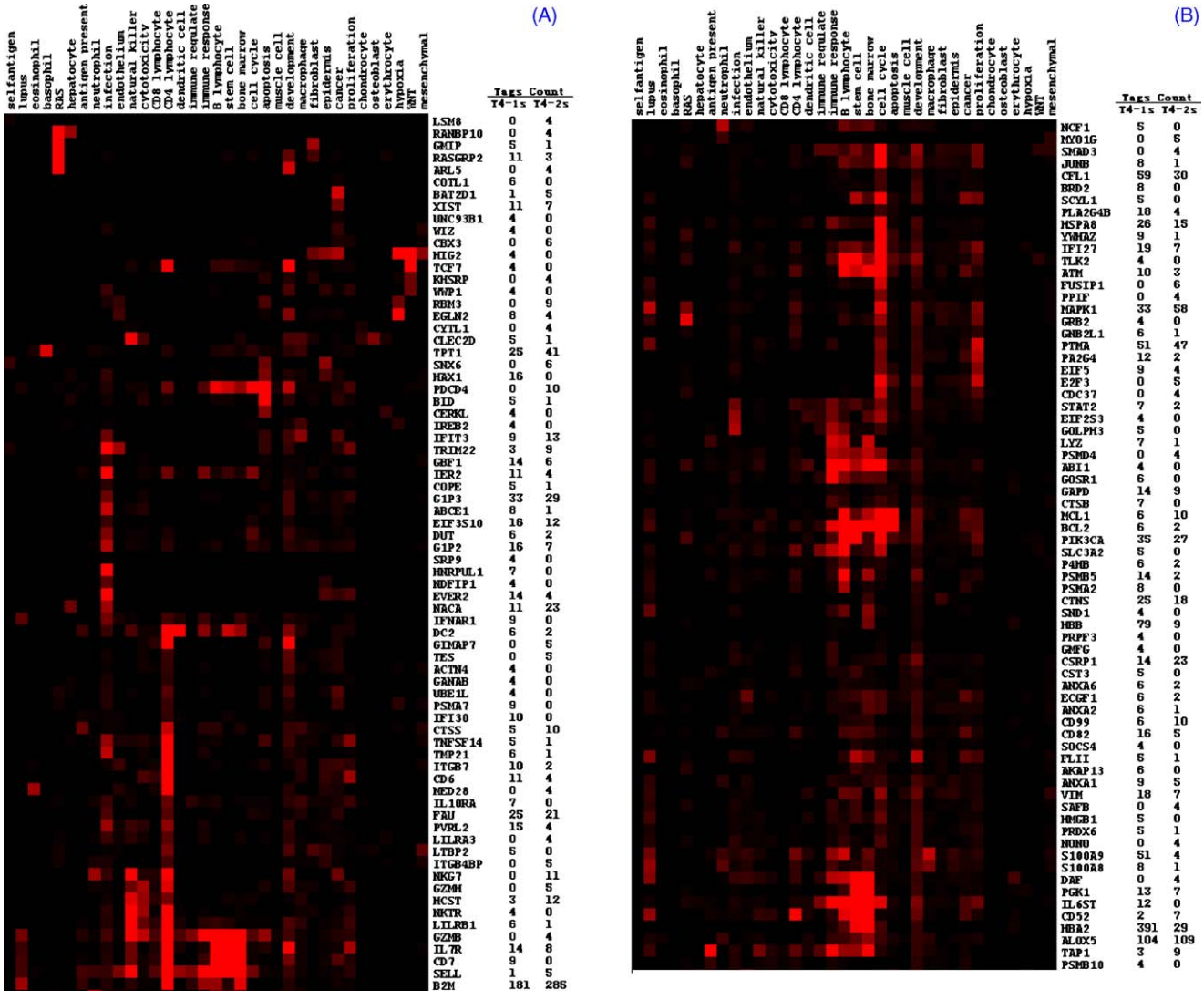

Fig. 5. Gene functional clustering analysis of LongSAGE data in T4-1s and T4-2s libraries. The genes expressions in the cells and possible functions were collected from the published articles of PubMed (http://www.ncbi.nih.gov/) and are shown as a color scale from black (no article involvement) to red (many articles involvement). The expression level of T4-1s and T4-2s libraries were listed at right. Genes related to: (A) CD4+ T lymphocytes, infection, development, Ras and Wnt signal pathways; (B) lupus, cell cycle control, immune response, development and proliferation; (C) development; and (D) lupus, apoaptosis, development and proliferation. Most of the genes play roles in the cellular development (A–D).

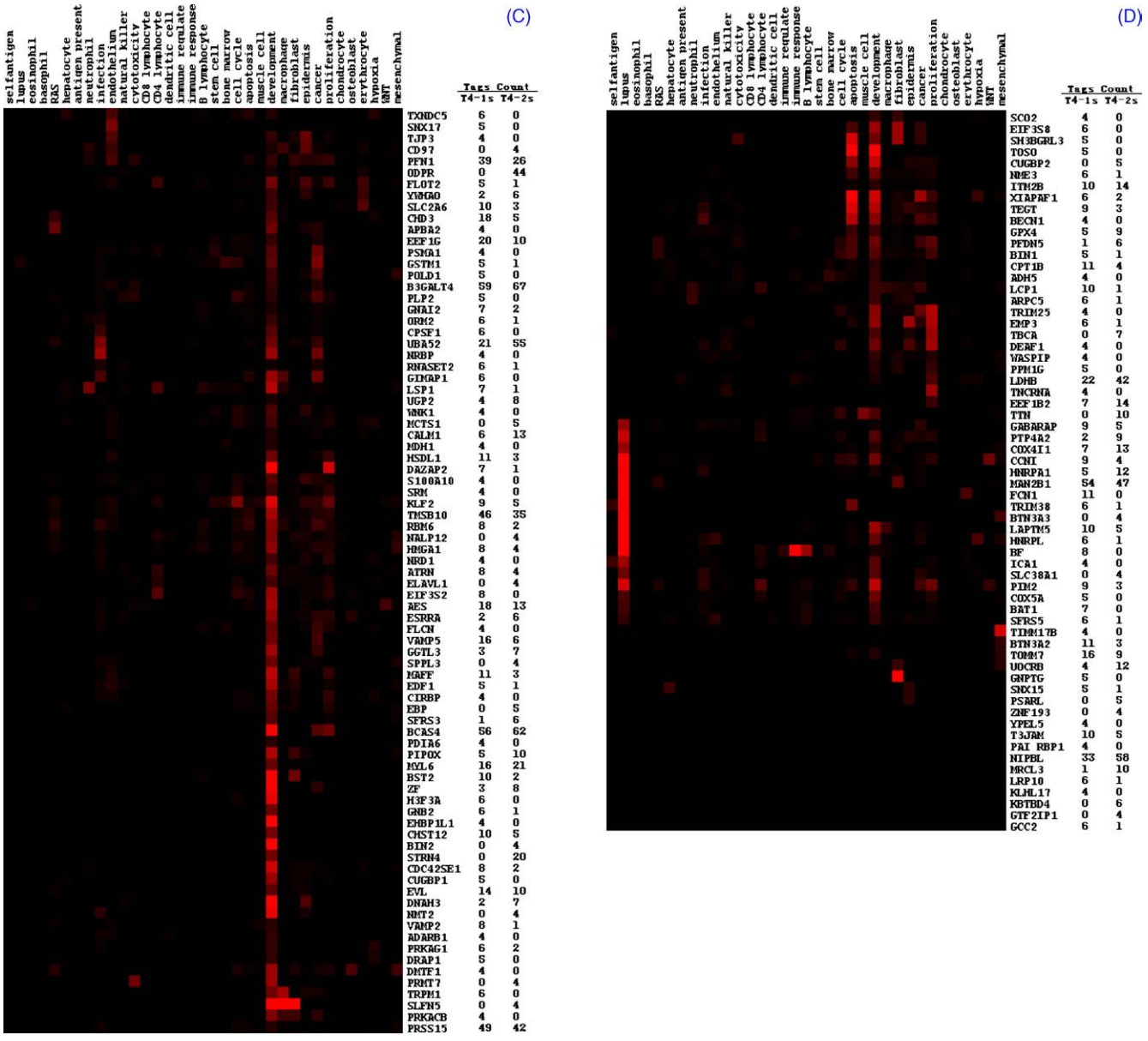

Fig. 5. (*Continued*).

inhibit expression of TCF7 which is required for the immature single positive to double positive transition at the stage of thymocytes development in thymus (Yu et al., 2004). BID, IER2, DUT and IFNAR1 are effectors of viral infection. IL10RA mainly participates in the modulation of immune responses. Proliferation of human CD4+ T cells expressing NKAR has been shown to be enhanced, particularly in response to low doses of antigen (Mandelboim et al., 1998; Strominger et al., 2003), and NKAR expressed in CD4+ cells of SLE might be the reason of autoimmunity. However, we do not know why PDCD4 expressed in T4-2s and did not express in T4-1s, though PDCD4 is commonly known to play a role in apoptosis.

RANBP10, GMIP, RASGRP2 and ARL5 were associated with Ras pathways (Fig. 5A). RANBP10 has been identified as a novel MET-interacting protein shares high sequence similarity with RANBPM, which activates the Ras/Erk signaling pathway by serving as an adaptor protein of MET to recruit SOS, but RANBP10 fails to stimulate MET-induced Ras/Erk signaling (Wang et al., 2004). The action of the RHOGAP protein GMIP may control the Ras signaling pathways (Aresta et al., 2002). RASGRP2, which is a calcium and diacylglycerol- responsive RAP1 exchange factor, is required to activate lymphocyte function-associated antigen-1 (LFA-1) (Quilliam et al., 2002). When RASGRP2 expressed in Jurkat cells associated with RAP1, it resulted in enhanced RAP1 activation and adhesion triggered by the TCR (Katagiri et al., 2004). Therefore, higher expression of RASGRP2 in T4-1s may contribute the hyperactivity of CD4+ T cells in the active stage of SLE. ARL5 is developmentally

regulated and may play a role in nuclear dynamics and/or signaling cascades during embryonic development (Lin et al., 2002). Non-expression of ARL5 in T4-1s indicates that there might be abnormal development of CD4+ T cells with SLE.

Wnt/beta-catenin signal transduction pathway mediates proliferation and differentiation of progenitor cells including T cell development (Mulroy et al., 2003; Staal et al., 2001). Dysregulation of the Wnt/beta-catenin signal transduction pathway has been implicated in the pathogenesis of tumors in the mammary gland, colon and other tissues (Kenny et al., 2005). We have found that HIG2 and TCF7 (TCF-1) expressed in T4-1s and did not express in T4-2s (Fig. 5A). HIG2 was first shown to be induced by both hypoxia and glucose deprivation in human cervical carcinoma cells (Denko et al., 2000), but its role in Wnt/beta-catenin signal transduction pathway is not determined. TCF7, which is critical to T cell development at particular checkpoints from T-lineage specification to peripheral T cell specialization (Rothenberg and Taghon, 2005), was identified by DNA microarrays in immature CD34+ thymocytes (Staal et al., 2004). TCF7 expressed in T4-1s and did not express in T4-2s means that CD4+ cells in SLE would not be well differentiated. The different expression of HIG2, TCF7, KHSRP, WWP1, SMAD3, TLK2, AES, CCNI and PIM2 (Fig. 5A–D) between T4-1s and T4-2s might be responsible for the autoimmunity of lupus by generating immature CD4+ T cells.

In the infection column (Fig. 5A–D), some genes are pertinent to infection. TRIM22 expressed in resting T cells in the absence of exogenous interferon treatment (Gongora et al., 2000). The expression of TRIM22 is induced by interferon. The down-regulating expression of TRIM22 in T4-1s implied that CD4+ cells were activated. It is not sure that SLE is associated with viral infection, but IER2, ABCE1, DUT, G1P2, G1P3, HNRPUL1, EVER2, IFNAR1, TNFSF14, TMP21, PVRL2 up-regulated expressions in T4-1s suggests that SLE would be susceptible to certain pathogens. For example, HNRPUL1 binds specifically to adenovirus E1B-55kDa oncoprotein (Gabler et al., 1998); TNFSF14 is also known as a herpes virus entry mediator (Chimma et al., 2004; Krummenacher et al., 2004).

Theoretically, autoimmunity of lupus may result from the hyperplasia of autogenous immunologic clone with T and B cells. It is well known that B cells of SLE produce a great deal of antinuclear antibodies that lead to break down multiple organs. Several enhanced genes in the T4-1s with proliferation suggest that there might be hyperplasia in some CD4+ T cells of SLE (Fig. 5B and D), and these genes are also involved in cell cycle control and development. JUNB belonging to the activating protein-1 (AP-1) family of transcription factors are key regulators of cellular proliferation, differentiation and invasion processes in many systems (Bamberger et al., 2004; Heinrich et al., 2004; Mehic et al., 2005), and they are overexpressed in T4-1s. Increased SCYL1 in T4-1s indicates activation and proliferation of CD4+ T cells, which is consistent with the study of Klapper et al. (2004). FLII (FLI-1) is found to enhance aggressive erythroleukemic clone (Truong et al., 2005) and be related to hematopoiesis (Masuya et al., 2005). An increase of FLII, IFI27, IL6ST, BCL2, PIK3CA, TRIM25, EMP3, DEAF1, TNCRNA and PIM2 in T4-1s denoted enhanced proliferation of CD4+ T cells of SLE. On the other hand, SMAD3, MAPK1, E2F3 and MCL1 are expressed at low levels in T4-1s (Fig. 5B). SMAD3, E2F3 and MCL1 isoform2 are recognized as activators of tumor suppressor genes, but their function with activation and proliferation of CD4+ T cells should be investigated further.

Genes related to cellular cycle control also had functions with development, differentiation, stem cells, immune responses and B lymphocytes (Fig. 5B). It is very difficult to take apart for genes with these functions since differentiation depends on cell cycle control and development. T and B lymphocyte are derived from hematopoietic stem cells (HSCs). ATM has been shown to have an essential function in the reconstitutive capacity of HSCs (Ito et al., 2004). CD52 is a glycosylphosphatidyl-inositol-linked glycoprotein expressed at high levels on normal T and B lymphocytes and at lower levels on monocytes, while being absent on granulocytes and bone marrow stem cell precursors. It has been found that the persistence of CD52 T cells in the peripheral blood in three out of 25 rheumatoid arthritis patients (Brett et al., 1996). Up-regulation of ATM and down-regulation of CD52 in the peripheral CD4+ T cells of lupus characterized by HSCs could be thought that self-reactive T cells for autoimmunity resembled HSCs' characteristics. If the immature T cells were released to peripheral blood, autoimmunity would come from the T cells abnormalities of immune responses. The highlighted expression genes are HBA2 and HBB, which were up-regulated in T4-1s and down-regulated in T4-2s. 391 tags of HBA2 were derived from 18 alternative matches in T4-1s, while 12 of 18 variants were negative and the others expressed at a low level (copies as 9, 15, 1, 1, 2 and 1, respectively) in T4-2s. For 79 HBB tags were composed of 15 types of 3′ end splicing variants in T4-1s, 10 variants were silenced and only 5 variants were detected (copies as 2, 1, 1, 1 and 4) in T4-2s (data not shown). Coincidently, light polypeptide ferritin gene (FTL) was also up-regulated in T4-1s. We do not know what is the possible role of HBA2 and HBB expressed in the CD4+ T lymphocytes, even though transferrin is thought to be required for early T cell differentiation (Macedo et al., 2004), and Haribhai et al. (2003) proposed that tolerogenic thresholds are T cell specific with extremely low levels of self-antigens exposure (e.g. Hb (64–76)) could generate a restricted repertoire of responding T cells in mice.

About one-sixth genes related to lupus were mentioned by the published articles (Fig. 5A, B and D). This means the majority of genes that we determined, their different expressions in the T4-1s and T4-2s libraries would be helpful for pathogenesis of lupus. In the mentioned genes for lupus, some of them are also combined with other functions. For instance, GZMB, SELL (CD62L) and TAP1 have a variety

role in lupus, natural killer, stem cell, T and B lymphocyte, cell growth, development, immune response and regulation (Fig. 5A and B). GZMB has been shown to increase expression with perforin in activated CD8+ T lymphocytes correlates with disease activity in patients with SLE (Blanco et al., 2005), but we detected four copies of GZMB in T4-2s and none in T4-1s. Low levels expression of SELL is related to disease activity of lupus susceptible mice (Blossom et al., 2004; Lian et al., 2004). Therefore, the broken-down homeostasis of immune tolerance may come from the concurrent abnormalities of cellular and humoral immunity.

In recent years, insights into the apoptotic process have provided important and clearer understanding of how the diverse manifestations of SLE may occur and how the disease might be triggered (Kaplan, 2004). Increased apoptosis has been described in a variety of SLE cells (Perniok et al., 1998). Apoptotic products of SLE cells are crucial to release self-intracellular antigens becoming immunogens capable of triggering and maintaining a strong and prolonged autoantibodies response. Gene's functional clustering shows that EIF3S8, SH3BGRL3, TOSO and CUGBP2 are attributed to apoptosis column, but their functions are not well characterized (Fig. 5D). The over-expression of NME3 (DR-nm23) inhibits differentiation and induces the apoptosis of myeloid precursor cell lines (Venturelli et al., 2000), and NME3 expressed at a higher level in T4-1s than T4-2s. Autophagic death, also called as type II programmed cell death, depends on the autophagy genes such as BECN1 (Boya et al., 2005; Shimizu et al., 2004), and BECN1 expressed in T4-1s and without in T4-2s. GPX4 is protected against oxidative stress-induced apoptosis (Ran et al., 2004), PFDN5 binding c-myc protein may play a pivotal role in apoptosis, BIN1 binds to and negatively regulates the activity of MYC (Telfer et al., 2005), and different expressions of these genes between T4-1s and T4-2s may reflect the apoptosis and differentiation of CD4+ lymphocytes. Nevertheless, XIAPAF1 and TEGT exert antiapoptotic effects, both of them up-regulated in T4-1s (Fig. 5D).

In conclusions, we have successfully established LongSAGE libraries of CD4+ T lymphocytes in the disease active state and nonactive state of an SLE patient. There were differences not only in the expression of genes matching to Unigene cluster but also in the genes' abundance between T4-1s and T4-2s libraries. The qualitative and quantitative comparison for genes clustering analysis showed that differently expressed genes were concentrated on a diverse cellular process including cell growth, development, differentiation, cell cycle control, apoptosis, Ras and Wnt/beta-catenin signal transduction pathways. CD4+ T cells could not be enough matured to maintain the tolerance and response in the immune system for SLE. Genes' expressions in T4-1s associated with apoptosis in T4-1s denoted that CD4+ T cell might enhance apoptosis of SLE and lead to excess uploading of self-antigens. All of these findings indicate that the immature CD4+ T lymphocytes may be responsible for the pathogenesis of SLE.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molimm.2005.07.039.

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