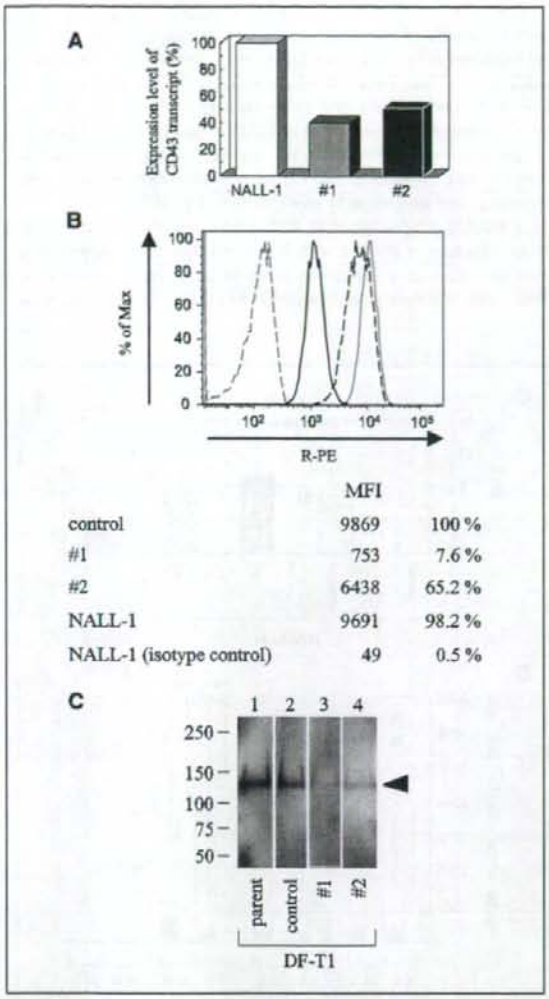


Figure 3. Suppression of CD43 expression on NALL-1 cells by shRNA for *CD43*. *A*, expression of *CD43* transcript in shRNA-introduced NALL-1 cells. The relative expression of *CD43* was calculated as the percentage of the value obtained for the parental NALL-1, which was set as 100. *B, top*, CD43 expression in NALL-1 cell sublines transfected with shRNA for *CD43*. The surface expression of CD43 was analyzed using DF-T1. *Dotted line*, control NALL-1 cells (scrambled shRNA-introduced NALL-1); *solid line*, NALL-1siCD43#1; *dark dashed line*, NALL-1siCD43#2; *gray dashed line*, isotype-matched control. *Bottom*, the relative expression of CD43 was also presented as MFI and percentage (%) compared with the positive control NALL-1scrambled cells. *C*, immunoblot analysis of NALL-1 cells transfected with shRNA. Lysates of shRNA-transfected NALL-1 cells were analyzed by Western blotting with anti-CD43 mAb DF-T1. *parent*, parental NALL-1 cells; *control*, NALL-1scrambled; *#1*, NALL-1siCD43#1; *#2*, NALL-1siCD43#2. *Left*, positions of molecular mass markers; *arrowhead*, position of CD43.

several cell adhesion molecules and gelatinase activity (Table 2). The levels of integrin β_1, VLA4α, integrin β_2, LFA1α, ICAM-1, L-selectin, CD44, CCR7, CXCR5, CCR6, CXCR3, CXCR4, *MMP2*, and *MMP9* and gelatinase activity did not decrease significantly in NALL-1siCD43#1 cells compared with NALL-1control cells. These results suggest that the down-regulation of CD43 results in an inhibition of cell migration from the vascular system to peripheral tissues and that CD43 plays a significant role in mediating the extravasation of NALL-1 cells.

Discussion

Thus far, PSGL-1, ESL-1, L-selectin, and CD44 have been reported as selectin counter-receptors. In BCP leukemia cells, the major selectin ligand carrier was first shown as a 150-kDa *O*-glycosylated protein (17, 18). L-selectin (33, 34) and CD44 (35, 36) are ruled out because of their molecular sizes, ~70 and ~85 kDa, respectively. ESL-1 is 150 kDa but an *N*-glycosylated protein (37). As demonstrated in the present study, PSGL-1 is essentially negative and the most feasible candidate of the major selectin ligand carrier on BCP-ALL cells is a sialomucin, CD43. In a study of T-cell recruitment to skin, the cutaneous lymphocyte-associated antigen (CLA) was reported as the only E-selectin/P-selectin ligand and located on PSGL-1 (38, 39). Recently, however, CD43 was reported as a ligand for E-selectin on CLA^+ T cells (32). CD43 was also found to be an E-selectin ligand in activated T cells (40). It is worth noting that PSGL-1 as well as CD43 are present under physiologic conditions in both human CLA^+ T cells and mouse Th1 cells (22, 32, 38–41). CD43 must function in cooperation with PSGL-1 and the central player may be PSGL-1 *in vivo*.

There are two major glycoforms of CD43 (135/115 kDa) in human T cells (42). The 115-kDa form is found on resting T, whereas the 135-kDa CD43 is expressed on activated T cells. Core 2–branched *O*-glycans are abundant in the larger glycoform and biosynthesis of the branch is regulated by the rate-limiting C2GnT1 (43). Its expression is up-regulated during T-cell and B-cell activation (42, 44). According to our previous investigations, C2GnT1 and carbohydrate selectin ligand are highly expressed in BCP-ALL cells and down-regulated simultaneously to 1 of 10 during differentiation, and the expression level of carbohydrate selectin ligand is regulated by C2GnT1 (12, 17–19). Applying our previous findings to the present results, C2GnT1 is thought to regulate the biosynthesis of core 2 branches on CD43 in BCP-ALL cells. The changes of CD43 glycoforms during pre-B-cell differentiation will be reported elsewhere.[8]

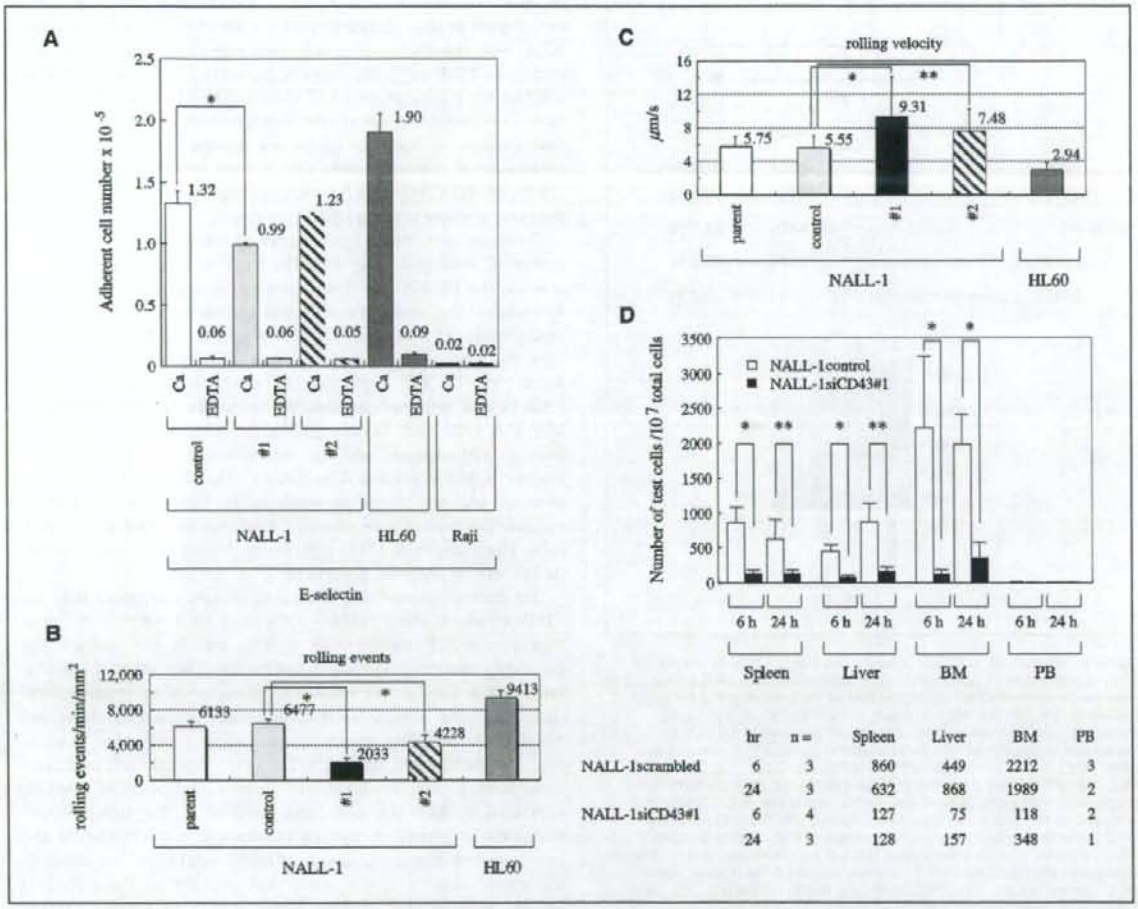
Our immunophenotypic observations and functional data on CD43 obtained using NALL-1 cells may be applicable to most B-precursor ALL patients. Of course, we do not exclude the possibility that the major carrier of carbohydrate selectin ligand is not CD43 or PSGL-1 but another *O*-glycoprotein on primary BCP-ALL cells. For selectin-related adhesion molecules, there are sulfated carbohydrate structures, including 6′-sulfo-sLe^X, 6-sulfo-sLe^X, 6,6′-disulfo-sLe^X, and sulfo-Le^X (45). Some of such structures have been proved as L-selectin ligands and may be possibly expressed in BCP-ALL cells and involved in the leukemic cell migration to peripheral tissues. However, it requires careful and extensive investigation to draw definitive conclusion. According to our recent data, it is suggested that primary precursor B cells express genuine sLe^X epitopes[9] and it may be involved in the trafficking of pre-B cells to BM.

[8] H. Sasaki, J. Kikuchi, H. Ohno, C. Nonomura, Y. Furukawa, and M. Nakamura, unpublished data.

[9] J. Kikuchi, H. Sasaki, C. Nonomura, H. Ohno, Y. Furukawa, and M. Nakamura, unpublished data.

Selectin-binding activity measured by flow cytometry may not necessarily reflect the actual function of a carbohydrate ligand and its carrier protein. That is, we detected P-selectin binding using flow cytometry but could not observe a significant adhesion capability of NALL-1 cells to P-selectin–immobilized surfaces in low-shear-stress cell adhesion assay (Fig. 1*B* and *C*). Likewise, we observed some discrepancies about the effects of knocking down the expression of CD43 and functional assays. Whereas NALL-1siCD43#1 and NALL-1siCD43#2 cells showed 60% and 49% decrease in the expression of CD43 transcript, the immunoreactivity for anti-CD43 mAb using flow cytometry exhibited 92% and 35% suppression, respectively (Fig. 3*A* and *B*). The knocking down of CD43 resulted in only 25% and 7% decrease in #1 and #2 cells on low-shear-force cell adhesion assay, respectively (Fig. 4*A*). The reactivity profile of the mAb in the flow cytometry assay may be very sensitive as for NALL-1siCD43#1 cells. Similarly, the reactivity profile of low-shear-force cell adhesion assay may be also sensitive for both #1 and #2 cells and rolling substrate E-selectin. Besides these, the knocking down effect of CD43 in NALL-1siCD43#1 (60%; Fig. 3*A*) was well correlated to the decrease in cell rolling events (69%; Fig. 4*B*), increase in cell rolling velocity (168%; Fig. 4*C*), and suppressed cell migration *in vivo* (70–75%; Fig. 4*D*).

For PSGL-1, a versatile mAb KPL-1 has been developed (46). It blocks adhesion of PSGL-1 with P-selectin. The development of a novel tool, such as a functional mAb, to block the interaction of CD43 with E-selectin is required to make further analyses



	hr	n =	Spleen	Liver	BM	PB
NALL-1scrambled	6	3	860	449	2212	3
	24	3	632	868	1989	2
NALL-1siCD43#1	6	4	127	75	118	2
	24	3	128	157	348	1

Figure 4. Cell adhesion analyses and *in vivo* leukemic cell migration assay using NALL-1 cell sublines transfected with shRNA for *CD43*. *A*, low-shear-force cell adhesion assay of the NALL-1 cell sublines. Cells were labeled with BCECF-AM and incubated at 37°C for 30 min under low shear stress in wells coated with selectin/Ig. *White columns*, NALL-1scrambled (*control*); *light gray columns*, NALL-1siCD43#1 (*#1*); *striped columns*, NALL-1siCD43#2 (*#2*); *dark gray columns*, HL60; *black columns*, Raji. *Columns*, average cell number of three wells; *bars*, SD. *, $P < 0.05$. *B* and *C*, rolling assay of the NALL-1 cell sublines on CHO-E cells under shear stress of 5 dyne/cm². The number of rolling cells (rolling events/min/mm²; *B*) was determined. *Columns*, mean; *bars*, SD. The rolling velocity (μm/s; *C*) was calculated. *Columns*, average of 25 cells traced; *bars*, SD. *White columns*, parental NALL-1; *light gray columns*, NALL-1scrambled; *black columns*, NALL-1siCD43#1; *striped columns*, NALL-1siCD43#2; *dark gray columns*, HL60. *, $P < 0.001$; **, $P < 0.005$. *D*, *in vivo* leukemic cell migration assay using immunodeficient mice. TRITC-labeled test cells and CFSE-labeled control cells (1:1) were injected to NOD/SCID mice. The cell number in peripheral blood (*PB*) and engrafted cell number in the cell suspension from spleen, liver, and BM were counted using flow cytometry. *Columns*, average cell number of the recovered test cells of independent experiments; *bars*, SD. *White columns*, NALL-1scrambled; *black columns*, NALL-1siCD43#1. *, $P < 0.01$; **, $P < 0.05$.

Table 2. Expression of cell adhesion molecules and gelatinase activity in the BCP-ALL cell line NALL-1 and its sublines

	NALL-1	NALL-1scrambled (NALL-1control)	NALL-1siCD43#1	Positive control cells*
A				
Integrin β_1	±	±	±	++++*
VLA4α	++++	++++	++++	n.t.
Integrin β_2	+++	++	+++	n.t.
LFA1α	++	++	++	n.t.
ICAM-1	+++	+++	+++	n.t.
L-selectin	++++	++++	++++	n.t.
CD44	++++	++++	++++	n.t.
CCR7	++	+	+	n.t.
CXCR5	±	±	±	+++*
CCR6	±	±	±	+++*
CXCR3	++	+	+	n.t.
CXCR4	+++	++	+++	n.t.
B				
MMP2	15 ± 8	18 ± 5	17 ± 7	100 ± 17
MMP9	7 ± 3	6 ± 2	7 ± 2	100 ± 12
C				
Gelatinase activity	10 ± 4	10 ± 3	9 ± 4	100 ± 26

NOTE: (A) Flow cytometric expression of cell adhesion molecules was examined using specific mAbs in the parental NALL-1, NALL-1siCD43#1, and NALL-1scrambled cells. The expression was presented in a semiquantitative manner: ++++, >75% of cells positive; +++, 35% to 75% positive; ++, 15% to 35% positive; +, 5% to 15% positive; ±, 1% to 5% positive; −, <1% of cells positive. (B) *MMP2* and *MMP9* expression was examined using real-time PCR in the parental NALL-1, NALL-1siCD43#1, and NALL-1scrambled cells. The relative expression is calculated as the percentage of the value obtained for the control HL60, which is set as 100, and presented as mean ± SD. (C) Gelatinase activity was measured using gelatin zymography in CXCL12-treated parental NALL-1, NALL-1siCD43#1, and NALL-1scrambled cells. The relative activity is calculated as the percentage of the value obtained for the control HL60, which is set as 100, and presented as mean ± SD.

*Positive control cells are HL60 for integrin β_1, Raji for CXCR5 and CCR6, and HL60 for *MMP2*, *MMP9*, and gelatinase activity, respectively.

possible. Recently, an anti-CD44 mAb is reported to have the ability to purge leukemic stem cells from niches (6, 7). The data presented here indicate that a functional anti-CD43 mAb could also be of potential use in purging BCP-ALL cells from microenvironmental niches.

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IMMUNOLOGY ORIGINAL ARTICLE

B-cell-activating factor inhibits CD20-mediated and B-cell receptor-mediated apoptosis in human B cells

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Summary

B-cell-activating factor (BAFF) is a survival and maturation factor for B cells belonging to the tumour necrosis factor superfamily. Among three identified functional receptors, the BAFF receptor (BAFF-R) is thought to be responsible for the effect of BAFF on B cells though details of how remain unclear. We determined that a hairy-cell leukaemia line, MLMA, expressed a relatively high level of BAFF-R and was susceptible to apoptosis mediated by either CD20 or B-cell antigen receptor (BCR). Using MLMA cells as an *in vitro* model of mature B cells, we found that treatment with BAFF could inhibit apoptosis mediated by both CD20 and BCR. We also observed, using immunoblot analysis and microarray analysis, that BAFF treatment induced activation of nuclear factor-κB2 following elevation of the expression level of *Bcl-2*, which may be involved in the molecular mechanism of BAFF-mediated inhibition of apoptosis. Interestingly, BAFF treatment was also found to induce the expression of a series of genes, such as that for CD40, related to cell survival, suggesting the involvement of a multiple mechanism in the BAFF-mediated anti-apoptotic effect. MLMA cells should provide a model for investigating the molecular basis of the effect of BAFF on B cells *in vitro* and will help to elucidate how B cells survive in the immune system in which BAFF-mediated signalling is involved.

Keywords: apoptosis; B-cell-activating factor; Bcl-2; B-cell receptor; CD20

Introduction

The immune system comprises a variety of immune effector cells, including T and B lymphocytes and antigen-presenting cells, such as dendritic cells and others; it protects individuals from infections and cancer. To maintain these sophisticated mechanisms, a very subtle balance between the life and death of the immune effector cells must be maintained to eliminate, by apoptosis, potentially harmful self-reactive lymphocytes and only allow the survival, development and activation of safe and protective immune cells. For this purpose, a number of molecules are involved in this regulatory system.[1]

B-cell-activating factor (BAFF, also termed BlyS, TALL-1, THANK and zTNF4) produced by monocytes, dendritic cells and some T cells is a member of the tumour necrosis factor (TNF) superfamily and is a type 2 transmembrane-bound protein that can also be expressed as a soluble ligand.[2] BAFF was first described as a factor that stimulates cell proliferation and the secretion of immunoglobulin in B cells.[3–7] Transgenic mice that overexpress BAFF in lymphoid tissues exhibited hyperplasia of the mature B-cell compartment.[8–10] In contrast, mice deficient in BAFF showed a deficit in peripheral B lymphocytes[10,11] and an almost complete loss of follicular and marginal zone B lymphocytes in secondary lymphoid organs. This suggests an absolute requirement for BAFF in normal B-cell development.[10] In contrast, a later examination of immunized BAFF-null mice validated the BAFF-independent nature of germinal centre formation and that antibody responses, including high-affinity responses, were attenuated, indicating that BAFF is required for maintenance, but not initiation, of the germinal centre reaction.[12] Based on the above evidence, BAFF is considered to be a survival and maturation factor for B lymphocytes and has emerged as a crucial factor that modulates B-cell tolerance and homeostasis.[2,13] However, the precise role of BAFF in B-cell development is

still controversial and it has been reported that the capacity of B lymphocytes to bind BAFF is correlated with their maturation state and that the effect of BAFF is dependent on the maturation stage of the B lymphocytes.[2,14]

Recent studies have further shown that BAFF affects not only B lymphocytes but also T lymphocytes.[15,16] The three distinct receptors for BAFF, namely the BAFF receptor (BAFF-R, also termed BR3), the B-cell maturation antigen (BCMA), and the transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), have been identified and BAFF binds with a similar high affinity to these receptors.[7,17–23] Among these receptors, however, BAFF-R is thought to be responsible for the survival and differentiation of B cells,[24] whereas the molecular basis of BAFF-mediated signalling remains unclear.

A number of systems inducing apoptosis in B cells are present to eliminate inappropriate clones, such as self-acting B cells. For example, it is reported that stimulation via particular surface molecules, including B-cell receptor antigen (BCR) and CD20, induces apoptosis in cultured B cells.[25,26] The balance between apoptosis-inducing systems and survival systems, such as CD20 and BAFF-mediated signalling, would be important for the maintenance of appropriate B-cell development, though details are not known.

To elucidate the molecular basis of the interaction between apoptosis-inducing signals and BAFF-mediated cell survival signals in B cells, we have employed a B-cell line that expresses BAFF-R and is sensitive to CD20-mediated and BCR-mediated apoptosis. In this paper, we present evidence that BAFF-mediated stimulation inhibits the apoptosis induced by both CD20-mediated and BCR-mediated signalling. The possible mechanisms involved in BAFF-mediated cell responses that regulate these apoptotic stimuli are discussed.

Materials and methods

Cells and reagents

The human hairy cell leukaemia cell line MLMA was obtained from the Japanese Cancer Research Resources Bank (JCRB, Tokyo, Japan). Cells were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum at 37° in a humidified 5% CO_2 atmosphere.

Recombinant human BAFF and a proliferation-inducing ligand (APRIL) were obtained from R&D Systems, Inc. (Minneapolis, MN), and used at a concentration of 400 ng/ml for cell stimulation unless otherwise described. The mouse monoclonal antibodies (mAbs) used for the immunofluorescence analysis were anti-CD10, anti-CD20, anti-CD21, anti-CD22, anti-CD24, anti-CD40, anti-human leucocyte antigen DR (HLA-DR; Beckman Coulter, Inc., Fullerton, CA); anti-CD19 (Becton Dickinson and Company, BD, Franklin Lakes, NJ); anti-κ, anti-λ, anti-μ, anti-δ, anti-γ (Dako, Denmark A/S); anti-BAFF-R (Santa Cruz Biotechnology, Santa Cruz, CA); and anti-CD45 (American Type Culture Collection, ATCC, Manassas, VA). The rat mAbs against BCMA (Vicky-1) and TACI (1A1) were purchased from Santa Cruz Biotechnology. The mouse mAbs used for the immunochemical analysis were anti-caspase-2, anti-caspase-3 and anti-glycogen synthase kinase-3β (GSK-3β; Becton Dickinson); anti-caspase-9 (Medical & Biological Laboratories Co., Ltd, Nagoya, Japan); anti-nuclear factor-κB (NF-κB) p52 (C-5), anti-Bcl-2 (100) from Santa Cruz; and anti-β-actin (AC-15) from Sigma-Aldrich Co. (St Louis, MO). The rabbit polyclonal antibodies used were anti-cleaved poly ADP-ribose polymerase (PARP), anti-cleaved caspase-3, anti-phospho-GSK-3β (Ser9) and anti-phospho-GSK-3α/β (Ser9, 21) from Cell Signaling Technology, Inc. (Danvers, MA). A goat anti-NF-κB p50 (C-19) from Santa Cruz was also used. Secondary antibodies, including fluorescein isothiocyanate- (FITC) and enzyme-conjugated antibodies, were purchased from either Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA) or Dako. To cross-link BCR, purified anti-μ rabbit polyclonal antibody (10 μg/ml) from Jackson ImmunoResearch Laboratories, Inc. was used. To cross-link CD20, a mouse anti-CD20 mAb from Beckman Coulter and a secondary anti-mouse immunoglobulin antibody from Jackson ImmunoResearch Laboratories, Inc. were used each at a concentration of 5 μg/ml.

Immunofluorescence analysis and detection of apoptosis

Cells were stained with FITC-labelled mAbs and analysed by flow cytometry (EPICS-XL, Beckman Coulter) as described previously.[27] To quantify the incidence of apoptosis, cells were incubated with FITC-labelled annexin V using a MEBCYTO-Apoptosis kit (Medical & Biological Laboratories Co., Ltd) and then analysed by flow cytometry according to the manufacturer's directions. Apoptotic cells were also detected by nuclear-staining with DAPI and examined by confocal microscopy as described previously.[28] The enzymatic activity of caspases -2, -3, -9 was assessed by using a colorimetric protease assay kit for each caspase (Medical & Biological Laboratories Co., Ltd) according to the manufacturer's protocol.

Immunoblotting

Immunoblotting was performed as described previously.[29] Briefly, cell lysates were prepared by solubilizing the cells in lysis buffer (containing 20 mM Na_2PO_4, pH 7·4, 150 mM NaCl, 1% Triton X-100, 1% aprotinin, 1 mM phenylmethylsulphonylfluoride, 100 mM NaF, and 2 mM Na_3VO_4), and the total protein concentration was determined using a Bio-Rad protein assay kit (Bio-Rad, Hercules, CA). For each cell lysate, 20 μg was separated by

sodium dodecyl sulphate–polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane using a semidry Transblot system (Bio-Rad). After blocking with 3% skimmed milk in phosphate-buffered saline, the membrane was incubated with the appropriate combination of primary and secondary antibodies as indicated, washed intensively, and examined using the enhanced chemiluminescence reagent system (ECL plus; GE Healthcare Bio-Sciences AB, Uppsala, Sweden).

DNA microarray analysis

The DNA microarray analysis was performed using GeneChip (Affymetrix, Santa Clara, CA). Total RNA isolated from MLMA cells treated with and without BAFF for 12 hr was reverse transcribed and labelled using One-Cycle Target Labeling and Control Reagents as instructed by the manufacturer (Affymetrix). The labelled probes were hybridized to Human Genome U133 Plus 2.0 Arrays (Affymetrix). The arrays were analysed using GeneChip Operating Software 1.2 (Affymetrix). Background subtraction and normalization were performed with GeneSpring GX 7.3 software (Agilent Technologies, Santa Clara, CA). Signal intensities were prenormalized based on the median of all measurements on that chip. To account for the difference in detection efficiency between the spots, prenormalized signal intensities on each gene were normalized to the median of prenormalized measurements for that gene. The data were filtered with the following steps. (1) Genes that were scored as absent in both samples were eliminated. (2) Genes with a signal intensity lower than 90 in both samples were eliminated. (3) Performing cluster analysis using filtering genes, genes were selected that exhibited increased expression or decreased expression in BAFF-treated cells.

Results

Immunophenotypic characterization of MLMA cells

While screening to identify human cell lines expressing BAFF-R, we found that MLMA cells expressed higher levels of BAFF-R than other human B-cell lines. Although the MLMA cell line is known to have been established from a patient with hairy-cell leukaemia, details were not reported. Therefore, we first examined the immunophenotypic characteristics of MLMA cells. Consistent with the JCRB records, flow cytometric analysis revealed that MLMA cells expressed high levels of μ heavy chain and low levels of δ heavy chain with expression of κ light chain (Fig. 1a). In addition to the CD19 and HLA-DR, MLMA cells were found to express mature B-cell

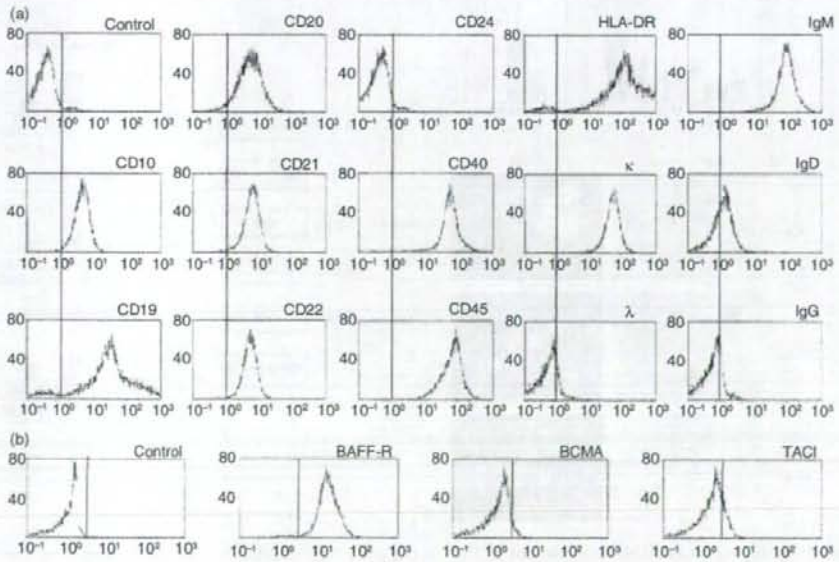

Figure 1. Immunophenotypic characterization of MLMA cells. (a) MLMA cells were stained with specific fluorescein isothiocyanate (FITC)-labelled monoclonal antibodies (mAbs) against B-cell differentiation antigens and analysed by flow cytometry. The *x*-axis represents fluorescence intensity and the *y*-axis the relative cell number; control was isotype-matched mouse immunoglobulin. (b) The expression of B-cell-activating factor receptor (BAFF-R), transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), and B-cell maturation antigen (BCMA) on MLMA cells was also examined as in (a).

antigens, including CD20, CD21, CD22 and CD40, but not CD24. Notably, MLMA cells showed the expression of CD10. When the expression of three types of receptors for BAFF was similarly examined, MLMA cells exhibited apparent expression of BAFF-R, while the levels of BCMA and TACI were found to be quite low (Fig. 1b). The data indicate that MLMA cells exhibit immunophenotypic characteristics of mature B cells expressing BAFF-R.

Cross-linking of BCR and CD20 induces apoptosis in MLMA cells

It has been well documented that cross-linking of BCR using anti-μ heavy chain antibodies induces apoptosis in some B cells *in vitro*.[25] Recent studies including our own have also shown that CD20 cross-linking mediates apoptosis in human B-cell lines in a manner involving raft-mediated signalling.[26,30] Therefore, we next examined whether cross-linking of either BCR or CD20 mediated apoptosis in MLMA cells. As shown in Fig. 2(a), when anti-μ antibodies were added to the culture, a time-dependent increase in the number of cells bound to annexin V was observed, suggesting the occurrence of apoptosis in MLMA cells after BCR cross-linking. The apoptosis was confirmed by the morphological appearance of nuclear fragmentation, a typical feature of apoptosis, detected by either Giemsa-staining or nuclear-staining with DAPI (Fig. 2b). Immunoblotting revealed the cleavage of caspases -9, -3 and -2 and of PARP after treatment with anti-μ antibodies (Fig. 2c), indicating that caspase activation was involved in the apoptosis. In the case of caspase-3, we also detected a 17 000 molecular weight cleaved fragment by using a specific antibody (Fig. 2c). In addition, elevation of the enzymatic activity of each caspase after cross-linking of BCR was detected by a colorimetric protease assay (Fig. 2d). We also examined the effect of anti-CD20 antibodies and found that CD20 cross-linking signalling induced apoptosis in MLMA cells (Fig. 2).

BAFF inhibits CD20-mediated and BCR-mediated apoptosis in MLMA cells

Next, we examined whether BAFF was able to inhibit apoptosis mediated by cross-linking of CD20 and BCR. As shown in Fig. 3(a), when BAFF was added to the

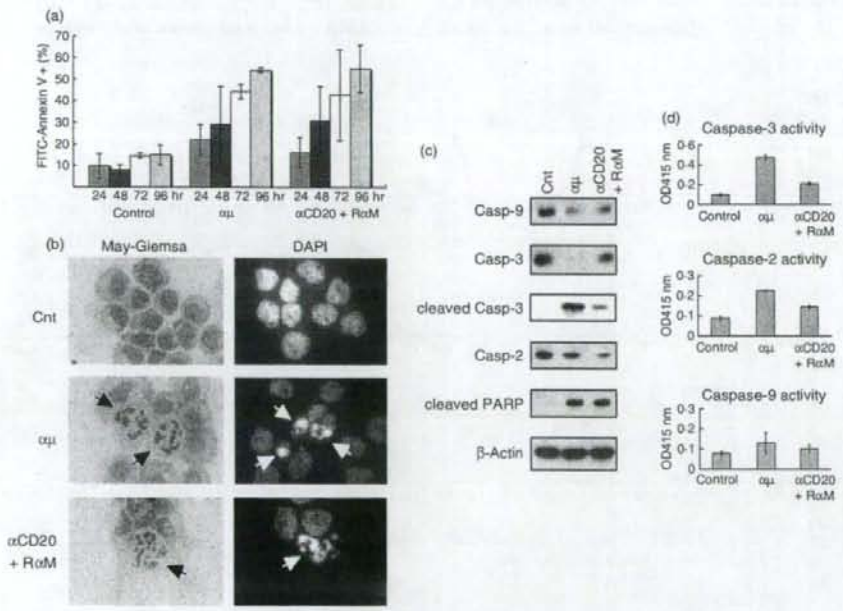

Figure 2. Induction of apoptosis in MLMA cells mediated by CD20 and B-cell antigen receptor. (a) MLMA cells were treated with either rabbit anti-μ heavy-chain polyclonal antibody (αμ, 10 μg/ml) or a combination of anti-CD20 monoclonal antibody (mAb; αCD20, 5 μg/ml) and secondary rabbit anti-mouse immunoglobulin antibody (RαM, 5 μg/ml) for 48 hr and binding with fluorescein isothiocyanate (FITC)-conjugated annexin V was examined by flow cytometry. Each experiment was performed in triplicate and the means + SD are indicated. (b) The same sample preparations as in (a) were cytocentrifuged and morphological appearance was examined by Giemsa-staining and nuclear staining with DAPI, using light microscopy and confocal microscopy, respectively. (c) Cell lysates were obtained from the same sample preparation as in (a) and the proforms of each caspase, cleaved caspase-3 and cleaved PARP were detected by immunoblotting.

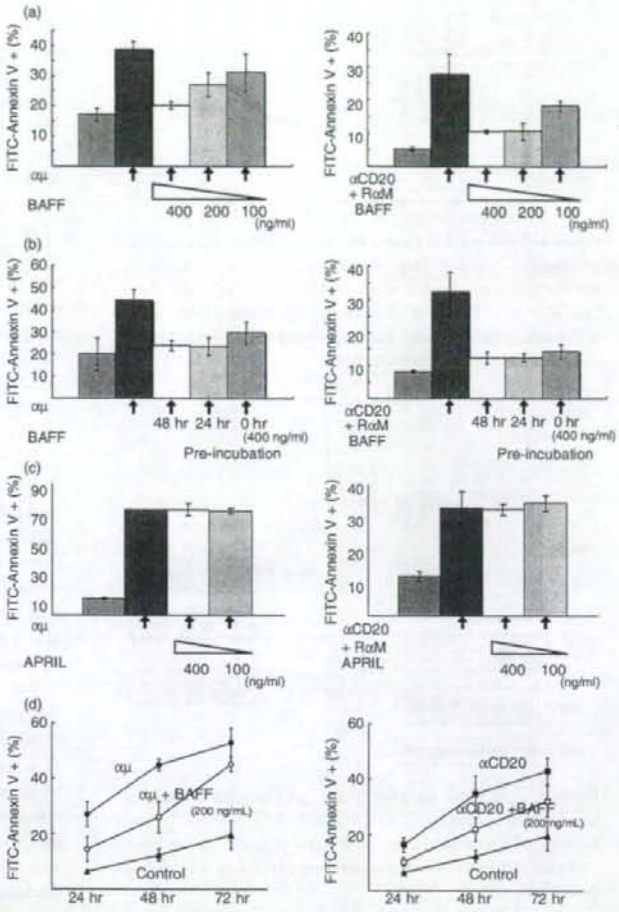

Figure 3. Effect of B-cell-activating factor (BAFF) on B-cell receptor (BCR)-induced and CD20-induced apoptosis in MLMA cells. (a) MLMA cells were treated with either rabbit anti-μ heavy-chain polyclonal antibody (mAb) (αμ, 10 μg/ml, left panel) or a combination of anti-CD20 mAb (αCD20, 5 μg/ml) and secondary rabbit anti-mouse immunoglobulin antibody (RαM, 5 μg/ml) (right panel) for 48 hr in the presence or absence of different concentrations of BAFF as indicated and binding with fluorescein isothiocyanate (FITC)-conjugated annexin V was examined as in Fig. 2(a). (b) MLMA cells preincubated with or without 400 ng/ml of BAFF for the indicated periods were treated with either αμ (left panel) or a combination of αCD20 and RαM (right panel) and examined as in (a). (c) The effect of APRIL on apoptosis induction was also examined as in (a). (d) MLMA cells were treated as in (a) and apoptosis was induced. The inhibitory effect of simultaneous addition of BAFF (200 ng/ml) against apoptosis was examined at different time-points as in (a).

culture, the incidence of apoptosis induced by both BCR-mediated and CD20-mediated stimuli was reduced as assessed by annexin V-binding. Although inhibition tended to be more effective with a higher dose of BAFF, the effect was not significant. We also examined the effect of pretreatment with BAFF on the inhibition of apoptosis but found none (Fig. 3b). In contrast, APRIL, another ligand for BCMA and TACI, did not affect apoptosis induced by the BCR-mediated and CD20-mediated stimuli, indicating the specificity of BAFF's effect (Fig. 3c). Therefore, we concluded that BAFF-mediated stimuli are able to inhibit apoptosis mediated by the cross-linking of either CD20 or BCR and simultaneous treatment with apoptosis-inducing stimuli is almost sufficient to achieve maximum BAFF-mediated inhibition of apoptosis, at least in these cases. However, the inhibitory effect of BAFF against apoptosis mediated by the cross-linking of either CD20 or BCR was only partial and it was more obvious when the inhibition of apoptosis was examined at several different time-points.

Cellular effect of BAFF involved in the inhibition of apoptosis in MLMA cells

We further examined the molecular basis of the BAFF-mediated inhibition of apoptosis in MLMA cells. First, we tested the effect of BAFF on the growth of MLMA cells. As shown in Fig. 4, when BAFF was added to the culture, the cell proliferation was slightly enhanced, as assessed by cell counting, suggesting that BAFF promotes the growth of MLMA cells.

Next, we examined the intracellular signalling induced in MLMA cells by BAFF treatment. As shown in Fig. 5(a), immunoblot analysis revealed cleavage of p100, the precursor of NF-κB2, and a increase in p52, the active form of NF-κB2 after BAFF treatment, suggesting that the

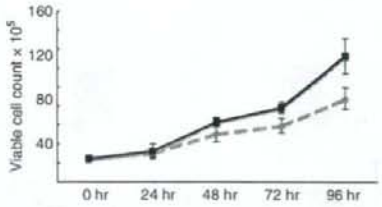

Figure 4. Effect of B-cell-activating factor (BAFF) on MLMA cell proliferation. Starting from a cell concentration at 5×10^5/ml, MLMA cells were cultured in the presence (solid line) and absence (dotted line) of 400 ng/ml of BAFF and cell numbers were counted at the time-points indicated. Each experiment was performed in triplicate and the means + SD are indicated.

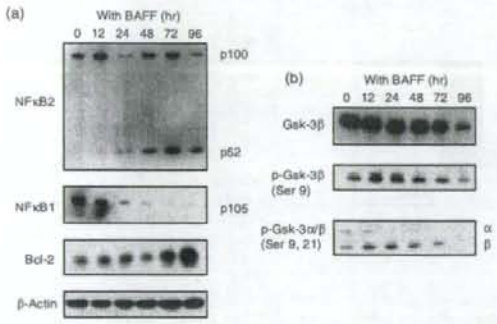

Figure 5. Intracellular signalling events and induction of Bcl-2 protein by B-cell-activating factor (BAFF). Cell lysates were prepared from MLMA cells treated with 400 ng/ml of BAFF for the periods indicated and an immunoblot analysis was performed using the antibodies indicated.

activation of NF-κB2 occurred after the treatment. We also observed the cleavage of the precursor of NF-κB1 after BAFF treatment (Fig. 5a). We further examined the activation of other molecules after treatment with BAFF and found that GSK-3β was transiently phosphorylated (Fig. 5b). In addition, we observed an elevation in the level of Bcl-2, an anti-apoptotic protein, after BAFF treatment.

To investigate the early responses to BAFF in MLMA cells, global screening of candidate genes whose expression is regulated by BAFF was performed by employing a microarray system. First, we selected up-regulated genes that are expressed in MLMA cells treated with BAFF for 12 hr at a level at least 1·5-fold higher than in untreated cells. Under these conditions, 178 probes were selected as up-regulated genes (Table 1). Consistent with the results of the immunoblot analysis presented in Fig. 5(a), the gene expression of Bcl-2 was found to be up-regulated by BAFF treatment (Table 1). Interestingly, the gene expression of CD40, a member of the TNF-receptor family involved in B-cell survival, was also increased after treatment with BAFF. The genes that are known to be involved in anti-apoptotic effect, including *Myb*, Epstein–Barr virus (EBV)-induced gene 3 (*EBI3*), and caspase 8 and FADD-like apoptosis regulator (*CFLAR*), were also up-regulated by BAFF treatment.

We further confirmed the increased CD40 protein expression by flow cytometry (Fig. 6a). Similarly, down-regulated genes that were expressed in BAFF-treated cells at a level at least 0·75-fold lower than in untreated cells were selected. As shown in Table 2, 517 probes were selected as down-regulated genes. The above results of global gene expression profiling suggest that the expression of various types of genes was influenced by BAFF stimulation in MLMA cells.

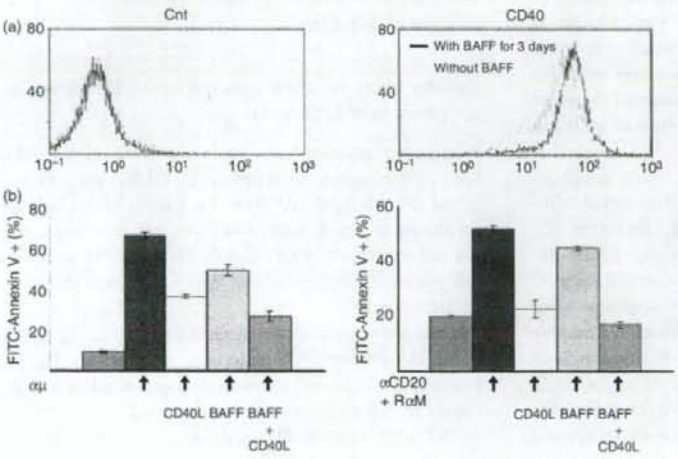

Figure 6. Effect of B-cell-activating factor (BAFF) on CD40 expression in MLMA cells. (a) MLMA cells cultured with or without BAFF for 3 days were stained with fluorescein isothiocyanate (FITC)-labelled monoclonal antibody (mAb) against CD40 and analysed by flow cytometry as in Fig. 1. (b) The inhibitory effect of CD40 stimulation on apoptosis induction was examined. MLMA cells were treated with 500 ng/ml of CD40-ligand in the presence of 2·5 ng/ml of interleukin-4 to stimulate CD40. The effects of either stimulation of CD40 alone or simultaneous stimulation of CD40 and BAFF receptor on apoptosis similarly induced as in Fig. 2 were examined.

Table 1. Up-regulated genes after BAFF stimulation

Affy ID	Gene name	Symbol	Fold-change
204798_at	V-myb myeloblastosis viral oncogene homolog	MYB	3·6934717
207861_at	Chemokine (C-C motif) ligand 22	CCL22	3·2195807
201669_s_at	Myristoylated alanine-rich protein kinase C substrate	MARCKS	3·0888734
213138_at	AT rich interactive domain 5A	ARID5A	2·9730885
203927_at	IkBe	NFKBIE	2·6444874
239412_at	Interferon regulatory factor 5	IRF5	2·6300144
205173_x_at	CD58 antigen	CD58	2·5103657
230543_at	Similar to Chloride intracellular channel protein 4	USP9X	2·4929807
201932_at	Leucine rich repeat containing 41	MUF1	2·3778253
203835_at	Leucine rich repeat containing 32	GARP	2·3439856
221912_s_at	Human DNA sequence from clone RP4-622L5	MGC1203	2·296463
205599_at	TNF receptor-associated factor 1	TRAF1	2·283286
218470_at	Tyrosyl-tRNA synthetase 2	CGI-04	2·2782216
203685_at	B-cell CLL/lymphoma 2	BCL2	2·2648098
202644_s_at	Tumor necrosis factor, alpha-induced protein 3	TNFAIP3	2·2458956
204897_at	Prostaglandin E receptor 4 (subtype EP4)	PTGER4	2·2327275
217728_at	S100 calcium binding protein A6	S100A6	2·188896
234339_s_at	Glioma tumor suppressor candidate region gene 2	GLTSCR2	2·1786015
226354_at	Lactamase, beta	LACTB	2·1238286
209680_s_at	Kinesin family member C1	KIFC1	2·1163168
206508_at	Tumor necrosis factor (ligand) superfamily, member 7	TNFSF7	2·0985012
223319_at	Gephyrin	GPHN	2·0934505
242312_x_at	AV736963 CB		2·0774355
207608_x_at	Cytochrome P450, family 1, subfamily A, polypeptide 2	CYP1A2	2·019507
229437_at	BIC transcript	BIC	1·9920377
224468_s_at	Multidrug resistance-related protein	MGC13170	1·9605879
214101_s_at	Aminopeptidase puromycin sensitive	NPEPPS	1·949555
208624_s_at	Eukaryotic translation initiation factor 4 gamma, 1	EIF4G1	1·9305304
218819_at	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 26	DDX26	1·925604
200648_s_at	Glutamate-ammonia ligase	GLUL	1·9081395
210686_x_at	Solute carrier family 25, member 16	GDA; GDC; ML7; hML7; HGT.1; D10S105E; MGC39851	1·903342
48659_at	Invasion inhibitory protein 45	FLJ12438	1·8990294
204283_at	Phenylalanine-tRNA synthetase 2	FARS2	1·8852582
1563796_s_at	KIAA1970 protein	KIAA1970	1·8749646
219424_at	Epstein–Barr virus induced gene 3	EBI3	1·8620349
213747_at	Antizyme inhibitor 1	OAZIN	1·8602847
205419_at	Epstein–Barr virus induced gene 2	EBI2	1·8580037
210978_s_at	Transgelin 2	TAGLN2	1·8418014
201502_s_at	IkBa	NFKBIA	1·8220907
207688_s_at	Inhibin, beta C	INHBC	1·8162365
208949_s_at	Lectin, galactoside-binding, soluble, 3	LGALS3	1·8159895
216252_x_at	Fas	FAS	1·7921783
203422_at	Polymerase (DNA directed), delta 1	POLD1	1·7856169
227299_at	Cyclin I	CCNI	1·7854006

Table 1. (*Continued*)

Affy ID	Gene name	Symbol	Fold-change
218872_at	Hypothetical protein FLJ20607	TSC	1·7839508
205749_at	Cytochrome P450, family 1, subfamily A, polypeptide 1	CYP1A1	1·7801132
210514_x_at	HLA-G histocompatibility antigen, class I, G	HLA-G	1·780091
211376_s_at	Chromosome 10 open reading frame 86	C10orf86	1·7799767
212063_at	CD44 antigen	CD44	1·778577
201404_x_at	Proteasome (prosome, macropain) subunit, beta type, 2	PSMB2	1·774633
209939_x_at	CASP8 and FADD-like apoptosis regulator	CFLAR	1·7721851
225775_at	Tetraspanin 33	MGC50844	1·7708977
213642_at	Ribosomal protein L27	RPL27	1·7626065
209100_at	Interferon-related developmental regulator 2	IFRD2	1·7502396
201572_x_at	DCMP deaminase	DCTD	1·7400836
212642_s_at	Human DNA sequence from clone RP1-67K17	HIVEP2	1·7382044
221866_at		TFEB	1·7346125
204562_at	Interferon regulatory factor 4	IRF4	1·7332152
230660_at	SERTA domain containing 4	SERTAD4	1·7324636
229671_s_at	Chromosome 21 open reading frame 45	C21orf45	1·7187352
201797_s_at	Valyl-tRNA synthetase	VARS2	1·7037842
212857_x_at	Similar to hypothetical protein DKFZp434P0316	PC4	1·7007011
225360_at	Hypothetical protein PP2447	PP2447	1·6819744
201565_s_at	Inhibitor of DNA binding 2	ID2	1·6807232
213113_s_at	Solute carrier family 43, member 3	SLC43A3	1·680492
212107_s_at	DEAH (Asp-Glu-Ala-His) box polypeptide 9	DHX9	1·6746678
204211_x_at	Eukaryotic translation initiation factor 2-alpha kinase 2	EIF2AK2	1·6744418
205153_s_at	CD40 antigen	CD40	1·6712306
214531_s_at	Sorting nexin 1	SNX1	1·6710757
226853_at	BMP2 inducible kinase	BMP2K	1·6657684
217734_s_at	WD repeat domain 6	WDR6	1·6582829
202418_at	Yip1 interacting factor homolog A	YIF1	1·6526384
203672_x_at	Thiopurine S-methyltransferase	TPMT	1·6522619
223287_s_at	Forkhead box P1	FOXP1	1·6494714
205621_at	AlkB, alkylation repair homolog	ALKBH	1·6476021
233310_at	Clone 25119 mRNA sequence		1·642435
201046_s_at	RAD23 homolog A	RAD23A	1·6409639
202161_at	Protein kinase N1	PKN1	1·6403072
219347_at	Nudix-type motif 15	NUDT15	1·6394255
226099_at	Elongation factor, RNA polymerase II, 2	ELL2	1·6393304
202715_at	Carbamoyl-phosphate synthetase 2,	CAD	1·6350683
214315_x_at	Calreticulin	CALR	1·6341119
227371_at	BAI1-associated protein 2-like 1	LOC55971	1·6332316
37028_at	Protein phosphatase 1, regulatory (inhibitor) subunit 15A	PPP1R15A	1·6298432
223079_s_at	Glutaminase	GLS	1·6280246
200613_at	Adaptor-related protein complex 2, mu 1 subunit	AP2M1	1·6246499
218611_at	Immediate early response 5	IER5	1·6235775
228993_s_at	Programmed cell death 4	PDCD4	1·6221018
224241_s_at	Homo sapiens cDNA clone IMAGE:2820510	PRO1855	1·6194851
205393_s_at	CHK1 checkpoint homolog	CHEK1	1·6177739
213826_s_at	H3 histone, family 3A	H3F3A	1·6139177
202819_s_at	Transcription elongation factor B (SIII), polypeptide 3	TCEB3	1·6136174
212064_x_at	MYC-associated zinc finger protein	MAZ	1·613094
218847_at	IGF-II mRNA-binding protein 2	IMP-2	1·6124035
201421_s_at	WD repeat domain 77	MEP50	1·6116982
230509_at	602041213F1 NCI_CGAP_Brn67	SNX22	1·610629
214383_x_at	Kelch domain containing 3	KLHDC3	1·604017
202265_at	Polycomb group ring finger 4	PCGF4	1·6002513

Table 1. (*Continued*)

Affy ID	Gene name	Symbol	Fold-change
200894_s_at	FK506 binding protein 4, 59 kDa	FKBP4	1·5997517
202024_at	ArsA arsenite transporter, ATP-binding, homolog 1	ASNA1	1·5976273
225625_at	Similar to hypothetical protein 9530023G02	MGC90512	1·5957043
224961_at	SCY1-like 2	SCYL2	1·5939683
222774_s_at	Neuropilin (NRP) and tolloid (TLL)-like 2	NETO2	1·5898373
225063_at	Ubiquitin-like 7 (bone marrow stromal cell-derived)	BMSC-UbP	1·5887783
218305_at	Importin 4	IPO4	1·5878817
204228_at	Peptidyl prolyl isomerase H (cyclophilin H)	PPIH	1·5876329
224966_s_at	Dihydrouridine synthase 3-like (S. cerevisiae)	LOC56931	1·5869961
239364_at	Ets variant gene 6 (TEL oncogene)	ETV6	1·5859513
206138_s_at	Phosphatidylinositol 4-kinase, catalytic, beta polypeptide	PIK4CB	1·5855292
209797_at	Transmembrane protein 4	TMEM4	1·5808252
204116_at	Interleukin 2 receptor, gamma	IL2RG	1·5777943
205965_at	Basic leucine zipper transcription factor, ATF-like	BATF	1·577366
201545_s_at	Poly(A) binding protein, nuclear 1	PABPN1	1·5768379
205235_s_at	M-phase phosphoprotein 1	MPHOSPH1	1·5718083
220924_s_at	Solute carrier family 38, member 2	SLC38A2	1·5694296
208858_s_at	Family with sequence similarity 62, member A	MBC2	1·5689234
203235_at	Thimet oligopeptidase 1	THOP1	1·5672989
215001_s_at	Glutamate-ammonia ligase (glutamine synthetase)	GS; GLNS	1·5667295
224571_at	Interferon regulatory factor 2 binding protein 2	IRF2BP2	1·566219
235759_at	EF-hand calcium binding protein 1	EFCBP1	1·5627139
218092_s_at	HIV-1 Rev binding protein	HRB	1·5555688
244413_at	Dendritic cell-associated lectin-1	DCAL1	1·5547262
209781_s_at	KH domain containing, RNA binding, signal transduction associated 3	KHDRBS3	1·5527176
211965_at	Zinc finger protein 36, C3H type-like 1	ZFP36L1	1·5514944
210740_s_at	Inositol 1,3,4-triphosphate 5/6 kinase	ITPK1	1·5482888
218097_s_at	CUE domain containing 2	CUEDC2	1·5461936
207618_s_at	BCS1-like	BCS1L	1·5440258
200628_s_at	Tryptophanyl-tRNA synthetase	WARS	1·5433701
201490_s_at	Peptidylprolyl isomerase F (cyclophilin F)	PPIF	1·5432048
222425_s_at	Polymerase (DNA-directed), delta interacting protein 2	POLDIP2	1·5430135
240277_at	Solute carrier family 30 (zinc transporter), member 7	SLC30A7	1·5403106
204882_at	Rho GTPase activating protein 25	ARHGAP25	1·5393674
214784_x_at	Exportin 6	XPO6	1·5390915
201801_s_at	Solute carrier family 29 (nucleoside transporters), member 1	SLC29A1	1·5378566
202307_s_at	Transporter 1, ATP-binding cassette, sub-family B	TAP1	1·5369159
224913_s_at	Translocase of inner mitochondrial membrane 50 homolog	TIMM50	1·5356098
226797_at	Mbt domain containing 1	MBTD1	1·5314596
202887_s_at	DNA-damage-inducible transcript 4	DDIT4	1·53085
207396_s_at	Asparagine-linked glycosylation 3 homolog	ALG3	1·5305443
228487_s_at	Ras responsive element binding protein 1	RREB1	1·5286149
201473_at	Jun B proto-oncogene	JUNB	1·5285981
222968_at	Chromosome 6 open reading frame 48	C6orf48	1·5267475
203879_at	Phosphoinositide-3-kinase, catalytic, delta polypeptide	PIK3CD	1·5265557
206181_at	Signaling lymphocytic activation molecule family member 1	SLAMF1	1·5258498
238567_at	Sphingosine-1-phosphate phosphotase 2	SGPP2	1·5248299
223427_s_at	Erythrocyte membrane protein band 4·1 like 4B	EPB41L4B	1·5245888
215450_at	Small nuclear ribonucleoprotein polypeptide E	SNRPE	1·5239984
210428_s_at	Hepatocyte growth factor-regulated tyrosine kinase substrate	HGS	1·5230072
202968_s_at	Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2	DYRK2	1·5226749
217289_s_at	glucose-6-phosphatase	G6PT; GSD1a	1·5194279
230466_s_at	Mesenchymal stem cell protein DSC96		1·5193534
229204_at	Heterochromatin protein 1, binding protein 3	HP1-BP74	1·5177599
223743_s_at	Mitochondrial ribosomal protein L4	MRPL4	1·5151922

Table 1. (*Continued*)

Affy ID	Gene name	Symbol	Fold-change
200706_s_at	Lipopolysaccharide-induced TNF factor	LITAF	1·5070926
217782_s_at	G protein pathway suppressor 1	GPS1	1·5069752
219279_at		DOCK10	1·5061336
215031_x_at	Ring finger protein 126	RNF126	1·5051547
203466_at	MpV17 transgene, murine homolog, glomerulosclerosis	MPV17	1·5044804
203114_at	Sjogren's syndrome/scleroderma autoantigen 1	SSSCA1	1·5035471
222037_at	MCM4 minichromosome maintenance deficient 4	MCM4	1·5032879
45749_at	Family with sequence similarity 65, member A	FLJ13725	1·5015459
213224_s_at	Hypothetical protein LOC92482	LOC92482	1·5014849
204171_at	Ribosomal protein S6 kinase, 70 kDa, polypeptide 1	RPS6KB1	1·5013521
219628_at	P53 target zinc finger protein	WIG1	1·5013262

Table 2. Down-regulated genes after BAFF stimulation

Affy ID	Gene name	Symbol	Fold-change
223697_x_at	Chromosome 9 open reading frame 64	C9orf64	0·18046121
237461_at	NACHT, leucine rich repeat and PYD containing 7	NALP7	0·1989845
243808_at	Cyclin-dependent kinase 6	CDK6	0·25961372
212027_at	wn02f07.x1 NCI_CGAP_Ut2	RBM25	0·27985397
211352_s_at	Similar to Hin-2	CAGH16	0·2816477
225569_at	Eukaryotic translation initiation factor 2C, 2	EIF2C2	0·3363239
230128_at	Homo sapiens cDNA: FLJ21578 fis, clone COL06726		0·33926746
204698_at	Interferon stimulated exonuclease gene 20 kDa	ISG20	0·33949938
222633_at	Transducin (beta)-like 1X-linked receptor 1	TBL1XR1	0·34330967
242601_at	Hypothetical protein LOC253012	LOC253012	0·35196936
207677_s_at	Neutrophil cytosolic factor 4, 40 kDa	NCF4	0·35400128
1553499_s_at	Serpin peptidase inhibitor, clade A, member 9	SERPINA9	0·37200212
212028_at	RNA binding motif protein 25	RBM25	0·38822186
240798_at	Cut-like 1, CCAAT displacement protein	CUTL1	0·39412326
202205_at	Vasodilator-stimulated phosphoprotein	VASP	0·39878875
212794_s_at	KIAA1033	KIAA1033	0·39967155
230831_at	FERM domain containing 5	MGC14161	0·41499138
222158_s_at	Chromosome 1 open reading frame 121	PNAS-4	0·41996363
213577_at	Squalene epoxidase	SQLE	0·42782143
1557953_at	Transcribed locus	ZNF36	0·43064588
1558678_s_at	Metastasis associated lung adenocarcinoma transcript 1	MALAT1	0·4366038
201291_s_at	Topoisomerase (DNA) II alpha 170 kDa	TOP2A	0·43767774
222186_at	Zinc finger, A20 domain containing 3	ZA20D3	0·43876088
225937_at	FP6778		0·44128555
205967_at	Histone 1, H4c	HIST1H4C	0·44577146
212592_at	Immunoglobulin J polypeptide	IGJ	0·45744386
213850_s_at	Splicing factor, arginine/serine-rich 2, interacting protein	SFRS2IP	0·4588066
201678_s_at	DC12 protein	DC12	0·46194622
225640_at	Hypothetical gene supported by AK091718		0·4679212
AFFX-M27830_M_at			0·4755289
225219_at	602071082F1 NCI_CGAP_Brn64	SMAD5	0·47642624
207057_at	Solute carrier family 16, member 7	SLC16A7	0·48003742
1569344_a_at	Homo sapiens, clone IMAGE:4044872, mRNA		0·48249447
223217_s_at	IkBz	NFKBIZ	0·48446876
201454_s_at	Aminopeptidase puromycin sensitive	NPEPPS	0·48679113
213906_at	V-myb myeloblastosis viral oncogene homolog-like 1	MYBL1	0·4908449
210970_s_at	Inhibitor of Bruton agammaglobulinemia tyrosine kinase	IBTK	0·49121413

Table 2. (*Continued*)

Affy ID	Gene name	Symbol	Fold-change
1255_g_at	guanylate cyclase activator 1A	GCAP	0·49559578
222858_s_at	Dual adaptor of phosphotyrosine and 3-phosphoinositides	DAPP1	0·4963491
204730_at	Regulating synaptic membrane exocytosis 3	RIMS3	0·5001501
207826_s_at	Inhibitor of DNA binding 3	ID3	0·50062287
33304_at	Interferon stimulated exonuclease gene 20 kDa	ISG20	0·50399923
211928_at	Dynein, cytoplasmic 1, heavy chain 1	DNCH1	0·5087544
242195_x_at	Numb homolog (Drosophila)-like	NUMBL	0·5093039
209257_s_at	Chondroitin sulfate proteoglycan 6	CSPG6	0·51205146
215990_s_at	B-cell CLL/lymphoma 6	BCL5	0·51233035
227396_at	Protein tyrosine phosphatase, receptor type, J	PTPRJ	0·5157389
228056_s_at	Napsin B aspartic peptidase pseudogene	NAPSB	0·5182605
228787_s_at	Breast carcinoma amplified sequence 4	BCAS4	0·51919734
225327_at	KIAA1370	FLJ10980	0·5194209
212368_at	Zinc finger protein 292	ZNF292	0·5197308
228343_at	POU domain, class 2, transcription factor 2	POU2F2	0·5240128
209138_x_at		IGLJ3	0·52593404
214016_s_at	Splicing factor proline/glutamine-rich	SFPQ	0·5273748
201236_s_at	BTG family, member 2	BTG2	0·5273795
202033_s_at	RB1-inducible coiled-coil 1	RB1CC1	0·5290374
219911_s_at	Solute carrier organic anion transporter family, member 4A1	SLCO4A1	0·5337818
204867_at	GTP cyclohydrolase I feedback regulator	GCHFR	0·53428966
209579_s_at	Methyl-CpG binding domain protein 4	MBD4	0·5378972
207761_s_at	DKFZP586A0522 protein	DKFZP586A0522	0·53842366
219517_at	Elongation factor RNA polymerase II-like 3	ELL3	0·53930795
207339_s_at	Lymphotoxin beta	LTB	0·53969586
228031_at	Hypothetical protein LOC149705	C20orf121	0·5410639
206219_s_at	Vav 1 oncogene	VAV1	0·54299057
231716_at	Membrane associated DNA binding protein	MNAB	0·5447174
213036_x_at	ATPase, Ca++ transporting, ubiquitous	SERCA3	0·5453293
230740_at	Transcribed locus	EHD3	0·5472777
230777_s_at	PR domain containing 15	PRDM15	0·54828
210679_x_at	Homo sapiens cDNA clone MGC:3878 IMAGE:3609162	BCL7A	0·55017775
229147_at	Ras association (RalGDS/AF-6) domain family 6	RASSF6	0·5539612
233746_x_at	Huntingtin interacting protein K	HYPK	0·55896664
224616_at	Dynein, cytoplasmic 1, light intermediate chain 2	DNCLI2	0·5595679
212677_s_at	RAB1A, member RAS oncogene family	RAB1A	0·5602901
213016_at	Bobby sox homolog	BBX	0·56184375
211383_s_at	WD repeat domain 37	WDR37	0·56346995
215504_x_at	Ankyrin repeat domain 10	ANKRD10	0·563754
212119_at	602149641F1 NIH_MGC_81	RHOQ	0·5639594
203819_s_at	IGF-II mRNA-binding protein 3	IMP-3	0·5641141
205124_at	MADS box transcription enhancer factor 2, polypeptide B	MEF2B	0·56775457
220071_x_at	Centrosomal protein 27 kDa	C15orf25	0·56932724
219396_s_at	Nei endonuclease VIII-like 1	NEIL1	0·5707603
226372_at	Carbohydrate (chondroitin 4) sulfotransferase 11	CHST11	0·57161444
232266_x_at	Homo sapiens cDNA FLJ14317 fis, clone PLACE3000401.	CDC2L5	0·57188374
211445_x_at	Nascent-polypeptide-associated complex alpha polypeptide pseudogene 1	FKSG17	0·5727707
224829_at	Cytoplasmic polyadenylation element binding protein 4	CPEB4	0·57316726
229353_s_at	Nuclear casein kinase and cyclin-dependent kinase substrate 1	NUCKS	0·5739766
215457_at	Actin related protein 2/3 complex, subunit 1A, 41 kDa	ARPC1A	0·5758453
1569594_a_at	Serologically defined colon cancer antigen 1	SDCCAG1	0·5770196
200596_s_at	Eukaryotic translation initiation factor 3, subunit 10 theta, 150/170 kDa	EIF3S10	0·5771801
209023_s_at	Stromal antigen 2	STAG2	0·57891893
203140_at	B-cell CLL/lymphoma 6	BCL6	0·5807235
217862_at	Protein inhibitor of activated STAT, 1	PIAS1	0·5808474

Table 2. (*Continued*)

Affy ID	Gene name	Symbol	Fold-change
227748_at	RNA binding motif protein, X-linked-like 1	KAT3	0·5812906
229429_x_at	LOC440667		0·582004
227740_at	U2AF homology motif (UHM) kinase 1	UHMK1	0·5825476
208615_s_at	Protein tyrosine phosphatase type IVA, member 2	PTP4A2	0·58321685
209360_s_at	Runt-related transcription factor 1	RUNX1	0·58370924
213734_at	WD repeat and SOCS box-containing 2	WSB2	0·5844417
202996_at	Polymerase (DNA-directed), delta 4	POLD4	0·58979243
212047_s_at	Ring finger protein 167	RNF167	0·59118843
218886_at	PAK1 interacting protein 1	PAK1IP1	0·59172606
215179_x_at	Placental growth factor	PGF	0·59284484
204141_at	Tubulin, beta 2A	TUBB2	0·5932073
212810_s_at	Solute carrier family 1, member 4	SLC1A4	0·59446627
201193_at	Isocitrate dehydrogenase 1 (NADP+), soluble	IDH1	0·59564257
228153_at	IBR domain containing 2	IBRDC2	0·59580594
64418_at	AP1 gamma subunit binding protein 1	AP1GBP1	0·5966325
203143_s_at	Transcribed locus	KIAA0040	0·5968683
209076_s_at	WDR45-like	WDR45L	0·5985668
204076_at	Ectonucleoside triphosphate diphosphohydrolase 4	ENTPD4	0·59857833
1558080_s_at	Hypothetical protein LOC144871	DNAJC3	0·5994704
203044_at	Carbohydrate (chondroitin) synthase 1	CHSY1	0·60100013
234762_x_at	Neurolysin (metallopeptidase M3 family)	NLN	0·6012079
207124_s_at	Guanine nucleotide binding protein (G protein), beta 5	GNB5	0·6012481
204449_at	Phosducin-like	PDCL	0·6038013
226508_at	Polyhomeotic like 3 (Drosophila)	PHC3	0·60685164
204681_s_at	Rap guanine nucleotide exchange factor (GEF) 5	RAPGEF5	0·60706514
203346_s_at	Metal response element binding transcription factor 2	M96	0·6077221
200998_s_at	Cytoskeleton-associated protein 4	CKAP4	0·608657
222816_s_at	Zinc finger, CCHC domain containing 2	ZCCHC2	0·6087468
219158_s_at	Synonyms: Ga19, NAT1, NATH, TBDN100	NARG1	0·6090036
201901_s_at	YY1 transcription factor	YY1	0·6101737
229072_at	RAB30, member RAS oncogene family	RAB30	0·6116257
212604_at	Mitochondrial ribosomal protein S31	MRPS31	0·61232865
214352_s_at	V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	KRAS2	0·6126175
231825_x_at	Activating transcription factor 7 interacting protein	ATF7IP	0·6132226
225204_at	T-cell activation protein phosphatase 2C	TA-PP2C	0·6143749
202379_s_at	Natural killer-tumor recognition sequence	NKTR	0·61503386
204853_at	Origin recognition complex, subunit 2-like	ORC2L	0·6158861
201138_s_at	Sjogren syndrome antigen B	SSB	0·6166977
225136_at	Pleckstrin homology domain containing, family A, member 2	PLEKHA2	0·6169427
201384_s_at	Neighbor of BRCA1 gene 1	M17S2	0·61722594
213620_s_at	Intercellular adhesion molecule 2	ICAM2	0·61761326
226158_at	Kelch-like 24	DRE1	0·6183106
205383_s_at	Zinc finger and BTB domain containing 20	ZBTB20	0·62210584
219148_at	PDZ binding kinase	PBK	0·6229036
227402_s_at	Chromosome 8 open reading frame 53	MGC14595	0·6240476
206641_at	Tumor necrosis factor receptor superfamily, member 17	TNFRSF17	0·62757266
202412_s_at	Ubiquitin specific peptidase 1	USP1	0·6286277
227224_at	Ral GEF with PH domain and SH3 binding motif 2	RALGPS2	0·62964123
231809_x_at	EST365840 MAGE resequences, MAGC	PDCD7	0·6322018
200797_s_at	Myeloid cell leukemia sequence 1	MCL1	0·6327875
204912_at	Interleukin 10 receptor, alpha	IL10RA	0·63407373
210754_s_at	V-yes-1 Yamaguchi sarcoma viral related oncogene homolog	LYN	0·6343251
235469_at	Similar to RIKEN cDNA 5830415L20	MGC40405	0·6344833
208415_x_at	Inhibitor of growth family, member 1	ING1	0·6353071
229656_s_at	Similar to echinoderm microtubule associated protein like 5		0·6356807

Table 2. (*Continued*)

Affy ID	Gene name	Symbol	Fold-change
224875_at	Hypothetical protein FLJ37562	FLJ37562	0·6361133
230110_at	Mucolipin 2	MCOLN2	0·6363045
213502_x_at	Similar to bK246H3·1	LOC91316	0·63717055
209272_at	NGFI-A binding protein 1	NAB1	0·6376612
214677_x_at	Immunoglobulin lambda joining 3	IGLC2	0·6377649
220999_s_at	synonym: PIR121; p53 inducible protein	CYFIP2	0·6382754
201320_at	SWI/SNF related, matrix associated, actin dependent regulator of chromatin	SMARCC2	0·63914317
223553_s_at	Docking protein 3	DOK3	0·63973147
214730_s_at	Golgi apparatus protein 1	GLG1	0·64044565
1555989_at	Dishevelled associated activator of morphogenesis 1	DAAM1	0·641099
210142_x_at	Flotillin 1	FLOT1	0·64127034
228098_s_at	Myosin regulatory light chain interacting protein	MYLIP	0·6423752
226464_at	Hypothetical protein MGC33365	MGC33365	0·64265686
227189_at	Copine V	CPNE5	0·64277476
228910_at	CD82 antigen	KAI1	0·643213
208246_x_at	hypothetical protein FLJ20006	FLJ20006	0·64384246
1565627_a_at	Leucine-rich repeat kinase 1	LRRK1	0·6438542
208070_s_at	REV3-like, catalytic subunit of DNA polymerase zeta	REV3L	0·64388376
226779_at	LMBR1 domain containing 2	DKFZp434H2226	0·64402026
212760_at	Ubiquitin protein ligase E3 component n-recognin 2	UBR2	0·6447363
232644_x_at	OCIA domain containing 1	OCIAD1	0·6447408
205922_at	Vanin 2	VNN2	0·6448793
209062_x_at	Nuclear receptor coactivator 3	NCOA3	0·64496595
200842_s_at	Glutamyl-prolyl-tRNA synthetase	EPRS	0·645458
212733_at	KIAA0226	KIAA0226	0·64567864
244887_at	Regulator of G-protein signalling 13	RGS13	0·6465569
205370_x_at	Dihydrolipoamide branched chain transacylase E2	DBT	0·6465768
219812_at	Stromal antigen 3	MGC2463	0·64680105
202378_s_at	Leptin receptor	LEPR	0·6468874
204285_s_at	Phorbol-12-myristate-13-acetate-induced protein 1	PMAIP1	0·6495961
228151_at	Transcribed locus		0·6497464
213007_at	KIAA1794	FLJ10719	0·6498637
222891_s_at	B-cell CLL/lymphoma 11A	BCL11A	0·64996225
220085_at	Helicase, lymphoid-specific	HELLS	0·6502575
220746_s_at	Receptor associated protein 80	RAP80	0·65041715
213111_at	Phosphatidylinositol-3-phosphate/phosphatidylinositol 5-kinase, type III	PIP5K3	0·65109867
203318_s_at	Zinc finger protein 148	ZNF148	0·6520053
202655_at	Arginine-rich, mutated in early stage tumors	ARMET	0·6522758
225273_at	KIAA1280 protein	KIAA1280	0·6529812
204709_s_at	Kinesin family member 23	KIF23	0·65339863
218358_at	Cysteine-rich with EGF-like domains 2	MGC11256	0·6534136
201917_s_at	Solute carrier family 25, member 36	FLJ10618	0·65368974
220933_s_at	Zinc finger, CCHC domain containing 6	ZCCHC6	0·6548457
212588_at	Protein tyrosine phosphatase, receptor type, C	PTPRC	0·6555439
215780_s_at	Human DNA sequence from clone RP1-30P20	SET	0·65566504
239748_x_at	yl95h12.s1 Soares infant brain 1NIB		0·6571978
209780_at	Putative homeodomain transcription factor 2	PHTF2	0·65742826
211040_x_at	G-2 and S-phase expressed 1	GTSE1	0·659053
206150_at	TAP binding protein-like	TNFRSF7	0·6612941
209049_s_at		PRKCBP1	0·66141385
217796_s_at	Nuclear protein localization 4	NPL4	0·6618553
222737_s_at	Bromodomain containing 7	BRD7	0·6626274
218306_s_at	Hect domain and RCC1 (CHC1)-like domain (RLD) 1	HERC1	0·662763
222420_s_at	Ubiquitin-conjugating enzyme E2H	UBE2H	0·66321975
210962_s_at	A kinase (PRKA) anchor protein (yotiao) 9	AKAP9	0·6633441

Table 2. (*Continued*)

Affy ID	Gene name	Symbol	Fold-change
1555275_a_at	Kelch-like 6 (Drosophila)	KLHL6	0·6639439
218348_s_at	Zinc finger CCCH-type containing 7A	ZC3HDC7	0·6641935
233329_s_at	Hypothetical protein LOC51315	LOC51315	0·66421455
225232_at	Myotubularin related protein 12	PIP3AP	0·66434306
230917_at	CDNA FLJ45450 fis, clone BRSTN2002691		0·664679
202181_at	KIAA0247	KIAA0247	0·66494757
210561_s_at	WD repeat and SOCS box-containing 1	WSB1	0·66532546
206272_at	S-phase response (cyclin-related)	SPHAR	0·66562426
201498_at	Ubiquitin specific peptidase 7 (herpes virus-associated)	USP7	0·6661961
235661_at	ye65a03.r1 Soares fetal liver spleen 1NFLS		0·666862
228087_at	LOC90693 protein	LOC90693	0·6674958
218150_at	ADP-ribosylation factor-like 5A	ARL5	0·6685538
203608_at		ALDH5A1	0·6689315
213460_x_at	Williams Beuren syndrome chromosome region 20C	WBSCR20C	0·6701285
202922_at	Glutamate-cysteine ligase, catalytic subunit	GCLC	0·67089856
222408_s_at	Yippee-like 5	YPEL5	0·671024
223391_at	Sphingosine-1-phosphate phosphatase 1	SGPP1	0·67116857
213166_x_at	Hypothetical protein FLJ14346	FLJ14346	0·67121977
219119_at	LSM8 homolog, U6 small nuclear RNA associated	LSM8	0·67156994
203297_s_at	Jumonji, AT rich interactive domain 2	JARID2	0·67171353
213940_s_at	Formin binding protein 1	FNBP1	0·67211777
224677_x_at	Chromosome 11 open reading frame 31	C11orf31	0·6722119
212066_s_at	Ubiquitin specific peptidase 34	USP34	0·67282677
201779_s_at	Ring finger protein 13	RNF13	0·67283076
243798_at	B-cell CLL/lymphoma 9-like	BCL9L	0·6730866
212023_s_at	Antigen identified by monoclonal antibody Ki-67	MKI67	0·673758
219502_at	Nei endonuclease VIII-like 3	FLJ10858	0·67435133
203556_at	Zinc fingers and homeoboxes 2	ZHX2	0·67446464
237475_x_at	qb48d05.x1 NCI_CGAP_Brn23		0·6744719
202704_at	Transducer of ERBB2, 1	TOB1	0·67514235
225701_at	AT-hook transcription factor	AKNA	0·6767782
219392_x_at	Proline rich 11	FLJ11029	0·67691547
205297_s_at	CD79B antigen	CD79B	0·67695534
226398_s_at	Chromosome 10 open reading frame 4	C10orf4	0·6774105
213064_at	Nuclear protein UKp68	FLJ11806	0·6776265
1559436_x_at	Arrestin, beta 2	ARRB2	0·67766494
212167_s_at	SWI/SNF related, matrix associated, actin dependent regulator of chromatin	SMARCB1	0·6779144
223268_at	LP4947	PTD012	0·6783802
217781_s_at	Zinc finger protein 106 homolog	ZFP106	0·6795011
1556059_s_at	Spen homolog, transcriptional regulator	SPEN	0·6800467
1552448_a_at	Homo sapiens chromosome 8 open reading frame 12 (C8orf12), mRNA.	C8orf12	0·6807321
224602_at	HCV F-transactivated protein 1	LOC401152	0·6808058
212944_at	Mitochondrial ribosomal protein S6	MRPS6	0·6811207
208737_at	ATPase, H+ transporting, lysosomal 13 kDa, V1 subunit G isoform 1	ATP6V1G1	0·6811667
211997_x_at	H3 histone, family 3B	H3F3B	0·68119144
212622_at	Transmembrane protein 41B	KIAA0033	0·68157566
203301_s_at	Cyclin D binding myb-like transcription factor 1	DMTF1	0·68273085
208899_x_at	ATPase, H+ transporting, lysosomal 34 kDa, V1 subunit D	ATP6V1D	0·6829173
202983_at	SWI/SNF related, matrix associated, actin dependent regulator of chromatin	SMARCA3	0·6829173
209250_at	Degenerative spermatocyte homolog 1, lipid desaturase	DEGS1	0·6831875
204581_at	CD22 antigen	CD22	0·6840824
225433_at	General transcription factor IIA, 1, 19/37 kDa	GTF2A1	0·685833
219076_s_at	Peroxisomal membrane protein 2, 22 kDa	PXMP2	0·6862011
208772_at	Ankyrin repeat and KH domain containing 1	ANKHD1	0·68652916
212571_at	Chromodomain helicase DNA binding protein 8	CHD8	0·68715703

Table 2. (*Continued*)

Affy ID	Gene name	Symbol	Fold-change
200920_s_at	B-cell translocation gene 1, anti-proliferative	BTG1	0·6878364
212126_at	Chromobox homolog 5	CBX5	0·687962
203752_s_at	Jun D proto-oncogene	JUND	0·68797
210105_s_at	FYN oncogene related to SRC, FGR, YES	FYN	0·68825364
221501_x_at	Hypothetical protein LOC339047	LOC339047	0·6883818
227696_at	Exosome component 6	EXOSC6	0·688953
201810_s_at	SH3-domain binding protein 5	SH3BP5	0·6889935
206513_at	Absent in melanoma 2	AIM2	0·689059
205484_at	Signaling threshold regulating transmembrane adaptor 1	SIT	0·68912697
225890_at	Chromosome 20 open reading frame 72	C20orf72	0·69149476
213154_s_at	Bicaudal D homolog 2	BICD2	0·6923916
217717_s_at	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein	YWHAB	0·69240123
212350_at	TBC1 domain family, member 1	TBC1D1	0·69268274
213128_s_at	Ubiquitin protein ligase E3A	UBE3A	0·6931148
212943_at	KIAA0528 gene product	KIAA0528	0·6943166
203434_s_at	Membrane metallo-endopeptidase	MME	0·69480205
232515_at	Ankyrin repeat and SOCS box-containing 3	ASB3	0·69493157
234984_at	Neural precursor cell expressed, developmentally down-regulated 1	NEDD1	0·6951
204446_s_at	Arachidonate 5-lipoxygenase	ALOX5	0·6954707
221918_at	PCTAIRE protein kinase 2	PCTK2	0·6954857
218384_at	Calcium regulated heat stable protein 1, 24 kDa	CARHSP1	0·6965451
223022_s_at	Chromosome 6 open reading frame 55	C6orf55	0·69656706
217249_x_at	Cytochrome c oxidase subunit VIIa pseudogene 2	COX7A3H	0·69701755
243539_at	Ring finger protein 11	RNF11	0·69720584
210592_s_at	Spermidine/spermine N1-acetyltransferase	SAT	0·69809985
217967_s_at	Chromosome 1 open reading frame 24	C1orf24	0·6989367
228006_at	Phosphatase and tensin homolog	PTEN	0·6992462
204710_s_at	WD repeat domain, phosphoinositide interacting 2	WIPI-2	0·699294
202760_s_at	Paralemmin 2	PALM2-AKAP2	0·6998125
204872_at	Transducin-like enhancer of split 4	TLE4	0·6999074
233702_x_at	Homo sapiens cDNA: FLJ20946 fis, clone ADSE01819.		0·70036465
222986_s_at	Scotin	SCOTIN	0·70078844
223445_at	Dystrobrevin binding protein 1	DTNBP1	0·7017351
203007_x_at	Lysophospholipase I	LYPLA1	0·7029054
235333_at	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 6	B4GALT6	0·7029247
213729_at	Formin binding protein 3	FNBP3	0·70293915
227124_at	MRNA full length insert cDNA clone EUROIMAGE 966164		0·70326364
205733_at	Bloom syndrome	BLM	0·7036139
219304_s_at	Platelet derived growth factor D	PDGFD	0·7036207
219237_s_at	DnaJ (Hsp40) homolog, subfamily B, member 14	FLJ14281	0·7041835
209358_at	TAF11 RNA polymerase II	TAF11	0·7044149
225545_at	Eukaryotic elongation factor-2 kinase	EEF2K	0·70445967
208146_s_at	Carboxypeptidase, vitellogenic-like	CPVL	0·7045879
210972_x_at	T-cell receptor rearranged alpha-chain V-region	TCRA	0·7055038
208579_x_at	H2B histone family, member S	H2BFS	0·7060805
212263_at	Quaking homolog, KH domain RNA binding	QKI	0·707485
202386_s_at	Limkain b1	LKAP	0·7075946
202113_s_at	sorting nexin 2	TRG-9	0·70800734
206323_x_at	Oligophrenin 1	OPHN1	0·70812446
202664_at	Wiskott-Aldrich syndrome protein interacting protein	WASPIP	0·70844746
233093_s_at	Baculoviral IAP repeat-containing 6	BIRC6	0·7087636
218662_s_at	Chromosome condensation protein G	HCAP-G	0·70939666
214508_x_at	CAMP responsive element modulator	CREM	0·7095808
213737_x_at	Transcribed locus	DKFZp434P162	0·7103367
201877_s_at	Protein phosphatase 2, regulatory subunit B (B56), gamma isoform	PPP2R5C	0·7103621

Table 2. (*Continued*)

Affy ID	Gene name	Symbol	Fold-change
212693_at	MDN1, midasin homolog (yeast)	MDN1	0·7104688
212314_at	KIAA0746 protein	KIAA0746	0·71076244
201247_at	Sterol regulatory element binding transcription factor 2	SREBF2	0·7107886
227993_at	Methionyl aminopeptidase 2	METAP2	0·7108942
216508_x_at	similar to nonhistone chromosomal protein HMG-1	WUGSC	0·71091264
227833_s_at	Methyl-CpG binding domain protein 6	MBD6	0·71103483
1566509_s_at	F-box protein 9	FBX9	0·7116769
203279_at	ER degradation enhancer, mannosidase alpha-like 1	EDEM1	0·7119141
235372_at	Fc receptor-like and mucin-like 1	FREB	0·71208745
241968_at	Transcribed locus		0·71281445
206296_x_at	Mitogen-activated protein kinase kinase kinase kinase 1	MAP4K1	0·7130861
212462_at	MYST histone acetyltransferase (monocytic leukemia) 4	MYST4	0·713116
233665_x_at	Mitochondrial translation optimization 1 homolog	MTO1	0·7134478
212209_at	Thyroid hormone receptor associated protein 2	THRAP2	0·7144091
235327_x_at	602284688F1 NIH_MGC_86	UBXD4	0·7144813
230618_s_at	BAT2 domain containing 1	XTP2	0·71461946
236641_at	Kinesin family member 14	KIF14	0·71463937
204224_s_at	GTP cyclohydrolase 1	GCH1	0·7147643
204531_s_at	Breast cancer 1, early onset	BRCA1	0·71507436
203787_at	Single-stranded DNA binding protein 2	SSBP2	0·7154888
212492_s_at	Jumonji domain containing 2B	JMJD2B	0·7159365
202817_s_at	Synovial sarcoma translocation, chromosome 18	SS18	0·7160071
212420_at	E74-like factor 1	ELF1	0·71618116
203338_at	Protein phosphatase 2, regulatory subunit B (B56), epsilon isoform	PPP2R5E	0·7164281
207540_s_at	Spleen tyrosine kinase	SYK	0·71644425
229943_at	Ret finger protein 2	RFP2	0·71658355
218671_s_at	ATPase inhibitory factor 1	ATPIF1	0·71663755
232909_s_at	Fetal Alzheimer antigen	FALZ	0·7174373
225816_at	PHD finger protein 17	PHF17	0·7179169
211713_x_at	KIAA0101	KIAA0101	0·71805906
229394_s_at	Glucocorticoid receptor DNA binding factor 1	GRLF1	0·71806455
212572_at	Serine/threonine kinase 38 like	STK38L	0·71829355
209382_at	Polymerase (RNA) III	POLR3C	0·7190526
225913_at	KIAA2002 protein	KIAA2002	0·7191407
210776_x_at	Transcription factor 3	TCF3	0·7192611
223053_x_at	Ssu72 RNA polymerase II CTD phosphatase homolog	HSPC182	0·71963733
226297_at	Homeodomain interacting protein kinase 3	HIPK3	0·7197105
1558801_at	Nicotinamide nucleotide transhydrogenase	NNT	0·71974134
222369_at	Hypothetical protein FLJ13848	FLJ13848	0·71995664
230352_at	Phosphoribosyl pyrophosphate synthetase 2	PRPS2	0·7200596
202365_at	Hypothetical protein MGC5139	MGC5139	0·7206042
205902_at	Potassium intermediate/small conductance calcium-activated channel	KCNN3	0·72095835
233936_s_at	Zinc finger protein 403	DIF3	0·7217099
201319_at	Myosin regulatory light chain MRCL3	MRCL3	0·72199404
229582_at	Chromosome 18 open reading frame 37	C18orf37	0·7221231
204115_at	Guanine nucleotide binding protein (G protein), gamma 11	GNG11	0·7221238
212010_s_at	Hypothetical protein H41	H41	0·7224393
218421_at	Ceramide kinase	CERK	0·722603
221500_s_at	Syntaxin 16	STX16	0·7226911
222433_at	Enabled homolog	ENAH	0·72285813
211936_at	Heat shock 70 kDa protein 5	HSPA5	0·7232242
203136_at	Rab acceptor 1	RABAC1	0·7233282
209902_at	Ataxia telangiectasia and Rad3 related	ATR	0·7234323
212780_at	Son of sevenless homolog 1	SOS1	0·723696
211503_s_at	RAB14, member RAS oncogene family	RAB14	0·72393346