containing 2% goat serum diluted in PBS. The cells were incubated with mouse monoclonal anti-NF-κB p65 antibody or an isotype control for 1 h at room temperature, then with AlexaFluor 488-conjugated goat anti-mouse antibody for 30 min at room temperature. Slides were coverslipped and examined using a fluorescence microscope (Olympus, Tokyo, Japan).

Western blotting analysis. For measurement of IκBa by Western blotting, RA-FLS at a density of 1.5×10^6/well were seeded into 6-well plates in culture medium for 24 h. After incubation with 10 ng/ml LIGHT for 40 min, cells were washed twice in ice-cold PBS and lysed in 100 µl of sodium dodecyl sulfate (SDS) sample buffer (62.5 mM Tris HCl, pH 6.8, 10% glycerol, 2% SDS, 5% mercaptoethanol, and 0.001% bromophenol blue). Cell lysates were separated by SDS-polyacrylamide gel electrophoresis in 12% polyacrylamide gels, and transferred onto nitrocellulose membranes (Invitrogen). After blocking, membranes were incubated with either anti-ß-actin or anti-IκBa antibody, overnight at 4°C, and then with secondary antibody conjugated to horseradish peroxidase (Dako), at room temperature for 1 h. The signals were visualized using chemiluminescence reagent (ECL; Amersham Biosciences, Little Chalfont, UK).

Statistical analysis. Comparisons of ≥ 3 populations were made using the Kruskal-Wallis test. Comparisons of 2 independent data sets were by Mann-Whitney U-test. P values less than 0.05 were considered statistically significant.

RESULTS

Increased expression of LIGHT in SF of patients with RA. To examine whether LIGHT is involved in the pathogenesis of RA, we analyzed the level of LIGHT in SF from 23 RA patients and 10 OA patients by ELISA. SF from OA patients were used as controls, because they were not available from healthy individuals. The concentration of LIGHT in SF from RA patients was significantly higher than in those from OA patients (Figure 1). The median levels of LIGHT in SF from RA and OA patients were 108.5 pg/ml and 7.8 pg/ml, respectively.

Expression of LIGHT and its receptors in RA synovial tissue and RA-FLS. Because RA patients had more LIGHT in their SF than OA patients, we investigated whether LIGHT and its membrane-bound receptors HVEM and LTßR were expressed in the RA and OA synovial tissues. Although quantitative real-time PCR analysis revealed that mRNA expression of LIGHT in synovial tissue was significantly higher in RA patients than in OA patients (Figure 2A), HVEM and LTßR levels were not different between RA and OA patients.

Further, we investigated the mRNA expression of LIGHT, HVEM, and LTßR in RA-FLS by quantitative real-time PCR. RA-FLS from all 7 patients expressed HVEM and LTßR mRNA, and the level of LTßR mRNA was significantly higher than that of HVEM mRNA, whereas no LIGHT expression was detected (Figure 2B).

Induction of RA-FLS proliferation by LIGHT. Previous studies reported that LIGHT induces cell proliferation in T lymphocytes[7,12] and vascular smooth muscle cells[13]. Since the expression of HVEM and LTßR in RA-FLS had been confirmed, we next evaluated the effect of LIGHT on the proliferation of RA-FLS using a BrdU assay. As shown in

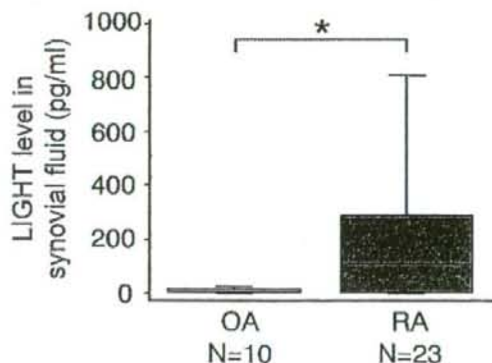

Figure 1. Levels of LIGHT in synovial fluid from RA patients and OA patients, determined by ELISA. Box plots represent 25th to 75th percentiles. Error bars represent 10th to 90th percentiles. Lines inside boxes represent the median. *$p < 0.05$.

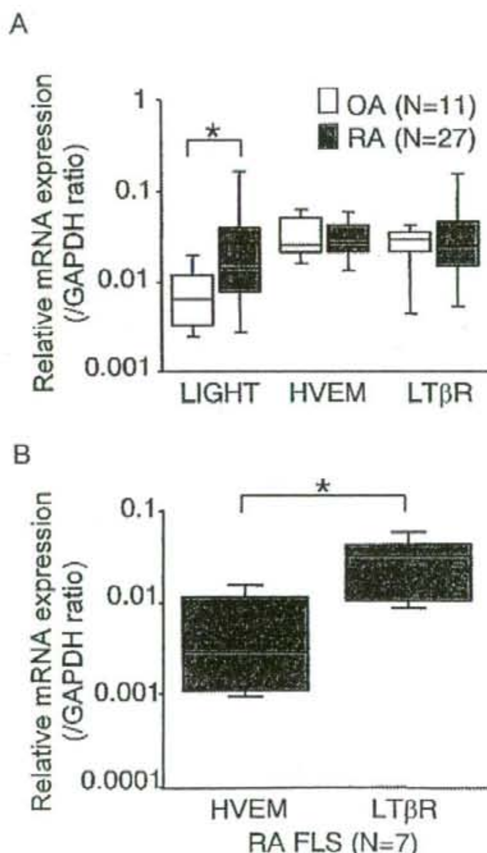

Figure 2. Expression of LIGHT, herpes virus entry mediator (HVEM), and lymphotoxin ß receptor (LTßR) mRNA in synovial tissues and fibroblast-like synoviocytes (FLS): A. In synovial tissues from RA patients and OA patients. B. In RA-FLS. Level was evaluated by real-time quantitative PCR; results are represented as relative ratios to GAPDH levels. *$p < 0.05$.

Figure 3A, treatment with LIGHT significantly enhanced *de novo* DNA synthesis in RA-FLS in a dose-dependent manner. LIGHT showed a stronger growth-promoting activity than PDGF, at lower concentrations.

To investigate the contributions of HVEM and LTßR to the LIGHT-induced proliferation of RA-FLS, we transfected RA-FLS with HVEM siRNA or LTßR siRNA. Quantitative real-time PCR analysis revealed that the HVEM mRNA level in HVEM siRNA-transfected RA-FLS was reduced by 75% compared with control siRNA-transfected RA-FLS (Figure 3B). Similarly, treatment of RA-FLS with LTßR siRNA led to a 75% reduction in the LTßR mRNA level compared with that in control siRNA-transfected RA-FLS (Figure 3B). Under these conditions, LIGHT-induced growth of RA-FLS was significantly decreased by LTßR siRNA, but not by HVEM siRNA, when compared with RA-FLS transfected with control siRNA (Figure 3C).

LIGHT induces expression of proinflammatory cytokines, chemokines, and adhesion molecules in FLS via LTβR. Previous studies reported that LIGHT induces secretion of various cytokines and augments the expression of adhesion molecules[13,15-17,19]. We examined the effects of LIGHT on inflammatory cytokine and chemokine production by RA-FLS. Treatment with LIGHT enhanced both mRNA and protein expression of IL-8, MCP-1, MIP-1α, and RANTES in RA-FLS, in a dose-dependent manner (Figures 4A, 4B). LIGHT induced IL-1ß, IL-6, and GM-CSF, but not TNF-α, eotaxin, or MIP-1ß (data not shown). Next, to assess whether LIGHT can induce the expression of cell-surface adhesion molecules on RA-FLS, we examined ICAM-1 and VCAM-1 expression on LIGHT-stimulated RA-FLS. LIGHT treatment significantly increased the expression of ICAM-1 mRNA in a dose-dependent manner (Figure 4C). Flow cytometry analysis revealed the augmented expression of ICAM-1 protein on the cell surface of RA-FLS stimulat-

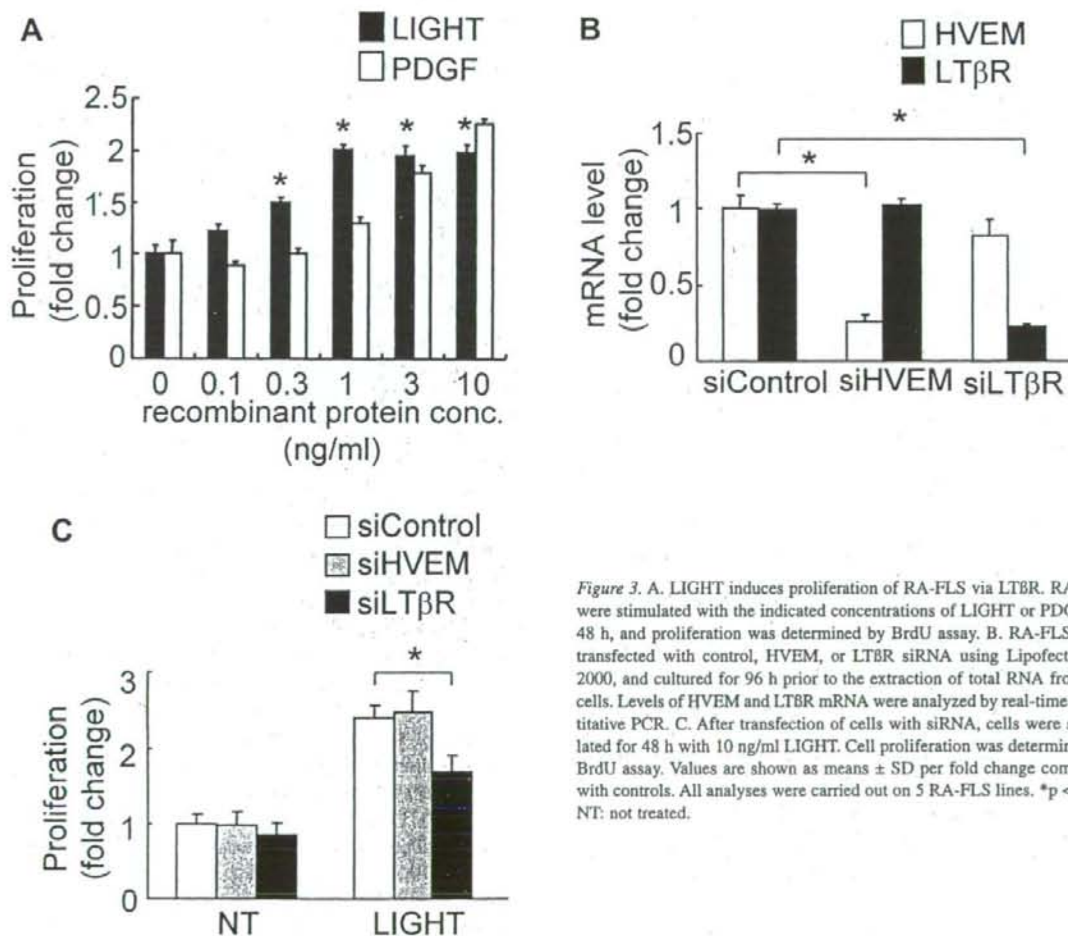

Figure 3. A. LIGHT induces proliferation of RA-FLS via LTßR. RA-FLS were stimulated with the indicated concentrations of LIGHT or PDGF for 48 h, and proliferation was determined by BrdU assay. B. RA-FLS were transfected with control, HVEM, or LTßR siRNA using Lipofectamine 2000, and cultured for 96 h prior to the extraction of total RNA from the cells. Levels of HVEM and LTßR mRNA were analyzed by real-time quantitative PCR. C. After transfection of cells with siRNA, cells were stimulated for 48 h with 10 ng/ml LIGHT. Cell proliferation was determined by BrdU assay. Values are shown as means ± SD per fold change compared with controls. All analyses were carried out on 5 RA-FLS lines. *$p < 0.05$. NT: not treated.

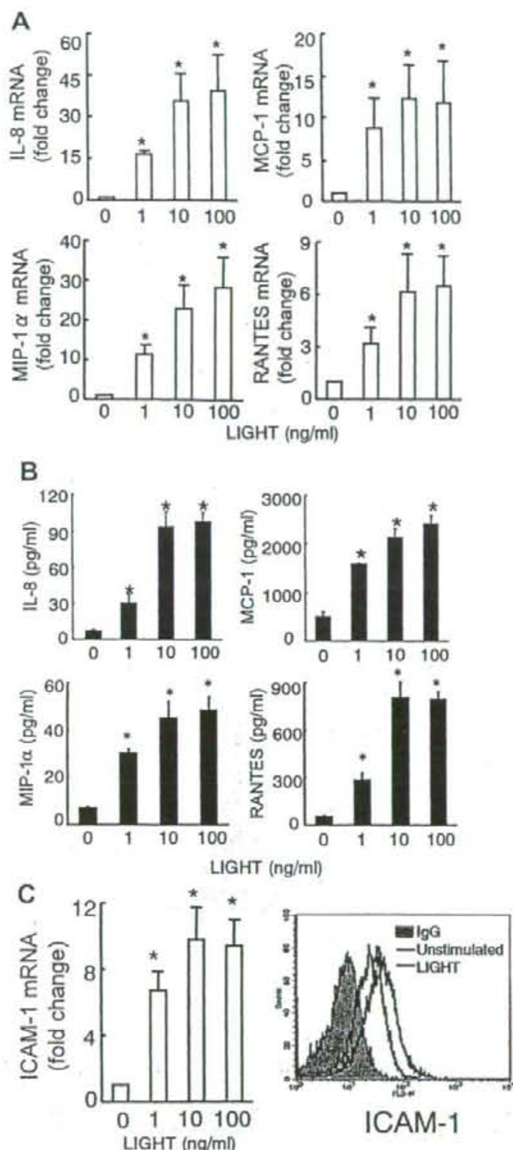

Figure 4. Upregulation of IL-8, MCP-1, MIP-1α, RANTES, and ICAM-1 expression in RA-FLS by LIGHT. A. RA-FLS were stimulated with the indicated concentrations of LIGHT for 3 h, and real-time quantitative PCR was performed to determine levels of IL-8, MCP-1, MIP-1α, and RANTES mRNA expression. Values are shown as means ± SD per fold change compared with controls. B. RA-FLS were stimulated with the indicated concentrations of LIGHT for 72 h. Concentrations of IL-8, MCP-1, MIP-1α, and RANTES in cell culture supernatants were determined by multiplex bead array assays. Values are shown as means ± SD pg/ml. C. RA-FLS were stimulated with the indicated concentrations of LIGHT for 3 h, and real-time quantitative PCR was performed to determine levels of ICAM-1 mRNA expression. ICAM-1 surface expression on RA-FLS was detected by flow cytometry after stimulation with 10 ng/ml LIGHT for 24 h. All analyses were carried out on 4 RA-FLS lines; flow cytometry profiles of one representative result are shown. $*p < 0.05$.

ed with LIGHT (Figure 4C). Similar increases in VCAM-1 mRNA and protein expression were also seen when stimulated with LIGHT (data not shown). Moreover, we investigated whether knockdown of HVEM or LTßR suppressed this series of LIGHT-induced gene expression in RA-FLS. Compared with control siRNA, LTßR siRNA, but not HVEM siRNA, significantly decreased the expression of IL-8, MCP-1, and ICAM-1 mRNA induced by LIGHT (Figure 5). Similarly, LTßR siRNA decreased the LIGHT-induced expression of IL-1ß, IL-6, GM-CSF, RANTES, and MIP-1α mRNA in FLS (data not shown).

Activation of NF-κB in RA-FLS via LTβR by LIGHT. It is known that activation of NF-κB has a key role in inflammatory disease[23]. Several studies have shown that LIGHT activates the transcription factor NF-κB in different cell types[7,9,13,24-26]. To investigate the involvement of NF-κB in LIGHT-induced gene expression, we examined the effect of the NF-κB inhibitor PDTC on the expression of IL-8, MCP-1, and ICAM-1 by real-time quantitative PCR. PDTC completely abolished the LIGHT-induced expression of IL-8, MCP-1, and ICAM-1 (Figure 6A). The LIGHT-induced expression of IL-1ß, IL-6, GM-CSF, RANTES, and MIP-1α mRNA in RA-FLS was also inhibited by PDTC treatment (data not shown). The concentration of PDTC used in these experiments had no cytotoxic effect, as demonstrated by cell viability studies using trypan blue exclusion, which showed that > 95% of cells remained viable over the entire period of the experiment (data not shown).

In the immunocytofluorescence analysis using anti-NF-κB p65 mAb, enhanced nuclear translocation of NF-κB p65 was observed in LIGHT-stimulated RA-FLS (Figure 6B). Further, Western blotting using anti-IκBa mAb showed that IκBa degradation was induced by LIGHT, and that IκBa degradation was inhibited by LTßR siRNA, but not by HVEM siRNA (Figure 6C).

DISCUSSION

We observed that LIGHT, but not HVEM or LTßR, is overexpressed in the synovial tissues of patients with RA compared with those of patients with OA. The expression of LIGHT was not detected in RA-FLS, which comprise one of the major components of the RA synovium. RA synovium is histologically characterized by prominent infiltration of macrophages and lymphocytes[27]. Although LIGHT has been supposed to be produced by activated T lymphocytes *in vitro*[4,5], a recent study reported that LIGHT was overexpressed in CD68-positive macrophages in RA synovial tissue compared with those in OA synovial tissue, and that expression levels of LIGHT were low in areas rich in lymphocytes[19]. Thus, macrophages rather than FLS and lymphocytes could be the major source of LIGHT in the RA synovium.

We further demonstrated that *in vitro*-cultured RA-FLS express HVEM and LTßR, which implies that RA-FLS are

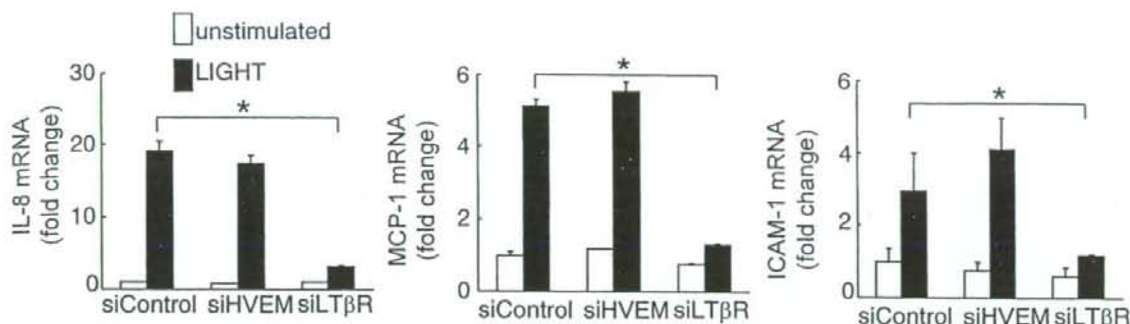

Figure 5. LIGHT-induced expression of IL-8, MCP-1, and ICAM-1 in RA-FLS via LTßR. RA-FLS were transfected with control, HVEM, or LTßR siRNA using Lipofectamine 2000. After 96 h incubation, cells were stimulated with 10 ng/ml LIGHT for an additional 3 h. Levels of IL-8, MCP-1, and ICAM-1 mRNA were analyzed by real-time quantitative PCR. Values are shown as means ± SD per fold change compared with controls. All analyses were carried out on 4 RA-FLS lines. *$p < 0.05$.

target cells of LIGHT. Indeed, we first showed that LIGHT had a stronger RA-FLS growth-promoting activity than PDGF, in lower concentrations. The proliferation of RA-FLS is one of the most critical pathological changes in RA. Thus, our findings suggest that increased expression of LIGHT might lead to the synovial hyperplasia of RA. Anticytokine therapies targeting TNF-α, IL-1ß, and IL-6 have been used to treat patients with RA, and it has been demonstrated that such treatments may suppress the accompanying bone destruction as well as the synovitis[28,29]. In addition, recent studies have indicated that LIGHT reduces Fas-mediated apoptosis in FLS[30], that LIGHT may function as a mediator of bone resorption through the induction of osteoclastogenesis[31], and that LTßR-Ig protein blocks the induction of experimental arthritis in mice[18]. Thus, a neutralizing antibody against LIGHT could be a useful tool for inhibition of synovial hyperplasia and bone destruction in RA.

The enhanced effects of LIGHT on RA-FLS proliferation were significantly inhibited by LTßR siRNA, but not by HVEM siRNA, suggesting that LTßR, rather than HVEM, is involved in the LIGHT-induced proliferation of RA-FLS. The exact mechanism by which LIGHT influences RA-FLS proliferation through LTßR is unknown. A potential mechanism underlying RA-FLS proliferation induced by LIGHT may involve cell-cycle regulators, including cyclin-dependent kinases (CDK). The mammal cell cycle is controlled by holoenzymes composed of a catalytic CDK and regulatory cyclin. The expression level of p21 was reduced in RA synovial linings and FLS compared with the level in patients with OA[32]. Overexpression of p21 or p16 by adenoviral-mediated delivery suppresses FLS growth *in vitro*[33,34]. Further, LIGHT induces cell proliferation, downregulates the CDK inhibitors p21, p27 and p53, and inversely upregulates cyclin D and Rb hyperphosphorylation in vascular smooth muscle cells[13]. Thus, it is possible that LIGHT promotes FLS proliferation by shortening the cell cycle of FLS in RA. Wang, *et al* reported that LTßR-null mice show reduced BrdU incorporation in dendritic cells[35]. This supports our claim that LTßR signaling is involved in the proliferation of RA-FLS.

We observed that LIGHT also induces the production of inflammatory cytokines and chemokines and expression of adhesion molecules on RA-FLS. Inflammatory cytokines and chemokines induce the migration of cells and release of mediators of inflammation and angiogenesis, and could be involved in the pathogenesis of RA[1,2,36]. The increased expression of ICAM-1 and VCAM-1 adhesion molecules on activated endothelial cells enhances the recruitment of monocytes, lymphocytes, and neutrophils, leading to inflammation. These findings indicate that LIGHT might play an important role in inflammation in the synovial lining layer, as well as in its hyperplasia. A recent study revealed that LIGHT upregulates the expression of ICAM-1, VCAM-1, and IL-6 in RA-FLS via NF-κB activation[30,37]. Although these reports are consistent with our present results, it has not been clear which of 2 receptors is involved in the induction of these genes in FLS. Our knockdown analysis using siRNA revealed that LIGHT induces proliferation and gene expression by signaling via LTßR, but not HVEM. Braun, *et al* have shown that LTßR is expressed on RA-FLS, and that LTα1ß2, a ligand for LTßR, induces expression of inflammatory cytokines, chemokines, and ICAM-1[38]. This supports our claim that LTßR signaling is involved in the activation of RA-FLS. The NF-κB transcription factor is certainly involved in cytokine- and chemokine-driven responses and is a point of convergence for several upstream proinflammatory pathways[23]. Indeed, NF-κB activation appears to be an important factor in RA, as the expression of NF-κB is enhanced in lining cells[39,40] and in the cartilage-pannus junction in the RA synovium[41]. In our study, treatment with PDTC blocked LIGHT-induced IL-8, MCP-1, and ICAM-1 expression, suggesting that the effects of LIGHT are mediated through NF-κB. The involvement of NF-κB in LIGHT-induced proinflammatory responses was further confirmed

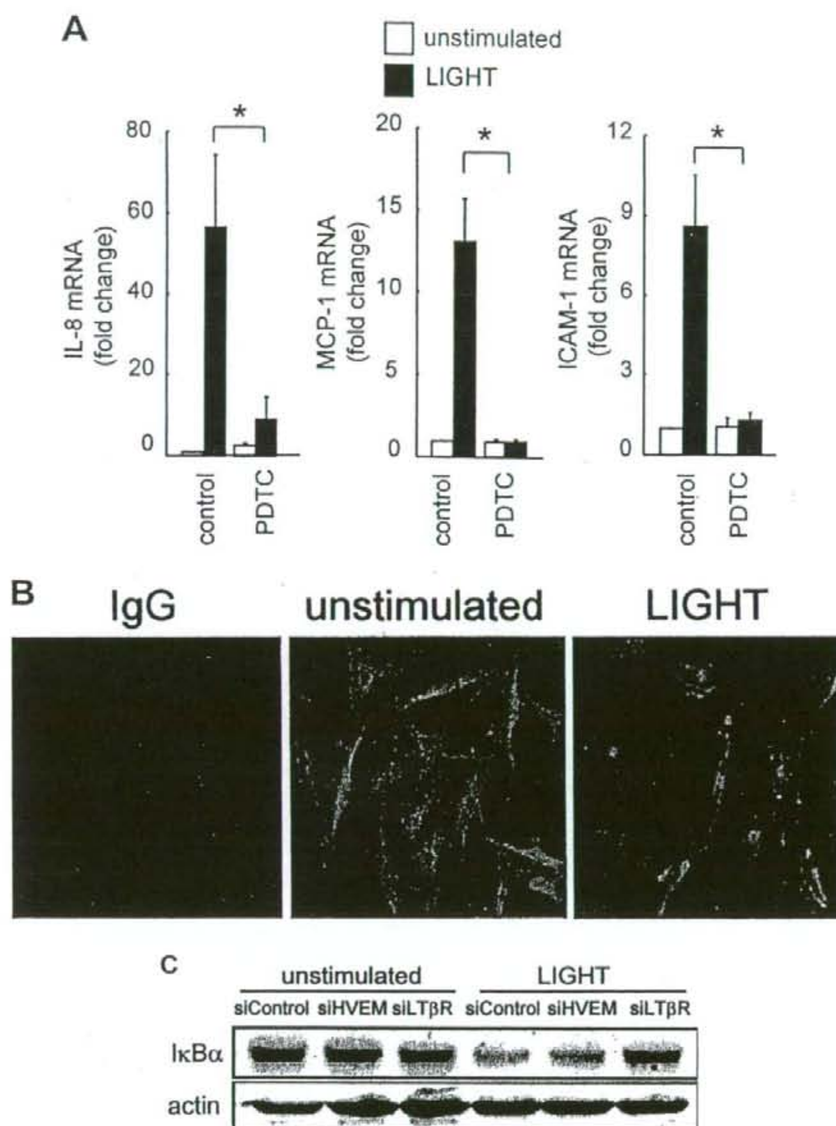

Figure 6. LIGHT-induced expression of IL-8, MCP-1, and ICAM-1 through NF-κB-mediated pathways. A. FLS were stimulated with 10 ng/ml LIGHT for 3 h with or without preincubation for 30 min with 30 μM PDTC. Levels of IL-8, MCP-1, and ICAM-1 mRNA were analyzed by real-time quantitative PCR. Values are shown as means ± SD per fold change compared with control. All analyses were carried out on 4 RA-FLS lines. *$p < 0.05$. B. Immunofluorescence staining for NF-κB p65 in RA-FLS. Control in which primary antibodies were replaced with control IgG (left panel); unstimulated RA-FLS (middle); and RA-FLS stimulated with 10 ng/ml LIGHT for 30 min (right). Results are representative of 2 experiments using 2 FLS lines. C. 96 h after siRNA transfection, cells were stimulated with 10 ng/ml LIGHT for 40 min. IκBa degradation was analyzed by immunoblotting. Results are representative of 2 experiments using 2 RA-FLS lines.

by the LIGHT-induced nuclear translocation of NF-κB p65. Moreover, LIGHT induced IκBa degradation in RA-FLS, an effect that was inhibited by LTßR siRNA, but not by HVEM siRNA. These findings are consistent with studies showing that LTßR ligation can lead to activation of NF-κB[24,42-45]. However, it is unknown why LIGHT prefers the LTßR signaling pathway in RA-FLS, even though HVEM is also expressed on these cells.

We have demonstrated that LIGHT is overexpressed in RA synovial tissues and SF. LIGHT induced increased production of inflammatory cytokines, chemokines, and adhesion molecules through NF-κB activation, as well as proliferation of RA-FLS. These findings indicate that LIGHT signaling via LTβR plays an important role in the pathogenesis of RA by affecting key processes such as the proliferation and activation of RA-FLS. Therefore, regulation of LIGHT-LTβR signaling may represent a new therapeutic target for the treatment of RA.

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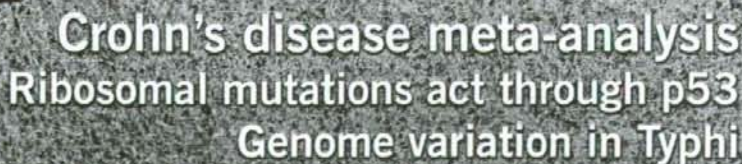

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Common variants in *DVWA* on chromosome 3p24.3 are associated with susceptibility to knee osteoarthritis

Yoshinari Miyamoto[1], Dongquan Shi[2], Masahiro Nakajima[1], Kouichi Ozaki[3], Akihiro Sudo[4], Akihiro Kotani[5], Atsumasa Uchida[4], Toshihiro Tanaka[3], Naoshi Fukui[6], Tatsuhiko Tsunoda[7], Atsushi Takahashi[8], Yusuke Nakamura[9], Qing Jiang[2] & Shiro Ikegawa[1]

Susceptibility to osteoarthritis, the most common human arthritis, is known to be influenced by genetic factors[1,2]. Through a genome-wide association study using ~100,000 SNPs, we have identified a previously unknown gene on chromosome 3p24.3, *DVWA*, which is associated with susceptibility to knee osteoarthritis. Expressed specifically in cartilage, *DVWA* encodes a 276-amino-acid protein with two regions corresponding to the von Willebrand factor type A domain (VWA domain)[3]. Several *DVWA* SNPs are significantly associated with knee osteoarthritis in two independent Japanese case-control cohorts. This association was replicated in a Japanese population cohort and a Han Chinese case-control cohort (combined $P = 7.3 \times 10^{-11}$). DVWA protein binds to β-tubulin, and the binding is influenced by two highly associated missense SNPs (rs11718863 and rs7639618) located in the VWA domain. The Tyr169-Cys260 isoform of DVWA, which is overrepresented in knee osteoarthritis, showed weaker interaction. Our findings reveal a new paradigm for study of osteoarthritis etiology and pathogenesis.

Osteoarthritis (MIM165720) is a common disorder that causes pain and restricted motion in joints, particularly the knee and hip[4]. About 6% of adults age 30 and older have frequent knee pain and radiographic osteoarthritis[5]. In the elderly, osteoarthritis frequently leads not only to disability but also to financial difficulty[6,7]. The critical need to manage osteoarthritis worldwide is the focus of the current 'Bone and Joint Decade' campaign. Susceptibility to osteoarthritis is influenced by genetic predisposition[8] and multiple susceptibility genes[1,2]. A few associated genes, including *FRZB*[9], *ASPN*[10] and *GDF5* (ref. 11), were identified using the candidate gene approach and confirmed in multiple populations, with functional data supporting their causality[1,12,13]. However, the genetic contribution to this complex disease is not entirely known.

The genome-wide association study is a powerful means for dissecting complex human diseases such as osteoarthritis. Susceptibility genes for several common diseases have been identified using this approach[14–16]. Notable advantages include its comprehensiveness and the potential for finding susceptibility genes with previously unknown function and relationship to the disease. As a part of the Japanese Millennium Project[14,17], we carried out a genome-wide association study for knee osteoarthritis.

To begin the study, we genotyped 94 knee osteoarthritis cases and 658 controls (set A) using 99,295 SNPs selected from the JSNP database[17]. After confirming the data quality, we compared the results of 79,763 SNPs between cases and controls by χ^2 tests for genotype, dominant, recessive and allele frequency models. We identified 2,153 SNPs that showed P values less than 0.01 in any of the four models. We further genotyped these SNPs using an independent population consisting of 646 knee osteoarthritis cases and 631 controls (set B). The SNP rs3773472 showed strong association (**Table 1**). This finding remained significant after Bonferroni correction ($0.000017 \times 2,153 = 0.037$). Therefore, we decided to examine SNPs around rs3773472. There were no other SNPs that showed significant association.

We referenced the International HapMap Project database (release 21a) and selected SNPs with D' value of >0.7 to rs3773472 and with a minor allele frequency of >0.1. The linkage disequilibrium (LD) block around rs3773472 contained 40 HapMap SNPs, two validated genes (*SH3BP5* and *CAPN7*) and one predicted gene (*LOC344875*). Next, we selected 12 tag SNPs (including rs3773472) that covered all 40 SNPs with an r^2 value of >0.9. After genotyping the tag SNPs using population set B, we found a more significantly associated SNP, rs7639618 ($P = 7.3 \times 10^{-8}$; **Table 2**). We examined potential

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Table 1 Association of rs3773472 with knee osteoarthritis in the genome-wide analysis

	Case					Control					
	Genotype					Genotype					
Population	CC	CG	GG	Sum	Allele G frequency	CC	CG	GG	Sum	Allele G frequency	P value for allele frequency
Set A	52	37	5	94	0.250	259	279	77	615	0.352	0.0059
Set B	324	272	50	646	0.288	240	314	74	628	0.368	0.000017
Set A+B combined	376	309	55	740	0.283	499	593	151	1243	0.360	0.00000065

confounding factors such as age, body mass index (BMI) and sex to evaluate whether they could generate pseudo-positive associations. No significant differences in mean age, BMI and sex distribution were apparent among rs7639618 genotypes (**Supplementary Table 1** online). We also checked for a population stratification effect using a genomic control method[18,19] but found this an unlikely explanation for the positive association (**Supplementary Table 2** online).

To confirm the association, we examined an independent population cohort (set C), which was divided into knee osteoarthritis and control groups on the basis of knee radiographs. Genotyping of rs7639618 in 242 knee osteoarthritis cases and 485 controls produced another significant result (**Table 3**). We further examined the association of rs7639618 in a Han Chinese population consisting of 417 knee osteoarthritis cases and 413 controls. The association was replicated in this distinct population ($P = 0.00072$; **Table 3**), further establishing the association of the SNP. The combined P was 7.3×10^{-11} with odds ratio of 1.43 (95% CI = 1.28–1.59).

In the NCBI genome database (build 36.2), rs7639618 lies within the *LOC344875* gene. The RefSeq transcript of *LOC344875* is based on *in silico* predictions and ESTs only, so we examined the full sequence of the expressed transcript with RACE and RT-PCR. We identified a previously unknown transcript (**Supplementary Fig. 1a** online), 2,250 bp in length and with a predicted protein of 276 amino acids. Protein motif analysis programs predicted that this protein lacks a signal peptide and contains two domains homologous with the VWA domain. We named this newly identified gene *DVWA* (double von Willebrand factor A domains). Because all 17 HapMap SNPs that associated with rs7639618 with $r^2 > 0.9$ are located in and around the *DVWA* region, we surmised that *DVWA*, rather than *SH3BP5* or *CAPN7*, was likely the gene associated with osteoarthritis.

To confirm the expression and size of the *DVWA* transcript, we carried out RNA analysis and identified a band corresponding to the predicted transcript length (**Supplementary Fig. 1b**). We also examined *DVWA* expression in various human tissues using real-time PCR. The highest expression was seen in cartilage tissues from both control individuals and individuals with osteoarthritis (**Supplementary Fig. 1c**), suggesting that *DVWA* function is associated with cartilage.

To locate the functional, osteoarthritis-associated SNP, we searched for SNPs in and around all exons of *DVWA* by direct sequencing of genomic DNA from 48 individuals with knee osteoarthritis. We found 4 previously unknown SNPs in addition to 21 known SNPs in the HapMap database (**Supplementary Table 3** online). After calculating pairwise r^2 values using all 25 SNPs in the *DVWA* region (**Supplementary Fig. 2** online), we selected 7 tag SNPs with $r^2 > 0.95$. Along with the four previously genotyped SNPs, three additional SNPs (rs1287464, rs9864422 and rs11718863) were selected and genotyped. This analysis revealed that rs9864422 and rs11718863 was significantly associated with knee osteoarthritis, similar to rs7639618 (**Supplementary Table 4** online). We also checked the association of these highly associated SNPs using set C and the combined set of B and C. The association of the SNPs had similar P values and odds ratios in both set C and the combined set (**Supplementary Table 5** online).

Table 2 Association of the selected tag SNPs with knee osteoarthritis

	Case					Control						
	Genotype					Genotype					Test for allele frequency	
dbSNP ID	11	12	22	Sum	Allele 2 frequency	11	12	22	Sum	Allele 2 frequency	P value	Odds ratio (95% CI)
rs618762	515	101	8	624	0.094	536	78	7	621	0.074	0.077	0.77 (0.58–1.03)
rs7639618	253	293	95	641	0.377	162	327	140	629	0.483	0.000000073	1.54 (1.32–1.81)
rs353093	169	316	142	627	0.478	117	317	190	624	0.558	0.000062	1.38 (1.18–1.61)
rs826428	457	166	15	638	0.154	483	129	16	628	0.128	0.066	0.81 (0.65–1.01)
rs3773475	273	302	69	644	0.342	196	328	100	624	0.423	0.000024	1.41 (1.20–1.66)
rs11713836	317	266	49	632	0.288	255	291	77	623	0.357	0.00021	1.37 (1.16–1.63)
rs1318937	253	301	77	631	0.361	206	311	104	621	0.418	0.0033	1.27 (1.08–1.50)
rs3732728	349	251	43	643	0.262	287	283	59	629	0.319	0.0016	1.32 (1.11–1.56)
rs2291853	192	318	135	645	0.456	148	325	156	629	0.506	0.011	1.22 (1.05–1.43)
rs3773472	324	272	50	646	0.288	240	314	74	628	0.368	0.000017	1.44 (1.22–1.70)
rs3773469	228	315	102	645	0.402	163	336	126	625	0.470	0.00054	1.32 (1.13–1.54)
rs1287467	479	144	16	639	0.138	475	148	6	629	0.127	0.43	0.91 (0.73–1.15)

Population set B was genotyped. Allele 1 and allele 2 indicate the major and minor allele in the knee osteoarthritis population, respectively, and 11, 12 and 22 indicate homozygote of allele 1 and heterozygote and homozygote of allele 2, respectively. Odds ratio shown is for allele 1 versus allele 2.

Table 3 Replication of association of rs7639618 with knee osteoarthritis in Japanese and Han Chinese populations

	Case					Control					Test for allele frequency	
	Genotype					Genotype						
Population	GG	GA	AA	Sum	Allele A frequency	GG	GA	AA	Sum	Allele A frequency	*P* value	Odds ratio (95% CI)
Japanese (set C)	99	107	36	242	0.370	166	222	95	483	0.427	0.038	1.27 (1.01–1.59)
Chinese	145	187	85	417	0.428	106	192	115	413	0.511	0.00072	1.40 (1.15–1.69)

We analyzed haplotype association using the first tag-SNP set of 12 SNPs from the entire LD block and the second tag-SNP set of 7 SNPs in the *DVWA* region (**Supplementary Table 6** online). Using the first tag-SNP set, we could not find any haplotypes that had more significant effects than rs7639618. Also, we examined the additional effect of the other SNPs combined with the SNP (adding incrementally) by comparing their conditional log-likelihoods calculated by THESIAS[20], and confirmed that there was no SNP-combination haplotype more significant than rs7639618. Using the second tag SNP set, we found that *P* values of the difference between each haplotype and others were not more significant than those of rs9864422 and rs7639618. Also, there was no SNP-combination haplotype more significant than rs9864422 and rs7639618 . Therefore, we selected the most associated SNPs, rs9864422, rs11718863 and rs7639618, as candidates for the osteoarthritis-associated SNP for further functional analyses.

To identify a functional (causal) SNP, we first assessed the function of rs9864422, located in *DVWA* intron 1, using a luciferase assay, but there was no allelic difference associated with the SNP in the promoter or enhancer activity of *DVWA*. The two missense SNPs, rs11718863 (encoding Y169N) and rs7639618 (encoding C260Y), are in almost complete LD with each other, so it is not possible at this time to determine which one is the causal SNP, and they may act as a haplotype rather than a single SNP. The SNPs yielded three haplotypes; one haplotype (Tyr169-Cys260) was significantly over-represented in osteoarthritis (**Supplementary Table 7** online).

To evaluate their potential effects on DVWA function, we set out to identify binding partners of DVWA protein. Immunoprecipitation analysis of cells transfected with S-tagged *DVWA* revealed a unique band (**Fig. 1a**). Matrix-assisted laser desorption/ionization–time of flight (MALDI/TOF) mass spectrometry analysis revealed that this band corresponded to β-tubulin, and we confirmed binding between DVWA and β-tubulin by immunoprecipitation and protein blot analysis (**Fig. 1b**). Next, we assessed the binding strength between β-tubulin and DVWA isoforms. We generated S-tagged recombinant proteins corresponding to four haplotypes of the two missense SNPs (**Fig. 1c**) and carried out solid-phase binding assays. All four DVWA isoforms bound to tubulin, but binding of DVWA Tyr169-Cys260 was significantly weaker than that of the other three isoforms (**Fig. 1d**).

We have identified a previously unknown gene, *DVWA*, which is associated with knee osteoarthritis across two distinct Asian populations. DVWA protein is predicted to have two domains homologous to the VWA domain, which typically is involved in cell adhesion and protein–protein interactions. Mutations in the VWA domains of *MATN3* cause osteoarthritis[21] and osteochondrodysplasia[22]. Although most VWA-containing proteins are extracellular, some reside within the cell and have roles in transcription, DNA repair and ribosomal and membrane transport[23].

DVWA physically interacts with β-tubulin. The strength of binding is influenced by alleles of two missense SNPs, both of which are significantly associated with osteoarthritis and lie within the predicted VWA domain. The Tyr169-Cys260 isoform showed weaker binding to β-tubulin, whereas the other three isoforms showed similar degrees of interaction. Therefore, it seems that the binding is influenced by the two SNPs acting together rather than by one SNP alone. Because the Tyr169-Cys260 isoform is over-represented in osteoarthritis and shows weaker binding to β-tubulin, we infer that the interaction between DVWA and tubulin might protect joints from osteoarthritis. We could not identify a functional impact of rs9864422, but we do not rule out the possibility of its contribution to the susceptibility to osteoarthritis. It may influence the transcriptional activity of *DVWA* or nearby genes such as *CAPN7*.

Tubulin proteins and microtubules have essential roles in protein trafficking and secretion. Microtubules are also reported to regulate chondrocyte differentiation[24]. The addition of colchicine, an agent that depolymerizes microtubules, reduces the amount of collagen and glycosaminoglycan in chondrocytes[24]. Further, cartilage in a rat model of osteoarthritis shows a significant reduction in tubulin[25]. These

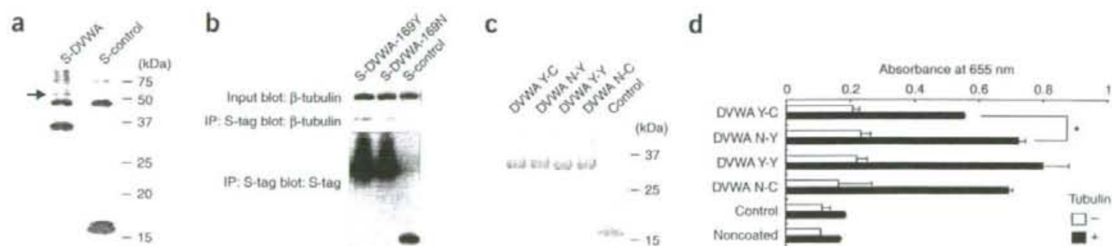

Figure 1 DVWA binds to β-tubulin. (**a**) SDS-PAGE and silver staining of samples immunoprecipitated with S-protein. Arrow indicates β-tubulin. (**b**) Confirmation of binding between DVWA and β-tubulin by protein blot. (**c**) Confirmation of DVWA recombinant proteins by SDS-PAGE. DVWA Y/N-C/Y denotes DVWA isoforms that have tyrosine (Y) or asparagine (N) at position 169, and cysteine (C) or tyrosine (Y) at position 260, respectively. (**d**) Binding strength of DVWA isoforms. Microplate wells coated with recombinant DVWA or controls were incubated with or without bovine tubulin. Data represent the mean ± s.d. in duplicate assays. The experiment was repeated three times with similar results. $*P < 0.01$ (Student's *t*-test).

observations suggest that tubulins and microtubules might be protective factors in osteoarthritis pathogenesis. We speculate that DVWA supports intracellular transport and affects osteoarthritis susceptibility by modulating the chondrogenic function of β-tubulin. Our findings will help identify osteoarthritis pathogenesis mechanisms and aid development of improved diagnosis, treatment and prevention methods.

METHODS

Subjects. We recruited individuals with knee osteoarthritis and control individuals in sets A and B through several medical institutes in Japan, as previously described[11]. All individuals in the osteoarthritis populations were over 40 years of age. Osteoarthritis was diagnosed on the basis of clinical and radiographic findings using previously described criteria[11,26,27]. Knee osteoarthritis populations include individuals with joint-space-narrowing grade 2 or higher[26]. For set C, we recruited population-based cohorts from inhabitants of Odai and Minami-ise town (previously Miyagawa village and Nansei town, respectively[10]) in the Mie prefecture in Japan. Each subject was classified into the knee osteoarthritis or control group on the basis of radiographic findings, as previously described[10]. The Han Chinese knee osteoarthritis and control populations were recruited as described previously[11,12] from the Center for Diagnosis and Treatment of Joint Disease and the Center of Physical Examination at Drum Tower Hospital. Clinical parameters for the populations in this study are shown in **Supplementary Table 8** online. There was no significant difference in mean age, BMI and sex distribution for the genotypes of rs7639618 among the Japanese and Chinese populations studied (**Supplementary Table 1**). We obtained written informed consent from each subject as approved by the ethical committees of the SNP Research Center at RIKEN, the Medical School of Nanjing University and participating clinical institutes.

Genotyping of SNPs. We extracted genomic DNA from peripheral blood leukocytes of affected individuals and controls using standard protocols. We genotyped SNPs using the multiplex PCR–based Invader assay[28] (Third Wave Technologies) or TaqMan SNP genotyping assays (Applied Biosystems), or by direct sequencing of PCR products using ABI 3700 DNA analyzers (Applied Biosystems), according to the manufacturers' protocols. We checked the quality of genotyping data of the initial genome-wide screening and the replication study, and omitted SNPs whose genotyping success rate was lower than 90%, or whose Hardy-Weinberg equilibrium P value of the control population was lower than 0.01.

Statistical analysis. We estimated haplotype frequencies using the EM algorithm. We carried out statistical analyses for the association, haplotype frequencies and Hardy-Weinberg equilibrium and for calculation of linkage disequilibrium coefficients (D' and r^2) using Haploview software 3.32 (ref. 29) and Microsoft Excel. We selected tag SNPs using Haploview with a pairwise tagging mode. We analyzed the effect of each haplotype to the disease using THESIAS[20]. Also, we analyzed effect of SNP-combination haplotypes by adding SNPs incrementally (in a stepwise manner) and by comparing their conditional log-likelihoods calculated by THESIAS with Akaike criterion (AIC).

Cell culture and RNA extraction. We cultured HEK293, chondrogenic HCS-2/8 and OUMS-27 cells in DMEM containing 10% FBS (FBS) at 37 °C under 5% CO_2. Normal human articular chondrocytes (NHAC-kn; Cambrex) were purchased and maintained in the supplied medium of the kit. We extracted mRNA for RACE, RT-PCR and RNA blot analysis from cultured cells using the FastTrack 2.0 Kit (Invitrogen). Total RNA from cultured cells was extracted using Isogen (Nippongene) and SV Total RNA Isolation System (Promega).

We obtained knee osteoarthritis cartilage from total knee arthroplasties (7 samples). Normal cartilage was obtained from the femoral heads of control individuals during surgery for femoral neck fractures (8 samples). None of the control individuals had a clinical history or radiographic signs of osteoarthritis. We extracted total RNAs from cartilage using the RNeasy Lipid Tissue Kit (Qiagen).

RACE, RT-PCR and real-time PCR. We carried out 5′ and 3′ RACE using the SMART RACE cDNA Amplification Kit (Clontech) according to the manufacturer's protocol. We used mRNA (1 µg) of NHAC to produce the RACE template. Multiple Tissue cDNA Panels (Clontech) were used to examine all tissues other than cartilage. cDNA from cartilage and cell lines was synthesized for RT-PCR and real-time PCR using Multiscribe reverse transcriptase and an oligo-dT primer (Applied Biosystems). We carried out quantitative real-time PCR using an ABI PRISM 7700 sequence detector with the Quantitect SYBR Green PCR Kit (Qiagen) in accordance with the manufacturers' instructions.

RNA blotting. We cloned the cDNA fragment corresponding to nucleotides 510–1,517 of *DVWA* into the pCR2.1TOPO vector (Invitrogen). The DIG-labeled probe was synthesized from the constructed vector using the DIG RNA Labeling Kit (Roche). We used 4 µg of NHAC, HCS-2/8 and OUMS-27 mRNA for gel electorophoresis. Transfer, hybridization and detection were done using the DIG Easy Hyb and DIG Wash and Block Buffer set (Roche) according to the manufacturer's instructions.

Luciferase assay. We determined the core promoter region of *DVWA* and cloned a native *DVWA* promoter (nucleotide −342 to +34; the number is from the transcription start site of *DVWA*) into pGL3-basic vector (Promega), which contained the firefly luciferase gene (*Luc*). We also cloned 675-bp fragments from the genomic sequence around rs9864422 (rs9864422 fragment) that contained either allele of the SNP. Using the native *DVWA* promoter, we constructed two kinds of vectors for each allele of rs9864422 that contained the rs9864422 fragments 5′ or 3′ to the luciferase gene: (from upstream) *DVWA* promoter-rs9864422 fragment-Luc and *DVWA* promoter-Luc- rs9864422 fragment. We constructed similar vectors containing the SV40 promoter instead of the *DVWA* promoter. We transfected cells (5×10^4) with 0.4 µg of the constructed pGL3 vectors and 4 ng of the pRL-TK vector as an internal control, using TransIT-293 (for HEK293) or TransIT-LT1 (for other cell lines) reagent (Mirus). After 48 h, we collected the cells and measured luciferase activity using the PicaGene Dual Sea Pansy system (Toyo Ink).

Immunoprecipitation. The entire coding sequence of *DVWA* was cloned into the pTriEx4 vector (Novagen), which expresses N-terminal S-tagged DVWA in mammalian cells. The vector or pTriEx4 alone (which expresses S-tagged artificial control protein) was transiently transfected into HCS-2/8 or HEK293 cells. Immunoprecipitation was done on cell lysates using S-protein agarose (Novagen) according to the manufacturer's instructions. Following SDS-PAGE, target protein bands were analyzed by MALDI/TOF mass spectrometry at APRO Life Science, or by protein blotting using antibody to β-tubulin (Santa Cruz) and S-protein-HRP (Novagen).

Recombinant protein and solid-phase binding assay. Rosetta (DE3) pLacI (Novagen) was transformed with pTriEx4-*DVWA* and cultured in the Overnight Express Autoinduction System (Novagen). We extracted recombinant DVWA protein using BugBuster Protein Extraction Reagent (Novagen), and refolded it from an insoluble fraction using Protein Refolding Kit (Novagen). Maxisorp ELISA plate (Nunc) wells were coated with 100 µl of 50 µg/ml recombinant S-tagged DVWA protein in 50 mM $NaHCO_3$ buffer (pH 9.6) at 4 °C overnight. Wells were blocked with 100 µl of 5% BSA in PBS; 5 µg of bovine tubulin (Cytoskeleton) was added in a total volume of 100 µl of 5% BSA in PBS and incubated overnight at 4 °C. Wells were washed three times with 20 mM Tris-HCl (pH 7.5), 137 mM NaCl and 0.05% Tween 20 (TBST) and incubated with β-tubulin-HRP antibody (Santa Cruz) for one hour. After washing five times with TBST, we assayed bound tubulin using the TMB Peroxidase EIA Substrate Kit (Biorad).

Accession codes. DNA Data Bank of Japan: *DVWA* mRNA sequence, AB299979. GenBank: *LOC344875*, XM_497913.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS
Y.M. carried out the Japanese knee osteoarthritis association study and *in vitro* functional assay together with M.N. and prepared the manuscript. D.S. carried out the Chinese association study. K.O., A.S., A.K., A.U., N.F., Y.N. and T.Tanaka managed DNA sample and clinical information and contributed data interpretation. A.T. and T.Tsunoda helped with statistic analysis. Q.J. managed the Chinese association study. S.I. planned and supervised the whole project.

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A Randomized Study Assessing the Efficacy of Communication Skill Training on Patients' Psychologic Distress and Coping

Nurses' Communication With Patients Just After Being Diagnosed With Cancer

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BACKGROUND. Although studies have shown the usefulness of improving health professionals' communication skills by training, to the authors' knowledge none have demonstrated the efficacy of communication skill training (CST) for health professionals in terms of improving patient outcomes. This study aimed to assess the efficacy of CST for nurses in improving psychologic distress and coping among patients after being informed of a cancer diagnosis.

METHODS. Nurses who mainly provide patients with psychologic and informational support after being informed of their cancer diagnosis by physicians at a cancer screening center were randomly assigned to either an experimental or a control group; patients were supported by either group of nurses. Patient selection criteria were: age >18 years with gastric, colorectal, or breast cancer that was not in advanced stage. Intervention consisted of 3 1-on-1 nurses' interviews (on the day of, 1 week after, and 1 month after diagnosis). Efficacy was assessed through patients' psychologic distress and coping by administering the Hospital Anxiety and Depression Scale (HADS) and Mental Adjustment to Cancer scale (MAC), at 3 time points (1 week, 1 month, and 3 months after diagnosis).

RESULTS. Eighty-nine patients participated. Repeated measures analysis of variance demonstrated a significant group-by-time decrease in patients' psychologic distress on HADS ($P = .03$), and significant group-by-time increase in fighting spirit and decrease of fatalism ($P = .01$ and $P = .04$, respectively), in addition to significant between-group difference of anxious preoccupation on the MAC ($P = .003$).

CONCLUSIONS. Support by nurses who completed the CST program was found to reduce psychologic distress and improved coping long term among patients informed of their cancer diagnosis. ***Cancer* 2008;113:1462–70.** *© 2008 American Cancer Society.*

KEYWORDS: communication skill training, psychological distress, coping, randomized study.

Communicating effectively in delivering bad news is reportedly an especially important skill for nurses in oncology, because they encounter many opportunities to confirm difficult information.[1,2] Nevertheless, the majortity of oncology nurses experience difficulty in communicating, for example, when supporting patients and families given bad news such as a cancer diagnosis.[3,4]

Communication skill training (CST) methods have been developed for nurses in Western countries to enable better communication skills (CS) in oncology practice,[4-7] and their effectiveness has been evaluated and summarized in several reviews.[8-12] Although

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these reviews found that participants' confidence and attitudes toward communication with patients improved after CST, to our knowledge, no studies have yet demonstrated the efficacy of CST for health professionals in improving patient outcomes such as psychologic distress and adjustment to cancer. Studies and reviews have suggested several reasons for this, including lack of randomized controlled design, evaluation of patient psychosocial outcomes, uniformity and sufficient duration of programs, and homogeneity of the study sample.[4-6,8-12] In light of these suggestions, we based our CST program on programs developed in the West and made it longer for nurses. In addition, our eligibility criteria were aimed at selecting homogenous groups of patients based on age, cancer site, cancer stage, and the type of bad news received (first-time cancer diagnosis). We then investigated the efficacy of CST for homogeneous patients' outcomes.

The purpose of the current study was to investigate in a randomized design whether a CST program to assist nurses would be useful to reduce psychologic distress and improve coping among patients informed of a cancer diagnosis just after a cancer screening test.

MATERIALS AND METHODS

Subjects

We chose cancer screening institutions in eastern Japan, which have a follow-up system by nurses after physicians have informed patients of their cancer diagnosis. At first, we approached 4 institutions and visited head nurses to ask them to participate. The head nurses who agreed allowed us to contact nurses who worked in the follow-up system. Consent was obtained in writing from each of them.

Patients were selected from among those who had undergone a cancer screening test and had the support of nurses after a physician consultation informing them of a cancer diagnosis. The study protocol was reviewed and approved by the Institutional Review Board and the Ethics Committee of Tokyo Metropolitan University.

Patient eligibility criteria for the study were 1) newly diagnosed and informed of cancer by a physician consultation after undergoing a cancer screening test within the study period (from January through December 2006); 2) nurse in charge present at the physician consultation; 3) age >18 years; 4) disease that was not advanced and was at an operable stage; 5) diagnosis of gastric, colorectal, or breast cancer, the 3 major sites most tested at the center, where nurses usually provide support for all these

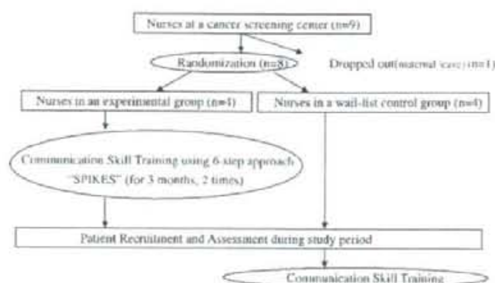

FIGURE 1. The nurses randomization procedure is shown. SPIKES indicates S, setting up the interview; P, assessing the patient's perception of the illness; I, obtaining an invitation by the patient to disclose information; K, giving knowledge and information to the patient; E, addressing the patient's emotions with empathic responses; and S, strategy and summary.

patients after cancer diagnosis; and 6) written informed consent.

Patients were excluded if they had not been informed of a cancer diagnosis by physicians and had severe psychologic status based on physician assessment.

Study Design

The efficacy of the CST program for nurses on patient outcomes was assessed by assigning nurses randomly either to the experimental groups or to the wait-listed control groups (Fig. 1). Before the study, nurses in the experimental group attended a 1-day CST program twice in the course of 3 months (October and December of 2005). Nurses assigned to the wait-listed control group were invited to participate in the CST program after the study period.

The study was explained during the study period to patients who met the eligibility criteria, and they were consecutively asked to participate by nurses in charge of each group. It was explained to the patients that: 1) the study was performed to develop a better nursing support style within the usual care method or a new method using CS by nurses; 2) the participants would be randomly assigned to either group using the usual method or a new method, and participants would be blinded to their assignment; and 3) they would have 3 interviews by nurses and 3 assessments. Patient recruitment and first-time assessment were initiated at T1 (1 week after cancer diagnosis), because the manager of the institution participating in this study (Tokyo Metropolitan Cancer Screening Center) did not approve of starting the patient assessment at T0 (on the day of cancer diagnosis). Patients in the experimental group were inter-

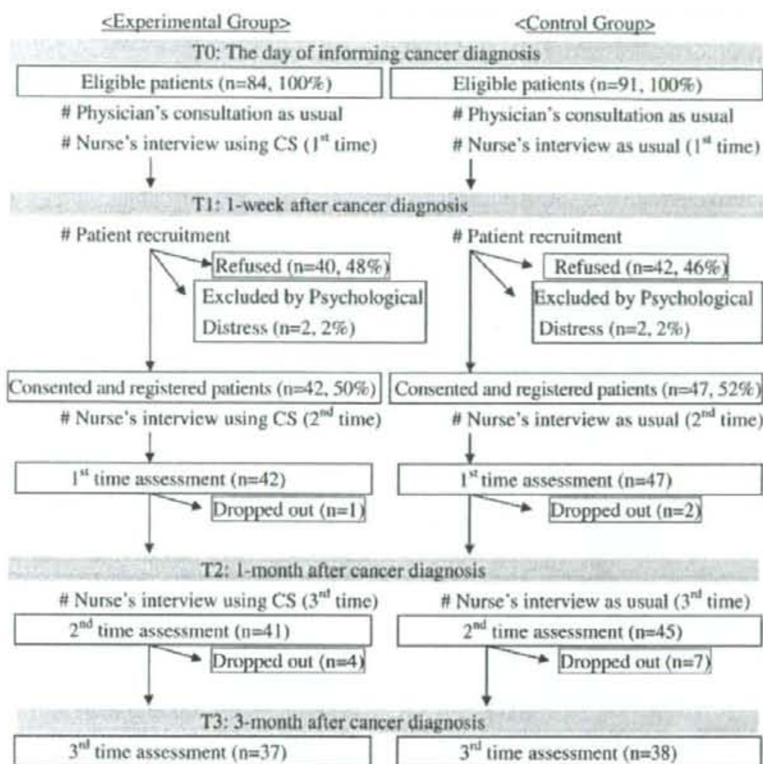

FIGURE 2. The patient recruitment and assessment procedure is shown. CS indicates communication skills.

viewed 3 times by nurses who attended the CST program, and controls were also interviewed at the same 3 times by nurses in the control group in the usual support system. In the conventional system, nurses in charge are always present at physician consultations when patients are informed of a cancer diagnosis. The physicians then entrust nurses with the support of patients thereafter. In the system, patients were routinely supported at 1 week and 1 month as well as on the day of the diagnosis. In our protocol, therefore, the interviews were scheduled on the day of cancer diagnosis (T0 [baseline]), 1 week after the diagnosis (T1), and 1 month after the diagnosis (T2), through 1-on-1 psychologic and informational support (Fig. 2).

CST Program for Nurses

The program consisted of 2 workshops: 1 at the start and the other at the end of the 3-month period. The program was based on those developed by Baile et al[13,14] and Fujimori et al.[15] The workshop was highly structured and brief, lasting 6 hours each time, and the study focused on CS for nurses. A step-by-step approach is used consisting of 6 steps, referred to by the acronym SPIKES: S, setting up the interview; P, assessing the patient's perception of the illness; I, obtaining an invitation by the patient to disclose information; K, giving knowledge and information to the patient; E, addressing the patient's emotions with empathic responses; and S, strategy and summary (Fig. 1).

The program began with a large group meeting for 2 hours, focusing on theoretic information, at which participants were lectured on the impact of communication between patients and health professionals, the principles of CS for breaking bad news, and how to handle distress in cancer patients. In addition, nurses were given original educational materials and a checklist to unify and confirm their CS.

Nurses were then divided into small groups of 2 or 3, in which 1 or 2 facilitators were assigned. One participant volunteered to play a nurse and another to play a patient in various scenarios involving a nurse's follow-up situation. We prepared the following 3 scenarios, which took into account a suitable Japanese medical system and culture: the first is a young female diagnosed with early-stage breast cancer; the second is a man diagnosed with advanced gastric cancer; and the third is an elderly person diagnosed with severe inoperable cancer whose daughter is the main caregiver. The "nurse" conducted a simulated interview with the "patient" according to the SPIKES steps, and other small group members acted as commentators. Facilitators urged the participants to discuss their concerns and uncertainties in these role-play scenarios. All 3 nurse facilitators had >5 years of counseling experience in oncology, and they also had received 2 days of training in facilitating workshops on CS.

Patients in the control group had the same interviews as the experimental group by control-group nurses in the usual support system.

Patients' Sociodemographic Data

Data regarding patient demographic and clinical characteristics (age, sex, employment status, marital status, number of family members living together, number of confidants, satisfaction with social support, cancer site, cancer stage, and whether they had undergone surgery) were obtained by reviewing patient records.

Patients' Psychologic Distress and Coping

Efficacy was assessed through patients' psychologic distress and coping by administering the Hospital Anxiety and Depression Scale (HADS) and Mental Adjustment to Cancer (MAC) scale 3 times: 1 week after diagnosis (T1), 1 month after (T2), and 3 months after (T3).

The HADS is a 14-item self-rating scale,[16] with each item rated on a scale of 0 to 3. Higher scores indicate a greater tendency to anxiety and depression. The Japanese version of HADS has been demonstrated to have adequate validity and reliability.[17,18]

The MAC scale is a 40-item self-rating scale used to assess cancer patients' coping style. It consists of 5 subscales: fighting spirit, anxious preoccupation, fatalism, helplessness/hopelessness, and avoidance. Each item is rated on a scale of 1 to 4, ranging from "definitely does not apply to me" to "definitely applies to me," with higher scores indicating a greater tendency to adopt that coping style. Previous studies indicated that the MAC scale has adequate validity and reliability.[19,20] The Japanese version of the MAC scale also showed adequate validity and reliability.[21]

Nurses, Physicians, and Interview Characteristics

Data regarding physicians' age and sex, nurses' age and sex, physicians' and nurses' experience in oncology practice (in years), time of physician consultation informing the patient of their cancer diagnosis, time of nurse interview after physician consultation, and whether the physician/nurse had some previous CST within the last year were obtained by reviewing the nurses' checklist.

For the experimental group, interviews were all audiotaped and transcribed. The transcripts were then assessed by 2 trained investigators to determine the number of CS used within the 6-step SPIKES. The 2 investigators rated each interview independently. If the results were different, the investigators had a discussion and decided the number of CS used in that interview. For the control group, we asked the nurses about the contents of their interview with the patients.

Statistical Analysis

Demographic and clinical data of the patients, nurses, and physicians as well as psychologic scores of each assessment time (T1-T3) were tested by the Student t test, chi-square test, or Mann-Whitney U test to assess comparability between the groups.

Effects of an intervention on each measure were assessed using repeated measures analysis of covariance (ANCOVA) to test for a difference between the experimental group and the control group over time, after adjusting for variables related with $P < .10$ between groups in the univariate analysis. Group and group-by-time changes were processed using ANCOVA. The P value was set at .05, and all data analyses were conducted using the SAS statistical software (version 9.1; SAS Institute, Inc, Cary, NC).

RESULTS

Sample of Institution, Nurses, and Physicians

A cancer institution, Tokyo Metropolitan Cancer Screening Center, consented to participate in the study. All of 9 follow-up nurses consented to participate in the study and completed the program. In this screening center, 18 other nurses provide technical support for potential cancer patients undergoing screening tests.

Because a nurse went on a maternity leave just after attending the CST program, 8 nurses were ran-

TABLE 1
Demographic Characteristics of Nurses and Physicians

	Mean±SD or No. (%)
Nurses' characteristics (n=8; 100%)	
Sex	7 (88)
Age, y	40.8±7.2
Clinical experience, years in oncology practice	17.2±6.87
Attendance to CST workshops within the last year	0 (0)
Physicians' characteristics (n=12; 100%)	
Sex	4 (33)
Age, y	42.6±9.4
Clinical experience, years in oncology practice	16.7±4.8
Attendance to CST workshops within the last year	0 (0)

SD indicates standard deviation; CST, communication skill training.

domly assigned to either group (4 to the experimental and 4 to the control) at the initiation of the study, and gathered patient data consecutively during the period. Twelve physicians provided consultations for registered patients in both groups. The demographic characteristics of nurses and physicians are listed in Table 1. Comparison of these characteristics in the experimental and control group demonstrated no statistically significant differences.

Patient Sample

Of the consecutive 175 eligible patients during the study period in both groups, 4 from both groups (2%) were excluded because of physicians' assessment of severe psychologic distress, and 82 (47%) refused to participate (57 because of the burden of participating in the study and 25 because of social barriers). Therefore, a total of 89 patients (51%; 42 in the experimental group, 47 in the control group) consented to participate after the explanation by the nurse in charge. Among these participants, 41 in the experimental group and 45 in the control group completed questionnaires more than twice (T1 and T2 assessments), including analyses of the study. Eleven patients (12.3%, 4 in the experimental group and 7 in the control group) dropped out after the second assessment, refusing further assessment (Fig. 2).

No statistically significant differences were found between subjects who consented to participate and those who refused to participate or dropped out with regard to age, social support, cancer stage, cancer site, and treatment. No statistically significant differences were found between the experimental and control groups within the patient demographic and clinical variables.

Interview Characteristics

In the experimental group, the mean number of CS used was 4.6 ± 1.5. All 4 trained nurses could conduct the interviews according to the 6-step SPIKES fairly well. In the 41 interviews by these 4 trained nurses for the experimental group patients, 22 (54%) interviews improved communication using all 6 steps. Ten (24%) of the remaining interviews did not sufficiently follow the second step "P: perception," 12 (29%) did not follow the third "I: invitation," and 9 (22%) did not follow the fourth "E: emotion." The other 3 steps were sufficiently followed in >80% of the total 41 interviews. In the interviews, the majority of patients expressed their negative experiences as well as satisfaction with the nurses' supportive guidance of communication. Examples of responses include "Although I could understand the cancer diagnosis given me by my physician, I was not sure what the physician said afterward. However, I could know what to do, after your calm and gradual help" and "Why did I have cancer? I panicked when I heard it. Your composed support was so helpful to me."

With regard to the interview characteristics between groups, the time of the nurse's interview at baseline (T0) was statistically different (mean time of experimental group: 21.9 ± 7.6 minutes; control group: 18.3 ± 8.0 minutes). Therefore, this variable was controlled in the multiple analysis of each of the outcomes. No other statistical differences were found between the groups with the variables in Table 2.

Conversely, all the control group nurses answered that they conducted interviews by passively reacting to patients' expressions. For example, they provided emotional support focused on what patients expressed, and they mentioned the cancer diagnosis again only when patients wished to confirm it. All 4 control group nurses also stated that they always communicated with patients in their own way, but had no confidence in it, because they never had a chance to learn the strategy.

Effect of Intervention on Patient Psychologic Distress and Coping

The repeated measures ANCOVAs, which controlled the time of the nurse's interview at baseline, revealed significant group-by-time differences in the depression score and the total HADS score ($P = .03$ and $P = .03$, respectively). No other difference over the study period was found for any HADS variables (Table 3).

On the MAC subscales, a significant between-group difference over the study period in the subscale of anxious preoccupation was revealed ($P = .003$). ANCOVA also revealed significant group-by-time differences in the fighting spirit and fatalism subscale scores ($P = .01$ and $P = .04$, respectively).

TABLE 2
Comparison of Patient Variables Between Groups

	Experimental, N=41 (100%), Mean±SD or No. (%)	Control, N=45 (100%), Mean±SD or No. (%)	P
Patient demographic characteristics			
Age, y	61.4±10.8	60.9±14.3	NS
Sex			NS
Male	16 (39.0)	18 (40.0)	
Female	25 (61.0)	27 (60.0)	
Employment status			NS
Working part or full time	25 (60.9)	25 (55.6)	
Unemployed	16 (39.1)	20 (44.4)	
Marital status			NS
Married	33 (80.5)	34 (75.6)	
Single/divorced/separated/widowed	8 (19.5)	11 (24.4)	
No. in family living together (including patients)	2.7 (1.6)	2.8 (1.4)	NS
No. of confidants	2.9±1.9	2.7±1.8	NS
Satisfaction with social support*	4.8±1.8	4.6±2.0	NS
Patient clinical characteristics			
Cancer site			NS
Gastric	21 (51)	19 (42)	
Colorectal	10 (24)	14 (31)	
Breast	10 (24)	12 (27)	
Cancer stage			NS
I	22 (54)	24 (53)	
II	14 (34)	18 (40)	
III	5 (12)	3 (7)	
Surgery undergone	38 (93)	43 (96)	NS
Interview characteristics			
Time of physicians' consultation informing of cancer diagnosis, min	16.1±11.6	6.0±10.0	NS
Time of nurses' interview after physicians' consultation, min			
T0	21.9±7.6	18.3±8.0	.04
T1	17.9±9.9	17.2±12.1	NS
T2	9.1±1.2	10.0±1.6	NS
No. of CS used within 6-step SPIKES through nurses' interview (range, 0-6)†	4.6±1.5	—	

SD indicates standard deviation; NS, not significant; T0, the day of the cancer diagnosis; T1, 1 week after the cancer diagnosis; T2, 1 month after the cancer diagnosis; CS, communication skills.

* Satisfaction with social support was estimated, with possible answers ranging from 1 (extremely dissatisfied) to 7 (extremely satisfied).

† Evaluation of communication skills of the nurse through the interview transcription by investigators (not evaluated for control-group nurses' interviews). SPIKES is an acronym for the following steps: S, setting up the interview; P, assessing the patient's perception of the illness; I, obtaining an invitation by the patient to disclose information; K, giving knowledge and information to the patient; E, addressing the patient's emotions with empathic responses; and S, strategy and summary.

No statistically significant between-group differences or group-by-time interactions were observed in other subscales (Table 4).

There were no significant differences among nurses in charge or physicians in both experimental and control groups in any variables measured by HADS and MAC for all 3 study periods (T1, T2, T3).

DISCUSSION

This randomized trial demonstrated the effects of a CST program for nurses on improving psychologic distress and coping style among patients who were informed of their cancer diagnosis just after a cancer screening test. To our knowledge, this is the first report to demonstrate the effect of CST for health professionals in improving cancer patient outcomes. One possible reason for this result may be the study design, which called for selecting homogeneous subjects.[4,8–12] We attempted to select homogeneous groups of patients and investigated the efficacy of CST for this population receiving the same bad news (first-time cancer diagnosis), and with the same cancer site and stage.

Another potential reason for the result may be the nurses' training schedule and Japanese nurses' basically lower CS in delivering bad news than in the West.[22] Several studies indicated that CST programs

TABLE 3
Effects of Nurses' Communication Skill on Patients' Psychologic Distress as Measured by the Hospital Anxiety and Depression Scale

	Time, Mean (SD)			Effects			
				Group		Group × Time	
Outcome (Range)	T1	T2	T3	F*	P	F*	P
Anxiety (0-21)†							
Experimental	4.7 (3.2)	4.2 (3.3)	3.3 (2.8)				
Control	4.5 (3.5)	5.2 (3.4)	4.4 (2.9)	1.02	.32	2.31	.11
Depression (0-21)‡							
Experimental	6.5 (3.1)	5.4 (3.0)	3.6 (2.7)				
Control	6.0 (3.5)	6.1 (3.4)	5.1 (3.6)	1.04	.31	3.65	.03
Total distress (0-42)§							
Experimental	11.3 (5.5)	9.6 (5.6)	6.9 (5.0)				
Control	10.6 (6.6)	11.3 (6.5)	9.5 (5.9)	1.22	.27	3.51	.03

SD indicates standard deviation; T1, 1 week after the cancer diagnosis; T2, 1 month after the cancer diagnosis; T3, 3 months after the cancer diagnosis.
* F statistic in repeated measures analysis of covariance, with the time of the nurses' interview at baseline as covariates.
† Higher scores indicate higher level of anxiety.
‡ Higher scores indicate higher level of depression.
§ Higher scores indicate higher level of psychological distress.

TABLE 4
Effects of Nurses' Communication Skill on Patients' Coping With Cancer Measured by the Mental Adjustment to Cancer Scale

	Time, Mean (SD)			Effects			
				Group		Group × Time	
Outcome (Range)	T1	T2	T3	F*	P	F*	P
Fighting spirit (16-64†)							
Experimental	48.2 (6.5)	51.9 (10.4)	50.4 (5.5)				
Control	50.9 (10.4)	49.4 (14.5)	51.1 (10.5)	0.10	.76	4.74	.01
Helplessness (6-24‡)							
Experimental	8.4 (2.0)	8.4 (2.9)	8.1 (2.2)				
Control	9.0 (3.1)	9.4 (3.9)	9.4 (3.5)	3.74	.06	2.43	.09
Anxious preoccupation (9-36‡)							
Experimental	22.2 (4.4)	22.1 (5.1)	20.7 (5.2)				
Control	23.9 (4.7)	23.8 (6.6)	22.8 (4.8)	9.25	.003	0.11	.89
Fatalism (8-32‡)							
Experimental	19.1 (4.2)	18.2 (4.8)	17.2 (4.9)				
Control	18.6 (5.0)	18.7 (5.6)	19.0 (4.7)	0.74	.39	3.30	.04
Avoidance (1-4‡)							
Experimental	2.4 (1.0)	2.4 (1.0)	2.2 (1.0)				
Control	2.1 (1.0)	2.1 (1.0)	2.2 (1.1)	0.81	.37	1.76	.18

SD indicates standard deviation; T1, 1 week after the cancer diagnosis; T2, 1 month after the cancer diagnosis; T3, 3 months after the cancer diagnosis.
* F statistic in repeated measures analysis of covariance, with the time of nurses' interview at baseline as covariates.
† Higher scores indicate more adaptive coping style.
‡ Higher scores indicate more maladaptive coping style.

must be longer and more intensive so that health professionals acquire satisfactory levels of skills.[4,11,23-25] A previous study reported that health professionals benefit by learning valuable communication strategies and reviewing demonstrations of skills in realistic clinical situations.[26] On the basis of these suggestions, we set a 3-month period for a CST program and nurses trained twice. In the current study, nurses may have been able to acquire CS by seeing examples of valuable communication strate-