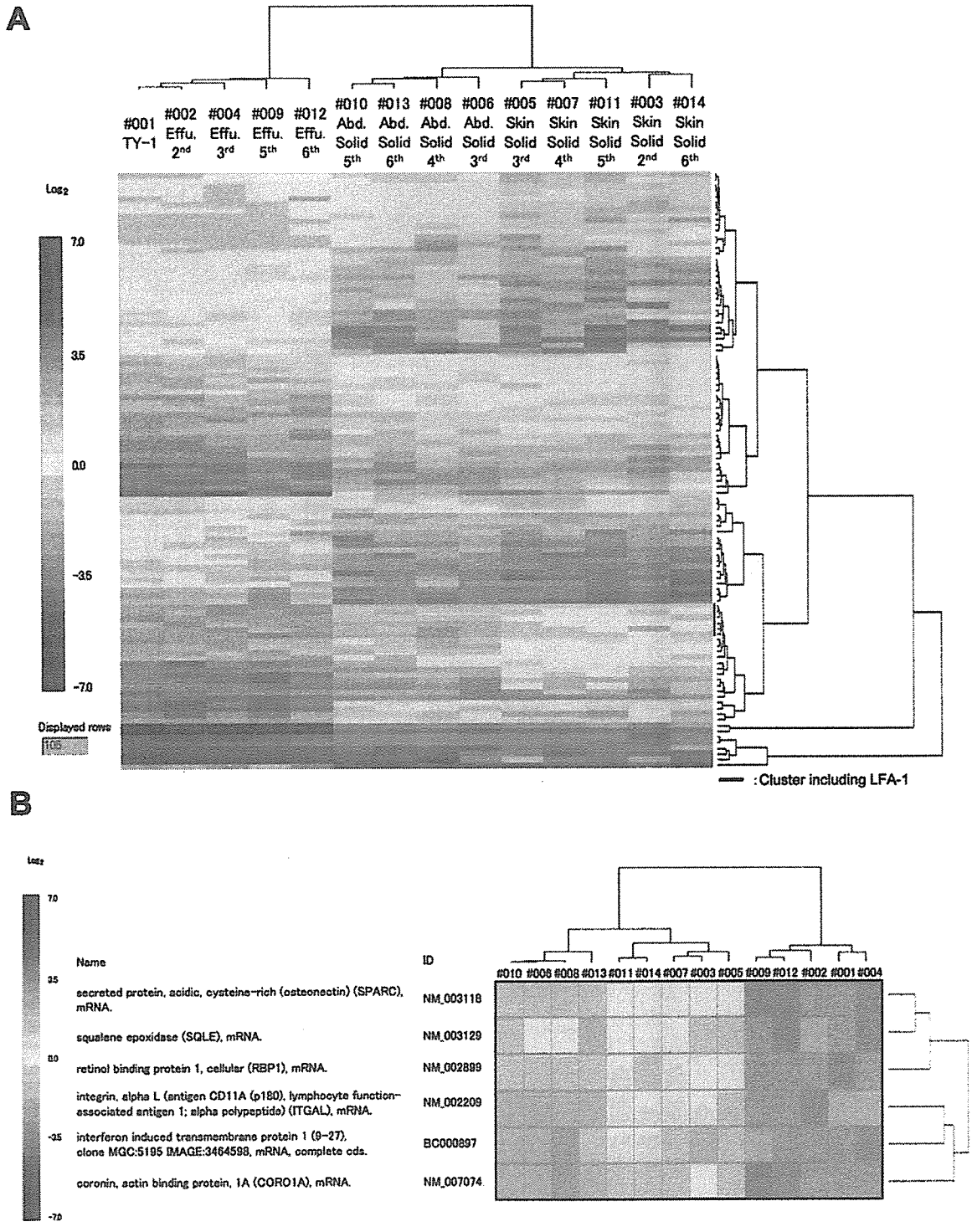

Figure 3. Cluster analysis and gene expression profiles in effusion and solid lymphomas. (A) Cluster analysis. The analysis was based on the expression profile of 105 genes identified as showing differences in expression between effusion and solid lymphomas by DNA microarray analyses. All 105 genes are shown and each row indicates the expression ratios of an individual gene in various samples. Red and blue colours indicate high and low expression, respectively, compared with the human common reference RNA sample. The sample numbers at the top correspond to the numbers in Figure 1B. The blue lines at the top and on the right indicate clusters. A violet line indicates a cluster including LFA-1. The raw data of the DNA microarray are available as supplementary Table 3 at http://www.interscience.wiley.com/jpages/0022-3417/suppmat/path.2012.html. (B) A cluster including LFA-1. Six genes are listed

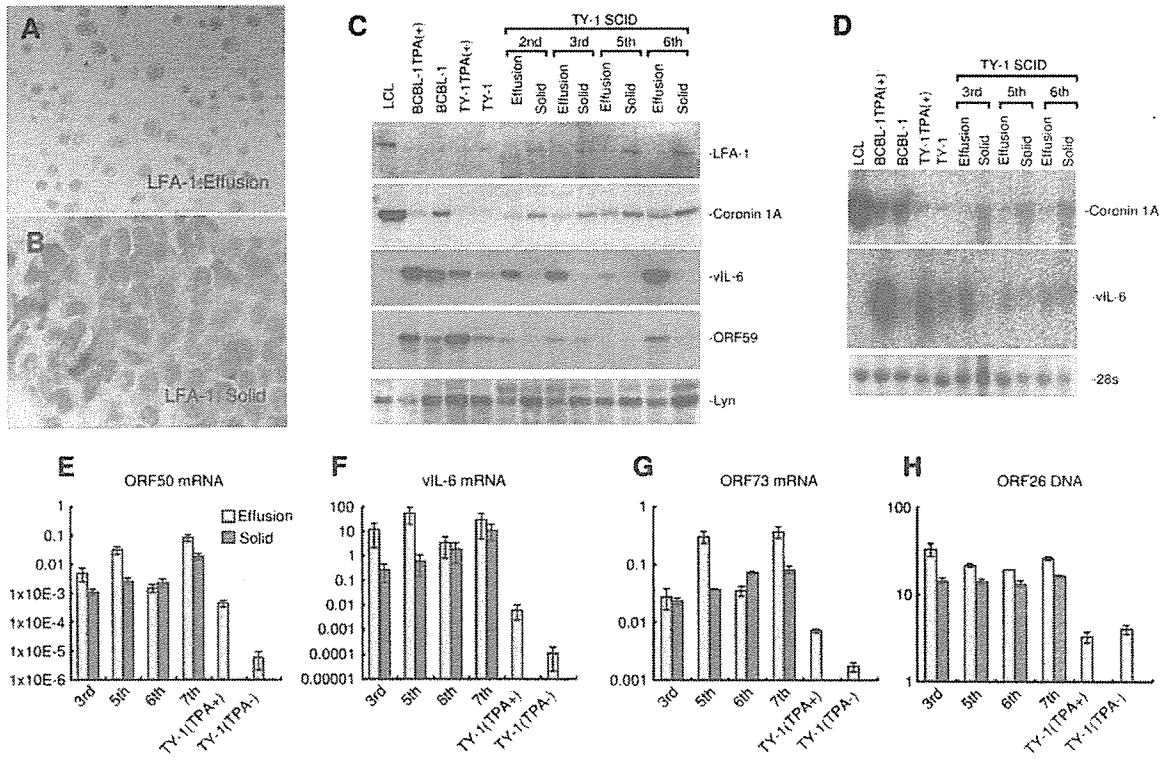

Figure 4. Expression of LFA-1, coronin 1A, and KSHV-encoded genes/proteins in effusion and solid lymphomas. (A, B) Immunohistochemistry of LFA-1. LFA-1 is expressed in solid lymphoma (B), but not in effusion lymphoma (A). (C) Western blotting. LFA-1, coronin 1A, KSHV-encoded vIL-6, and ORF59 were detected. Lyn was examined as an internal control. LCL = lymphoblastoid cell line. (D) Northern blotting. Coronin 1A and KSHV-encoded vIL-6 were detected. 28S ribosomal RNA is shown at the bottom. (E–G) Real-time reverse transcriptase (RT)-PCR for the KSHV-encoded ORF50, vIL-6, and ORF73 genes. The y-axis shows the copy numbers per cell. Effusion and solid lymphomas in the 3rd (sample Nos #004 and #005 in Figure 1B), 5th (#009 and #011), 6th (#012 and #014), and 7th (#015 and #017) passages were examined. The ratios to *GAPDH* mRNA are shown. The error bars indicate the standard deviations. (H) Real-time PCR for virus ORF26 DNA. The ratios to *GAPDH* DNA are shown. The y-axis shows the copy numbers per cell

model show a similar protein expression pattern to KSHV-associated solid lymphoma in humans [10], the results suggest that KSHV-associated solid lymphoma may also be categorized as a different disease entity from PEL in humans. Further studies of human cases are required to clarify the distinctive profiles. In addition, the genes and proteins with different expression profiles between effusion and solid lymphomas may be associated with the formation of effusion lymphoma or invasive features of solid lymphoma. Although some of the genes and proteins are categorized as adhesion molecules, many others have different or unknown functions (Table 1). The list of genes with different expression profiles identified in the present study will be useful for future analyses.

Signal transduction involving LFA-1 activation has been extensively investigated in T cells [15–17,37,38]. Activation of LFA-1 on the cell surface plays roles in various lymphocyte functions, especially adhesion. However, activation is different from expression. PEL cells do not express LFA-1 at all. LFA-1 expression is common among subtypes of lymphoma, but its down-regulation is rare and has only been reported in a few cases of lymphoma. For example, a few cell lines derived from adult T cell leukaemia/lymphoma do not express LFA-1, but do express other adhesion molecules, such as LFA-3 and intercellular adhesion molecule-1 (ICAM-1) [22,23]. Although some adhesion molecules are expressed by PEL cells [39], low expression of LFA-1 seems to be common in PEL cells, and such a restricted expression of LFA-1 may be important for the formation of effusion lymphoma [11,24–26]. Our mouse model of effusion and solid lymphomas was established by inoculating a single cell line originating from a single clone, implying that the origins of the two types of lymphoma were identical. Therefore, what factor could alter gene expression in this model? Endogenous factors, such as autocrine stimulation of cytokines, and exogenous factors, such as viral infection, can be considered. Cytokines and chemokines are known to be associated with the expression of adhesion molecules in cells [38], and to have various effects on adhesion molecules, including their induction and suppression. In our animal model, effusion lymphoma cells grew as peritoneal effusions that should have contained abundant cytokines. For the solid lymphoma inoculated into subcutaneous sites of SCID mice, different kinds of cytokines should

be present between the peritoneal cavity and subcutaneous tissue. In addition, the cells inoculated into the skin attached and stimulated each other in the subcutaneous space, which may induce the expression of adhesion molecules and structural proteins. Viral infection sometimes alters the expression of adhesion molecules [20,21]. Our results indicated that vIL-6 was expressed at a higher level in effusion lymphoma than in solid lymphoma. vIL-6 plays an important role in the pathogenesis of PEL and Kaposi's sarcoma [40–42]. Although the relationships between vIL-6 and adhesion molecules remain unknown, our data clearly demonstrate reciprocal expression patterns for vIL-6 and LFA-1. Further studies are required to clarify the association of vIL-6 expression with down-regulation of LFA-1.

Coronin was first identified as an actin-binding protein in *Dictyostelium discoideum* [43]. It has a WD-repeat motif that is involved in cell migration, cytokinesis, and phagocytosis [44]. Coronin 1A, a mammalian homologue of coronin, is expressed in haematopoietic tissues [43]. It contains five consecutive WD-repeat motifs within its *N*-terminal region and a leucine zipper domain at the *C*-terminus. In mouse T lymphocytes, coronin is involved in the dynamics of the actin cytoskeleton in response to T cell receptor stimulation and cell activation [45]. Our data demonstrated a much higher coronin 1A expression level in solid lymphoma than in effusion lymphoma, and its expression pattern was similar to that of LFA-1. To investigate the expression of coronin 1A in human clinical samples of solid lymphoma, we performed immunohistochemistry on solid lymphoma samples from 12 cases. Coronin 1A was found to be expressed in all samples (data not shown), suggesting that its expression is common in solid lymphoma. Since LFA-1 is also activated by T cell receptor stimulation [37], coronin 1A may be activated by a similar signal transduction mechanism to LFA-1 in solid lymphoma. Interestingly, coronin 1A expression was elevated in BCBL-1 cells, regardless of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) stimulation, but not in TY-1 cells (Figure 4). However, TY-1 cells expressed coronin 1A in the form of a solid lymphoma, suggesting that coronin 1A expression is not strictly associated with TPA stimulation. Furthermore, LFA-1 was not induced in BCBL-1 and TY-1 cells by TPA. Therefore, these data suggest the presence of a specific signal transduction mechanism that induces both LFA-1 and coronin 1A.

Proteomics and DNA microarray analyses are useful tools for comparing gene and protein expression profiles, respectively. However, few studies have used both methods to investigate differences in the gene and protein expression profiles of the same samples [46–48]. The combination of proteomics and DNA microarray analyses of the same samples provided us with new insights into the relationship between mRNA production and protein synthesis. Although coronin 1A was identified by both methods, other molecules were only identified by one method. However, when compared precisely, the results of the proteomics and DNA microarray analyses appeared to correlate with each other well (supplementary Table 1, available at http://www.interscience.wiley.com/jpages/0022-3417/suppmat/path.2012.html). Both methods detected similar trends of expression between effusion and solid lymphomas for 12 of 13 molecules (supplementary Table 1). These data imply that protein synthesis partly reflects, but does not correlate strictly with, mRNA production. Therefore, the combination of proteomics and DNA microarray analyses will provide useful information for elucidating accurate expression profiles of molecules.

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Supplementary material

Supplementary material may be found at the web address http://www.interscience.wiley.com/jpages/0022-3417/suppmat/path.2012.html.

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