Glycosylphosphatidyl inositol-anchored protein (*GPI-80*) gene expression is correlated with human thymoma stage

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Thymoma is one of the most common solid tumors in the mediastinum. Because there is no typical cell line for human thymoma, the development and use of molecular-based therapy for thymoma will require detailed molecular-genetic analysis of patients' tissues. Recent reports showed that genetic aberrations in thymoma were most frequently seen in chromosome 6q regions. We investigated the use of oligonucleotide arrays to monitor *in vivo* expression levels of genes in chromosome 6 regions in early- (stage I or II) and late- (stage IVa) stage thymoma tissues from patients. These *in vivo* gene expression profiles were verified by real-time quantitative reverse transcription polymerase chain reaction (RT-PCR) using LightCycler for 48 thymoma patients and sandwich ELISA for 33 thymoma patients. Using both methods, a candidate gene was identified which was overexpressed in stage IV thymoma. This was a known glycosylphosphatidyl inositol (GPI)-anchored protein (GPI-80), which is highly homologous with Vanin-1, a mouse thymus homing protein. Serum level of GPI-80 was confirmed to be elevated in stage IV thymoma compared with in stage I thymoma by using sandwich ELISA. The combined use of oligonucleotide microarray, real-time RT-PCR, and ELISA analyses provides a powerful new approach to elucidate the *in vivo* molecular events surrounding the development and progression of thymoma. (Cancer Sci 2003; 94: 809–813)

Thymoma is a neoplasm of thymic epithelial cells mixed with lymphocytes. Although thymomas are usually encapsulated, some are characterized by locally invasive growth, pleural dissemination, or extrathoracic metastasis. However, there seems to be no cytological difference between noninvasive thymoma and invasive/metastatic thymoma.[1–3] Several approaches have been made to differentiate non-invasive thymoma from invasive/metastatic thymoma, but the issue still remains controversial. Malignancy is generally evaluated in terms of the tumor's macroscopic invasiveness seen at the time of surgery.

The elucidation of the genetic events underlying the initiation and progression of human thymoma has been hampered by limitations inherent in both *in vitro* and *in vivo* methods of study. The most significant limitation of *in vitro*-based systems is that there is no typical cell line for human thymoma. Also, genetic information derived from cell lines may not accurately reflect the molecular events taking place in the tissues from which they were derived. Examining tumors for alterations in gene expression is a potentially useful approach to identifying molecular differences between early- and late-stage thymomas. The advent of high-density oligonucleotide microarray technology,[4] with its capacity for simultaneous monitoring of thousands of genes, provides a unique opportunity for high-throughput genetic analysis of tumors.[5–12]

A recent report showed that chromosome 6, especially 6q25, suffers frequent aberrations in thymoma.[13]

We examined differential gene expression at chromosome 6 in patients with invasive/non-invasive thymoma by means of the Biochip (microarray) method and real-time reverse transcription polymerase chain reaction (RT-PCR) analysis using LightCycler,[14] and identified glycosylphosphatidyl inositol (GPI)-anchored protein (GPI-80) as being overexpressed in advanced thymoma. We analyzed the data in relation to clinicopathological factors. We also examined serum GPI-80 levels by using sandwich ELISA for soluble GPI-80.

Materials and Methods

Patients. The study groups included 48 (26 male: 22 female) tissues from thymoma patients who had undergone surgery at the Department of Surgery II, Nagoya City University Medical School between 1996 and 2000. The thymomas were classified according to Masaoka's staging system.[15] Of the 48 cases, 7 were diagnosed with myasthenia gravis. All tumor samples were collected at resection and immediately frozen. To evaluate serum GPI-80 in thymomas, 33 serum samples from thymoma patients who had undergone surgery between 1994–1996 were included. Written informed consent was obtained from these patients. The sandwich ELISA system used to detect serum GPI-80 has been reported previously.[16]

Treatment of raw data from Affymetrix oligonucleotide arrays. Total RNA was extracted using an Isogen kit (Nippon Gene, Tokyo) according to the manufacturer's instructions. The cRNA transcript labeling reaction was performed using the Enzo Bioarray High Yield RNA transcript labeling kit (Enzo Diagnostics, Inc., Farmingdale, NY) and purified using RNeasy spin columns (Qiagen, Valencia, CA) and then hybridized to the Affymetrix Hum95000 array (Santa Clara, CA) for 16 h as described.[5, 8] The Affymetrix Hum95000 array contains about 12,625 features, each containing a DNA 20-mer oligonucleotide. Sequences from 12,625 full-length human cDNAs or expressed sequence tags (ESTs) that have some similarity to other eukaryotic genes are represented on a set of chips. In the following, we refer to either a full-length gene or an EST that is represented on the chip as EST. About 20 feature pairs represent each EST on the array. Each feature contains a 25-bp sequence, which is either a perfect match (PM), or a single central-base mismatch (MM). The hybridization signal fluctuates between different features that represent different 25-mer oligonucleotide segments of the same EST. Biochip data were analyzed by Genespring software (Silicon Genetics, Redwood City, CA)

Immunohistochemistry. Mouse monoclonal antibody against GPI-80 (clone 3H9) was produced by Sendo *et al*. at Yamagata

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University.[16, 17] Frozen sections (6 μm) were cut on a cryostat, placed on 3-aminopropyltriethoxysilane (APS)-coated glass microscope slides and air-dried at room temperature for 30 min. Endogenous peroxidase activity was blocked with 0.3% H_2O_2. Non-specific binding was blocked by Block Ace Solution (Dako Japan Co., Kyoto), and the slides were incubated with the monoclonal antibody against GPI-80 (1:2800) overnight at 4°C. Envision Kit (Dako Japan Co.) and 3,3-diaminobenzidine (DAB) substrate were used to visualize the antibody binding, and the sections were counterstained with hematoxylin.[17]

RT-PCR assays for GPI-80. RNA concentration was determined by absorbance measurement with a spectrophotometer and adjusted to a concentration of 200 ng/ml. RNA (1 μg) was reverse transcribed by Superscript II enzyme (Gibco BRL, Gaithersburg, MD) with 0.5 mg of oligo$(dT)_{12-16}$ (Amersham Pharmacia Biotech, Inc., Piscataway, NJ). The reaction mixture was incubated at 42°C for 50 min followed by incubation at 72°C for 15 min. To ensure the fidelity of mRNA extraction and reverse transcription, all samples were subjected to PCR amplification with oligonucleotide primers specific for the constitutively expressed gene *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) and data were normalized with respect to this value. All PCR reactions were performed using LightCycler-FastStart DNA Master SYBR Green I kit (Roche Molecular Biochemicals, Mannheim, Germany) and quantified. The primer sequences for the *GPI-80* gene were as follows: the forward primer, 5-GACTCCACATGTCCTCCTAA-3 and the reverse primer, 5-ACATGGAAATCTTTCACCAG-3. The cycling conditions were as follows: initial denaturation at 95°C for 10 min, followed by 50 cycles of 95°C for 15 s, 53°C for 5 s, and 72°C for 10 s. The primer sequences for the other genes are shown in Table 1. Amplified cDNAs were separated on 1% agarose gels, and the bands were visualized with ethidium bromide.

Statistical methods. Statistical analysis was done using the Stat-View software package (Abacus Concepts, Inc., Berkeley, CA). The Mann-Whitney *U* test was used to evaluate the significance of differences in expression in paired groups. Differences among the means of the stage and pathological subtypes in the patients with thymomas were examined using Fisher's method. Correlation between the mRNA levels and age was evaluated by means of Spearman's rank test. The criterion of significance was taken to be a *P* value of less than 0.05.

Results

cDNA array analysis. To demonstrate the feasibility of applying the technology to thymoma specimens, we examined differential gene expression at chromosome 6 between advanced thymoma (2 stage IVa cases) and early thymoma (1 stage I and 1 stage II cases) samples. Comparative differential gene expression analysis of advanced stage thymoma versus early stage thymoma revealed that 11 genes had significantly altered levels of expression by 2-fold or greater at chromosome 6. Of these 11 genes, 7 were on the p-arm and 4 on the q-arm (Table 2).

Validation of array data with real-time RT-PCR assays using LightCycler. To confirm the reliability of our array data, we measured the expression levels of four of the genes differentially expressed at chromosome 6q using LightCycler. Fig. 1 shows the differential expression pattern and the quantitative expression level of each of the four genes at chromosome 6q as determined by LightCycler between 22 stage I and 11 stage IV thymomas.

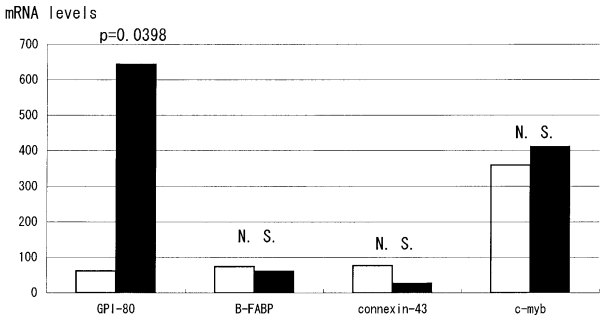

Fig. 1. RT-PCR analysis for 4 genes at chromosome 6q in stage I (□) and stage IV (■) thymomas. N.S.: not significant.

Table 1. Primer sequences for four Biochip-identified genes at chromosome 6q

	Forward	Reverse
GPI-80	GACTCCACATGTCCTCCTAA	ACATGGAAATCTTTCACCAG
B-FABP	CATTCAAGAACACGGAGATT	ACAAGTTTGTCTCCATCCAG
connexin 43	TGAACTTGCCTTTTCATTTT	CAGTTGAGTAGGCTTGAACC
c-myb	ATTCTTCATCAGCAGCATCT	CTTGGGGAAATTACTCCTTT

GPI-80, glycosylphosphatidyl inositol-anchored protein 80; *B-FABP*, brain fatty acid binding protein.

Table 2. Representative list of genes differentially expressed at chromosome 6 between invasive and non-invasive thymoma

Clone ID	GB	Description	Fold change	Map
34498_at	D899974	glycosylphosphatidyl inositol-anchored protein (GPI-80)	233.25	q23–24
39317_at	D86324	CMP-N-acetylneuraminic acid hydroxylase	7.495878	p22–23
1476_s_at	U22376	c-myb	6.480358	q22–23
31850_at	M90656	γ-glutamylcysteine synthetase	6.405943	p12
39331_at	X79535	β-tubulin	4.879784	p21.3
37561_at	AL031778	nuclear transcription factor Y	3.245321	p21.3
1261_i_at	M16594	glutathione S-transferase Ha subunit 2	3.153584	p12.2
38052_at	M14539	factor XIII	2.995713	p25.3–24
35185_at	AJ002962	fatty acid binding protein 7	2.931442	q22–23
36773_f_at	M81141	MHC class II HLA-DQ β	2.797017	p21.3
32531_at	X52947	connexin 43	2.71037	q21–23.2

GB, gene bank number; CMP, cytidine monophosphate.

A significant difference was observed for one of the four genes.

In the 48 thymomas studied, the GPI-80/GAPDH mRNA ratio was 132.568±30.908 (mean±SD). The relationship between the gene expression of *GPI-80* in thymoma and the patients' clinicopathological factors is shown in Table 3. There was no significant difference in the GPI-80 expression among patient groups stratified according to age and gender. There was no significant difference in GPI-80 expression between tumors in patients with myasthenia gravis (142.302±200.658) and in those without (252.384±907.296) (P=0.7469). The values of the GPI-80/GAPDH mRNA ratio were as follows: stage I, 60.439±87.468, stage II, 143.589±423.468, stage III, 160.229±241.280 and stage IV, 706.685±1676.985. There was a significant difference in GPI-80 expression between the stage IV and stage I (P=0.0398). Using Spearman's rank correlation, there was a correlation between GPI-80 expression and Masaoka's stages (P=0.0206). There was no significant difference of GPI-80 expression among the World Health Organization (WHO) classifications.

On the other hand, the values of connexin 43/GAPDH mRNA ratio were stage I, 75.960±122.890 and stage IV, 26.721±47.571, those of FABP/GAPDH mRNA ratio were stage I, 73.823±218.727 and stage IV, 60.295±179.141, and those of c-myb/GAPDH mRNA ratio were stage I, 358.772±213.495 and stage IV, 410.917±552.913. There was no significant difference in expression of these genes between patient groups stratified according to the clinical stages of thymoma.

Serum level of GPI-80 using sandwich ELISA. In 33 thymomas studied, the serum GPI-80 level was 37.912±39.814 ng/ml (mean±SD). The relationship between the serum GPI-80 level in thymoma and the patients' clinicopathological factors is shown in Table 4. There was no significant difference in serum GPI-80 levels between the normal controls (23.736±20.016 ng/ml) and thymoma (P=0.2208). There was no significant difference in serum GPI-80 level between patient groups stratified according to age and gender. There was no significant difference in the serum GPI-80 level between thymoma patients with myasthenia gravis (38.480±39.602 ng/ml) or without it (37.810±40.574 ng/ml) (P=0.6879). The serum GPI-80 levels were as follows: stage I, 16.879±10.484 ng/ml, stage II, 34.292±27.215 ng/ml, stage III, 32.668±18.479 ng/ml and stage IV, 62.235±58.922 ng/ml. There was a significant difference in the serum GPI-80 level between stage IV and stage I (P=0.0133) (Fig. 2). Using Spearman's rank correlation, there was a correlation between serum GPI-80 levels and Masaoka's stages (P=0.0161). There was no significant difference of GPI-80 levels among the WHO classifications. The serum GPI-80 levels in myasthenia gravis patients without thymoma were not elevated (39.455±35.082, n=9) (Fig. 2).

Although 14 cases overlapped between mRNA and protein analyses, the correlation of the levels was only marginal (R^2=0.8409, P=0.0931). To check GPI-80 protein expression in thymic cancer, samples from thymic cancers were tested. In thymic cancers, the serum level of GPI-80 (30.975±18.434 ng/ml) was not significantly different from the normal control value.

GPI-80 protein expression by immunohistochemistry. An immunohistochemical (IHC) approach was used to localize GPI-80

Table 3. Clinico-pathological data of 48 thymoma patients

Factors	GPI-80/GAPDH expression		
	Number (%)	mRNA levels	*P* value
Age			
50 Y≤	15 (68.8%)	455.597±1460.009	0.6550
50 Y>	33 (31.3%)	137.058±270.782	
Gender			
male	26 (54.2%)	343.751±1126.941	0.6550
female	22 (45.8%)	109.971±199.320	
MG			
MG+	7 (14.6%)	142.302±200.658	0.7469
MG−	41 (85.4%)	252.384±907.296	
Stage			
I	22 (45.8%)	60.439±87.468	IV vs. I
II	9 (18.8%)	143.589±423.468	0.0398
III	6 (12.5%)	160.229±241.280	
IV	11 (22.9%)	706.685±1676.985	
Pathoogical subtype			
polygonal	32 (66.7%)	305.726±1004.390	NS
mixed	11 (22.9%)	26.699±55.518	
spindle	5 (10.4%)	255.991±568.428	
Lymphocyte infiltration			
PD	23 (47.9%)	336.264±1185.532	NS
moderate	16 (33.3%)	138.406±176.613	
scant	9 (18.8%)	156.480±419.290	
WHO classification			
A	5 (10.4%)	255.591±568.428	NS
AB	11 (22.9%)	26.699±55.518	
B1	16 (33.3%)	477.432±1411.180	
B2	11 (22.9%)	183.268±192.127	
B3	5 (10.4%)	25.673±29.203	

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Y, years old; MG, myasthenia gravis; PD, predominant; NS, not significant.

Table 4. Clinico-pathological data of 33 thymoma patients

Factors	Serum GPI-80		
	Number (%)	GPI-80 levels (ng/ml)	*P* value
Age			
50 Y≤	7 (21.2%)	36.992±19.757	0.3326
50 Y>	26 (78.8%)	38.159±43.989	
Gender			
male	11 (33.3%)	29.693±23.228	0.7025
female	22 (66.7%)	42.021±45.885	
MG			
MG+	5 (15.2%)	38.480±39.602	0.6879
MG−	28 (84.8%)	37.810±40.574	
Stage			
I	9 (27.3%)	16.879±10.484	IV vs. I
II	12 (36.4%)	34.292±27.215	0.0133
III	2 (6.1%)	32.668±18.479	
IV	10 (30.3%)	62.235±58.922	
Pathological subtype			
polygonal	19 (57.6%)	45.111±48.540	NS
mixed	8 (24.2%)	32.634±25.244	
spindle	6 (18.2%)	22.150±15.202	
Lymphocyte infiltration			
PD	9 (27.3%)	48.046±63.171	NS
moderate	16 (48.5%)	32.708±25.804	
scant	8 (24.2%)	36.919±32.616	
WHO classification			
A	6 (18.2%)	22.150±15.202	NS
AB	8 (24.2%)	32.634±25.244	
B1	8 (24.2%)	51.528±66.603	
B2	8 (24.2%)	36.175±25.284	
B3	3 (9.1%)	51.831±53.756	

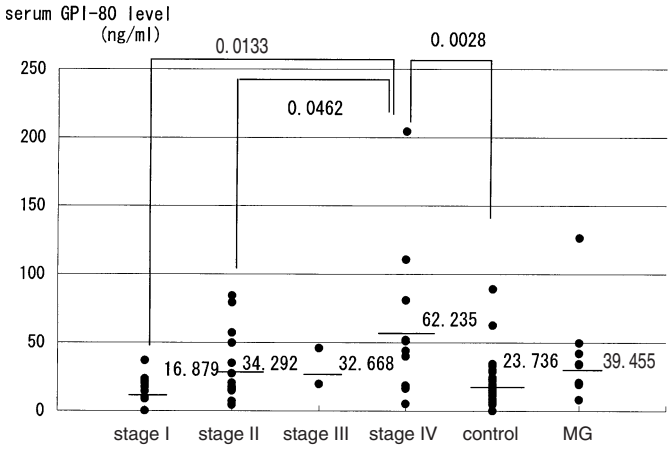

Fig. 2. Serum GPI-80 levels in thymoma and myasthenia gravis (MG) patients.

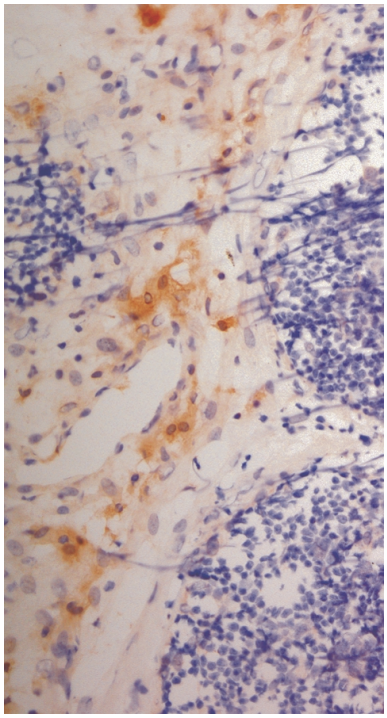

Fig. 3. GPI-80 protein expression evaluated by immunohistochemistry. GPI-80 protein expression was seen in stromal cells just surrounding the tumor.

protein expression in 30 thymomas. Thymoma cells and normal lymphocytes were not stained, but stromal cells just surrounding thymoma cells were stained (Fig. 3). There was no significant difference of GPI-80 mRNA level between GPI-80 IHC-positive (181.458±311.811) and GPI-80 IHC-negative (114.504±274.158) tissues (P=0.7150), although the tissues were taken from different parts for these analyses.

Discussion

This report represents the first investigation involving the genome-wide examination of changes in gene expression at chromosome 6 in clinical samples of early- and late-stage thymoma. In our cDNA microarray analysis, we identified differentially expressed genes. We validated the differential expression of several of the genes identified through Biochip analysis, by means of quantitative RT-PCR of RNA from 48 thymoma tumors. We found that GPI-80 was significantly higher in invasive thymoma (stage IV thymoma) than in stage I thymoma.

It has previously been shown that the GPI-80 is a possible regulatory molecule of cell adhesion and migration.[18–20] GPI-80 protein has a high sequence homology with Vanin-1,[21] which is expressed on perivascular thymic stromal cells and is involved in thymus homing in mice.[22] There is about 60% molecular homology between GPI-80 and Vanin-1, and these two molecules share a similar function in the sense that both may be involved in the regulation of leukocyte trafficking. In addition, GPI-80 may play an important role in the regulation of β2-integrin-dependent adhesion.[21, 23–25] Yoshitake *et al*. reported that cross-linking of GPI-80 induced up-regulation of CD11b/CD18 expression on neutrophil surfaces and shedding of L-selectin.[26] Because an inhibitor of PI-3 kinase blocks GPI-80-mediated tyrosine phosphorylation of pp34, PI-3 kinase may be important for the activation of Src protein tyrosine kinases (PTKs) upon cross-linking of GPI-80.[20]

Human thymoma is a peculiar epithelial neoplasm in which abnormal epithelium supports the differentiation of polyclonal T-lymphocytes.[27–30] Immunophenotypic studies have shown that most lymphocytes in thymoma are identical to normal cortical thymocytes.[27–29] It has already been demonstrated by conventional histology that thymomas contain lymphocytes with a stimulated or activated appearance, and some mitoses are also seen.[27–29] However, in our study, neither mRNA nor protein level of GPI-80 in thymoma correlated with lymphocyte infiltration of thymoma. Thus, we believe that expression of the gene was not correlated with normal lymphocytes.

The exact mechanism underlying the overexpression of GPI-80 during thymoma progression is unknown. Interestingly, GPI-80 protein level was not elevated in thymic cancers. Overexpression of GPI-80 at an advanced stage might be specific to thymoma. GPI-80 was reported to be expressed mainly on the surface of neutrophils and, to a lesser extent, on monocytes.[21, 23] In addition, GPI-80 is found on the plasma membranes and in the secretory vesicles of human neutrophils. Thus, GPI-80 is a cell surface or secretory protein. Vanin-1 protein, which has a high sequence homology with GPI-80,[21] is expressed on perivascular thymic stromal cells and our immunohistochemistry also showed that GPI-80 protein was expressed on stromal cells. If the stromal cells, but not the tumor cells, are the origin of GPI overexpression, there may be no rational basis for searching for abnormalities in the chromosome 6q region. However, the overexpression of both GPI-80 mRNA and protein in advanced thymoma and the nearly significant expression correlation between GPI-80 mRNA and protein suggested that GPI-80 expression might somehow be correlated with thymoma tumors.

We have demonstrated that *in vivo* subpopulations of neoplastic cells from multiple stages of thymoma progression can be simultaneously screened for thousands of genes. Here, we examined the feasibility of using Biochip high-throughput cDNA arrays to study *in vivo* gene expression profiling, and we confirmed through the use of hybridizations, RT-PCR assay using LightCycler, ELISA, and immunohistochemistry that this approach produced valid data. We believe that this *in vivo* functional genomic approach not only provides an evolving opportunity to rapidly and directly monitor *in vivo* gene expression in human thymoma, but also promises to provide novel insights into fundamental cancer biology. Furthermore, the application of this approach to clinical thymoma specimens may provide a key to rapid advances in thymoma prevention, detection, diagnosis, and therapeutics.

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1. Lardinois D, Rechsteiner R, Lang RH, Gugger M, Betticher D, von Briel C, Krueger T, Ris HB. Prognostic relevance of Masaoka and Muller-Hermelink classification in patients with thymic tumors. *Ann Thorac Surg* 2000; **69**: 1550–5.
2. Rios A, Torres J, Galindo PJ, Roca MJ, Rodriguez JM, Sola J, Parrilla P. Prognostic factors in thymic epithelial neoplasms. *Eur J Cardiothorac Surg* 2002; **21**: 307–13.
3. Sperling B, Marschall J, Kennedy R, Pahwa P, Chibbar R. Thymoma: a review of the clinical and pathological findings in 65 cases. *Can J Surg* 2003; **46**: 37–42.
4. Iyer VR, Eisen MB, Ross DT, Schuler G, Moore T, Lee JCF, Trent JM, Staudt LM, Hudson J Jr, Boguski MS, Lashkari D, Shalon D, Botstein D, Brown PO. The transcriptional program in the response of human fibroblasts to serum. *Science* 1999; **283**: 83–7.
5. Lockhart DJ, Dong H, Byrne MC, Follettie MT, Gallo MV, Chee MS, Mittmann M, Wang C, Kobayashi M, Horton H, Brown EL. Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nat Biotechnol* 1996; **14**: 1675–80.
6. DeRisi J, Penland L, Brown PO, Bittner ML, Meltzer PS, Ray M, Chen Y, Su YA, Trent JM. Use of a cDNA microarray to analyze gene expression patterns in human cancer. *Nat Genet* 1996; **14**: 457–60.
7. Pietu G, Alibert O, Guichard V, Lamy B, Bois F, Leroy E, Mariage-Sampson R, Houlgatte R, Soularue P, Auffray C. Novel gene transcripts preferentially expressed in human muscles revealed by quantitative hybridization of a high density cDNA array. *Genome Res* 1996; **6**: 492–503.
8. Wodicka L, Dong H, Mittmann M, Ho MH, Lockhart DJ. Genome-wide expression monitoring in *Saccharomyces cerevisiae*. *Nat Biotechnol* 1997; **15**: 1359–67.
9. DeRisi JL, Iyer VR, Brown PO. Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* 1997; **76**: 680–6.
10. Chu S, DeRisi J, Eisen M, Mulholland J, Botstein D, Brow PO, Herskowitz I. The transcriptional program of sporulation in budding yeast. *Science* 1998; **282**: 699–705.
11. Marton MJ, DeRisi JL, Bennett HA, Iyer VR, Meyer MR, Roberts CJ, Stoughton R, Burchard J, Slad D, Dai H, Bassett DE Jr, Hartwell LH, Brown PO, Friend SH. Drug target validation and identification of secondary drug target effects using DNA microarrays. *Nat Med* 1998; **4**: 1293–301.
12. Kallioniemi OP. Biochip technologies in cancer research. *Ann Med* 2001; **33**: 142–7.
13. Inoue M, Marx A, Zettl A, Strobel P, Muller-Hermelink H-K, Starostik P. Chromosome 6 suffers frequent and multiple aberrations in thymoma. *Am J Pathol* 2002; **161**: 1507–13.
14. Wittwer CT, Ririe KM, Andrew RV, David DA, Gungry RA, Balis UJ. The LightCycler: a microvolume multisample fluorimeter with rapid temperature control. *Biotechniques* 1997; **22**: 176–81.
15. Masaoka A, Monden Y, Nakahara K, Tanioka T. Follow up study of thymomas with special reference to their clinical stages. *Cancer* 1981; **48**: 2485–92.
16. Huang J, Takeda Y, Watanabe T, Sendo F. A sandwich ELISA for detection of soluble GPI-80, a glycosylphosphatidyl-inositol (GPI)-anchored protein on human leukocyte involved in regulation of neutrophil adherence and migration—its release from activated neutrophil and presence in synovial fluid of rheumatoid arthritis patients. *Microbiol Immunol* 2001; **45**: 467–71.
17. Koike S, Takeda Y, Hozumi Y, Okazaki S, Aoyagi M, Sendo F. Immunohistochemical localization in human tissues of GPI-80, a novel glycosylphosphatidyl inositol-anchored protein that may regulate neutrophil extravasation. *Cell Tissue Res* 2002; **307**: 91–9.
18. Sendo F, Araki Y. Regulation of leukocyte adherence and migration by glycosyl phosphatidyl-inositol-anchored proteins. *J Leukoc Biol* 1999; **66**: 369–74.
19. Dahlgren C, Karlsson A, Sendo F. Neutrophil secretory vesicles are the intracellular reservoir for GPI-80, a protein with adhesion-regulating potential. *J Leukoc Biol* 2001; **69**: 57–62.
20. Yu Y, Araki Y, Sendo F. Tyrosine phosphorylation of a 34-kD protein induced by cross-linking a novel glycosylphosphatidylinositol-anchored glycoprotein (GPI-80) on human neutrophils that may regulate their adherence and migration. *IUBMB Life* 2000; **49**: 43–7.
21. Suzuki K, Watanabe T, Sakurai S, Ohtake K, Kinoshita T, Araki A, Fujita T, Takei H, Takeda Y, Sato Y, Yamashita T, Araki Y, Sendo F. A novel glycosylphosphatidyl inositol-anchored protein on human leukocytes: a possible role for regulation of neutrophil adherence and migration. *J Immunol* 1999; **162**: 4277–84.
22. Aurrand-Lions M, Galland F, Bazin H, Zakharyev VM, Imhof BA, Naquet P. Vanin-1, a novel GPI-linked perivascular molecule involved in thymus homing. *Immunity* 1996; **5**: 391–405.
23. Ohtake K, Takei H, Watanabe T, Sato Y, Sendo F. A monoclonal antibody modulates neutrophil adherence while enhancing cell motility. *Microbiol Immunol* 1997; **41**: 67–72.
24. Suzuki H, Takei H, Ohtake K, Watanabe T, Sendo F. External calcium-dependent F-actin-independent and pertussis toxin-insensitive novel neutrophil locomotion induced by a mAb. *Int Immunol* 1997; **9**: 763–9.
25. Nakamura-Seto Y, Sasaki K, Watanabe H, Araki Y, Sendo F. Clustering on the forward surfaces of migration neutrophils of a novel GPI-anchored protein that may regulate neutrophil adherence and migration. *J Leukoc Biol* 2000; **68**: 650–4.
26. Yoshitake H, Takeda Y, Nitto T, Sendo F. Cross-linking of GPI-80, a possible regulatory molecule of cell adhesion, induces up-regulation of CD11b/CD18 expression of neutrophil surfaces and shedding of L-selectin. *J Leukoc Biol* 2002; **71**: 205–11.
27. Aisemberg AC, Wilkes B, Harris NL, Frist WH. The predominant lymphocyte in most thymomas and in nonneoplastic thymus from patients with myasthenia gravis is the cortical thymocyte. *Clin Immunol Immunopathol* 1985; **35**: 130–6.
28. Eimoto T, Teshima K, Shirakusa T, Takeshita M, Okamura H, Naito H, Mitsui T, Kikuchi M. Heterogeneity of epithelial cells and reactive components in thymomas: an ultrastructural and immunohistochemical study. *Ultrastruct Pathol* 1986; **10**: 157–73.
29. Chilosi M, Iannucci A, Menestrina F, Lestani M, Scarpa A, Bonetti F, Fiore-Donati L, Dipasquale B, Pizzolo G, Palestro G, Tridente G, Janossy G. Immunohistochemical evidence of activated thymocyte proliferation in thymoma. Its possible role in the pathogenesis of autoimmune diseases. *Am J Pathol* 1987; **128**: 464–70.
30. Levine GD, Polliack A. The T-cell nature of the lymphocytes in two human epithelial thymomas: a comparative immunologic, scanning and transmission electron microscopic study. *Clin Immunol Immunopathol* 1975; **4**: 199–208.