Expression profiling of esophageal squamous cell carcinoma patients treated with definitive chemoradiotherapy: Clinical implications

AKIO ASHIDA¹, NARIKAZU BOKU², KAZUHIKO AOYAGI¹, HIROSHI SATO¹, YASUHIRO TSUBOSA¹, KEIKO MINASHI², MANABU MUTO², ATSUSHI OHTSU², ATSUSHI OCHIAI³, TERUHIKO YOSHIDA¹, SHIGEAKI YOSHIDA² and HIROKI SASAKI¹

¹Genetics Division, National Cancer Center Research Institute, 1-1 Tsukiji 5-chome, Chuo-ku, Tokyo 104-0045; ²GI Oncology Division, and ³Pathology Division, Hospital East, National Cancer Center, Kashiwa-shi, Chiba 277-8577, Japan

Received December 12, 2005; Accepted February 8, 2006

Abstract. In esophageal squamous cell carcinoma (ESCC), chemoradiotherapy (CRT) has a curative potential even in cases of locally advanced carcinoma. However, only about half of the patients benefit from CRT, and an accurate prediction of sensitivity to CRT is eagerly awaited. Using microarrays, we analyzed gene-expression patterns of pretreatment biopsy specimens from 33 patients with CRT alone including longterm survivors, more than 3 years (14 cases) and short-term survivors, less than 1 year (11 cases). The expression patterns of about 12,600 genes were used to identify genes correlated with survival terms. Fifty-seven genes correlating with shortterm survival and 120 genes with long-term survival were identified. The genes involved in the immune response were characteristically upregulated in the long-term survivors, and an immunohistochemical staining confirmed an increased CD8positive T cell number in the long-term survivors over that in the short-term survivors. In the short-term survivors, on the other hand, increased expression of the genes involved in drug resistance was observed. Our gene list should contribute to the elucidation of the mechanisms of CRT response and contains useful markers for predicting the prognosis of individual ESCC patients treated with CRT alone.

Introduction

Esophageal cancer in East Asian countries including Japan and China, and in some parts of Europe consists mainly of squamous cell carcinomas located mostly in the thoracic esophagus, while adenocarcinoma in the distal part of the

E-mail: hksasaki@gan2.res.ncc.go.jp

esophagus has increasingly become the major pathological type found in Europe and North America. Overall, esophageal squamous cell carcinoma (ESCC) is the sixth most frequent cancer in the world, and is a highly virulent disease. Although surgery is the standard therapy for locally confined ESCC, results of surgery alone remain poor, with 5-year survival rates of 6-24% in Western countries (1). Recent improvement in surgical results following radical node dissection has been reported at some Japanese institutions, with 5-year survival rates of 31-55% (2,3). In a Japanese prospective randomized study that compared surgery alone with surgery followed by adjuvant chemotherapy, the group that received surgery with radical dissection alone showed a 5-year survival rate of 45%, although the study did not show a survival advantage over treatment with adjuvant chemotherapy (4). The success of radical surgery led many Japanese surgeons to extend the indications of surgery to locally advanced carcinoma. On the other hand, a combination of 5-fluorouracil (5-FU) and cisplatin (CDDP) has become a standard world regimen, not only because of its clinical outcome, but also because of the synergism between the two agents and their radiosensitizing effects (5-7). Recent studies clearly indicated that definitive chemoradiotherapy (CRT) seemed to have a curative potential even in cases of locally advanced carcinoma (8), although the treatment may be associated with significant toxicity (9). The reported 5-year survival rate is 27% or 29% (9,10). Despite recent progress in the molecular genetics of ESCC, little is known about the mechanisms of sensitivity to CRT, and a prediction of the outcome of CRT by clinicopathological terms still remains very difficult. We conducted gene expression profiling using an oligonucleotide microarray of pretreatment biopsy samples from 33 ESCC patients treated with CRT alone. The purpose of the study was to explore the underlying mechanisms of CRT responses and to contribute to the development of the markers, which aid to select the best therapeutic modality prior to the initiation of therapy.

Materials and methods

RNA isolation from biopsy specimens. Patients received protracted infusion of 5-FU 400 mg/m²/24 h on days 1-5 and

Correspondence to: Dr H. Sasaki, Genetics Division, National Cancer Center Research Institute, 1-1 Tsukiji 5-chome, Chuo-ku, Tokyo 104-0045, Japan

Key words: microarray, esophageal cancer, chemoradiotherapy, prognosis

8-12, 2-h infusion of CDDP 40 mg/m² on days 1 and 8, and concurrent radiation therapy at a dose of 30 Gy in 15 fractions over 3 weeks. Filgrastim was prophylactically administered to all patients. This schedule was repeated twice every 5 weeks, for a total radiation dose of 60 Gy, followed by two courses of 5-FU (800 mg/m²/24 h for 5 days) and CDDP (80 mg/m² on day 1). Biopsy samples, which were obtained from patients before CRT, were immediately frozen at -80°C until use. The samples were homogenized in Isogen lysis buffer (Nippon Gene Co., Ltd., Toyama, Japan) at room temperature, extracted with chloroform, and precipitated with 20 μ g glycogen in isopropanol. The RNA pellet was resuspended in RNase-free water, and treated with RNase-free DNase I in the presence of RNase inhibitor followed by phenol extraction and precipitation in isopropanol. The pellet was resuspended in 15 μ l of RNasefree water; one-third of the total RNA from each sample was used for quality analysis of RNA by RNA LabChip (Caliper Technologies Corp., CA), and an appropriate amount from the remaining two-thirds was used for oligonucleotide microarray analysis. This study with biopsy specimens was approved by our institutional review boards.

Microarray analysis. We used human U95A oligonucleotide probe arrays (Affymetrix, Santa Clara, CA) for analysis of mRNA expression levels corresponding to 12,600 transcripts. The procedures were conducted according to the supplier protocols, and are thus described briefly. Each 5 μ g of total RNA was used to generate a cRNA probe. Ten microgram of fragmented cRNA was hybridized to the microarrays in 200 μ l of a hybridization cocktail at 37°C for 16 h in a rotisserie oven set at 60 rpm. The arrays were then washed with a low stringent wash buffer (6X SSPE) at 25°C, followed by stringent wash buffer [100 mM MES (pH 6.7), 0.1 M NaCl, and 0.01% Tween-20] at 50°C, stained with streptavidin phycoerythrin (Molecular Probes), washed again with 6X SSPE, stained with biotinylated anti-streptavidin IgG, followed by a second staining with streptavidin phycoerythrin and a third wash with 6X SSPE. The arrays were scanned using a GeneArray scanner (Affymetrix) at $3-\mu m$ resolution, and quantitatively the canned image analyzed with computer software Microarray Suite 4.0 (Affymetrix). For normalization of the data to compare mRNA expression levels among the samples, we unified 1000 as an average of AD scores corresponding to signal intensities of all probe sets in each sample. We selected genes whose expression levels were higher than an average of signal intensity in one group (14 long-term survivors or 11 short-term survivors) plus its standard deviation (SD) in more than 60% of another group. The two-dimensional hierarchical clustering analysis of the 177 selected genes differentially expressed in longand short-term survivors was performed by the Cluster and Treeview programs (11).

RNA slot blot analysis. To verify differences in expression indicated by microarray analysis, we performed slot blot analysis of cRNAs produced from total RNA extracted from 14 long-term survivors and 12 short-term survivors. A SuperScript Choice System (Invitrogen Corp., CA) was used for cDNA synthesis prior to generating cRNA. A MEGA-script *in vitro* Transcription Kit (Ambion Inc., TX) was used for cRNA production by *in vitro* transcription using T7 RNA

polymerase. Each 0.5 μ g cRNA was denatured and blotted to a NitroPlus membrane (Micron Separations, Inc.). The filter was hybridized with radiolabeled probes. Hybridization was carried out in 50% formamide, 5X standard saline citrate (SSC) (1X standard saline citrate = 0.15 M NaCl, 0.015 M sodium citrate), 5X Denhardt's solution, 5 mM EDTA, 0.1% sodium dodecyl sulfate (SDS), 10% dextran sulfate, and 100 μ g/ml denatured salmon sperm DNA at 42°C for 16 h. The filter was washed twice in 0.1X SSC and 0.1% SDS at room temperature for 10 min each and then washed at 65°C for 30 min, and exposed to Kodak XAR film at -80°C.

Immunohistochemical analysis. Biopsy specimens were embedded in paraffin, sectioned, and treated with phosphatebuffered saline (PBS). The specimen was then treated with a blocking reagent containing 10% goat serum and incubated with an anti-CD8 antibody in PBS also containing 3% goat serum. After incubation with the secondary antibody, a streptavidin-peroxidase solution was applied to the specimen followed by a development solution containing diaminobenzidine.

Results

Classification of ESCC patients who received CRT alone and microarray analysis. The clinicopathological characteristics of 33 esophageal carcinoma patients who received CRT alone are summarized in Table I. All cases were diagnosed as squamous cell carcinoma, and all patients were treated according to the same protocol as described in Materials and methods. Twenty-five of the 33 patients were classified into two groups on the basis of the duration of survival: long-term survivors, more than 3 years (14 cases, A-1-1 to A-1-14); short-term survivors, less than 1 year (11 cases, D-1-1 to D-1-11). The two groups showed no significant differences with respect to clinicopathological features. Of the remaining 8 patients, 6 (A-2-1 to A-2-4, D-2-1, and D-2-3) survived for 2-3 years until the end of the follow-up period, and 2 patients (D-2-2 and D-2-4) survived for 1-2 years. Approximately 10-30 μ g of total RNA was isolated from each biopsy sample of these 33 ESCC patients, and all RNA samples were confirmed to be of good quality by visualization of ribosomal RNAs using the capillary electrophoresis method as described in Materials and methods and considered sufficient for microarray analysis. The 33 RNA samples were converted to cRNA, labeled by biotin and hybridized to Affymetrix Human Genome U95Av2 Array according to the protocol recommended by the manufacturer (Affymetrix, CA, USA). The array analyzed mRNA expression levels corresponding to 12,600 transcripts. First, we conducted an unsupervised clustering analysis using the 316 genes expressed (i.e., gave 'Presence' call by data analysis) in all the 33 samples. Three patient clusters appeared (Fig. 1). Cluster 1 (7 cases) consisted of 5 long-term survivors (A-1-1, A-1-7, A-1-9, A-1-11, and A-1-13), and 6 of the 7 cases (86%) in this cluster survived for more than 2 years. Cluster 2 (15 cases) consisted of 9 short-term survivors (D-1-1 to D-1-5, and D-1-7 to D-1-10), and 73% of the cases of this cluster (11 of 15) survived for less than 2 years. Cluster 3 (11 cases) consisted of 6 long-term survivors (A-1-2, A-1-5, A-1-6, A-1-10, A-1-11, and A-1-14), and 10 of the 11 cases (91%) of

Table I. Clinical outcome and disease stage of 33 patients.

No.	Age	Sex	Т	Ν	М	TNM stage	CR ^a	Outcome ^b
A-1-1	56	М	3	1	0	3	CR	A (>2171)
A-1-2	61	М	3	1	1	4	CR	A (>2233)
A-1-3	68	М	3	1	0	3	CR	A (>2012)
A-1-4	48	М	3	0	0	2A	CR	A (>1891)
A-1-5	55	М	3	0	0	2A	CR	A (>2076)
A-1-6	54	М	2	1	0	2B	CR	A (>1857)
A-1-7	67	М	3	0	0	2A	CR	A (>1901)
A-1-8	68	М	3	1	0	3	CR	A (>1733)
A-1-9	50	М	3	1	0	3	CR	A (>1527)
A-1-10	65	М	3	1	0	3	CR	A (>1367)
A-1-11	72	М	3	0	0	2A	CR	A (>1366)
A-1-12	64	F	3	1	0	3	CR	A (>1346)
A-1-13	58	F	3	1	0	3	CR	A (>1233)
A-1-14	72	М	3	0	0	2A	CR	A (>1136)
D-1-1	72	F	3	1	0	3	Non-CR	D (379)
D-1-2	60	М	3	1	0	3	Non-CR	D (251)
D-1-3	61	М	3	1	1	4	Non-CR	D (227)
D-1-4	49	М	3	1	1	4	Non-CR	D (239)
D-1-5	53	М	3	0	0	2A	Non-CR	D (364)
D-1-6	50	М	3	1	1	4	Non-CR	D (226)
D-1-7	55	М	2	1	0	2B	Non-CR	D (280)
D-1-8	70	М	3	0	1	4	Non-CR	D (299)
D-1-9	72	М	3	1	1	4	Non-CR	D (156)
D-1-10	75	М	3	0	0	2A	Non-CR	D (309)
D-1-11	67	М	3	1	0	3	Non-CR	D (236)
A-2-1	71	М	3	0	0	2A	CR	A (>912)
A-2-2	70	М	3	1	0	3	CR	A (>1018)
A-2-3	44	М	3	0	0	2A	CR	A (>919)
A-2-4	55	М	3	0	0	2A	CR	A (>959)
D-2-1	71	М	3	1	0	3	CR	D (664)
D-2-2	64	М	2	1	0	2B	CR	D (299)
D-2-3	61	М	3	1	0	3	CR	D (968)
D-2-4	59	F	3	0	0	2A	CR	D (521)

^aA CR was defined as the complete disappearance of all measurable and assessable disease for a minimum of 4-weeks. ^bDisease-free survival (days).

this cluster survived for more than 2 years. In summary, 11 (79%) of the 14 long-term survivors (A-1-1 to A-1-14) or 16 (80%) of the 20 cases that survived for more than 2 years were grouped into clusters 1 and 3, whereas cluster 2 was characterized by a poorer prognosis, gathering 9 (82%) of the 11 short-term survivors (D-1-1 to D-1-11) or 10 (77%) of 13 cases that survived for less than 2 years. These unsupervised data on expression profiling suggested the presence of distinct subclasses within the 33 patient groups, and the subclasses may be associated with the survival term.

To compile a gene list for prognostic marker candidates for CRT, we selected genes by comparing expression levels between the two groups, 14 long-term survivors and 11 shortterm survivors. A gene was selected if more than 60% of the samples in one group expressed the gene at a higher level than an average of signal intensity plus its standard deviation (SD) in the other group. By this procedure, 57 genes associated with short-term survival and 120 genes with long-term survival were identified (Table II). To investigate whether the subclass exists among long-term survivors or short-term survivors, we

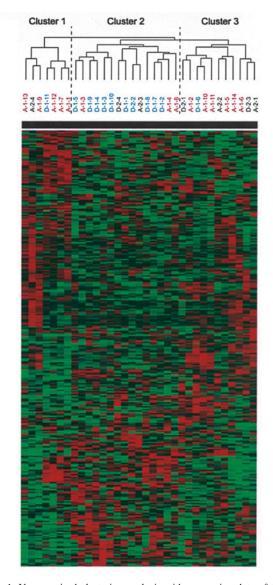


Figure 1. Unsupervised clustering analysis with expression data of the 316 genes in the 33 ESCC patients treated with CRT alone. Expression data of the 316 genes expressed in all the 33 samples were analyzed by the Cluster and Treeview programs (11). The results showed a significant association between the expression profile and the survival term. The long-term survivors (red), the short-term survivors (blue).

carried out a two-dimensional hierarchical clustering analysis of the 177 selected genes. A dendrogram of the clustering analysis was generated, and is shown in Fig. 2. Two patient clusters (vertical bar) and three gene clusters (horizontal bar) were recognized. One of the two patient clusters consisted of 10 of 11 short-term survivors, and the other patient cluster included all of the 14 long-term survivors. Only one case (D-1-11) was found in an inappropriate cluster. Among the gene clusters, the 11 short-term survivors appear to compose a single gene cluster (A in Fig. 2), whereas two major gene clusters, B and C, exist for the 14 long-term survivors.

Genes differentially expressed between long- and short-term survivors. We next analyzed the known functional annotations of 177 genes identified as differentially expressed genes between the long- and short-term survivors. More than 20 out of 120 genes over-expressed in the long-term survivors were found to have some functions in the immune response (Table II).

Table II. Over-ex		

25AB028959KIAA10226M86667NAP1L127M32334ICAM228L05148ZAP7029M31767MGMT30M54914FSHB31M60483PP2CA32M90356BTF3L333X96969SLC14A234Z50853CLPP35X75252PBP36AB029036TRIM3337AB014596FBXW1H38M58458RPS4X39Z25749RPS740AF070638CGI-5741Z46973PIK3C342L33842IMPDH243J04132CD3Z44S72869D10S17045M31315F1246U70451MYD8847L19185PRDX248D11466PIGA49D89667MM-150D21089XPC51U19144GAGE352AL050135RFX553AB018344DDX4654AF052155SEC13L155U19145GAGE556X15940RPL3157AF039555VSNL1	No.	GenBank	Gene symbo	
1 U19142 GAGE1 2 L76191 IRAK1 3 X53777 RPL17 4 AF023612 DIM1 5 M96860 DPP6 6 AF031416 IKBKB 7 Z48482 MMP15 8 S61953 ERBB3 9 Y18448 BSN 10 W26628 MRPL9 11 M24194 GNB2L1 12 D63478 NICE-4 13 AF054187 NACA 14 U84570 C21orf2 15 J03592 SLC25A 16 U87954 PA2G4 17 Z48481 MMP14 18 AB016902 MLLT4 19 AI381790 APM2 20 M24194 GNB2L1 21 M62982 ALOX12 22 Y00764 UQCRH 23 X78710 MTF1 24 H16917	Long-term			
2 L76191 IRAK1 3 X53777 RPL17 4 AF023612 DIM1 5 M96860 DPP6 6 AF031416 IKBKB 7 Z48482 MMP15 8 S61953 ERB3 9 Y18448 BSN 10 W26628 MRPL9 11 M24194 GNB2L1 12 D63478 NICE-4 13 AF054187 NACA 14 U84570 C21of2 15 J03592 SLC25A 16 U87954 PA2G4 17 Z48481 MMP14 18 AB016902 MLLT4 19 AI381790 APM2 20 M24194 GNB2L1 21 M62982 ALOX12 22 Y00764 UQCRH 23 X78710 MTF1 24 H16917 KIAA05 25 AB028959 <t< td=""><td>survivor</td><td></td><td></td></t<>	survivor			
3 X53777 RPL17 4 AF023612 DIM1 5 M96860 DPP6 6 AF031416 IKBKB 7 Z48482 MMP15 8 S61953 ERBB3 9 Y18448 BSN 10 W26628 MRPL9 11 M24194 GNB2L1 12 D63478 NICE-4 13 AF054187 NACA 14 U84570 C21orf2 15 J03592 SLC25A 16 U87954 PA2G4 17 Z48481 MMP14 18 AB016902 MLLT4 19 A1381790 APM2 20 M24194 GNB2L1 21 M62982 ALOX12 22 Y00764 UQCRH 23 X78710 MTF1 24 H16917 KIAA050 25 AB028959 KIAA100 26 M66667	-	U19142	GAGE1	
4 AF023612 DIM1 5 M96860 DPP6 6 AF031416 IKBKB 7 Z48482 MMP15 8 S61953 ERB3 9 Y18448 BSN 10 W26628 MRPL9 11 M24194 GNB2L1 12 D63478 NICE-4 13 AF054187 NACA 14 U84570 C21orf2 15 J03592 SLC25A 16 U87954 PA264 17 Z48481 MMP14 18 AB016902 MLLT4 19 AI381790 APM2 20 M24194 GNB2L1 21 M62982 ALOX12 22 Y00764 UQCRH 23 X78710 MTF1 24 H16917 KIAA055 25 AB028959 KIAA100 26 M86667 NAP1L1 27 M32334 ICAM2 28 L05148 ZAP70 29 <td></td> <td>L76191</td> <td>IRAK1</td>		L76191	IRAK1	
5 M96860 DPP6 6 AF031416 IKBKB 7 Z48482 MMP15 8 S61953 ERBB3 9 Y18448 BSN 10 W26628 MRPL9 11 M24194 GNB2L1 12 D63478 NICE44 13 AF054187 NACA 14 U84570 C21orf2 15 J03592 SLC25A 16 U87954 PA2G4 17 Z48481 MMP14 18 AB016902 MLLT4 19 AI381790 APM2 20 M24194 GNB2L1 21 M62982 ALOX12 22 Y00764 UQCRH 23 X78710 MTF1 24 H16917 KIA4050 25 AB028959 KIA410 26 M86667 NAP1L1 27 M32334 ICAM2 28 L05148		X53777	RPL17	
6 AF031416 IKBKB 7 Z48482 MMP15 8 S61953 ERBB3 9 Y18448 BSN 10 W26628 MRPL9 11 M24194 GNB2L1 12 D63478 NICE-4 13 AF054187 NACA 14 U84570 C21orf2 15 J03592 SLC25A 16 U87954 PA2G4 17 Z48481 MMP14 18 AB016902 MLLT4 19 AI381790 APM2 20 M24194 GNB2L1 21 M62982 ALOX12 22 Y00764 UQCRH 23 X78710 MTF1 24 H16917 KIAA050 25 AB028959 KIAA102 26 M86667 NAP1L1 27 M3234 ICAM2 28 L05148 ZAP70 29 M31767		AF023612	DIM1	
7 Z48482 MMP15 8 S61953 ERBB3 9 Y18448 BSN 10 W26628 MRPL9 11 M24194 GNB2L1 12 D63478 NICE-4 13 AF054187 NACA 14 U84570 C21orf2 15 J03592 SLC25A 16 U87954 PA2G4 17 Z48481 MMP14 18 AB016902 MLLT4 19 AI381790 APM2 20 M24194 GNB2L1 21 M62982 ALOX12 22 Y00764 UQCRH 23 X78710 MTF1 24 H16917 KIAA050 25 AB028959 KIAA102 26 M86667 NAP1L1 27 M32334 ICAM2 28 L05148 ZAP70 29 M31767 MGMT 30 M54914 FSHB 31 M60443 PPP2CA 32 </td <td></td> <td>M96860</td> <td>DPP6</td>		M96860	DPP6	
8 S61953 ERBB3 9 Y18448 BSN 10 W26628 MRPL9 11 M24194 GNB2L1 12 D63478 NICE-4 13 AF054187 NACA 14 U84570 C21orf2 15 J03592 SLC25A 16 U87954 PA2G4 17 Z48481 MMP14 18 AB016902 MLLT4 19 A1381790 APM2 20 M24194 GNB2L1 21 M62982 ALOX12 22 Y00764 UQCRH 23 X78710 MTF1 24 H16917 KIAA050 25 AB028959 KIAA105 26 M86667 NAP1L1 27 M32334 ICAM2 28 L05148 ZAP70 29 M31767 MGMT 31 M60483 PPP2CA 32 M90356		AF031416	IKBKB	
9 Y18448 BSN 10 W26628 MRPL9 11 M24194 GNB2L1 12 D63478 NICE-4 13 AF054187 NACA 14 U84570 C21orf2 15 J03592 SLC25A 16 U87954 PA2G4 17 Z48481 MMP14 18 AB016902 MLLT4 19 A1381790 APM2 20 M24194 GNB2L1 21 M62982 ALOX12 22 Y00764 UQCRH 23 X78710 MTF1 24 H16917 KIAA056 25 AB028959 KIAA100 26 M86667 NAP1L1 27 M32334 ICAM2 28 L05148 ZAP70 29 M31767 MGMT 30 M54914 FSHB 31 M60483 PPP2CA 32 M90356		Z48482	MMP15	
10 W26628 MRPL9 11 M24194 GNB2L1 12 D63478 NICE-4 13 AF054187 NACA 14 U84570 C21orf2 15 J03592 SLC25A 16 U87954 PA2G4 17 Z48481 MMP14 18 AB016902 MLLT4 19 AI381790 APM2 20 M24194 GNB2L1 21 M62982 ALOX12 22 Y00764 UQCRH 23 X78710 MTF1 24 H16917 KIAA050 25 AB028959 KIAA102 26 M86667 NAPIL1 27 M32334 ICAM2 28 L05148 ZAP70 29 M31767 MGMT 30 M54914 FSHB 31 M60483 PPP2CA 32 M90356 BT3L3 33 X96969 <td></td> <td>S61953</td> <td>ERBB3</td>		S61953	ERBB3	
11 M24194 GNB2L1 12 D63478 NICE-4 13 AF054187 NACA 14 U84570 C21orf2 15 J03592 SLC25A 16 U87954 PA2G4 17 Z48481 MMP14 18 AB016902 MLLT4 19 AI381790 APM2 20 M24194 GNB2L1 21 M62982 ALOX12 22 Y00764 UQCRH 23 X78710 MTF1 24 H16917 KIAA050 25 AB028959 KIAA102 26 M86667 NAPIL1 27 M32334 ICAM2 28 L05148 ZAP70 29 M31767 MGMT 30 M54914 FSHB 31 M60483 PPP2CA 33 X96969 SLC14A2 34 Z50853 CLPP 35 X75252 PBP 36 AB029036 TRIM33 <				
12 D63478 NICE-4 13 AF054187 NACA 14 U84570 C21orf2 15 J03592 SLC25A 16 U87954 PA2G4 17 Z48481 MMP14 18 AB016902 MLLT4 19 AI381790 APM2 20 M24194 GNB2L1 21 M62982 ALOX12 22 Y00764 UQCRH 23 X78710 MTF1 24 H16917 KIAA050 25 AB028959 KIAA102 26 M86667 NAPIL1 27 M32334 ICAM2 28 L05148 ZAP70 29 M31767 MGMT 30 M54914 FSHB 31 M60483 PPP2CA 32 M90356 BTF3L3 33 X96969 SLC14A2 24 L33842 IMPDH2 35 X75252				
13 AF054187 NACA 14 U84570 C21orf2 15 J03592 SLC25A 16 U87954 PA2G4 17 Z48481 MMP14 18 AB016902 MLLT4 19 AI381790 APM2 20 M24194 GNB2L1 21 M62982 ALOX12 22 Y00764 UQCRH 23 X78710 MTF1 24 H16917 KIAA05 25 AB028959 KIAA102 26 M86667 NAPIL1 27 M32334 ICAM2 28 L05148 ZAP70 29 M31767 MGMT 30 M54914 FSHB 31 M60483 PPP2CA 32 M90356 BTF3L3 33 X96969 SLC14A2 34 Z50853 CLPP 35 X75252 PBP 36 AB029036 TRIM33 37 AB014596 FBXWI1				
14 U84570 C21orf2 15 J03592 SLC25A0 16 U87954 PA2G4 17 Z48481 MMP14 18 AB016902 MLLT4 19 AI381790 APM2 20 M24194 GNB2L1 21 M62982 ALOX12 22 Y00764 UQCRH 23 X78710 MTF1 24 H16917 KIAA050 25 AB028959 KIAA102 26 M86667 NAPIL1 27 M32334 ICAM2 28 L05148 ZAP70 29 M31767 MGMT 30 M54914 FSHB 31 M60483 PPP2CA 32 M90356 BTF3L3 33 X96969 SLC14A2 34 Z50853 CLPP 35 X75252 PBP 36 AB029036 TRIM33 37 AB014596 FBXW11 38 M58458 RPS4X				
15 J03592 SLC25A4 16 U87954 PA2G4 17 Z48481 MMP14 18 AB016902 MLLT4 19 AI381790 APM2 20 M24194 GNB2L1 21 M62982 ALOX12 22 Y00764 UQCRH 23 X78710 MTF1 24 H16917 KIAA050 25 AB028959 KIAA100 26 M86667 NAP1L1 27 M32334 ICAM2 28 L05148 ZAP70 29 M31767 MGMT 30 M54914 FSHB 31 M60483 PP2CA 32 M90356 BTF3L3 33 X96969 SLC14A2 34 Z50853 CLPP 35 X75252 PBP 36 AB029036 TRIM33 37 AB014596 FBXW1H 38 M58458 RPS4X 39 Z25749 RPS7 <td< td=""><td></td><td></td><td></td></td<>				
16 U87954 PA2G4 17 Z48481 MMP14 18 AB016902 MLLT4 19 AI381790 APM2 20 M24194 GNB2L1 21 M62982 ALOX12 22 Y00764 UQCRH 23 X78710 MTF1 24 H16917 KIAA050 25 AB028959 KIAA102 26 M86667 NAP1L1 27 M32334 ICAM2 28 L05148 ZAP70 29 M31767 MGMT 30 M54914 FSHB 31 M60483 PPP2CA 32 M90356 BTF3L3 33 X96969 SLC14A2 34 Z50853 CLPP 35 X75252 PBP 36 AB029036 TRIM33 37 AB014596 FBXW11 38 M58458 RPS4X 39 Z25749 RPS7 40 AF070638 CG1-57 <				
17 Z48481 MMP14 18 AB016902 MLLT4 19 AI381790 APM2 20 M24194 GNB2L1 21 M62982 ALOX12 22 Y00764 UQCRH 23 X78710 MTF1 24 H16917 KIAA050 25 AB028959 KIAA100 26 M86667 NAP1L1 27 M32334 ICAM2 28 L05148 ZAP70 29 M31767 MGMT 30 M54914 FSHB 31 M60483 PP2CA 32 M90356 BTF3L3 33 X96969 SLC14A2 34 Z50853 CLPP 35 X75252 PBP 36 AB029036 TRIM33 37 AB014596 FBXW11 38 M58458 RPS4X 39 Z25749 RPS7 40 AF070638 CGI-57 41 Z46973 PIK3C3 <				
18 AB016902 MLLT4 19 AI381790 APM2 20 M24194 GNB2L1 21 M62982 ALOX12 22 Y00764 UQCRH 23 X78710 MTF1 24 H16917 KIAA050 25 AB028959 KIAA102 26 M86667 NAP1L1 27 M32334 ICAM2 28 L05148 ZAP70 29 M31767 MGMT 30 M54914 FSHB 31 M60483 PPP2CA 32 M90356 BTF3L3 33 X96969 SLC14A2 34 Z50853 CLPP 35 X75252 PBP 36 AB029036 TRIM33 37 AB014596 FBXW1H 38 M58458 RPS4X 39 Z25749 RPS7 40 AF070638 CGI-57 41 Z46973 PIK3C3 42 L33842 IMPDH2				
19 AI381790 APM2 20 M24194 GNB2L1 21 M62982 ALOX12 22 Y00764 UQCRH 23 X78710 MTF1 24 H16917 KIAA050 25 AB028959 KIAA102 26 M86667 NAP1L1 27 M32334 ICAM2 28 L05148 ZAP70 29 M31767 MGMT 30 M54914 FSHB 31 M60483 PP2CA 32 M90356 BTF3L3 33 X96969 SLC14A2 34 Z50853 CLPP 35 X75252 PBP 36 AB029036 TRIM33 37 AB014596 FBXW11 38 M58458 RPS4X 39 Z25749 RPS7 40 AF070638 CGI-57 41 Z46973 PIK3C3 42 L33842 IMPDH2 43 J04132 CD3Z <td< td=""><td></td><td></td><td></td></td<>				
20 M24194 GNB2L1 21 M62982 ALOX12 22 Y00764 UQCRH 23 X78710 MTF1 24 H16917 KIAA050 25 AB028959 KIAA100 26 M86667 NAP1L1 27 M32334 ICAM2 28 L05148 ZAP70 29 M31767 MGMT 30 M54914 FSHB 31 M60483 PP2CA 32 M90356 BTF3L3 33 X96969 SLC14A2 34 Z50853 CLPP 35 X75252 PBP 36 AB029036 TRIM33 37 AB014596 FBXWIN 38 M58458 RPS4X 39 Z25749 RPS7 40 AF070638 CGI-57 41 Z46973 PIK3C3 42 L33842 IMPDH2 43 J04132 CD3Z 44 S72869 D105170 <t< td=""><td></td><td></td><td></td></t<>				
21 M62982 ALOX12 22 Y00764 UQCRH 23 X78710 MTF1 24 H16917 KIAA050 25 AB028959 KIAA102 26 M86667 NAP1L1 27 M32334 ICAM2 28 L05148 ZAP70 29 M31767 MGMT 30 M54914 FSHB 31 M60483 PPP2CA 32 M90356 BTF3L3 33 X96969 SLC14A2 34 Z50853 CLPP 35 X75252 PBP 36 AB029036 TRIM33 37 AB014596 FBXW1H 38 M58458 RPS4X 39 Z25749 RPS7 40 AF070638 CGI-57 41 Z46973 PIK3C3 42 L33842 IMPDH2 43 J04132 CD3Z 44 S72869 D108170 45 M31315 F12				
22 Y00764 UQCRH 23 X78710 MTF1 24 H16917 KIAA050 25 AB028959 KIAA102 26 M86667 NAP1L1 27 M32334 ICAM2 28 L05148 ZAP70 29 M31767 MGMT 30 M54914 FSHB 31 M60483 PPP2CA 32 M90356 BTF3L3 33 X96969 SLC14A2 34 Z50853 CLPP 35 X75252 PBP 36 AB029036 TRIM33 37 AB014596 FBXWH 38 M58458 RPS4X 39 Z25749 RPS7 40 AF070638 CGI-57 41 Z46973 PIK3C3 42 L33842 IMPDH2 43 J04132 CD3Z 44 S72869 D10S170 45 M31315 F12 46 U70451 MYD88 47				
23 X78710 MTF1 24 H16917 KIAA050 25 AB028959 KIAA102 26 M86667 NAP1L1 27 M32334 ICAM2 28 L05148 ZAP70 29 M31767 MGMT 30 M54914 FSHB 31 M60483 PPP2CA 32 M90356 BTF3L3 33 X96969 SLC14A2 34 Z50853 CLPP 35 X75252 PBP 36 AB029036 TRIM33 37 AB014596 FBXW11 38 M58458 RPS4X 39 Z25749 RPS7 40 AF070638 CGI-57 41 Z46973 PIK3C3 42 L33842 IMPDH2 43 J04132 CD3Z 44 S72869 D10S170 45 M31315 F12 46 U70451 MYD88 47 L19185 PRDX2 4				
24 H16917 KIAA050 25 AB028959 KIAA103 26 M86667 NAP1L1 27 M32334 ICAM2 28 L05148 ZAP70 29 M31767 MGMT 30 M54914 FSHB 31 M60483 PP2CA 32 M90356 BTF3L3 33 X96969 SLC14A2 34 Z50853 CLPP 35 X75252 PBP 36 AB029036 TRIM33 37 AB014596 FBXW1H 38 M58458 RPS4X 39 Z25749 RPS7 40 AF070638 CGI-57 41 Z46973 PIK3C3 42 L33842 IMPDH2 43 J04132 CD3Z 44 S72869 D10S170 45 M31315 F12 46 U70451 MYD88 47 L19185 PRDX2 48 D11466 PIGA 49				
25 AB028959 KIAA102 26 M86667 NAP1L1 27 M32334 ICAM2 28 L05148 ZAP70 29 M31767 MGMT 30 M54914 FSHB 31 M60483 PPP2CA 32 M90356 BTF3L3 33 X96969 SLC14A2 34 Z50853 CLPP 35 X75252 PBP 36 AB029036 TRIM33 37 AB014596 FBXW1H 38 M58458 RPS4X 39 Z25749 RPS7 40 AF070638 CGI-57 41 Z46973 PIK3C3 42 L33842 IMPDH2 43 J04132 CD3Z 44 S72869 D10S170 45 M31315 F12 46 U70451 MYD88 47 L19185 PRDX2 48 D11466 PIGA 49 D89667 MM-1 50 </td <td></td> <td></td> <td></td>				
26 M86667 NAP1L1 27 M32334 ICAM2 28 L05148 ZAP70 29 M31767 MGMT 30 M54914 FSHB 31 M60483 PP2CA 32 M90356 BTF3L3 33 X96969 SLC14A2 34 Z50853 CLPP 35 X75252 PBP 36 AB029036 TRIM33 37 AB014596 FBXW1H 38 M58458 RPS4X 39 Z25749 RPS7 40 AF070638 CGI-57 41 Z46973 PIK3C3 42 L33842 IMPDH2 43 J04132 CD3Z 44 S72869 D10S170 45 M31315 F12 46 U70451 MYD88 47 L19185 PRDX2 48 D11466 PIGA 49 D89667 MM-1 50 D21089 XPC 51			KIAA0563	
27 M32334 ICAM2 28 L05148 ZAP70 29 M31767 MGMT 30 M54914 FSHB 31 M60483 PPP2CA 32 M90356 BTF3L3 33 X96969 SLC14A2 34 Z50853 CLPP 35 X75252 PBP 36 AB029036 TRIM33 37 AB014596 FBXW1B 38 M58458 RPS4X 39 Z25749 RPS7 40 AF070638 CGI-57 41 Z46973 PIK3C3 42 L33842 IMPDH2 43 J04132 CD3Z 44 S72869 D10S170 45 M31315 F12 46 U70451 MYD88 47 L19185 PRDX2 48 D11466 PIGA 49 D89667 MM-1 50 D21089 XPC 51 U19144 GAGE3 52			KIAA1036	
28 L05148 ZAP70 29 M31767 MGMT 30 M54914 FSHB 31 M60483 PPP2CA 32 M90356 BTF3L3 33 X96969 SLC14A2 34 Z50853 CLPP 35 X75252 PBP 36 AB029036 TRIM33 37 AB014596 FBXW1H 38 M58458 RPS4X 39 Z25749 RPS7 40 AF070638 CGI-57 41 Z46973 PIK3C3 42 L33842 IMPDH2 43 J04132 CD3Z 44 S72869 D10S170 45 M31315 F12 46 U70451 MYD88 47 L19185 PRDX2 48 D11466 PIGA 49 D89667 MM-1 50 D21089 XPC 51 U19144 GAGE3 52 AL050135 RFX5 53				
29 M31767 MGMT 30 M54914 FSHB 31 M60483 PPP2CA 32 M90356 BTF3L3 33 X96969 SLC14A2 34 Z50853 CLPP 35 X75252 PBP 36 AB029036 TRIM33 37 AB014596 FBXW1H 38 M58458 RPS4X 39 Z25749 RPS7 40 AF070638 CGI-57 41 Z46973 PIK3C3 42 L33842 IMPDH2 43 J04132 CD3Z 44 S72869 D10S170 45 M31315 F12 46 U70451 MYD88 47 L19185 PRDX2 48 D11466 PIGA 49 D89667 MM-1 50 D21089 XPC 51 U19144 GAGE3 52 AL050135 RFX5 53 AB018344 DDX46 54				
30 M54914 FSHB 31 M60483 PPP2CA 32 M90356 BTF3L3 33 X96969 SLC14A2 34 Z50853 CLPP 35 X75252 PBP 36 AB029036 TRIM33 37 AB014596 FBXW1H 38 M58458 RPS4X 39 Z25749 RPS7 40 AF070638 CGI-57 41 Z46973 PIK3C3 42 L33842 IMPDH2 43 J04132 CD3Z 44 S72869 D10S170 45 M31315 F12 46 U70451 MYD88 47 L19185 PRDX2 48 D11466 PIGA 49 D89667 MM-1 50 D21089 XPC 51 U19144 GAGE3 52 AL050135 RFX5 53 AB018344 DDX46 54 AF052155 SEC13L1 55<				
31 M60483 PPP2CA 32 M90356 BTF3L3 33 X96969 SLC14A3 34 Z50853 CLPP 35 X75252 PBP 36 AB029036 TRIM33 37 AB014596 FBXW1H 38 M58458 RPS4X 39 Z25749 RPS7 40 AF070638 CGI-57 41 Z46973 PIK3C3 42 L33842 IMPDH2 43 J04132 CD3Z 44 S72869 D10S170 45 M31315 F12 46 U70451 MYD88 47 L19185 PRDX2 48 D11466 PIGA 49 D89667 MM-1 50 D21089 XPC 51 U19144 GAGE3 52 AL050135 RFX5 53 AB018344 DDX46 54 AF052155 SEC13L1 55 U19145 GAGE5 56				
32 M90356 BTF3L3 33 X96969 SLC14A2 34 Z50853 CLPP 35 X75252 PBP 36 AB029036 TRIM33 37 AB014596 FBXW11 38 M58458 RPS4X 39 Z25749 RPS7 40 AF070638 CGI-57 41 Z46973 PIK3C3 42 L33842 IMPDH2 43 J04132 CD3Z 44 S72869 D10S170 45 M31315 F12 46 U70451 MYD88 47 L19185 PRDX2 48 D11466 PIGA 49 D89667 MM-1 50 D21089 XPC 51 U19144 GAGE3 52 AL050135 RFX5 53 AB018344 DDX46 54 AF052155 SEC13L1 55 U19145 GAGE5 56 X15940 RPL31 57<				
33 X96969 SLC14A2 34 Z50853 CLPP 35 X75252 PBP 36 AB029036 TRIM33 37 AB014596 FBXW11 38 M58458 RPS4X 39 Z25749 RPS7 40 AF070638 CGI-57 41 Z46973 PIK3C3 42 L33842 IMPDH2 43 J04132 CD3Z 44 S72869 D10S170 45 M31315 F12 46 U70451 MYD88 47 L19185 PRDX2 48 D11466 PIGA 49 D89667 MM-1 50 D21089 XPC 51 U19144 GAGE3 52 AL050135 RFX5 53 AB018344 DDX46 54 AF052155 SEC13L1 55 U19145 GAGE5 56 X15940 RPL31 57 AF039555 VSNL1 <td></td> <td></td> <td>-</td>			-	
34 Z50853 CLPP 35 X75252 PBP 36 AB029036 TRIM33 37 AB014596 FBXW1B 38 M58458 RPS4X 39 Z25749 RPS7 40 AF070638 CGI-57 41 Z46973 PIK3C3 42 L33842 IMPDH2 43 J04132 CD3Z 44 S72869 D10S170 45 M31315 F12 46 U70451 MYD88 47 L19185 PRDX2 48 D11466 PIGA 49 D89667 MM-1 50 D21089 XPC 51 U19144 GAGE3 52 AL050135 RFX5 53 AB018344 DDX46 54 AF052155 SEC13L1 55 U19145 GAGE5 56 X15940 RPL31 57 AF039555 VSNL1				
35 X75252 PBP 36 AB029036 TRIM33 37 AB014596 FBXW1B 38 M58458 RPS4X 39 Z25749 RPS7 40 AF070638 CGI-57 41 Z46973 PIK3C3 42 L33842 IMPDH2 43 J04132 CD3Z 44 S72869 D10S170 45 M31315 F12 46 U70451 MYD88 47 L19185 PRDX2 48 D11466 PIGA 49 D89667 MM-1 50 D21089 XPC 51 U19144 GAGE3 52 AL050135 RFX5 53 AB018344 DDX46 54 AF052155 SEC13L1 55 U19145 GAGE5 56 X15940 RPL31 57 AF039555 VSNL1				
36AB029036TRIM3337AB014596FBXW1I38M58458RPS4X39Z25749RPS740AF070638CGI-5741Z46973PIK3C342L33842IMPDH243J04132CD3Z44S72869D10S17045M31315F1246U70451MYD8847L19185PRDX248D11466PIGA49D89667MM-150D21089XPC51U19144GAGE352AL050135RFX553AB018344DDX4654AF052155SEC13L155U19145GAGE556X15940RPL3157AF039555VSNL1				
37 AB014596 FBXW1H 38 M58458 RPS4X 39 Z25749 RPS7 40 AF070638 CGI-57 41 Z46973 PIK3C3 42 L33842 IMPDH2 43 J04132 CD3Z 44 S72869 D10S170 45 M31315 F12 46 U70451 MYD88 47 L19185 PRDX2 48 D11466 PIGA 49 D89667 MM-1 50 D21089 XPC 51 U19144 GAGE3 52 AL050135 RFX5 53 AB018344 DDX46 54 AF052155 SEC13L1 55 U19145 GAGE5 56 X15940 RPL31 57 AF039555 VSNL1				
38 M58458 RPS4X 39 Z25749 RPS7 40 AF070638 CGI-57 41 Z46973 PIK3C3 42 L33842 IMPDH2 43 J04132 CD3Z 44 S72869 D10S170 45 M31315 F12 46 U70451 MYD88 47 L19185 PRDX2 48 D11466 PIGA 49 D89667 MM-1 50 D21089 XPC 51 U19144 GAGE3 52 AL050135 RFX5 53 AB018344 DDX46 54 AF052155 SEC13L1 55 U19145 GAGE5 56 X15940 RPL31 57 AF039555 VSNL1				
39 Z25749 RPS7 40 AF070638 CGI-57 41 Z46973 PIK3C3 42 L33842 IMPDH2 43 J04132 CD3Z 44 S72869 D10S170 45 M31315 F12 46 U70451 MYD88 47 L19185 PRDX2 48 D11466 PIGA 49 D89667 MM-1 50 D21089 XPC 51 U19144 GAGE3 52 AL050135 RFX5 53 AB018344 DDX46 54 AF052155 SEC13L1 55 U19145 GAGE5 56 X15940 RPL31 57 AF039555 VSNL1				
40AF070638CGI-5741Z46973PIK3C342L33842IMPDH243J04132CD3Z44S72869D10S17045M31315F1246U70451MYD8847L19185PRDX248D11466PIGA49D89667MM-150D21089XPC51U19144GAGE352AL050135RFX553AB018344DDX4654AF052155SEC13L155U19145GAGE556X15940RPL3157AF039555VSNL1				
41Z46973PIK3C342L33842IMPDH243J04132CD3Z44S72869D10S17045M31315F1246U70451MYD8847L19185PRDX248D11466PIGA49D89667MM-150D21089XPC51U19144GAGE352AL050135RFX553AB018344DDX4654AF052155SEC13L155U19145GAGE556X15940RPL3157AF039555VSNL1				
42 L33842 IMPDH2 43 J04132 CD3Z 44 S72869 D10S170 45 M31315 F12 46 U70451 MYD88 47 L19185 PRDX2 48 D11466 PIGA 49 D89667 MM-1 50 D21089 XPC 51 U19144 GAGE3 52 AL050135 RFX5 53 AB018344 DDX46 54 AF052155 SEC13L1 55 U19145 GAGE5 56 X15940 RPL31 57 AF039555 VSNL1				
43J04132CD3Z44S72869D10S17045M31315F1246U70451MYD8847L19185PRDX248D11466PIGA49D89667MM-150D21089XPC51U19144GAGE352AL050135RFX553AB018344DDX4654AF052155SEC13L155U19145GAGE556X15940RPL3157AF039555VSNL1		,		
44S72869D10S17045M31315F1246U70451MYD8847L19185PRDX248D11466PIGA49D89667MM-150D21089XPC51U19144GAGE352AL050135RFX553AB018344DDX4654AF052155SEC13L155U19145GAGE556X15940RPL3157AF039555VSNL1				
45M31315F1246U70451MYD8847L19185PRDX248D11466PIGA49D89667MM-150D21089XPC51U19144GAGE352AL050135RFX553AB018344DDX4654AF052155SEC13L155U19145GAGE556X15940RPL3157AF039555VSNL1				
46U70451MYD8847L19185PRDX248D11466PIGA49D89667MM-150D21089XPC51U19144GAGE352AL050135RFX553AB018344DDX4654AF052155SEC13L155U19145GAGE556X15940RPL3157AF039555VSNL1				
47L19185PRDX248D11466PIGA49D89667MM-150D21089XPC51U19144GAGE352AL050135RFX553AB018344DDX4654AF052155SEC13L155U19145GAGE556X15940RPL3157AF039555VSNL1				
48D11466PIGA49D89667MM-150D21089XPC51U19144GAGE352AL050135RFX553AB018344DDX4654AF052155SEC13L155U19145GAGE556X15940RPL3157AF039555VSNL1				
49D89667MM-150D21089XPC51U19144GAGE352AL050135RFX553AB018344DDX4654AF052155SEC13L155U19145GAGE556X15940RPL3157AF039555VSNL1				
50D21089XPC51U19144GAGE352AL050135RFX553AB018344DDX4654AF052155SEC13L155U19145GAGE556X15940RPL3157AF039555VSNL1				
51U19144GAGE352AL050135RFX553AB018344DDX4654AF052155SEC13L155U19145GAGE556X15940RPL3157AF039555VSNL1				
52 AL050135 RFX5 53 AB018344 DDX46 54 AF052155 SEC13L1 55 U19145 GAGE5 56 X15940 RPL31 57 AF039555 VSNL1			-	
53 AB018344 DDX46 54 AF052155 SEC13L1 55 U19145 GAGE5 56 X15940 RPL31 57 AF039555 VSNL1				
54AF052155SEC13L155U19145GAGE556X15940RPL3157AF039555VSNL1				
55U19145GAGE556X15940RPL3157AF039555VSNL1				
56X15940RPL3157AF039555VSNL1				
57 AF039555 VSNL1				
		879522	RPS27A	
			CYP4F12	
60 X78926 ZNF268			-	

Table II.	Continued.
-----------	------------

Table II. Continued.

62 AB002332 CLOCK 2 Z80782 H 63 AF054174 H2AFY 3 AF035287 SE 64 Z11692 EEF2 4 Y08614 XI 65 AA846749 APOM 5 AL0499444 DI 66 D83702 CRY1 6 Z3738 HH 67 U2256 LSS 7 U35451 CI 68 AD007960 SH3GELB1 8 AL050089 BA 69 J02625 CYP2E1 9 AF009615 AI 70 AL080119 PAI-R8P1 10 X59892 W 71 M24398 PTMS 11 U51920 SR 73 M32313 SRD5A1 12 D13629 KI 74 L18960 EIF1A 13 Z80779 HI 75 D87002 IGL 14 A8023187 KI 76 S76992 VA2 15 U33204 PL 77 M95678 PL6 17 <t< th=""><th>Gene symbol</th><th>GenBank</th><th>No.</th><th>Gene symbol</th><th>GenBank</th><th>No.</th></t<>	Gene symbol	GenBank	No.	Gene symbol	GenBank	No.
61 U91329 KIFIC I AF03028 TI 62 AB00332 CLOCK 2 Z80782 HI 63 AF054174 H2AFY 3 AF035287 SE 64 Z11692 EEF2 4 Y08614 M4 65 AA846749 APO35287 SE Af035287 SE 66 D83702 CRY1 6 Z83738 HI 67 U22526 LSS 7 U35451 CC 68 AB007960 SH3GLB1 8 AL050089 BA 70 AL080119 PA1-RBP1 O X59892 W 71 M24398 PTMS 10 X59892 W 72 AB16034 NOLA2 11 U51920 SR 73 M32313 SRD5A1 12 D13629 K 74 L18960 EIF1A 14 AB023187 K 75 D87002 VAV2						
62 AB002332 CLOCK 2 Z80782 HI 63 AF0054174 H2AFY 3 AF035287 SI 64 AT1692 EFE2 4 Y08614 XI 65 AA846749 APOM 5 AL049944 DD 66 DB3702 CRY1 6 Z83738 HI 67 U22526 LSS 7 U35451 CF 68 AB007960 SH3GLB1 8 AL050089 BK 70 AL080119 PA1-R8P1 10 X59892 W 71 M24398 PTMS 11 U51920 SR 73 M32313 SRD5A1 12 D13629 KI 74 L18960 EIF1A 13 Z80779 HI 75 D87002 IGL 14 AB02317 KI 76 S7692 VA2 15 U33204 PI 77 M95678 PLCB2 1				VIE10	1101220	
63 AF054174 H2AFY 3 AF035287 SET 64 Z11692 EEF2 4 Y08614 X1 65 AA846749 APOM 5 AL049944 DD 66 D83702 CRY1 6 Z83738 HI 67 U22526 L.SS 7 U35451 CF 68 AB007960 SH3GLB1 8 AL030089 BA 69 J02625 CYP2E1 9 AF009615 AA 70 AL080119 PA1-RBP1 10 X59892 W 71 M23313 SRD5A1 12 D13629 KX 73 M32313 SRD5A1 12 D13629 KX 74 L18960 EF1A 13 Z8079 FH 75 D87002 IGL 14 AB023187 KX 77 M95678 PLCB2 17 AB80844 GF 78 K63931 RPL6	T1A-2					
64 Z11692 EEP2 4 Y08611 X1 65 AA846749 APOM 5 AL049944 Di 66 D83702 CRY1 6 Z83738 HI 67 U22526 LSS 7 U35451 CF 68 AB007960 SH3GLB1 8 AL050089 BØ 69 J02625 CYP2E1 9 AF009615 AI 70 AL080119 PAL-R8P1 10 X59892 W 71 M24398 PTMS 11 U5120 SR 73 M32313 SRD5A1 12 D13629 KI 74 L18960 EIF1A 13 Z80779 HI 75 D57002 IGL 15 U33204 PI 77 M95678 PLC52 16 D38551 R 78 K69391 RPL6 17 A1888084 G6 81 M38378 SYN1 20<	HIST1H2BI		2			
65 AA846749 APOM 5 AL049944 Di 66 DB3702 CRY1 6 Z83738 HI 67 U22526 LSS 7 U35451 CE 68 Ab007960 SH13CLB1 8 AL050089 B/ 69 J02625 CYP2E1 9 AF009615 AI 70 AL080119 PA1-RBP1 10 X59892 W 71 M24398 PTMS 11 U31920 SR 73 M32313 SRD5A1 12 D13629 KX 74 L18960 EIF1A 14 AB023187 KK 75 D87002 IGL 15 U33204 PL 76 S76992 VAV2 15 U38204 PL 77 M95678 PLCB2 16 D38551 R/ 78 X69391 RPL6 17 AL88084 GI 78 X69305 RPS12 <t< td=""><td>SDFR1</td><td></td><td></td><td></td><td></td><td></td></t<>	SDFR1					
66 D83702 CRY1 6 D83702 CRY1 6 67 U22326 LSS 7 U35451 CE 68 AB007960 SH3GLB1 8 ALD50089 B/ 69 J02625 CYP2E1 9 AF009015 AI 70 AL080119 PAI-R8P1 10 X59892 W 71 M24398 PTMS 11 U51920 SR 73 M32313 SRD5A1 12 D13629 K1 74 L18960 EIFIA 13 Z80779 H 75 D87002 IGI 14 AB033187 KI 76 S76992 VAV2 16 D38551 R/ 77 M95678 PLCB2 17 AI888084 GF 78 X69391 PL6 17 AI88804 GF 79 M86546 PBX1 18 X63679 TF 81 M500233 KP	XPO1					
67 U22526 LSS 7 U35451 CF 68 AB007960 SH3GCB1 8 AL050089 B/ 69 J02625 CYP2E1 9 AF009615 AI 70 AL080119 PAI-R8P1 10 X59892 W 71 M2398 PTMS 11 U51920 SR 72 AI816034 NOLA2 12 D13629 KT 73 M32313 SRD5A1 12 D13629 KT 75 D87002 IGL 14 AB023187 KT 76 S76992 VAV2 16 D38551 RZ 78 X69391 RPL6 17 AI88084 GF 78 X69391 RPL6 17 AI88084 GF 78 X69391 RPL6 17 AI88084 GF 80 AB002533 KPNA4 19 D83485 GF 80 AB02535	DKFZP564G2022					
68 AB007960 SH3GLB1 1 CD0110 CH 69 J02625 CYP2E1 8 ALD50089 BZ 70 AL080119 PAI-RBP1 J0 X59892 W 71 M24398 PTMS I1 U51920 SR 73 M32313 SRD5A1 12 D13629 KT 74 L18960 EIFIA 13 Z80779 HT 75 D87002 IGL 14 AB023187 KT 76 S76992 VAV2 16 D38551 RZ 77 M95678 PLCB2 16 D38551 RZ 78 X69391 RPL6 17 Al88084 GT 79 M86546 PBX1 18 X63679 TB 81 M58378 SYN1 20 AL120559 AL 82 X53505 RPS12 21 U29332 IF 83 Y09445 TBX5 <td< td=""><td>HIST1H2BM</td><td></td><td></td><td></td><td></td><td></td></td<>	HIST1H2BM					
69 J02625 CYP2E1 3 AE00305 JA 70 AL080119 PAI-RBP1 10 X59892 W 71 M23398 PTMS 11 U51920 SR 73 M32313 SRD5A1 13 Z8079 HI 74 L18960 EIF1A 13 Z8079 HI 75 D87002 IGL 14 AB023187 KI 76 S76992 VAV2 15 U53204 PL 77 M95678 PLCB2 16 D38551 R/ 78 X69391 RPL6 17 AI88084 GF 79 M86546 PBX1 18 X63679 TF 80 AB002533 KPNA4 19 D83485 GF 81 M58378 SYN1 20 AL120559 AF 82 X53505 RYN12 21 U29332 GF 83 Y09445 TBX5 22<	CBX1					
70 AL080119 PAL-RP1 9 AL080119 PAL-RP1 9 AL080119 AL 71 M24398 PTMS 10 US920 WR 72 Al816034 NOLA2 11 US920 KR 73 M32313 SRD5A1 13 Z80779 HI 74 L18960 EIF1A 14 AB023187 KI 75 D87002 IGL 14 AB023187 KI 76 S76992 VAV2 15 US3204 PL 77 M95678 PLCB2 16 D38551 RZ 78 X69391 RPL6 17 Al880844 GF 79 M66546 PBX1 18 X63679 TF 80 AB002533 KPNA4 19 D83485 GF 81 M5378 SYN1 20 AL120559 AF 82 X53505 RPS12 21 U29332 CZ	BAZ1A					
71 M24398 PTMS 10 M3232 M 72 AI816034 NOLA2 11 US1920 SR 73 M32313 SRD5A1 13 Z80779 HI 74 L18960 EIF1A 13 Z80779 HI 75 D87002 IGL 14 AB023187 KI 76 S76992 VAV2 15 US3204 PL 77 M95678 PLCB2 16 D38551 R4 78 K69391 RPL6 1 AI888084 GF 79 M86546 PBX1 18 X63679 TF 80 AB002533 KPN4 19 D83485 GF 81 M58378 SYN1 20 AL120559 AF 82 X5305 RPS12 21 U29332 FF 83 Y09445 TBX5 22 AF013759 CZ 84 AF112472 CAMK2B 23	ADAM10					
72 AI816034 NOLA2 11 D13629 KX 73 M32313 SRD5A1 13 Z80779 HI 74 L18960 EIF1A 14 AB023187 KI 75 D87002 IGL 14 AB023187 KI 76 S76992 VAV2 15 U35204 PL 77 M95678 PLCB2 16 D38551 R/ 79 M86546 PBX1 18 X63679 TF 80 AB002533 KPNA4 19 D3485 GF 81 M58378 SYN1 20 AL120559 AI 82 X5305 RPS12 21 U29332 FF 83 Y09445 TBX5 22 AF013759 C/ 84 AF025654 RNGTT 24 AA142964 LC 86 AB007447 FLN29 25 AL049851 C/ 87 AF006621 SL	WARS					
73M32313SRD5A112D1029KL74L18960EIF1A13Z80779HI75D87002IGL15U53204PL76S76992VAV215U53204PL77M95678PLCB216D38551R/78X69391RPL617A188084GI79M86546PBX118X63679TF80AB002533KPNA419D83485GI81M58378SYN120AL120559AI82X5305RPS1221U29332FF83Y09445TBX522AF013759CC84AF112472CAMK2B23X82103CC85AF025654RNGTT24AA142964LC86AB007447FLN2925AL049851C/87AF006621SLC30A926U76421AI88U35139NDN27A1365215RF89M23323CD3E28AF084523CF90U79259DJ159A19.329M35878IG91X04828GNA1230AF085692AI92Z22865DPT31D38521PS93AF037195RGS1432L02426PS94W72733KIAA153633U81006TN95AL006744REV3L34A122352HI96AB005047 <td>SRP54</td> <td></td> <td></td> <td></td> <td></td> <td></td>	SRP54					
74L18960EIF1A15 2807.97 PI75D87002IGL14AB023187KI76S76992VAV215U33204PI77M95678PLCB216D38551RJ78X69391RPL617AI888084GI79M86546PBX118X63679TF80AB0025333KPNA419D83485GI81M58378SYN120AL120559AI82X53505RPS1221U29332FF83Y09445TBX522AF013759CZ84AF112472CAMK2B23X82103CC85AF025654RNGTT24AA142964LC86AB007447FLN2925AL049851CZ87AF006621SLC30A926U76421AI88U35139NDN27A1365215RI89M23323CD3E28AF084523CB90U79259DJ159A19.329M58878IG91X04828GNA1230AF085692AI92Z2865DPT31D38521PS94W72733KIAA153633U81006TN95AL096744REV3L34AJ223352HI96AB007896KIAA0436AA04324NP97AB007896KIAA0436AB014562PF98L24564	KTN1					
75 D87002 IGL 14 AB023187 NI 76 S76992 VAV2 15 U53204 PL 77 M95678 PLCB2 16 D38551 R7 78 X69391 RPL6 17 Al888084 GI 79 M86546 PBX1 18 X63679 TR 80 AB002533 KPNA4 19 D83485 GI 81 M58378 SYN1 20 AL120559 AF 82 X53505 RPS12 21 U29332 FF 83 Y09445 TBX5 22 AF013759 C/ 84 AF112472 CAMK2B 23 X82103 CO 85 AF025654 RNGTT 24 AA142964 LC 86 AB007447 FL29 25 AL049851 C/ 87 AF006621 SLC30A9 26 U76421 AI 88 U35139 NDN	HIST1H2BF					
76S76992VAV215U53204PL77M95678PLCB216D38551R/278X69391RPL617A1888084GF79M86546PBX118X63679TF80AB002533KPNA419D83485GF81M58378SYN120AL120559AF82X53505RPS1221U29332FF83Y09445TBX522AF013759C/284AF112472CAMK2B23X82103CC85AF025654RNGTT24AA142964LC86AB007447FLN2925AL049851C/387AF006621SLC30A926U76421AF88U35139NDN27A1365215RF89M23323CD3E28AF084523CF90U79259DJ159A19.329M35878IG91X04828GNA1230AF085692AI92Z22865DPT31D38521PS93AF037195RGS1432L02426PF94W72733KIAA153633U81006TK95AL096744REV3L34AJ223352HI96AB005047SH3BP535AF043324NN97AB07896KIAA043666AB014562PF98L24564RRAD37AA16780TF99 </td <td>KIAA0970</td> <td></td> <td></td> <td></td> <td></td> <td></td>	KIAA0970					
77M95678PLCB216D38551R778X69391RPL617Al888084GI79M86546PBX118X63679TF80AB002533KPNA419D83485GI81M58378SYN120AL120559AI82X53505RPS1221U29332FF83Y09445TBX522AF013759C/84AF112472CAMK2B23X82103CC85AF025654RNGTT24AA142964LC86AB007447FLN2925AL049851C/87AF006621SLC30A926U76421AI88U35139NDN27AI365215RF89M23323CD3E28AF084523CF90U79259D159A19.329M35878IG91X04828GNAI230AF085692AF92Z22865DPT31D38521PS93AF037195RGS1432L02426PS94W72733KIAA153633U81006TN95AL096744REV3L34AI223352HI96AB005047SH3BP535AF04324NN97AB007896KIAA043636AB014562PF98L24564RRAD37AA176780TR100Z22555SCA8B139L04282ZN101<	PLEC1					
78 X69391 RPL6 17 Al888084 Gi 79 M86546 PBX1 18 X63679 TR 80 AB002533 KPNA4 19 D83485 GI 81 M58378 SYN1 20 AL120559 AI 82 X53505 RPS12 21 U29332 FF 83 Y09445 TBX5 22 AF013759 C/ 84 AF112472 CAMK2B 23 X82103 CC 85 AF025654 RNGTT 24 AA142964 LC 86 AB007447 FLN29 25 AL049851 C/ 87 AF006621 SLC30A9 26 U76421 AI 89 M23323 CD3E 28 AF084523 CF 90 U79259 DJ159A19.3 29 M35878 IG 91 X04828 GNA12 30 AF085692 AF 92 Z22865 DF	RAD21					
79 M86546 PBX1 18 X63679 TR 80 AB002533 KPNA4 19 D83485 GR 81 M58378 SYN1 20 AL120559 AF 82 X53505 RPS12 21 U29332 FF 83 Y09445 TBX5 22 AF013759 C/ 84 AF112472 CAMK2B 23 X82103 CC 85 AF025654 RNGTT 24 AA142964 LC 86 AB007447 FLN29 25 AL049851 C/ 87 AF006621 SLC30A9 26 U76421 AF 88 U35139 NDN 27 AI365215 RF 89 M23323 CD3E 28 AF084523 CF 90 U79259 D159A19.3 29 M35878 IG 91 X04828 GNA12 30 AF085692 AH 92 Z2865 DPT </td <td>GPR107</td> <td>AI888084</td> <td></td> <td></td> <td></td> <td></td>	GPR107	AI888084				
80 AB002533 KPNA4 19 D83485 GF 81 M58378 SYN1 20 AL120559 AF 82 X53505 RPS12 21 U29332 FF 83 Y09445 TBX5 22 AF013759 CZ 84 AF112472 CAMK2B 23 X82103 CC 85 AF025654 RNGTT 24 AA142964 LC 86 AB007447 FLN29 25 AL049851 CZ 87 AF006621 SLC30A9 26 U76421 AF 88 U35139 NDN 27 AI365215 RF 89 M23323 CD3E 28 AF084523 CF 90 U79259 D1159A19.3 29 M35878 IG 91 X04828 GNA12 30 AF085692 AF 92 Z22865 DPT 31 D38521 PS 94 W72733 KISA	TRAM1	X63679				
81 M58378 SYN1 20 AL120559 AI 82 X53505 RPS12 21 U29332 FF 83 Y09445 TBX5 22 AF013759 C/ 84 AF112472 CAMK2B 23 X82103 CC 85 AF025654 RNGTT 24 AA142964 LC 86 AB007447 FLN29 25 AL049851 C/ 87 AF006621 SLC30A9 26 U76421 AI 88 U35139 NDN 27 AI365215 RF 89 M23323 CD3E 28 AF084523 CF 90 U79259 D1159A19.3 29 M35878 IG 91 X04828 GNA12 30 AF085692 AI 92 Z22865 DPT 31 D38521 PS 93 AF037195 RGS14 32 L02426 PS 94 W72733 KIAA	GRP58	D83485	19			
82 X53505 RPS12 21 U29332 FF 83 Y09445 TBX5 22 AF013759 C4 84 AF112472 CAMK2B 23 X82103 C2 85 AF025654 RNGTT 24 AA142964 LC 86 AB007447 FLN29 25 AL049851 C4 88 U35139 NDN 27 Al365215 RF 89 M23323 CD3E 28 AF084523 CF 90 U79259 D1159A19.3 29 M35878 IG 91 X04828 GNA12 30 AF085692 AI 92 Z22865 DPT 31 D38578 IG 93 AF037195 RGS14 32 L02426 PS 94 W72733 KIAA1536 33 U81006 TN 95 AL096744 REV31 34 A1223352 HI 96 AB005047	ARPP-19	AL120559	20			
83 Y0945 TBX5 22 AF013759 C4 84 AF112472 CAMK2B 23 X82103 C0 85 AF025654 RNGTT 24 AA142964 L0 86 AB007447 FLN29 25 AL049851 C4 87 AF006621 SLC30A9 26 U76421 AI 88 U35139 NDN 27 Al365215 RF 89 M23323 CD3E 28 AF084523 CF 90 U79259 DJ159A19.3 29 M35878 IG 91 X04828 GNA12 30 AF085692 AI 92 Z22865 DPT 31 D38521 PS 93 AF037195 RGS14 32 L02426 PS 94 W72733 KIAA0436 36 AB014562 PF 95 AL096744 REV31 34 AJ223352 HI 96 AB007896	FHL2	U29332	21			
84 AF112472 CAMK2B 23 X82103 CC 85 AF025654 RNGTT 24 AA142964 LC 86 AB007447 FLN29 25 AL049851 C/A 87 AF006621 SLC30A9 26 U76421 AI 88 U35139 NDN 27 AI365215 RF 89 M23323 CD3E 28 AF084523 CF 90 U79259 DJ159A19.3 29 M35878 IG 91 X04828 GNAI2 30 AF085692 AF 92 Z22865 DPT 31 D38521 PS 93 AF037195 RG14 32 L02426 PS 94 W72733 KIAA1536 33 U81006 TN 95 AL096744 REV3L 34 AJ223352 HI 96 AB005047 SH3BP5 35 AF043324 NN 97 AB005047	CALU	AF013759	22			
85 AF025654 RNGTT 24 AA142964 LC 86 AB007447 FLN29 25 AL049851 CA 87 AF006621 SLC30A9 26 U76421 AI 88 U35139 NDN 27 AI365215 RH 89 M23323 CD3E 28 AF084523 CF 90 U79259 DJ159A19.3 29 M35878 IG 91 X04828 GNA12 30 AF085692 AH 92 Z22865 DPT 31 D38521 PS 93 AF037195 RGS14 32 L02426 PS 94 W72733 KIAA1536 33 U81006 TN 95 AL096744 REV3L 34 AJ22352 HI 96 AB005047 SH3BP5 35 AF043324 NN 98 L24564 RRAD 37 AA176780 TF 99 W26677 <	COPB	X82103	23			
86 AB007447 FLN29 25 AL049851 C4 87 AF006621 SLC30A9 26 U76421 AI 88 U35139 NDN 27 AI365215 RF 89 M23323 CD3E 28 AF084523 CF 90 U79259 DJ159A19.3 29 M35878 IG 91 X04828 GNA12 30 AF085692 AF 92 Z22865 DPT 31 D38521 PS 93 AF037195 RGS14 32 L02426 PS 94 W72733 KIAA1536 33 U81006 TM 95 AL096744 REV3L 34 AJ223352 HI 96 AB007896 KIAA0436 36 AB014562 PF 98 L24564 RRAD 37 AA176780 TR 99 W26677 FLJ35827 38 AJ010901 MI 100 Z22555	LOC285148	AA142964	24			
87 AF006621 SLC30A9 26 U76421 AI 88 U35139 NDN 27 AI365215 RF 89 M23323 CD3E 28 AF084523 CF 90 U79259 DJ159A19.3 29 M35878 IG 91 X04828 GNAI2 30 AF085692 AF 92 Z22865 DPT 31 D38521 PS 93 AF037195 RGS14 32 L02426 PS 94 W72733 KIAA1536 33 U81006 TN 95 AL096744 REV3L 34 AJ23352 HI 96 AB005047 SH3BP5 35 AF043324 NN 97 AB007896 KIAA0436 36 AB014562 PF 98 L24564 RRAD 37 AA176780 TR 99 W26677 FLJ35827 38 AJ010901 MI 100 Z2555	CARD10	AL049851	25			
88 U35139 NDN 27 AI365215 RF 89 M23323 CD3E 28 AF084523 CF 90 U79259 DJ159A19.3 29 M35878 IG 91 X04828 GNA12 30 AF085692 AF 92 Z22865 DPT 31 D38521 PS 93 AF037195 RGS14 32 L02426 PS 94 W72733 KIAA1536 33 U81006 TN 95 AL096744 REV3L 34 AJ223352 HI 96 AB005047 SH3BP5 35 AF043324 NM 97 AB007896 KIAA0436 36 AB014562 PF 98 L24564 RRAD 37 AA176780 TR 99 W26677 FLJ35827 38 AJ010901 MI 100 Z22555 SCARB1 39 L04282 ZM 101 M64174	ADARB1	U76421	26			
90U79259DJ159A19.329M35878IG91X04828GNA1230AF085692AI92Z22865DPT31D38521PS93AF037195RGS1432L02426PS94W72733KIAA153633U81006TN95AL096744REV3L34AJ223352HI96AB005047SH3BP535AF043324NN97AB007896KIAA043636AB014562PF98L24564RRAD37AA176780TF99W26677FLJ3582738AJ010901MI100Z22555SCARB139L04282ZN101M64174JAK140M57730EF102U88629ELL241AF038844DU103S77812FLT141AF038844DU104U26455ATM42AB014529AF105X72631NR1D143J03060GF108AF010242SYNPO46AF006010DI109M81141HLA-DQB147AF012072EI110M17016GZMB48AB002357KI111U69645ZNF3249L24123NF112X54871RAB5B50X76534GF113U15552F1AF011468ST114AB014597ANKRD1752X13466AF11	RRAS2	AI365215	27	NDN		
91X04828GNA1230AF085692AH92Z22865DPT31D38521PS93AF037195RGS1432L02426PS94W72733KIAA153633U81006TM95AL096744REV3L34AJ223352HI96AB005047SH3BP535AF043324NM97AB007896KIAA043636AB014562PH98L24564RRAD37AA176780TR99W26677FLJ3582738AJ010901MI100Z22555SCARB139L04282ZD101M64174JAK140M57730EH102U88629ELL241AF038844DU103S77812FLT142AB014529AH104U26455ATM43J03060GH105X72631NR1D143J03060GH106M83088PGM144M13194ER107M28393PRF145X55885KI108AF010242SYNPO46AF006010DI109M81141HLA-DQB147AF012072EI110M17016GZMB48AB002357KI111U69645ZNF3249L24123NR112X54871RAB5B50X76534GH113U1552HSU1555251AF011468ST <t< td=""><td>CREG</td><td>AF084523</td><td>28</td><td>CD3E</td><td>M23323</td><td>89</td></t<>	CREG	AF084523	28	CD3E	M23323	89
92Z22865DPT31D38521PS93AF037195RGS1432L02426PS94W72733KIAA153633U81006TN95AL096744REV3L34A1223352HI96AB005047SH3BP535AF043324NN97AB007896KIAA043636AB014562PH98L24564RRAD37AA176780TR99W26677FLJ3582738AJ010901MI100Z22555SCARB139L04282ZN101M64174JAK140M57730EH102U88629ELL241AF038844DU103S77812FLT141AF038844DU104U26455ATM42AB014529AH105X72631NR1D143J03060GH106M83088PGM144M13194EH107M28393PRF145X55885KI108AF010242SYNPO46AF006010DI109M81141HLA-DQB147AF012072EH110M17016GZMB48AB002357KI111U69645ZNF3249L2423NH112X54871RAB5B50X76534GH113U1552HSU1552251AF011468ST114AB014597ANKRD1752X13466AH <t< td=""><td>IGFBP3</td><td>M35878</td><td>29</td><td>DJ159A19.3</td><td>U79259</td><td>90</td></t<>	IGFBP3	M35878	29	DJ159A19.3	U79259	90
93AF037195RGS1432L02426PS94W72733KIAA153633U81006TN95AL096744REV3L34AJ223352HI96AB005047SH3BP535AF043324NN97AB007896KIAA043636AB014562PH98L24564RRAD37AA176780TR99W26677FLJ3582738AJ010901MI100Z22555SCARB139L04282ZN101M64174JAK140M57730EH102U88629ELL241AF038844DU103S77812FLT141AF038844DU104U26455ATM42AB014529AH105X72631NR1D143J03060GH106M83088PGM144M13194ER107M28393PRF145X55885KI108AF010242SYNPO46AF006010DI109M81141HLA-DQB147AF012072EI110M17016GZMB48AB002357KI111U69645ZNF3249L24123NH112X54871RA55B50X76534GH113U1552HSU155251AF011468ST114AB014597ANKRD1752X13466AF115M64322PTPN753U28249FX <td>ABCC3</td> <td>AF085692</td> <td>30</td> <td>GNAI2</td> <td>X04828</td> <td>91</td>	ABCC3	AF085692	30	GNAI2	X04828	91
94W72733KIAA153633U81006TM95AL096744REV3L34AJ223352HI96AB005047SH3BP535AF043324NM97AB007896KIAA043636AB014562PH98L24564RRAD37AA176780TR99W26677FLJ3582738AJ010901MI100Z22555SCARB139L04282ZN101M64174JAK140M57730EF102U88629ELL241AF038844DU103S77812FLT142AB014529AH104U26455ATM43J03060GH105X72631NR1D143J03060GH106M83088PGM144M13194ER107M28393PRF145X55885KI108AF010242SYNPO46AF006010DI109M81141HLA-DQB147AF012072EI110M17016GZMB48AB002357KI111U69645ZNF3249L24123NF112X54871RAB5B50X76534GF113U1555251AF011468ST114AB014597ANKRD1752X13466AF115M64322PTPN753U28249FX116AB018333SASH154U49020M	PSME4	D38521	31	DPT	Z22865	
94 W72733 KIAA1536 33 U81006 TM 95 AL096744 REV3L 34 AJ223352 HI 96 AB005047 SH3BP5 35 AF043324 NM 97 AB007896 KIAA0436 36 AB014562 PF 98 L24564 RRAD 37 AA176780 TR 99 W26677 FLJ35827 38 AJ010901 MI 100 Z22555 SCARB1 39 L04282 ZM 101 M64174 JAK1 40 M57730 EF 102 U88629 ELL2 41 AF038844 DU 103 S77812 FLT1 42 AB014529 AF 104 U26455 ATM 43 J03060 GF 105 X72631 NR1D1 43 J03060 GF 106 M83088 PGM1 44 M13194 EF 107 M28393 <	PSMC1	L02426	32			
95 AL096744 REV3L 34 AJ223352 HI 96 AB005047 SH3BP5 35 AF043324 NN 97 AB007896 KIAA0436 36 AB014562 PH 98 L24564 RRAD 37 AA176780 TR 99 W26677 FLJ35827 38 AJ010901 MI 100 Z22555 SCARB1 39 L04282 ZN 101 M64174 JAK1 40 M57730 EH 102 U88629 ELL2 41 AF038844 DU 103 S77812 FLT1 42 AB014529 AH 104 U26455 ATM 43 J03060 GH 105 X72631 NR1D1 43 J03060 GH 106 M83088 PGM1 44 M13194 ER 107 M28393 PRF1 45 X55885 KI 108 AF010242 <t< td=""><td>TM9SF2</td><td>U81006</td><td></td><td></td><td></td><td></td></t<>	TM9SF2	U81006				
96 AB005047 SH3BP5 35 AF043324 NN 97 AB007896 KIAA0436 36 AB014562 PH 98 L24564 RRAD 37 AA176780 TR 99 W26677 FLJ35827 38 AJ010901 M1 100 Z22555 SCARB1 39 L04282 ZN 101 M64174 JAK1 40 M57730 EF 102 U88629 ELL2 41 AF038844 DU 103 S77812 FLT1 42 AB014529 AF 104 U26455 ATM 42 AB014529 AF 105 X72631 NR1D1 43 J03060 GF 106 M83088 PGM1 44 M13194 ER 107 M28393 PRF1 45 X55885 KI 108 AF010242 SYNPO 46 AF006010 DI 109 M81141 <	HIST1H2BK				AL096744	
97AB007896KIAA043636AB014562PH98L24564RRAD37AA176780TR99W26677FLJ3582738AJ010901MI100Z22555SCARB139L04282ZN101M64174JAK140M57730EF102U88629ELL241AF038844DU103S77812FLT142AB014529AF104U26455ATM42AB014529AF105X72631NR1D143J03060GF106M83088PGM144M13194ER107M28393PRF145X55885KI108AF010242SYNPO46AF006010DI109M81141HLA-DQB147AF012072EI110M17016GZMB48AB002357KI111U69645ZNF3249L24123NF112X54871RAB5B50X76534GF113U15552HSU1555251AF011468ST114AB014597ANKRD1752X13466AF115M64322PTPN753U28249FX116AB018333SASH154U49020MI	NMT1					
98 L24564 RRAD 37 AA176780 TR 99 W26677 FLJ35827 38 AJ010901 MI 100 Z22555 SCARB1 39 L04282 ZN 101 M64174 JAK1 40 M57730 EF 102 U88629 ELL2 41 AF038844 DU 103 S77812 FLT1 42 AB014529 AF 104 U26455 ATM 42 AB014529 AF 104 U26455 ATM 43 J03060 GF 105 X72631 NR1D1 43 J03060 GF 106 M83088 PGM1 44 M13194 EF 107 M28393 PRF1 45 X55885 KI 108 AF010242 SYNPO 46 AF006010 DI 109 M81141 HLA-DQB1 47 AF012072 EI 110 M169645 ZNF	PHF2					
99W26677FLJ3582738AJ010901MI100Z22555SCARB139L04282ZN101M64174JAK140M57730EF102U88629ELL241AF038844DU103S77812FLT142AB014529AF104U26455ATM43J03060GF105X72631NR1D143J03060GF106M83088PGM144M13194ER107M28393PRF145X55885KI108AF010242SYNPO46AF006010DI109M81141HLA-DQB147AF012072EI110M17016GZMB48AB002357KI111U69645ZNF3249L24123NF112X54871RAB5B50X76534GF113U15552HSU1555251AF011468ST114AB014597ANKRD1752X13466AF115M64322PTPN753U28249FX116AB018333SASH154U49020MI	TRIM44					
100Z22555SCARB139L04282ZN101M64174JAK140M57730EF102U88629ELL241AF038844DU103S77812FLT142AB014529AF104U26455ATM43J03060GF105X72631NR1D144M13194EF106M83088PGM144M13194EF107M28393PRF145X55885KI108AF010242SYNPO46AF006010DI109M81141HLA-DQB147AF012072EI110M17016GZMB48AB002357KI111U69645ZNF3249L24123NF112X54871RAB5B50X76534GF113U15552HSU1555251AF011468ST114AB014597ANKRD1752X13466AF115M64322PTPN753U28249FX116AB018333SASH154U49020MI	MUC4					
101M64174JAK140M57730EF102U88629ELL241AF038844DU103S77812FLT142AB014529AF104U26455ATM42AB014529AF105X72631NR1D143J03060GF106M83088PGM144M13194EF107M28393PRF145X55885KI108AF010242SYNPO46AF006010DI109M81141HLA-DQB147AF012072EI110M17016GZMB48AB002357KI111U69645ZNF3249L24123NF112X54871RAB5B50X76534GF113U15552HSU1555251AF011468ST114AB014597ANKRD1752X13466AF115M64322PTPN753U28249FX116AB018333SASH154U49020MI	ZNF148					
102U88629ELL241AF038844DU103S77812FLT142AB014529AB104U26455ATM42AB014529AB105X72631NR1D143J03060GB106M83088PGM144M13194ER107M28393PRF145X55885KI108AF010242SYNPO46AF006010DI109M81141HLA-DQB147AF012072EI110M17016GZMB48AB002357KI111U69645ZNF3249L24123NB112X54871RAB5B50X76534GB113U15552HSU1555251AF011468ST114AB014597ANKRD1752X13466AF115M64322PTPN753U28249FX116AB018333SASH154U49020MI	EFNA1					
103S7/812FL1142AB014529AH104U26455ATM43J03060GH105X72631NR1D143J03060GH106M83088PGM144M13194ER107M28393PRF145X55885KI108AF010242SYNPO46AF006010DI109M81141HLA-DQB147AF012072EI110M17016GZMB48AB002357KI111U69645ZNF3249L24123NH112X54871RAB5B50X76534GH113U15552HSU1555251AF011468ST114AB014597ANKRD1752X13466AH115M64322PTPN753U28249FX116AB018333SASH154U49020MI	DUSP14					
104026433ATM43J03060GH105X72631NR1D144M13194ER106M83088PGM144M13194ER107M28393PRF145X55885KI108AF010242SYNPO46AF006010DI109M81141HLA-DQB147AF012072EI110M17016GZMB48AB002357KI111U69645ZNF3249L24123NH112X54871RAB5B50X76534GH113U15552HSU1555251AF011468ST114AB014597ANKRD1752X13466AH115M64322PTPN753U28249FX116AB018333SASH154U49020MI	AKAP11					
105 A72051 NRTD1 44 M13194 ER 106 M83088 PGM1 45 X55885 KI 107 M28393 PRF1 45 X55885 KI 108 AF010242 SYNPO 46 AF006010 DI 109 M81141 HLA-DQB1 47 AF012072 EI 110 M17016 GZMB 48 AB002357 KI 111 U69645 ZNF32 49 L24123 NF 112 X54871 RAB5B 50 X76534 GF 113 U15552 HSU15552 51 AF011468 ST 114 AB014597 ANKRD17 52 X13466 AF 115 M64322 PTPN7 53 U28249 FX 116 AB018333 SASH1 54 U49020 MI	GBA					
100M83088PGM145X55885KI107M28393PRF145X55885KI108AF010242SYNPO46AF006010DI109M81141HLA-DQB147AF012072EI110M17016GZMB48AB002357KI111U69645ZNF3249L24123NF112X54871RAB5B50X76534GF113U15552HSU1555251AF011468ST114AB014597ANKRD1752X13466AF115M64322PTPN753U28249FX116AB018333SASH154U49020MI	ERCC1					
107M28393FKF146AF006010DI108AF010242SYNPO46AF006010DI109M81141HLA-DQB147AF012072EI110M17016GZMB48AB002357KI111U69645ZNF3249L24123NF112X54871RAB5B50X76534GF113U15552HSU1555251AF011468ST114AB014597ANKRD1752X13466AF115M64322PTPN753U28249FX116AB018333SASH154U49020MI	KDELR1					
108AF010242STRTO47AF012072EII109M81141HLA-DQB147AF012072EII110M17016GZMB48AB002357KI111U69645ZNF3249L24123NF112X54871RAB5B50X76534GF113U15552HSU1555251AF011468ST114AB014597ANKRD1752X13466AF115M64322PTPN753U28249FX116AB018333SASH154U49020MI	DD5					
105M61141MEDQB148AB002357KI110M17016GZMB48AB002357KI111U69645ZNF3249L24123NH112X54871RAB5B50X76534GH113U15552HSU1555251AF011468ST114AB014597ANKRD1752X13466AH115M64322PTPN753U28249FX116AB018333SASH154U49020MI	EIF4G3					
110M17010OLMB49L24123NH111U69645ZNF3249L24123NH112X54871RAB5B50X76534GH113U15552HSU1555251AF011468ST114AB014597ANKRD1752X13466AH115M64322PTPN753U28249FX116AB018333SASH154U49020MI	KIF3B					
111CoordsLAR52X76534GH112X54871RAB5B50X76534GH113U15552HSU1555251AF011468ST114AB014597ANKRD1752X13466AH115M64322PTPN753U28249FX116AB018333SASH154U49020MI	NFE2L1					
113U15552HSU1555251AF011468ST114AB014597ANKRD1752X13466AF115M64322PTPN753U28249FX116AB018333SASH154U49020MI	GPNMB					
114AB014597ANKRD1752X13466AF115M64322PTPN753U28249FX116AB018333SASH154U49020MI	STK6					
115M64322PTPN753U28249FX116AB018333SASH154U49020MI	APP					
116 AB018333 SASH1 54 U49020 MI	FXYD3					
	MEF2A					
	PDHX	U82328		PTGDS	M98539	117
	OCRL					
	MGC15523					
120 AF030234 SFRS2IP	110013323	A1010070	· · ·			

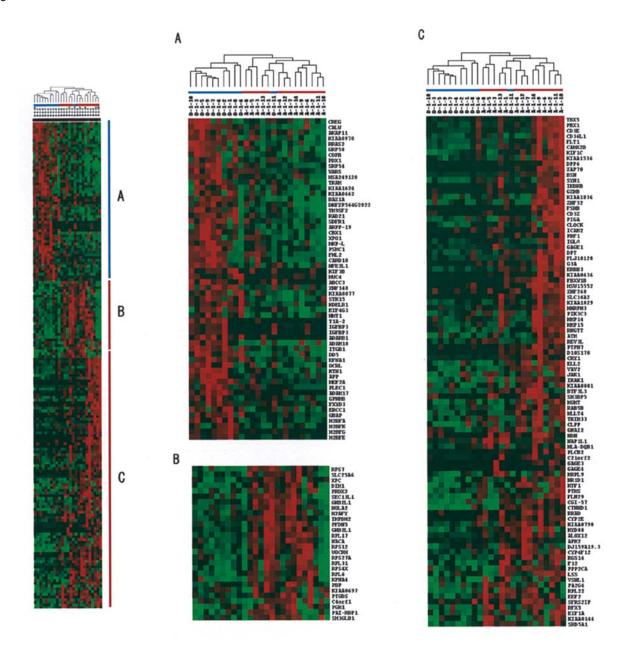


Figure 2. Supervised clustering analysis with expression data of the 177 genes differentially expressed between the 14 long-term survivors and the 11 short-term survivors. A dendrogram of the clustering analysis was generated with the same programs as used in Fig. 1. Two patient clusters (vertical bar) and the three major gene clusters (horizontal bar) are present. Three gene clusters are indicated as an insert. The long-term survivors (red), the short-term survivors (blue).

For example, GZMB/Granzyme B, Perforin1 and ICAM2 encoding crucial effectors for cytolysis by natural killer cells and cytotoxic T cells or for recruitment of those cells were found over-expressed in 14, 10, and 9 of the 14 long-term survivors, respectively. Similarly, of the 14 long-term survivors, upregulations were noted for GAGE1, 3 and 5 encoding tumor antigen mRNAs in 11, 12, and 10 patients, IRAK1, *MyD88* and *IKBKB* encoding factors for IL-1 receptor-mediated NFκB activation in 11, 9, and 9 patients, VAV2 and ZAP70 encoding crucial effectors for T cell activation by APC-T cell interaction in 9 and 9 patients, and the MHC class II gene in 9 patients, respectively. These data suggest that cytotoxic T cell activation occurred preferentially in the long-term survivors. It is also noted that a vascular endothelial cell receptor gene FLT1 was over-expressed in 7 of the 14 longterm survivors.

In contrast, there was only a limited number of genes involved in T cell activation in the 11 short-term survivors. As shown in Table II, 57 genes associated with the shortterm survivors were identified. *ABCC3/MRP3* and *ERCC1* which are involved in drug resistance were found overexpressed in 9 and 8 of the 11 short-term survivors. Among the other over-expressed genes in the short-term survivors, *BAZ1A* and *SRP54*, which were located at chromosome 14q12-13, have been reported amplified in ESCCs (12).

To verify the differences in expression indicated by microarray analysis, we performed slot blot analysis of cRNAs produced from total RNA extracted from 8 of the 14 long-term survivors and 8 of the 11 short-term survivors, whose RNAs were available in both microarray and slot blot analyses. Microarray data and the slot blot data of some of the above-mentioned genes are shown in Fig. 3. By slot blot analysis, over-expression

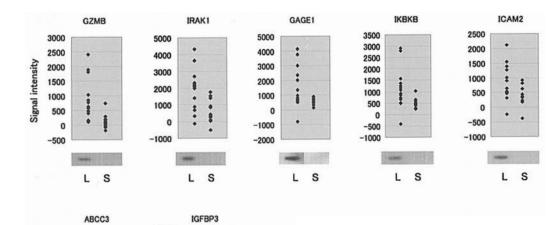
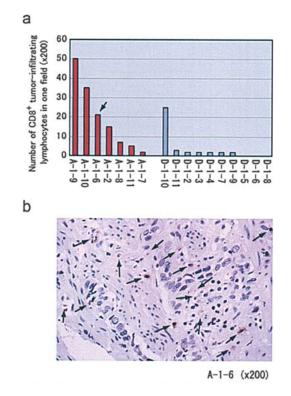


Figure 3. Microarray data and the slot blot data of the 7 genes differentially expressed between the long-term survivors and the short-term survivors. Microarray data of the 7 genes (*GZMB, ICAM, GAGE1, IRAK1*, and *IKBKB* over-expressed in long-term survivors; *ABCC3* and *IGFBP3* over-expressed in short-term survivors) are plotted (upper). The cRNA slot blot data of the 7 genes in two sets of total RNAs extracted from 8 of the 14 long-term survivors (L) and 8 of the 11 short-term survivors (S), whose RNAs were available in both microarray and slot blot analyses (lower). Over-expression of *GZMB, ICAM, GAGE1, IRAK1*, and *IKBKB* was observed in the long-term survivors. In the short-term survivors, over-expression of *ABCC3* and *IGFBP3* was confirmed.



2500

2000

1500

1000

500

-500

-1000

-1500

0

5

S

L

Signal intensity

90000

80000 70000

60000

50000

40000

30000

20000

10000

-10000

0

S

L

Figure 4. Immunohistochemical analysis of tumor infiltrating T-lymphocytes in long- and short-term survivors. (a), The CD8-positive T cell number in a field (x200) in 7 long-term survivors were 50, 35, 21, 15, 7, 5 and 2, respectively, whereas those in 11 short-term survivors were 25, 3, 2, 2, 2, 2, 0, 0, 0, and 0, respectively. (b), A representative result showing presence of tumor infiltrating CD8-positive T cells in long-term survivors is shown.

of *GZMB*, *ICAM*, *GAGE1*, *IRAK1*, and *IKBKB* was observed in the long-term survivors. In the short-term survivors, overexpression of *ABCC3* and *IGFBP3* was confirmed. Comparison of the number of tumor infiltrating T-lymphocytes between long- and short-term survivors. Next, using paraffinembedded tissues of 7 biopsy samples of the 14 long-term survivors and all of the 11 short-term survivors, which were available for immunohistochemistry, we compared the number of tumor infiltrating lymphocytes between the long-term survivors and the short-term survivors. As shown in Fig. 4, the CD8-positive T cell numbers in a field (x200) in 7 longterm survivors were 50, 35, 21, 15, 7, 5 and 2, respectively, whereas those in 11 short-term survivors were 25, 3, 2, 2, 2, 2, 2, 0, 0, 0, and 0, respectively. The CD8-positive T cell number in the long-term survivors was significantly higher than that in the short-term survivors, although A-1-7 and D-1-10 were exceptions. However, D-1-10, the short-term survivor with 25 CD8-positive T cells in a field, showed no over-expression of any of the 14 above-mentioned genes for T cell activation, whereas the genes were over-expressed in all of the long-term survivors except A-1-7. These data suggested that the increased number of activated T cells in a tumor is significantly associated with a good prognosis for patients with ESCC treated by CRT.

Discussion

The clustering analysis on gene expression profiles (Fig. 2) together with the known functions of the genes selected as prognostic marker candidates (Table II) suggested that multiple factors are involved in the sensitivity and resistance to CRT in ESCC. The early studies in the basic radiation biology of tumor tissues, both *in vitro* and *in vivo* showed that well-oxygenated cells are radiosensitive (13-15). Recently, it has been reported that microvessel density in laryngeal SCCs and ESCCs is a useful factor for predicting radiosensitivity (16,17). These vascularity-oxygenation-radio-

sensitivity relationships seem to be supported also by our analysis, in which we noted over-expression of a tumor vessel marker gene *FLT1* in the long-term survivors.

Second, it has been recognized that doses of radiation, lower than or equal to those that cause direct cytolysis, may alter the phenotype of target tissue by up-regulating gene products that may make tumor cells more susceptible to Tcell-mediated immune attack (18,19). Our present results (Fig. 4) suggest that the effect of CRT is correlated with the number of CD8-positive T cells in a tumor in each patient. Therefore, genes for T cell activation and for tumor vessel formation may become good markers for identifying potentially long-term survivors.

On the other hand, identification of poor-responders to CRT is also clinically important in the decision process for treatment modality. The genes which might be associated with the short-term survival include those for drug resistance or apoptosis resistance and some genes such as *BAZ1A* and *SRP54* showing gene amplification in ESCC.

Establishment of a CRT response prediction algorithm requires ascertainment of another set of validation samples. However, our analysis suggests the feasibility of such class prediction, and the gene list may contribute to the understanding of the underlying molecular architecture of the heterogeneity of CRT responses and the selection of useful markers for predicting the prognosis of individual ESCC patients treated with CRT alone.

Acknowledgments

This work was supported in part by the program for promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NiBio), in part by a Grant-in-Aid for the Third Comprehensive 10-Year Strategy for Cancer Control and for Cancer Research (15-5 and 16-16) from the Ministry of Health, Labour and Welfare of Japan, and in part by a Research Grant of the Princess Takamatsu Cancer Research Fund. A.A. was an awardee of Research Resident Fellowships from the Foundation for Promotion of Cancer Research.

References

- 1. Roth JA and Putnam JB Jr: Surgery for cancer of the esophagus. Semin Oncol 21: 453-461, 1994.
- Akiyama H, Tsurumaru M, Udagawa H and Kajiyama Y: Radical lymph node dissection for cancer of the thoracic esophagus. Ann Sug 220: 364-373, 1994.
- 3. Baba M, Aikou T, Yoshinaga H, Natsugoe S, Fukumoto T, Shimazu H and Akazawa K: Long-term results of subtotal esophagectomy with three-field lymphadenectomy for carcinoma of the thoracic esophagus. Ann Sug 219: 310-316, 1994.

- 4. Ando N, Iizuka T, Kakegawa T, Isono K, Watanabe H, Ide H, Tanaka O, Shinoda M, Takiyama W, Arimori M, Ishida K and Tsugane S: A randomized trial of surgery with and without chemotherapy for localized squamous carcinoma of the thoracic esophagus: the Japan Clinical Oncology Group Study. J Thorac Cardiovasc Surg 114: 205-209, 1997.
- Scanlon KJ, Newman YL and Priest DG: Biochemical basis for cisplatin and 5-fluorouracil synergism in human ovarian carcinoma cells. Proc Natl Acad Sci USA 83: 8923-8925, 1986.
- Byfield JE: Combined modality infusional chemotherapy with radiation. In: Cancer Chemotherapy by Infusion. 2nd edition. Lokich JJ (ed). Percepta Press, Chicago, pp521-551, 1990.
 Douple EB and Richmond RC: A review of interactions between
- Douple EB and Richmond RC: A review of interactions between platinum coordination complexes and ionizing radiation: implication for cancer therapy. In: Cisplatin: Current Status and New Developments. Prestayko AW, Crook ST and Karter SK (eds). Academic, Orlando, pp125-147, 1980.
- Ohtsu A, Boku N, Muro K, Chin K, Muto M, Yoshida S, Satake M, Ishikura S, Ogino T, Miyata Y, Seki S, Kaneko K and Nakamura A: Definitive chemoradiotherapy for T4 and/or M1 lymph node squamous cell carcinoma of the esophagus. J Clin Oncol 17: 12915-12921, 1999.
- 9. Ishikura S, Nihei K, Ohtsu A, Boku N, Hironaka S, Mera K, Muto M, Ogino T and Yoshida S: Long-term toxicity after definitive chemoradiotherapy for squamous cell carcinoma of the thoracic esophagus. J Clin Oncol 21: 2697-2702, 2003.
- Al-Sarraf M, Martz K, Herskovic A, Leichman L, Brindle JS, Vaitkevicius VK, Cooper J, Byhardt R, Davis L and Emami B: Progress report of combined chemoradiotherapy versus radiotherapy alone in patients with esophageal cancer: an intergroup study. J Clin Oncol 15: 277-284, 1997.
- Eisen MB, Spellman PT, Brown PO and Botstein D: Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci USA 95: 14863-14868, 1998.
- 12. Yasui K, Imoto I, Fukuda Y, Pimkhaokham A, Yang ZO, Naruto T, Shimada Y, Nakamura Y and Inazawa J: Identification of target genes within an amplicon at 14q12-q13 in esophageal squamous cell carcinoma. Genes Chromosome Cancer 32: 112-118, 2001.
- Suit HD and Suchato C: Hyperbaric oxygen and radiotherapy of a fibrosarcoma and of a squamous-cell carcinoma of C3H mice. Radiology 89: 713-719, 1967.
- 14. Moulder JE and Rockwell S: Tumor hypoxia: its impact on cancer therapy. Cancer Metastasis Rev 5: 313-341, 1987.
- Rockwell S and Moulder JE: Hypoxic fractions of human tumors xenografted into mice: a review. Int J Radiat Oncol Biol Physiol 19: 197-202, 1990.
- 16. Kamijo T, Yokose T, Hasebe T, Yonou H, Sasaki S, Hayashi R, Ebihara S, Miyahara H, Hosoi H and Ochiai A: Potential role of microvessel density in predicting radiosensitivity of T1 and T2 stage laryngeal squamous cell carcinoma treated with radiotherapy. Clin Cancer Res 6: 3159-3165, 2000.
- 17. Hironaga S, Hasebe T, Kamijo T, Ohtsu A, Boku N, Yoshida S, Saitoh H and Ochiai A: Biopsy specimen microvessel density is a useful prognostic marker in patients with T(2-4)M(0) esophageal cancer treated with chemoradiotherapy. Clin Cancer Res 8: 124-130, 2002.
- Chakraborty M, Abrams SI, Champhausen K, Liu K, Scott T, Coleman CN and Hodge JW: Irradiation of tumor cells upregulates Fas and enhances CTL lytic activity and CTL adoptive immunotherapy. J Immunol 170: 6338-6347, 2003.
- Charlie TG, Claudia P, Mala C, Kwong-Yok T, Jeffrey S and James WH: Sublethal irradiation of human tumor cells modulates phenotype resulting in enhanced killing by cytotoxic T lymphocytes. Cancer Res 64: 7985-7994, 2004.