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Differential expression of S100 gene family in human esophageal squamous cell carcinoma

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Abstract *Purpose*: To study the differential expression of the S100 gene family at the RNA level in human esophageal squamous cell carcinoma (ESCC), and to find the relationship of the S100 gene family with ESCC. *Methods*: Firstly, the specific primers were designed for the different S100 genes with Software Primer 3, which required that both primer sequences of each S100 gene were from two different exons respectively. Then, the differential expression of 16 S100 genes was examined by semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) in 62 cases of ESCC versus the corresponding normal esophageal mucosa. All RT-PCR products were analyzed by 1.5% agarose gel. With Fluor-S MultiImager and Multi-Analyst software, the electrophoresis images were evaluated with statistics analysis using SAS 8.1 software. *Results*: Eleven out of 16 S100 genes were significantly downregulated ($p < 0.05$) in ESCC versus the normal counterparts such as *S100A1, S100A2, S100A4, S100A8*, *S100A9*, *S100A10, S100A11, S100A12, S100A14, S100B,*and *S100P* genes. Only the *S100A7* gene in the S100 family was markedly upregulated ($p < 0.05$). Moreover, the *S100B* gene was significantly correlated with histological differentiation of ESCC ($p = 0.0247$), and the deregulation of some S100 genes was closely correlated ($p < 0.05$), such as *S100A10*/*S100A11*, *S100A2*/*S100A8*, *S100A2*/*S100A14*, *S100A8*/*S100A14*, and *S100A2*/*S100P* etc. *Conclusions*: The S100 gene family is closely associated with ESCC.

Keywords S100 gene family · Human esophageal squamous cell carcinoma · Histological differentiation

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Introduction

Esophageal squamous cell carcinoma (ESCC) is one of the most common human cancers in the world, especially in developing countries. Despite the recent advances in surgical techniques, chemotherapy, radiotherapy, and palliative measures, the 5-year survival rate is only approximately 5% (Ribeiro et al. 1996). In developed countries, the incidence rates of ESCC are about 1.5–5.0 per 100,000, whereas in some regions of China, the relative risk is as high as 300–500 fold (Togawa et al. 1994).

The first S100 calcium-binding protein was isolated from bovine brain in 1965 by Moore (1965). Subsequent studies identified many members of this family based on their homology of amino acid sequences and their feature of calcium-binding properties. The S100 family became a major interest because of its deregulated expression in human diseases, especially in cancer; the cluster organization of S100 genes on human chromosome 1q21, which is a region frequently rearranged in tumors (Watson et al. 1998); and the widespread application of S100 protein antibodies for tumor diagnosis by immunohistochemistry. Up to now, 21 genes encoding S100 calcium-binding proteins have been identified, and a cluster of 16 S100 genes is mapped to the human chromosome 1q21 region (Gendler et al. 1990; Schafer et al. 1995; Weterman et al. 1996; Pietas et al. 2002). Early studies have shown that S100A2, S100A4, S100A12, and S100P proteins were deregulated in esophageal cancer tissues and cell lines and could influence cell differentiation and cell cycle (Kyriazanos et al. 2002; Ninomiya et al. 2001; Hitomi et al. 1998; Sato

et al. 2002). Moreover, it has been reported that many S100 genes were expressed at high levels in stratified epithelia of the upper digestive tract. All these factors indicate that the S100 gene family may be correlated with ESCC. However no study about their relationship has been reported yet. In this study, we focused on the differential expression of 16 S100 genes in 62 pairs of ESCC samples (cancer tissues and their corresponding normal counterparts).

Materials and Methods

Patients and samples

Esophageal cancer and matched adjacent normal mucosa were taken from surgical specimens of 62 patients who had not been treated with radiotherapy or chemotherapy before surgery. Cancer sample was taken from the tumor tissue where there was no hemorrhage or putrescence, whereas the matched normal mucosa was taken from the surgical cutting edge, which was approximately 3–5 cm away from the cancerous lesion. Fresh samples were dissected by hand to remove mixed connective tissues and stored in liquid nitrogen immediately. The clinical diagnosis of all 62 patients was approved by histological diagnosis after surgery. Among them, 45 patients were diagnosed and underwent surgery at the Xinxiang Central Hospital in Henan Province and 17 at Peking Union Hospital.

RNA extraction and reverse transcription

Total RNA was extracted with TRIzol Reagent (G_{IBCO}BRL, New York, NY, USA) according to the manufacturer's instructions. Five micrograms total RNA of each sample was used to synthesize the first strand cDNA in a 20 μl volume with Super-Script First-Strand Synthesis System for RT-PCR Kit (G_{IBCO}BRL). The single-strand cDNA synthesized was used as the template for PCR.

Primer design

It was very important to choose the most specific primers for each S100 gene because of their high homologies to each other. The sequences of the PCR primer pairs of 16 S100 members listed in Table 1 were designed as follows: (1) Selection and design of primer by Software Primer 3 according to standard criteria (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi); (2) test of the primer specificity through nucleotide BLAST comparison

Table 1 Primers used for RT-PCR and conditions of polymerase chain reaction (PCR). *F* forward; *R* reverse; *Dir* direction; *Ref* accession number from GenBank; *Anne* annealing temperature

S100 genes	Dir*	Primer sequences (5'-3')	Start point	Product size	Ref*	Anne (°C)*	PCR cycles
S100A1	F	AGGAGCTGAAAGAGCTGCTG	208	279	NM_006271	65.5	29
	R	AGGGATAAGTGGGGTGAGGT	486				
S100A2	F	CACTACCTTCCACAAGTACT	79	229	NM_005978	56	27
	R	GAAGTCATTGCACATGAC	307				
S100A3	F	GCAGGCGGTAGCTGCCATC	101	255	NM_002960	59	29
	R	TTGAAGTACTCGTGGCAGTAG	355				
S100A4	F	CAAGTACTCGGGCAAAGAGG	120	250	NM_002961	58	27
	R	CTTCCTGGGCTGCTTATCTG	369				
S100A6	F	GGACCGCTATAAGGCCAGTC	2	285	NM_014624	60	27
	R	GGTCCAAGTCTTCCATCAGC	286				
S100A7	F	TGAGCAACACTCAAGCTGAG	73	336	NM_002963	63	27
	R	GGGTCTCTGGAGGCCCATTG	408				
S100A8	F	TGTCAGCCGTCTTTCAGAAG	9	286	BC005928	58	27
	R	ACGCCCATCTTTATCACCAG	294				
S100A9	F	GGGAATTCAAAGAGCTGGTG	149	267	NM_002965	58	26
	R	CACTGTGATCTTGGCCACTG	415				
S100A10	F	CATCTCAAATGGAACACGCC	116	321	NM_002966	56	27
	R	TATCAGGGAGGAGCGAACT	436				
S100A11	F	ATCGAGTCCCTGATTGCTGT	160	331	NM_005620	59	27
	R	AGAAAGGCTGGAAGGAAAGG	490				
S100A12	F	TTGAAGAGCATCTGGAGGG	79	269	NM_005621	56	27
	R	CTACTCTTTGTGGGTGTGG	347				
S100A13	F	ACCACCTTCTTCACCTTTGC	125	254	NM_005979	60	28
	R	AGGCGGCTTTACTTCTTCCT	378				
S100A14	F	CATGAGCCATCAGCTCCTCT	23	276	NM_020672	59	27
	R	TTCTCTTCCAGGCCACAGTT	298				
S100B	F	ATGTCTGAGCTGGAGAAGG	73	338	NM_006272	58	28
	R	CTGTCTGCTTTCTTGCATG	410				
S100P	F	AGGTGCTGATGGAGAAGGAG	122	223	NM_005980	60	28
	R	ATGGCTCTGCAGGAATCTGT	344				
S100Z	F	TGGAGTGGTCAGTTCTGCTG	307	230	NM_130772	62	32
	R	CGTTGTCCTTATTGGCATCC	536				

analysis with all known human sequences; and (3) test of whether both primer sequences of each paired primer were from two different exons in order to distinguish the cDNA and residual genomic DNA in total RNA.

Semiquantitative PCR and image analysis

Every PCR reaction was performed in 25 μl final volume containing 1×PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM each dNTPs, 1.25 U Taq DNA polymerase (TakaRa, Dalian, China), 1 μl RT product, 0.3–0.5 μM of each primer. The housekeeping gene glyceraladehyde-3-phosphate dehydrogenase (*GAPDH*) was used as an internal control. The conditions of PCR reaction were as follows: initial denaturalization for 4 min at 94°C, amplification for 26–32 cycles (30 s at 94°C, 30 s at 55–65°C, 30 s at 72°C), which were varying in different genes and are listed in Table 1, and final extension for 4 min at 72°C. The RT-PCR reaction products were analyzed by electrophoresis on 1.5% agarose gel. Then, the electrophoresis images were scanned with Fluor-S MultiImager (Bio-Rad, California, USA), and the original intensities of specific bands were quantified by Multi-Analyst software (Bio-Rad). The final data were obtained after normalization by the intensity of *GAPDH*, which included the expression amount of every gene in each case of ESCC (R_C), the corresponding normal esophageal mucosa (R_N), and the ratio of R_C to R_N ($R_{C/N}$).

Statistical analysis

All data were analyzed with SAS 8.1 software for Windows 98. With the Wilcoxon paired signed-rank test (nonparametric variance for pair comparisons), the difference between R_N and R_C or the differential expression of the 16 S100 genes in ESCC versus normal tissues was examined. After every $R_{C/N}$ was obtained, the correlation of each S100 gene with the histological differentiation (including well, moderate, and poor differentiation) of ESCC was analyzed with the Kruskal-Wallis test. When the $R_{C/N}$ of two different S100 genes in the same case were used as variables, the possible correlations between every two S100 genes were carried out with the Pearson test. A p value of <0.05 was considered as significant.

Results

Primer design

With Software Primer 3 and BLAST analysis, 16 specific primer pairs were obtained (Table 1) that could discriminate the different members of the S100 gene family. At the same time, both primer sequences of each S100 gene were from two different exons respectively so that each primer pair also was able to discriminate the cDNA and genomic DNA. Thus, residual genomic DNA in total RNA could not influence the RT-PCR results.

Semiquantitative RT-PCR

With semiquantitative RT-PCR, the differential expression of 16 S100 genes was examined in 62 cases of ESCC. The results evaluated with the Wilcoxon paired sign-rank test showed that 12 S100 genes were significantly deregulated in ESCC versus the normal counterparts, which were *S100A1* ($p=0.0007$), *S100A2* ($p<0.0001$), *S100A4* ($p=0.0003$), *S100A7* ($p=0.0200$), *S100A8* ($p<0.0001$), *S100A9* ($p<0.0001$), *S100A10* ($p<0.0001$), *S100A11* ($p<0.0001$), *S100A12* ($p<0.0001$), *S100A14* ($p<0.0001$), *S100B* ($p=0.0006$), and *S100P* ($p<0.0001$). However, the expression of other genes such as *S100A3*,*S100A6*, *S100A13*, and *S100Z* was

Table 2 The differential expression of S100 genes

S100 genes	Differential expression				
	Up cases*	NS cases*	Down cases*	S	*p* value
S100A1	8(12.90%)	23(37.10%)	31(50.00%)	467.5	0.0007
S100A2	9(14.52%)	19(30.65%)	34(54.84%)	529.5	<0.0001
S100A3	17(27.42%)	26(41.94%)	19(30.65%)	−23.5	NS*
S100A4	8(12.90%)	21(33.87%)	33(53.23%)	498.5	0.0003
S100A6	18(29.03%)	31(50.00%)	13(20.97%)	−193.0	NS
S100A7	35(54.45%)	7(11.29%)	20(32.26%)	−328.5	0.0200
S100A8	6(9.68%)	5(8.06%)	51(82.26%)	830.5	<0.0001
S100A9	4(6.45%)	10(16.13%)	48(77.42%)	871.5	<0.0001
S100A10	5(8.06%)	20(32.26%)	37(59.68%)	768.5	<0.0001
S100A11	7(11.29%)	24(38.71%)	31(50.00%)	610.5	<0.0001
S100A12	10(16.13%)	13(20.97%)	39(62.90%)	544.5	<0.0001
S100A13	15(24.19%)	29(46.77%)	18(29.03%)	−24.5	NS
S100A14	5(8.06%)	8(12.90%)	49(79.03%)	864.5	<0.0001
S100B	8(12.90%)	23(37.10%)	31(50.00%)	472	0.0006
S100P	8(12.90%)	18(29.03%)	36(58.06%)	660.5	<0.0001
S100Z	14(22.58%)	24(38.71%)	24(38.71%)	230.5	NS

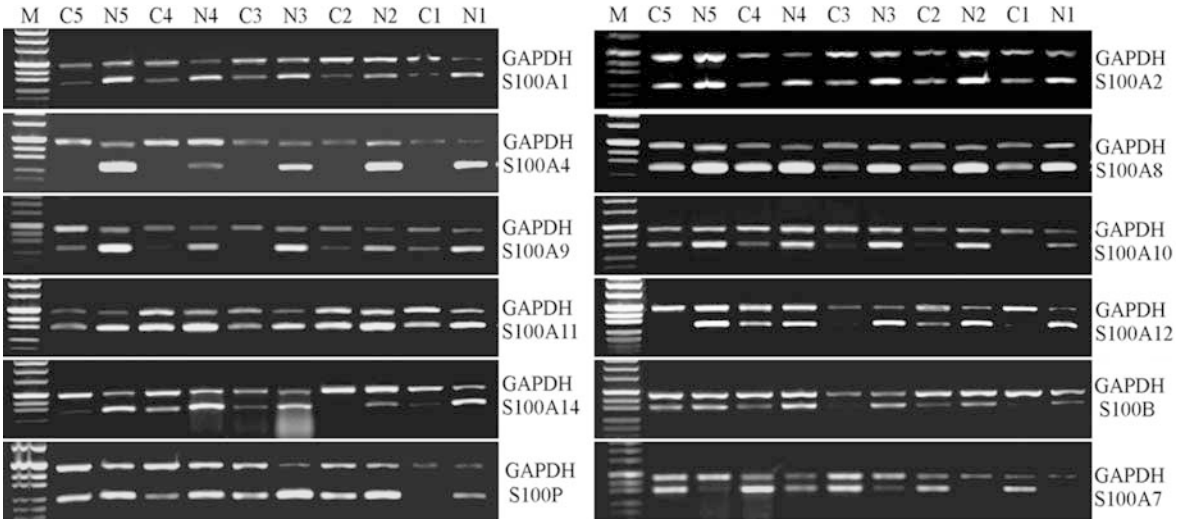


Fig. 1 Partial electrophoresis images of semiquantitative RT-PCR results. (*M*: marker; *N*: normal; *C*: cancer) The expression of *GAPDH* was used as internal control, and the size of the amplified fragment was 452 bp. The pUC Mix Marker 8 (MBI, Lithuania) was used as molecular-weight marker

not significantly different in ESCC and the counterparts (Table 2). Partial electrophoresis images of semiquantitative RT-PCR were shown in Fig. 1. At the same time, the criteria of a gene overexpression or underexpression in ESCC were decided by three independent researchers. The criteria were that a gene was considered as upregulated in one case of ESCC when $R_{C/N}$ was higher than 4/3, as downregulated when $R_{C/N}$ was lower than 3/4, and the alteration of the gene was not considered as significant when $R_{C/N}$ was between 3/4 and 4/3 (including 3/4 and 4/3). With this criteria, the number of underexpression cases was found in significant downregulated genes such as *S100A8* (51 cases, 82.3%), *S100A14*(49 cases, 79.0%), *S100A9* (48 cases, 77.4%), *S100A12* (39 cases, 62.9%), *S100A10* (37 cases, 59.7%), *S100P* (36 cases, 58.1%), *S100A2*(34 cases, 54.8%), *S100A4* (33 cases, 53.2%), *S100A1* (31 cases, 50.0%), *S100A11* (31 cases, 50.0%), and *S100B* (31 cases, 50.0%). That of overexpression cases was found in significant upregulated gene, the *S100A7* gene (35 cases, 56.5%) in ESCC (Table 2, Fig. 2).

Correlation of the S100 gene expression with the histological differentiation

In 62 cases of ESCC, there were 39 cases with well-known histological differentiation. The correlation analyses showed that only the *S100B* gene was significantly correlated with the histological differentiation ($p = 0.0247$), and its mean rank score was the lowest in moderately (15.82) and the highest in poorly differentiated ESCC (28.00). At the same time, the expression of *S100A3*, *S100A4*, *S100A7*, *S100A12*, and *S100P* genes showed increasing tendency and the *S100A13* gene showed decreasing tendency from poorly to well via moderately differentiated ESCC, but the difference was not significant (data not shown).

Correlation analysis of the deregulation of 16 S100 genes

Fig. 3 showed that the deregulation of many S100 genes in ESCC was significantly correlated ($p < 0.05$). Among *S100A2*, *S100A8*, *S100A14*, and *S100P*genes, the deregulation of each other was markedly correlated ($p < 0.0001$), and their correlation coefficients (r) were 0.6829 (*S100A2*/*S100A8*), 0.6818 (*S100A2*/*S100A14*), 0.6549 (*S100A2*/*S100P*), 0.6744 (*S100A8*/*S100A14*),

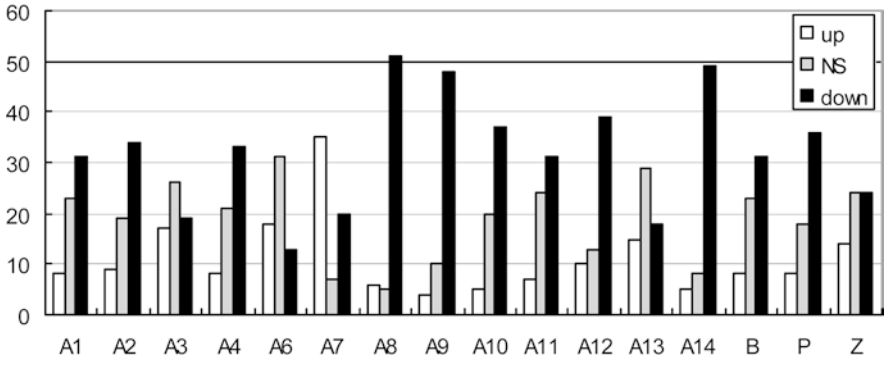
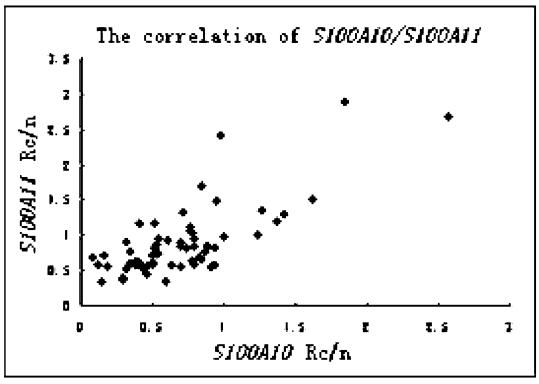


Fig. 2 Differential expression of 16 S100 genes in 62 cases of esophageal squamous cell carcinoma (ESCC) examined by RT-PCR (*up*: up-regulation, *white bars*; *NS*: not significant, *grey bars*; *down*: downregulation, *black bars*). Every column expressed the number of upregulated or downregulated or insignificantly deregulated cases



		A1	A2	A3	A4	A6	A7	A8	A9	A10	A11	A12	A13	A14	B	P
A1	r	1														
	p															
A2	r	NS	1													
	p															
A3	r	NS	NS	1												
	p															
A4	r	NS	NS	NS	1											
	p															
A6	r	NS	NS	NS	NS	1										
	p															
A7	r	NS	NS	NS	NS	NS	1									
	P															
A8	r	NS	0.6829	NS	NS	NS	NS	1								
	p		<0.0001													
A9	r	NS	0.3587	NS	NS	NS	0.4639	0.5394	1							
	p		0.0042				0.0001	<0.0001								
A10	r	NS	0.2754	NS	NS	NS	0.5900	0.4204	0.5982	1						
	p		0.0303				<0.0001	0.0007	<0.0001							
A11	r	NS	NS	NS	NS	NS	0.4841	0.4919	0.4645	0.7559	1					
	p						<0.0001	<0.0001	0.0001	<0.0001						
A12	r	NS	NS	NS	NS	NS	0.2771	0.3886	0.3473	0.3984	0.4616	1				
	p						0.0292	0.0018	0.0057	0.0013	0.0002					
A13	r	NS	NS	NS	NS	NS	NS	NS	0.3178	0.3019	0.2885	NS	1			
	p								0.0118	0.0171	0.0229					
A14	r	NS	0.6818	NS	NS	NS	NS	0.6744	0.5427	0.4545	0.3452	0.3136	NS	1		
	p		<0.0001					<0.0001	<0.0001	0.0002	0.006	0.0131				
B	r	0.2576	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.3225	NS	1	
	P	0.0433											0.0106			
P	r	NS	0.6549	NS	NS	NS	NS	0.6422	0.3727	0.4249	0.3804	0.2972	NS	0.5801	NS	1
	P		<0.0001					<0.0001	0.0028	0.0006	0.0023	0.0190		<0.0001		
Z	r	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.3209	NS	0.3389	NS
	p												0.0110		0.0070	

Fig. 3 Correlation analysis of differential expression of 16 S100 genes. *r*: correlation coefficient; *p*: p value; *NS*: not significant. The figure at the top right corner was the scanning spot of the correlation of *S100A10* and *S100A11* ($r = 0.7559$, $p < 0.0001$). In this figure, each spot expressed the $R_{C/N}$ of *S100A10* and *S100A11* genes in each case of esophageal squamous cell carcinoma (ESCC)

0.6422 (*S100A8*/*S100P*), and 0.5801 (*S100A14*/*S100P*) respectively. In addition, *S100A10*/*S100A11* ($r = 0.7559$), *S100A9*/*S100A10* ($r = 0.5928$), *S100A7*/*S100A10* ($r = 0.5900$), *S100A9*/*S100A14* ($r = 0.5427$), and *S100A8*/*S100A9* ($r = 0.5374$) etc., deregulation of these was also significantly correlated ($p < 0.0001$) (Fig 3).

Discussion

The S100 gene family is a family encoding calcium-binding proteins, which contains one canonical EF-hand in the C-terminal half and a specific motif in the N-terminal half. Early studies have shown that some S100 members are associated with ESCC, such as S100A2, S100A4, S100A12, and S100P proteins. In this work, the differential expression of 16 S100 genes was examined in ESCC at mRNA level by RT-PCR. The results showed that 11 genes were significantly downregulated ($p < 0.05$), and only the *S100A7* gene was markedly upregulated ($p < 0.05$) in ESCC. This indicates that the S100 gene family is closely correlated with ESCC and that some deregulated S100 genes may be important ESCC-associated genes.

Chromosome 1q21 is a region of the structural and numerical aberration in many tumors (Lestou et al. 2003; Chen et al. 2003; Wong et al. 2003; Watson et al. 1998). It also may be closely associated with the development of ESCC due to the following reasons: (1) The chromosome 1q21 has been known to be involved in chromosome rearrangement in various human tumors (Watson et al. 1998); (2) the chromosome region 1q21–1q22 contains the high-density CpG island (Wright et al. 2001); (3) ten genes were significantly deregulated out of the 13 S100 genes that were clustered in 1q21 among the total 16 S100 genes in our work; (4) other genes in 1q21, such as *SPRR3* and *TGM-3*, were also deregulated in ESCC (Chen et al. 2000; Chen et al. 2002).

The S100A2 protein was proposed as a valuable prognostic marker in tumors including ESCC (Kyriazanos et al. 2002). Due to the underexpression in some tumors and the stable expression in normal epithelia of the *S100A2* gene, it was considered as a candidate tumor-suppressor gene (Nagy et al. 2001; Liu et al. 2000; Hitomi et al. 1996). Our results have shown that the *S100A2* gene was significantly downregulated (54.8%, $p < 0.0001$) in ESCC as compared with normal mucosa

so that it also might be a candidate tumor-suppressor gene in ESCC. It has been shown that the S100A4 protein was upregulated in ESCC versus normal counterparts, which was contrary with our result that the *S100A4* gene was downregulated (Ninomiya et al. 2001). With regard to the *S100A4* primer specificity, plentiful cases, and proper statistical analysis, our result was convincing.

The reason for inconsistent expression of S100A4 at the protein and gene level has not yet been reported. The inconsistency might indicate that the modulation of translation from mRNA to protein was important and complex in the process of tumorigenesis. S100P and S100A12 have been found to be downregulated in ESCC at the protein level and produced by esophageal epithelial cells in the process of cell differentiation. They were considered to be associated with the differentiation of ESCC (Hitomi et al. 1998; Sato et al. 2002). However, our results only indicated that their expression at the RNA level were downregulated ($p < 0.05$), and their mean rank score of $R_{C/N}$ took on an increasing tendency from poorly to well via moderately differentiated ESCC. The S100P protein-positive cells were located mainly in the second to fourth layers just above the basal cells (Sato et al. 2002), and the S100A12 protein was located in the superbasal squamous epithelial cells undergoing differentiation but not in the cells in the proliferating basal layer (Hitomi et al. 1996). All these factors suggest that the deregulation of S100P and S100A12 proteins may be an earlier event in the terminal differentiation of human ESCC. However, most cases we collected were a later stage of ESCC; therefore, the correlation of *S100P* and *S100A12*genes with histological differentiation could not be detected. In addition, the limited cases of well- and poorly differentiated ESCC (ten and six cases respectively) and subjectivity of histological differentiation diagnosis might have an effect on the results.

The *S100B* gene was first detected significantly downregulated (50.0%, $p = 0.0006$) in ESCC. Its underexpression may be accordant with its possible tumor-suppressor activity that S100B could protect p53 from thermal denaturation and aggregation and cooperate with p53 to cause cell growth arrest and apoptosis (Baudier et al. 1992; Scotto et al. 1998). Moreover, the *S100B* gene was significantly correlated with the histological differentiation ($p = 0.0247$). Its expression was the lowest in moderately differentiated ESCC, next to well-differentiated ESCC, and highest in poorly differentiated ESCC. This just confirmed the hypothesis of S100B possible tumor-promoting activity that S100B could actually block p53 based on structural and functional analysis of the S100B-p53 interaction (Rustandi et al. 2000; Lin et al. 2001). According to these authors, the decrease of *S100B* gene expression in ESCC would result in the increase of p53 function and lead to a decreased cell proliferation rate. It was believed that the proliferation rate of poorly differentiated cells was higher than that of well- and moderately differentiated cells. So, in poorly differentiated ESCC, *S100B* gene expression should be higher. All these factors suggest that S100B may play a different role in different differentiation stages of ESCC.

S100A1, *S100A7*, *S100A8*, *S100A9*, *S100A10*, *S100A11*, and *S100A14* genes were also first detected significantly deregulated in ESCC. The precise mechanism of their deregulated expression in ESCC was still unknown; the deregulation may influence tumorigenesis by affecting cell cycle or proliferation, cell differentiation, and apoptosis. It has been reported that nuclear S100A11 inhibited cell growth. In normal cells, S100A11 protein was phosphorylated and moved to nuclei from cytoplasm to inhibit DNA synthesis. Whereas in immortalized cells, S100A11 was neither phosphorylated nor imported to the nuclei but remained in the cytoplasm so that it could not inhibit DNA synthesis (Sakaguchi et al. 2000). In B16 melanoma cells, the S100A4 protein has been shown to sequester p53 to form a complex of S100A4 with p53. The complex could abrogate cell G1-S checkpoint control and result in the increase of the size of the S-phase fraction (Parker et al. 1994). Moreover, the cells induced by the S100A4 protein to enter S-phase could successfully negotiate the G2-M checkpoint control to transit into mitosis (Cajone et al. 1999). All these factors suggest that in the initiation and development of ESCC, it may be an important mechanism that the S100 family influences on the cell cycle. None of these genes were significantly correlated with the histological differentiation, but some genes might be similar with *S100P* and *S100A12* genes and might be associated with the earlier differentiation of ESCC cells.

Early studies have revealed that S100A8 and S100A9 proteins can form heterodimer, which was preferred within cells (Strupat et al. 2000; Propper et al. 1999). This suggested that the deregulation of the *S100A8* gene would be consistent with that of *S100A9*, which has been confirmed by our data. Our data also showed that there was significant correlation in many genes such as *S100A2*/*S100A8*, *S100A2*/*S100A14*, and *S100A2*/*S100P* genes, etc. The correlation coefficients of some genes were more than 0.5349, which was the correlation coefficient of *S100A8* and *S100A9* genes (Fig. 3). This meant that the interaction of these genes might be closer than that of *S100A8*and *S100A9* genes. All these correlations indicate that some S100 genes may be modulated by some common factors or modulate each other in ESCC. Although the precise modes of interaction remained unknown, the correlation of some S100 genes provided an important clue for illustrating the function of S100 members and the roles they played in the mechanisms of ESCC initiation and progression. Considering the close association of the chromosome 1q21 region and the S100 gene family with ESCC and the possible interaction of S100 members, it would be worthwhile to further analyze the S100 genes and proteins in ESCC.

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