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Reduction in the local expression of *complement component 6* (*C6*) and *7* (*C7*) mRNAs in oesophageal carcinoma

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Abstract

Differential displays of tumour/normal pair specimens of human oesophagus identified *complement component 7* (*C7*) as being enhanced in normal tissues, but remarkably reduced in carcinoma tissues. *In situ* hybridisation confirmed the localisation of *C7* mRNA in normal oesophageal epithelial cells and its disappearance in tumour cells. When mRNA expressions of other components were examined by means of semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) in 10 tumour/normal pair specimens, significant reductions in *C6* and *C7* mRNAs were observed, while *C3* and *C5* mRNAs were enhanced in both normal and tumour tissues. A similar reduction was observed in colon and kidney cancers using the tumour/normal expression array analysis. Gene deletion of *C7* was not found in the cell lines by Southern blot analysis. Our findings suggest a possible relationship between oesophageal tumorigenesis and reduced expression of *C6* and *C7* mRNAs, which is probably caused by a change in gene expression regulation and not by genetic loss of the locus.

Keywords: Differential display; *C7*; *C6*; Down-regulation; Oesophageal carcinoma

1. Introduction

The complement system is a powerful immune effector that can eliminate foreign cells, including virally-infected cells and cancer cells [1]. This system is reportedly activated through three pathways, the classical pathway [2], the alternative pathway [3], and the lectin complement pathway [4]. The classical pathway is triggered by the antigen–antibody complex, while the latter two pathways are initiated on a foreign surface in the absence of antibodies. The activation of either pathway results in the formation of the C3/C5 convertases, which cleave C5 into C5a and C5b to initiate the formation of the terminal complement complex consisting of C5b, C6, C7, C8 and C9, which is also known as the membrane attack complex (MAC) [5]. In the assembly of the terminal complement components, C7 plays a pivotal role because its attachment to C5b-6 leads to the initial insertion of the complex into cell membrane. Furthermore, cytolytically inactive C5b-7 has chemotactic activity for polymorphonuclear leucocytes and induces further cellular inflammatory response as well as C5b-9 [6].

Cancer cells carrying tumour-associated antigens or transformed membrane glycoproteins on their cell surface are exposed to complement proteins in blood during the process of invasion and metastasis [7], but some of them are resistant to cytolysis and are thus able to progress. Such resistance has been explained mainly in terms of membrane-associated and/or soluble complement regulators. Overexpression of membrane-bound inhibitory proteins, such as CD35, CD46, CD55 and CD59, have been found on tumour cells of colon, breast, ovary, uterine, prostate and central nervous system (CNS) malignancies [8–13]. These molecules regulate the activation at the level of the generation of C3/C5 convertase (CD35, CD46 and CD55), or interfere with the formation of MAC by binding C5b-8 (CD59). Blocking experiments have showed that the neutralisation of CD55 or CD59 by specific antibodies significantly

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increased the susceptibility of tumour cells to complement-mediated lysis [14–16]. Furthermore, certain tumours express proteases that degrade complement proteins, such as C3 [17], or ecto-protein kinases which can phosphorylate and inactivate C9 [18]. However, there is little information on the expression of complement components themselves in tumour cells or tissues. As complement components have been considered to be plasma proteins of mainly hepatic origin, study of the local expression of complement components is limited.

Along with the accumulation of genetic changes during tumorigenesis of the human oesophagus, the mRNA expressions of related genes may also change. Isolation of such genes could provide not only a better understanding of the biological features of tumour development, but also provide new diagnostic markers and targets for cancer therapy. In this study, the mRNA expression of complement components which participate in the terminal pathway were examined by means of semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR).

2. Materials and methods

2.1. Clinical samples

Histologically confirmed squamous cell carcinomas of the oesophagus from 10 patients were included in this study. Informed consent was obtained from all of the patients who were treated by curative surgical resection at the Kanazawa University Hospital during 1997–1998. The tumour and the corresponding normal specimens were immediately frozen in liquid nitrogen after resection, and kept at −80°C until use.

2.2. Cells

Nine cell lines established from the oesophageal carcinomas (TE-4, -6 and -10: from well-differentiated squamous carcinoma, TE-12, -14 and -15: from moderately-differentiated squamous carcinoma, and TE-2, -9 and -13: from poorly-differentiated squamous carcinoma) were provided by Dr Nishihira [19]. Cell lines were grown in a monolayer culture in Roswell Park Memorial Institute (RPMI)-1640 (Nissui, Tokyo, Japan) with fetal bovine serum (JRH Bioscience, Lenexa, KS, USA) at 37°C in a 5% CO_2/95% humidified-air atmosphere.

2.3. Rat oesophageal epithelia

The oesophagus was dissected from male rats (Wister, 4 weeks old: Clea, Tokyo, Japan) immediately after sacrifice, and the epithelium was scraped off with a spatula and subjected to RNA preparation.

2.4. RNA and Genomic DNA preparation

Total RNA was prepared with the guanidium-isothiocyanated and caesium chloride method [20] from clinical samples, confluently cultured cells and rat oesophageal epithelium. Genomic DNA from cultured cells was obtained according to the standard procedure [20].

2.5. Differential display

To circumvent individual differences of clinical specimens, pairs of normal and tumour mRNAs from 5 patients were simultaneously analysed using differential displays using the RNA Image kit (GeneHunter Corp., Brookline, MA, USA). According to the manufacturer's instruction, 0.2 μg of total RNA was reverse transcribed in the RT buffer containing 20 μl of 0.2 μM anchored poly-dT primer 5′-AAGCT11V-3′, 20 μM dNTPs, and 1 U MMuLV reverse transcriptase (GeneHunter Corp.). One microlitre aliquot was subjected to PCR amplification with one of the anchored poly-dT-primer and an arbitrary primer included in the kit in 20 μl of 25 μM deoxynucleotide triphosphates (dNTPs), PCR buffer, [α-^{32}P] deoxycytosine triphosphate (dCTP) (0.25 μl/reaction, specific activity 110TBq/mmol, Amersham Pharmacia Biotech, Buckinghamshire, UK), and 1U of *Taq* polymerase (Takara, Shiga, Japan). Amplified ^{32}P-labelled cDNA was electrophoresed on 6% polyacrylamide gels, which were then exposed to X-ray films overnight. Any bands of interest were excised, extracted, PCR re-amplified and subcloned into pGEM-T vectors (Promega, Madison, WI, USA). The obtained clones were sequenced with a dRhodamine Terminator Cycle Sequencing Kit (Applied Biosystems, Norwalk, CT, USA) and analysed using the GenBank BLAST and FAST homology search programs. The cloned cDNA was also utilised to generate the probe for Northern blot.

2.6. Northern blot analysis

Twenty micrograms of total RNA from tumour and normal tissues of 2 different patients were separated on 1% agarose-formaldehyde gel and blotted via capillary transfer onto a nitrocellulose filter membrane. The cDNA insert of the isolated clone was radiolabelled with [^{32}P]-dCTP using a DNA labelling system (Amersham). Hybridisation was performed in HB-N [20] at 42°C overnight and the blot was washed in 2×SSC/0.1% sodium dodecyl sulphate (SDS) at room temperature and then washed twice in 0.5×SSC/0.1%SDS at 65°C. After drying, the filter was mounted on an imaging plate (BAS-III: Fuji, Kanagawa, Japan), and exposed overnight on an image analyser BAS-2000 (Fuji).

2.7. In situ *hybridisation*

The coding region of *C7* mRNA was RT-PCR amplified using the primer sets for the quantification of *C7* mRNA and cloned into the pGEM-T vector. The bidirectional cRNA probes were synthesised using either T7 or SP6 RNA polymerase (Takara) and DIG Labeling Kit (Boehringer, Mannheim, Germany). Frozen specimens were cut into 4 μm sections, mounted on coated glass slides, and fixed with 4% paraformaldehyde in 0.1 M phosphate buffer for 15 mm. The sections were then treated with 0.2 N HCl, acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine, pH 8.0, dehydrated with ethanol series, and air dried. After prehybridisation with the hybridisation solution containing 50% formamide, 10% dextran sulphate, 1×Denhardt [20], 600 mM NaC1, 0.25% SDS, 150 μg/ml yeast tRNA at 50°C for 2 h, each slide was allowed to hybridise with approximately 0.5 μg/ml of the cRNA probe at 50°C overnight. The slides were washed briefly in 5×SSC at ambient temperature in 50% formamide and 2×SSC at 50°C for 30 min, treated with 10 μg/ml RNase A at 37°C for 30 min and again washed once with 2×SSC and twice with 0.2×SSC for 20 min each at 50°C. After hybridised probes were detected with the Nucleic Acid Detection Kit (Boehringer), the slides were rinsed with 10 mM Tris–HCl, pH 8.0 and 1 mM ethylene diamine tetra acetic acid (EDTA), and fixed.

2.8. *RT-PCR*

RT-PCR was utilised for the quantification of complement component mRNAs in clinical specimens, as described elsewhere [21]. Preliminary experiments disclosed that cycles providing the exponential increase for *C7*, *C3*, *C5* and *C6* were 24 to 30 cycles in normal tissue and from 28 cycles in tumour tissue. Products for *GAPDH* increased linearly from 20 to 26 cycles in both normal and tumour tissues.

Therefore, the quantitation of complement component mRNAs and *GAPDH* mRNA for each sample was performed by the duplicate PCR respectively at 26 and 28 cycles and at 22 and 24 cycles. When linearity of PCR amplification was confirmed, the expression levels of complement component mRNAs were standardised by those of *GAPDH* mRNA. The sequences of the primers used were shown in Table 1. RT-PCR was also utilised to confirm the steady state expression of *C7* mRNA in rat oesophageal epithelium. Since rat *C7* mRNA had not yet been cloned, the primers were designed according to the conserved sequences between those of human and porcine (Table 1). The obtained cDNA displayed high homology to human and porcine C7 (86 and 84%, respectively), hence its sequence was registered as a part of the rat *C7* coding sequence (accession No: AF309948).

2.9. *SDS-PAGE and western blot analysis*

The culture media of confluent cells cultivated without FBS were concentrated approximately 1000 times by lyophilisation and subjected to SDS-PAGE on a 10% gel under non-reducing conditions [20]. After electrophoresis, the proteins were transferred onto nitrocellulose membrane (Millipore Corp., Bedford, MA, USA) using a semi-dry system (Bio-Rad Laboratories, Richmond, CA, USA). The membranes were, respectively, reacted with goat polyclonal antihuman C7 and

Table 1
Sequence and nucleotide position of the primers[a]

Gene (accession no.)	Primer sequence		Position (nt)	Product size (bp)
C7 (NM000587)	sense	ATGTCAGCGcTGGGAGAAACT	2103–2123	411
	anti sense	CAAGGCCITATGCTGGTGACA	2493–2513	
For rat *C7* (AF309948)	sense	CAGAATTCTGTCCATCACCTC	[b]1703–4723	433
	antisense	GAATTCTGCAG1TFCTCCCAG	[b]2113–2133	
C3 (NM000064)	sense	TCGGATGACAAGGTCACCCT	4627–4826	408
	antisense	GACAACCATGCTCTCGGTGA	5015–5034	
C5 (NM001735)	sense	CAGTCCTGCCACTITCACAGT	4476–4496	
	antisense	CATGTFGTGTCTCTAGGCCAG	4953–4973	498
C6 (NM000065)	sense	TCCFGGACACCACCCATTTCA	2403–2423	559
	antisense	GCTAGGCCAAACACTTTCCAG	2941–2961	
C8 (NM000562)	sense	GCCTGTGAGCAAACACAGACA	1716–1736	426
	antisense	GTGCTOTAAGTAGGACAGAGC	2121–2141	
C9 (NM001737)	sense	TGTTTGTGTGCCTGCCCATTC	1580–1600	358
	antisense	GGGATAAAGCAGTTCTGGCGT	1917–1937	
GAPDH (M33197)	sense	CATGGGGAAGGTGAAGGTCGG	60–80	352
	antisense	TTGGCTCCCCCCTGCAAATGAG	390–411	

nt, nucleotide; bp, base pairs.

[a] Since rat *C7* had not been cloned, primer sequences were designed according to the conserved sequences between [b]human and porcine C7. All the primer sets were designed to anneal at 61°C.

C6 antibodies (ICN Pharmaceuticals, Inc., Costa Mesa, CA, USA) at a 1:1000 dilution overnight at 4°C. The membranes were subsequently reacted with and detected with avidine biotin alkalyphosphatase.

2.10. Southern blot analysis

Ten micrograms of genomic DNA of TE-6, -14 and-15 and normal peripheral-blood mononuclear cells was digested with *TaqI* and *DraI*. In addition to undigested DNA, these DNAs were electrophoresed and transferred to a nitrocellulose membrane. The cDNA fragment obtained by RT-PCR was used as the probe and labelled with [^{32}P]-dCTP by random prime labelling [20]. Hybridisation and image analysis were performed as described in the northern blot analysis. The expected size of hybridisation was 4.1 kb for the *TaqI* fragments and 1.6, 0.4 and 0.25 kb for the *DraI* fragments.

2.11. Tumour/normal expression array hybridisation

Differential gene expression in other human tumours was investigated using Matched Tumor/Normal Expression Array (Clontech, Palo Alto, CA, USA). Labelled cDNA probe was hybridised in a solution of ExpressHyb™ at 65°C overnight and the membrane was washed each twice in 2×SSC/1%SDS and 0.1×SSC/0.5% SDS at 65°C according to the company's instruction. The probe synthesis and image analysis was performed as described in northern blot analysis.

2.12. Statistical analysis

The statistical significance of differences in the mRNA expression between the normal/tumour pair were calculated using Wilcoxon's signed rank test for RT-PCR and one sample sign test for the Matched Tumor/Normal Expression Array. The significance of correlation between T/N ratios was investigated using the *t*-test.

3. Results

3.1. Isolation of differentially expressed gene

When the PCR products of differential displays using RNAs from cases 1 to S were electrophoresed, each lane consisted of approximately 40 bands (Fig. 1a). Most bands showed the same intensities between the tumour and the normal samples in all 5 cases, but one band, which was seen in all normal samples, was substantially reduced in all the tumour samples. Cloning and sequencing analysis identified that the band was 210 bp in size and its sequence was identical to that of the 3′ non-coding region of *C7* mRNA (accession No: NM000587). The differential expression of C7 mRNA was confirmed by northern blot for two cases (cases 1 and 3) of normal/tumour pair specimens (Fig. 1b). A single band of 3.9 kb in size was observed in the normal tissues, but not in the tumour counterparts. *C7* mRNA was also detected in rat oesophageal epithelium (Fig. 1c).

3.2. Localisation of C7 mRNA expression

C7 mRNA was detected in the epithelial cells of the normal oesophagus (Fig. 2a). Its signal intensity was especially strong in the parabasal cells and weakened towards the surface. No positive cells were observed in the submucosa. In tumour tissue, where normal epithelial cells were not included, no signal was detected either in the tumour cells (T) or in the interstitial cells (I) (Fig. 2b).

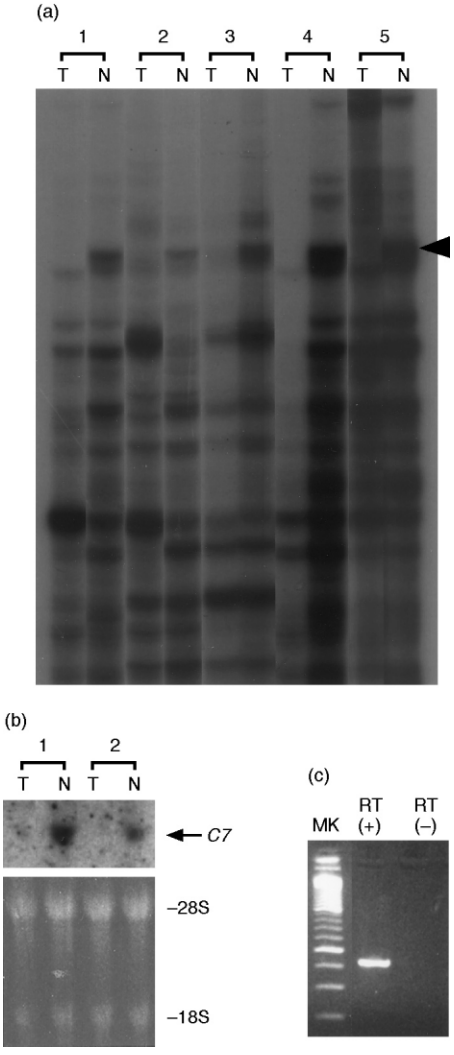

Fig 1. Differential expression *C7* mRNA in normal/tumour pair specimens. *C7* cDNA (arrow) was obtained using differential display for 5 patients (a) and Northern blot for 2 patients (b). Reverse transcriptase-polymerase chain reaction (RT-PCR) amplification for *C7* mRNA in normal rat oesophagus (c).

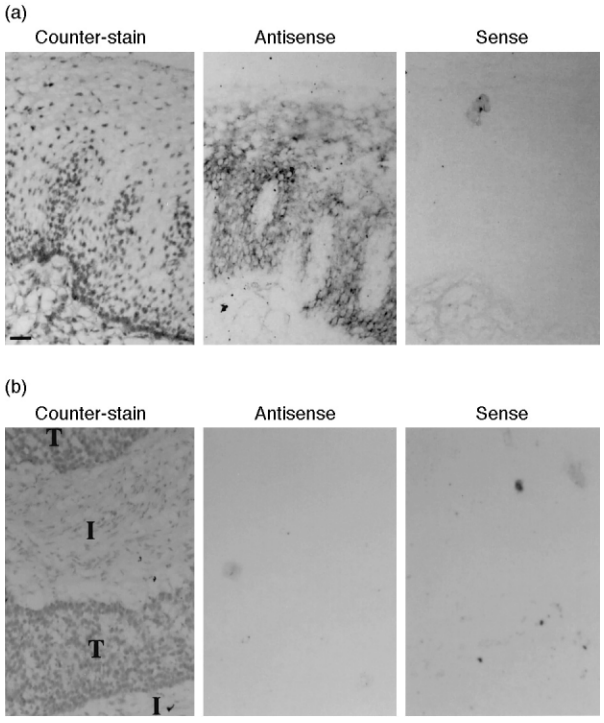

Fig. 2. Localisation of *C7* mRNAs in normal oesophageal tissue (a) and oesophageal carcinoma tissue (b) determined by *in situ* hybridisation. Serial sections were stained by haematoxylin, antisense probe and sense probe. The bar indicates 50 μm.

3.3. Quantification of locally expressed complement component mRNAs

The expression of complement component mRNAs was measured by means of semiquantitative RT-PCR (Table 2). In 10 cases of matched tumour and normal specimens, *C7* mRNA levels in tumour tissues were more than 10 times lower than those of their normal counterparts in 4 cases, more than twice as low in 4 cases, and less than twice as low in 2 cases. *C6* mRNA levels in the tumour tissues were not so reduced as *C7* mRNA levels, but their reduction was also statistically significant ($P<0.01$) and paralleled those in *C7* since a significant correlation was found between the T/N ratios of the *C7* and *C6* mRNAs ($r=0.842$, $P<0.01$). The expression levels of *C5* and *C3* mRNAs did not differ significantly between tumour and normal tissues ($P=0.17$, $P=0.88$). However, amplification for *C8* or *C9* mRNAs was not observed even after 34 cycles of amplification (data not shown).

The same rounds of PCR amplification were performed to evaluate the expression of the complement in the components cell lines derived from oesophageal carcinoma (Fig. 3). Significant expression of *C7* mRNA equivalent to that in normal tissue was observed in TE-10, TE-12, TE-2, and TE-9, but in the remaining five cell lines the expression was faint or almost absent. *C6* mRNA expression was less detectable than *C7* mRNA, with only slight expression in the TE-12 and TE-13 cell lines. Ubiquitons expression of *C3* and *C5* mRNAs was confirmed in all cell lines.

3.4. Detection of C6 and C7 proteins

Immunohistochemistry could not detect C7 or C6 proteins in the oesophageal mucosa under a variety of staining conditions (data not shown). When the culture media of the TE-10 and TE-12 cell lines were concentrated and subjected to western blot analysis, constitutive secretion of C7, as well as C6, were demonstrated (Fig. 4).

Table 2
Expression of complement component mRNAs in clinical samples

	C7 mRNA/*GAPDH*			*C6* mRNA/*GAPDH*			*C5* mRNA/*GAPDH*			*C3* mRNA/*GAPDH*		
Case	N	T	T/N ratio	N	T	T/N ratio	N	T	T/N ratio	N	T	T/N ratio
1	0.99	0.00	0.00	0.27	0.00	0.00	0.89	0.99	1.11	1.11	1.55	1.40
2	0.67	0.01	0.01	0.35	0.08	0.23	0.85	0.44	0.52	0.48	0.66	1.38
3	0.92	0.02	0.02	0.76	0.05	0.07	0.72	1.36	1.89	0.95	0.66	0.69
4	0.77	0.04	0.05	0.88	0.11	0.13	0.58	1.41	2.43	1.11	2.23	2.01
5	0.73	0.47	0.64	0.67	0.40	0.60	0.69	1.56	2.26	0.81	0.87	1.07
6	1.34	0.15	0.11	0.56	0.04	0.07	0.46	0.44	0.96	0.64	0.47	0.73
7	1.31	0.36	0.27	0.55	0.09	0.16	0.25	0.29	1.16	1.31	0.67	0.51
8	0.15	0.11	0.73	0.23	0.38	1.65	0.44	0.62	1.41	0.31	0.44	1.42
9	1.25	0.25	0.20	0.33	0.21	0.64	0.54	0.77	1.43	0.66	0.67	1.02
10	0.76	0.23	0.30	0.56	0.35	0.63	0.99	0.45	0.45	0.65	0.38	0.58
Mean	0.89± 0.36	0.16± 0.16	0.24± 0.26	0.52± 0.22	0.17± 0.15	0.42± 0.50	0.64± 0.23	0.83± 0.47	1.36± 0.67	0.80± 0.31	0.86± 0.58	1.08± 0.47
Range	(0.15, 1.34)	(0.00, 0.47)	(0.00, 0.73)	(0.23, 0.88)	(0.00, 0.40)	(0.00, 1.65)	(0.25, 0.99)	(0.29, 1.56)	(0.45, 2.43)	(0.31, 1.31)	(0.38, 2.23)	(0.51, 2.01)
P^a		0.01			0.01			0.17			0.88	

Expression is given as $\times 10^{-1}$ of *GAPDH* mRNA expression and represents the mean of duplicate measurements.

[a] Statistical differences between normal (N) and tumour (T) tissues were calculated using Wilcoxon's signed rank test.

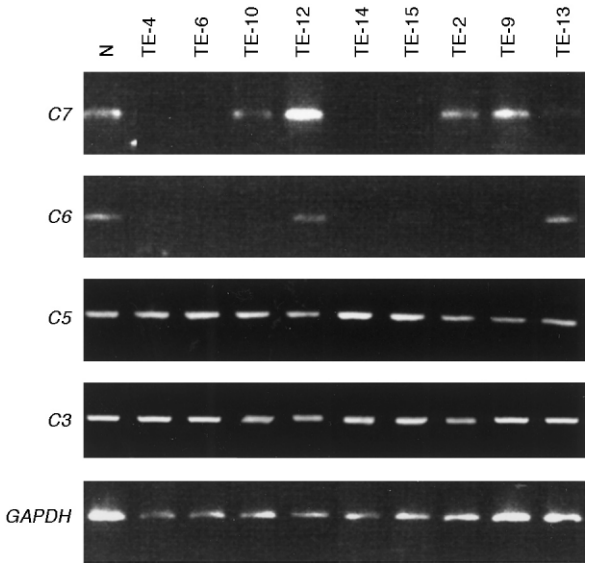

Fig. 3. mRNA expression of complement components in nine oesophageal carcinoma cell lines. PCR amplification after 28 cycles for the complement components and after 24 cycles for *GAPDH* is demonstrated. N, normal oesophageal tissue.

3.5. *Analysis of* C7 *genomic deletion*

To assess whether such reduction was caused by genomic loss, Southern blot analysis of the *C7* gene was performed (Fig. 5). The *C7* gene was present in the TE cell lines whose mRNA expression was undetectable. Moreover, their intensities of hybridisation were the same as that of normal peripheral-blood mononuclear cells indicating that these cells contained both alleles.

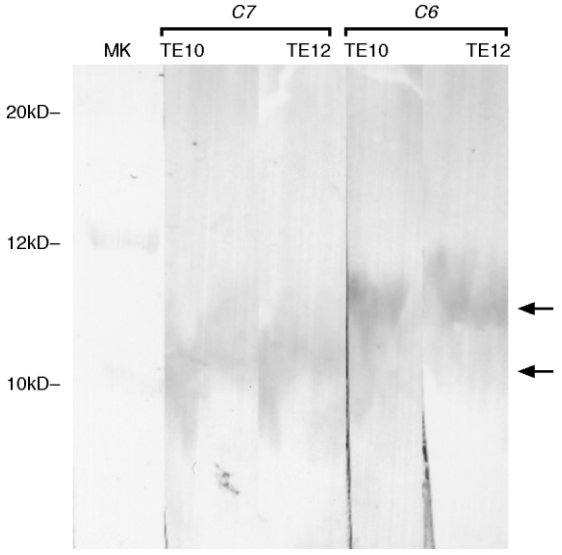

Fig. 4. Immunoblot analysis for C6 and C7 secreted by TE-10 and TE-12 cell lines. The bands of C6 and C7 are indicated by arrows. MK, molecular weight markers.

3.6. C7 *mRNA expression in other cancers*

The differential expression of *C7* mRNA expression was examined in other cancers by means of Matched Tumor/Normal Expression Array (Fig. 6). Since the amount of spotted cDNA had been normalised individually between each tumour/normal pair by housekeeping genes, the differential gene expression was evaluated by comparisons of the intensities of paired tumour/normal spots. Thus, the T/N ratios were calculated and analysed. A significant reduction in *C7* mRNA expression was observed in kidney and colon cancers.

4. Discussion

Differential display was used to identify cancer-related genes and *C7* expression was notably reduced in oesophageal tumour tissue compared with that in its corresponding normal tissue. Constitutive expression of *C3*, *C5*, and *C6* mRNAs in normal tissue was also confirmed, but only *C6* mRNA was reduced simultaneously with *C7* in tumour tissues.

The local synthesis of complement components has been studied through allotypic changes in circulating C7 from transplant recipients to donors, and approximately 30% of C7 and 10% of C3 were estimated to have been derived from non-marrow extrahepatic sources [22]. At the cellular level, C3 was observed without inflammatory

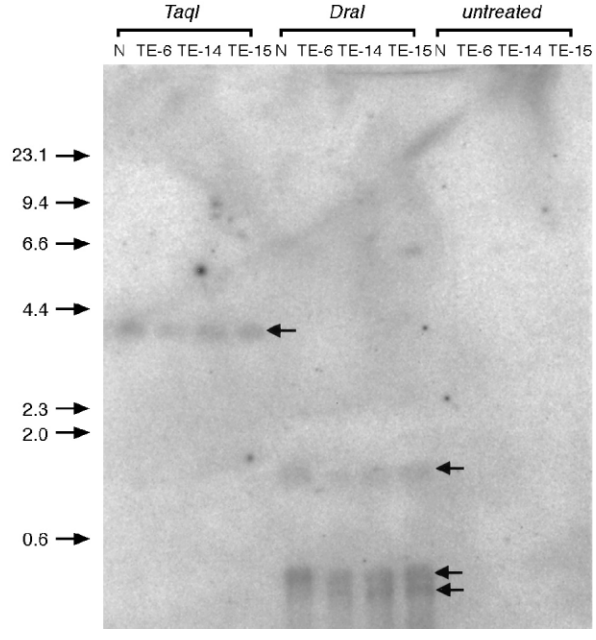

Fig. 5. Southern blot analysis for *C7* gene. Genomic DNA from TE-6, -14 and -15 cell lines and normal peripheral-blood mononuclear cells (N) was digested or undigested with restriction enzymes. Size markers are shown in the left margin.

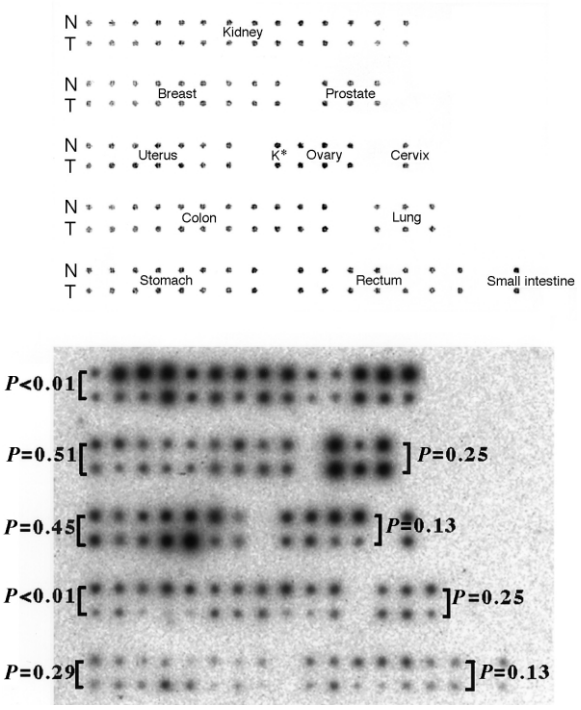

Fig. 6. *C7* mRNA expression on the Matched Tumor/Normal Expression Array. The T/N ratios of expression level were compared independently in each tumour/normal pair. Statistical significance was examined by one sample sign test.

stimuli in keratinocytes in skin [23] and colorectal mucosa [24]. In the colon, *C3* mRNA expression was also observed in tumour tissues such as adenomas and carcinomas [24], so that this observation is compatible with our findings that *C3* mRNA expression was abundant in both normal and tumour tissues. Constitutive expression of C5-C9 was also observed in glial cells in the cerebral cortex [25]. Recently, porcine *C7* mRNA was cloned and its expression was widely distributed in various organs, such as lung, intestine, liver, heart and bone marrow, although the precise cellular localisation has not yet been determined [26]. Our study revealed the endogenous expression of *C7* mRNA in keratinocytes, but not in any submucosal cells, such as tissue-resident macrophages or fibroblasts. Co-expression of C3, CS and C6 was also confirmed. Partial *C7* mRNA was detected in normal rat oesophagus, indicating that the expression of complement component mRNAs in normal human specimens reflects its constitutive expression, but was not induced during operative procedures. Immunohistochemistry was insufficient to detect local C7 protein in the oesophageal mucosa. A similar observation was reported even in the hepatocytes [27] and kidney [28] which are thought to be the major source of C7. It is possible that complement components may be secreted immediately after synthesis [27,28]. By means of western blot and immunostaining, C6 as well as C7 proteins were detected in the culture media of the TE-10 and 12 cell lines, suggesting that oesophageal keratinocytes can not only transcribe *C6* and *C7* mRNAs, but also translate them into proteins.

The complement system is thought to be involved in cancer immune surveillance. Deposition of C5b-9 complex as well as of C3 have been detected on nests of tumour cells and in the necrotic area of tumour tissues [29]. However, tumour cells show resistance to complement-mediated killing by several protective mechanisms: overexpression of complement-inhibitory proteins, secretion of C3-cleaving cysteine proteinase, and removal of the MAC from their surface as a result of rapid vesiculation or internalisation [30]. Furthermore, reductions in C6 and C7 expression in tumour tissues may limit the formation of MAC, thus conferring additional resistance against the complement attack. Finally, their reduction may hamper cell trafficking and sensitisation to the cellular immune effectors since C5b-7 has a chemotactic activity for polymorphonuclear leucocytes [6]. These evasive mechanisms may exist in colon and kidney cancers as well as in oesophageal carcinoma.

Interestingly the *C7* and *C6* genes are located closely together on chromosome 5p13, no more than 160 kbp apart [31]. Recently, on 5pl3, a novel tumour suppressor locus, Del-27, was isolated by genomic difference cloning of lung carcinoma cell lines [32].

When genomic deletion around this locus was investigated by means of genomic Southern blot analysis, the gene was retained in all cell lines without mRNA expression. The simultaneous downregulation of *C7* and *C6* mRNAs thus seems to be caused transcriptionally rather than by genomic deletion.

In this study, local expression of *C3*, *C5*, *C6* and *C7* mRNAs was confirmed in oesophageal epithelia. Among them, *C7* and *C6*, both playing important roles not only in the formation of MAC but also in the chemotaxis of cellular immune effectors, were specifically transcriptionally downregulated in tumour cells. It is conceivable that such downregulation may cause a reduction in the amounts of local complement components and contribute to cancer resistance to the complement attack, as well as membrane-bound complement inhibitors [8–13]. Exploring the mechanism causing their downregulation may lead to the elucidation of the tumour-related alteration in gene expression.

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