Soft and Hard Keratin Expression in Epstein-Barr-Virus-associated Gastric Carcinoma

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Abstract. *Background: Epstein-Barr-virus-associated gastric carcinoma (EBVaGC) is a distinct subset of gastric carcinoma (GC). The expressions of cytokeratins (CK)7, 8, 18, 19 and 20 and truncated basic hair keratin 1 (hHb1-ΔN) were investigated in GC to clarify the characteristics of EBVaGC. Materials and Methods: For immunohistochemical evaluation, 173 GC tissues were examined and 31 GC tissues and 5 GC cell lines were used for quantitative real-time RT-PCR (qrt-RT-PCR) and RT-PCR. Results: EBVaGC showed significantly lower immunohistochemical positivity of CK7 (–/±/+/++/+++; 27/15/4/1/1) compared to EBV-negative GC (12/29/27/44/13), even after stratification by histological types. The qrt-RT-PCR test demonstrated decreased amounts of CK7, 18 and 19 mRNAs in EBVaGC. Two among 5 GC cell lines showed a decrease of CK7 mRNA level after recombinant EBV infection. hHb1-ΔN expression was not specific to EBVaGC. Conclusion: Abnormalities of CK7, 18 and 19 expressions, especially a decreased amount of CK7 expression, are characteristics of EBV-associated epithelial malignancies and might be important in carcinogenesis.*

Epstein-Barr-virus (EBV)-associated gastric carcinoma (GC) is a distinct subset of GC, accounting for 10% or less of total GC. EBV-associated GC (EBVaGC) is defined as

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GC harboring a monoclonal EBV. Its histology is a characteristic, moderately-differentiated tubular or poorly-differentiated solid type of histology admixed with lymphocytic infiltration and, in its extreme end, the histology resembles lympho-epithelioma of the nasopharynx, *i.e.*, nasopharyngeal carcinoma (NPC). EBV-infected neoplastic gastric epithelia also show unique cell-biological features, such as production of IL-1β (1) and IGF-1 (2), abnormal expression of variant forms of CD44 (3) and decreased levels of apoptosis (4). However, there have been few attempts to investigate the expression of cytoskeleton-related proteins in EBVaGC, which might be potentially related to its unique histology or biological behavior.

The cytoskeleton of mammalian cells consists of three major protein families: actin-containing microfilaments, tubulin-containing microtubules and intermediate filaments. Keratins are the intermediate filament proteins, expressed in the epithelial cells, and can be divided into two types, soft and hard (5). Soft keratins, or epithelial cytokeratins (CKs), are typically expressed in the various types of epithelia, while hard keratins and hair keratins, are constituents of hard keratinizing structures such as hairs and nails. Soft keratins comprise 20 members (CK1-20) classified into two types, type I (CK9-20) and II (CK1-8). Regardless of the number of keratins found in a given epithelial cell, the ratio of type I and type II keratins is 1:1. For example, hepatocytes express CK8/18, whereas intestinal epithelial cells express CK7/8 (type II) and CK18/19/20 (type I) at variable levels.

CK expression in the stomach seems to be determined not only by the cell lineage, but also by regulation during development and/or tissue repair. CK7 is expressed transiently in the fetal stomach from the 14th to 16th

Table I. *Summary of patients and tumors.*

	for IHC stain		for qrt-RT-PCR	
	EBV-associated GC	EBV-negative GC	EBV-associated GC	EBV-negative GC
Patient	46	115	11	20
Age	60±12[a]	65±12	64±16	65±10
Gender (Male/Female)	37/9	79/36	6/5	15/5
Tumor	48	125	11	20
Size	45±36 mm[a]	47±33 mm	58±34 mm	68±39 mm
Location (U/M/L)[b]	19/23/6	26/63/36	3/8/0	5/7/8
Intestinal/ Diffuse type	29/19	76/49	6/5	11/10
tub1/tub2/pap	6/22/1	34/37/5	4/2/0	4/3/3
por/sig/muc	19/0/0	32/16/1	5/0/0	5/5/0
Vascular invasion	19	64	10	17
Lymphatic invasion	21	74	10	18
Lymph node metastasis	16 (/45)	55 (/112)	5 (/10)	12
Advanced tumor	24	54	10	13

[a]mean±standard error of the mean
[b]U: upper, M: middle, L: lower location.
Tumors for IHC stain included 13 cases of double GC and 1 case of triple GC. Three patients had double GCs with and without EBV.

gestational week, at the start of gastric pit development, and disappears thereafter (6). CK19 expression is observed in the stomach up to the 11th week of gestation and reappears weakly from the 18 to 24th week (7). CK7 expression is absent or minimal in the adult stomach, but reappears in gastritis. Recent studies also suggest that keratin protein expression is affected by EBV infection in the gastric epithelial cells. Kim *et al.* reported that EBVaGC might be correlated to a decreased level of CK7 expression (8). Nishikawa *et al.* (9) observed that EBV infection up-regulated a hard keratin, truncated-type of human basic hair keratin 1 (hHb1). The truncated-type of *hHb1* (*hHb1-ΔN*) is transcribed as mRNA with a 709-base deletion in the 5'-side of the full length of *hHb1*, 1825 bases, and is thereafter translated into protein.

In this study, the soft and hard keratin expression profiles of both surgical GC tissues and GC cell lines were further evaluated in order to clarify the association of cytokeratin abnormalities with EBV infection, considering other clinicopathological factors.

Materials and Methods

Tissue analysis. For the immunohistochemical (IHC) evaluation of CK protein expression, specimens of stomachs resected for the treatment of gastric cancer at the University of Tokyo and Jichi Medical School Hospitals, Japan, between 1990 and 1997, were used. Surgically-resected specimens were fixed with 10% formalin and embedded in paraffin. Histological and pathological data were evaluated according to the Japanese Classification of Gastric Carcinoma (10) and Lauren's classification (11). EBVaGCs were screened by *in situ* hybridization targeting EBV-encoded small RNA with an EBER-PNA probe (DakoCytomation, Denmark), which was applied to the tumor sections. The positive signal was visualized by PNA ISH detection kit (DakoCytomation).

Forty-eight EBVaGCs and 125 EBV-negative GCs (EBVnGCs) were retrieved from the archives. The series included relatively many cases of EBVaGCs, with the aim of finding the difference between GC with and without association of EBV. Otherwise, EBVnGCs were selected without any bias. Thirteen cases contained double GC and 1 case triple GC. Three cases had double GCs, consisting of one EBVaGC and the other EBVnGC in each case. Nodal involvement could not be determined in 17 cases, since the lymph nodes were not dissected. The clinicopathological features of GC, with and without association of EBV, are summarized in Table I.

Fresh tumor tissues were obtained from 31 cases, which were subjected to quantitative real-time (qrt-) RT-PCR for mRNA expression of CK and to RT-PCR for *hHb1-ΔN* (Table I). These 31 cases included 11 EBVaGC and 20 EBVnGC. Sixteen tumors were examined by both IHC stain and qrt-RT-PCR analysis.

Establishment of EBV-infected GC cell lines. Five GC cell lines, MKN1, MKN7, MKN74, NU-GC-3 and TMK-1, were infected with recombinant EBV using the cell-to-cell contact method (12). A Burkitt lymphoma cell line, Akata, with the recombinant EBV, in which the neomycin resistance gene (*Neo^r*) was inserted into BXLF1 (13), was used as a source of recombinant EBV. EBV infection in the GC cell lines was confirmed by *EBER1 in situ* hybridization and by Southern blotting with an EBV terminal repeat probe.

IHC evaluation of CK protein expression. CK protein expression was evaluated by IHC, which was applied to the formalin-fixed paraffin-embedded specimens. For IHC evaluation, the following antibodies were used: anti-CK7, OV-TL12/30, anti-CK8, 35βH11, anti-CK18, DC10, anti-CK19, RCK108 (DakoCytomation), anti-CK20, ks20.8 (Novocastra Laboratories, UK) and anti-Ki67, MIB-1 (Dako-Cytomation). The optimal dilutions were 1:100 for OV-TL 12/30, RCK108 and ks20.8 and 1:200 for 35βH11, DC10 and MIB-1. The optimal antigen retrieval was found to be 10 min autoclaving in 0.01 M citrate buffer (pH 6.0), except for 35βH11 (0.2 mg/mL proteinase K treatment for 20 min). At least one representative section was evaluated for each tumor. Four-micrometer sections on silane-coated slides were serially subjected to antigen retrieval, incubation with specific antibodies and immunostaining by streptoavidin-biotin-peroxidase method, using a LSAB2 kit (DakoCytomation), and were then counter-stained with hematoxylin. By referring to normal tissue sections, it was confirmed that the preservation of paraffin blocks for up to 15 years did not change the positivity of the IHC stain. Negative control studies included substituting each antibody with the non-immune serum.

Table II. *Summary of IHC staining of GC tissues.*

	EBV-associated GC -/±/+/++/+++	EBV-negative GC -/±/+/++/+++	
CK7	27/15/4/1/1	12/29/27/44/13	$p<0.0001^{*,b}$
CK8	0/2/6/19/21	0/9/11/41/64	NS[c]
CK18	0/0/0/3/45	1/0/4/17/103	NS
CK19	0/7/6/19/16	5/20/24/50/26	NS
CK20	35/9/4/0/0	69/38/10/7/1	NS
MIB1-LI	54.7±15.2%[a]	50.5±18.3%	NS

[a]mean±standard error of the mean.
[b]CK7 positivity is significantly lower in EBVaGC than in EBVnGC.
[c]NS: no significant difference.

The double-staining of CK7 and Ki-67 was performed as follows: the Ki-67 antigen was initially stained with DAB, followed by autoclaving for 10 min for inactivation of the Ki-67 antibody and the secondary anti-mouse antibody. The section was then immunostained with anti-CK7 antibody and the reaction product was stained with TrueBlue (DakoCytomation). Finally, the sections were counter-stained with Kernechtrot (Wako Pure Chemical Industries, Japan). In a pilot study, it was confirmed that the sensitivities for the Ki-67 antigen and CK7 were not changed between double IHC staining and each single IHC staining.

The results of IHC were evaluated without knowledge of the clinical data. The specimens were graded into 5 groups according to the extent of positivity of carcinomas as follows; –: no positive stain, ±: less than 10%, +: more than 10%, ++: more than 30%, +++: more than 70% of the tumor cells showed positive stain, respectively. As for MIB-1 positivity, 10 high-power fields were selected at random and at least 1000 tumor cells were evaluated to obtain the rate of positively-stained tumor cells (MIB1-labeling index, MIB1-LI).

Qrt-RT-PCR evaluation of CK mRNA expression. Total tissue RNA was extracted with TRIzol reagent (Invitrogen, CA, USA) and treated with DNaseI. Each RNA was confirmed not to be contaminated with DNA by PCR using a GAPDH primer set; sense 5'-GAAGGTGAAGGTCGGAGTC-3', anti-sense 5'-GAAG ATGGTGATGGGATTTC-3', which yielded a 225bp-sized product. First-strand cDNA was synthesized using the Ready-To-Go™ T-Primed First-Strand Kit (Amersham Biosciences, UK).

Qrt-RT-PCR was performed using a SYBR Green method with the QuantiTect SYBR Green PCR Kit (Qiagen, Netherlands) and iCycler iQ real-time PCR detection system (Bio-Rad Laboratories, CA, USA), according to the manufacturers' instructions. The primer sets and amplification conditions were according to Dimmler *et al.* (14).

Each amplicon was inserted to the pCR2.1 vector with a TOPO-TA cloning kit (Invitrogen) and used as a standard for each assay. Each PCR assay was repeated three times for each sample. Estimated quantities of *CKs* mRNA were standardized as the ratio to *GAPDH* mRNA for each sample.

RT-PCR evaluation of hHb1 expression. Two primer sets reported to amplify *hHb1* cDNA; hHB1-5 for amplifying the 5'-side of *hHB1*

cDNA and hHB1-3 for the 3'-side (9), were used. Total RNA extracted from the scalp was used as a positive control for RT-PCR. When the full length of *hHb1*, 1825 bases, was expressed, the amplicon could be obtained with both hHb1-5 and hHb1-3 primer sets. When *hHb1-ΔN*, 1116 bases, was expressed, the amplicon could be obtained only with an hHb1-3 primer set.

Statistical analysis. The difference of IHC protein expression between the two groups, EBVaGC and EBVnGC, was tested by a Wilcoxon test. The difference of mRNA expression was tested by *t*-test. The correlations between CK-positivities and other clinicopathological factors were tested by Wilcoxon tests or Kruskal-Wallis tests. The correlation between *hHb1-ΔN* expression and EBV status was tested by a Chi-square test. All statistics were computed with the SAS System for Windows release 6.12 (SAS Institute, NC, USA).

Results

Keratin expression in GC with or without association of EBV IHC evaluation of CK protein expression: IHC stainings of CKs in GC are presented in Figure 1 and the results are summarized in Table II. Neoplastic cells of GC showed stronger positivity of CKs compared to the non-tumorous stomach. When CK expression was compared between EBVaGC and EBVnGC, CK7 positivity was generally weaker in EBVaGC, and the Wilcoxon test demonstrated that the difference was statistically significant. To further confirm the relationship between EBV infection and decreased expression of CK7, positivity was compared in each histological type of Lauren and the Japanese Classification of Gastric Carcinoma. Since there was no case of signet-ring cell carcinoma or mucinous carcinoma and only 1 case of papillary carcinoma, these histological types were excluded from the comparison. However, EBVaGC showed lower CK7 positivity in each histological type, compared to the corresponding EBVnGC.

Clinicopathological factors correlating with CK expression were further evaluated in GC with and without association of EBV, by Chi-square tests (Figure 2). While there was no factor affecting CK expression in EBVaGC, correlation was discernible in EBVnGC, especially in the case of location of the carcinoma. The upper location was correlated with decreased expressions of CK8, the middle location with increased expression of CK8 and 20 and the lower location with decreased expression of CK20. A decreased level of CK19 was correlated with vascular invasion and lymph node metastasis. MIB1-LI was not related to the positivities of any CKs.

Qrt-RT-PCR evaluation of CK expression: The results of qrt-RT-CR of tissue specimens are summarized in Table III. *CKs* mRNA per 1000 copies of *GAPDH* mRNA was calculated. The expression of *CK8* mRNA was relatively lower than the other *CK* mRNA expressions. The expressions of *CK7*, *CK18* and *CK19* mRNAs were
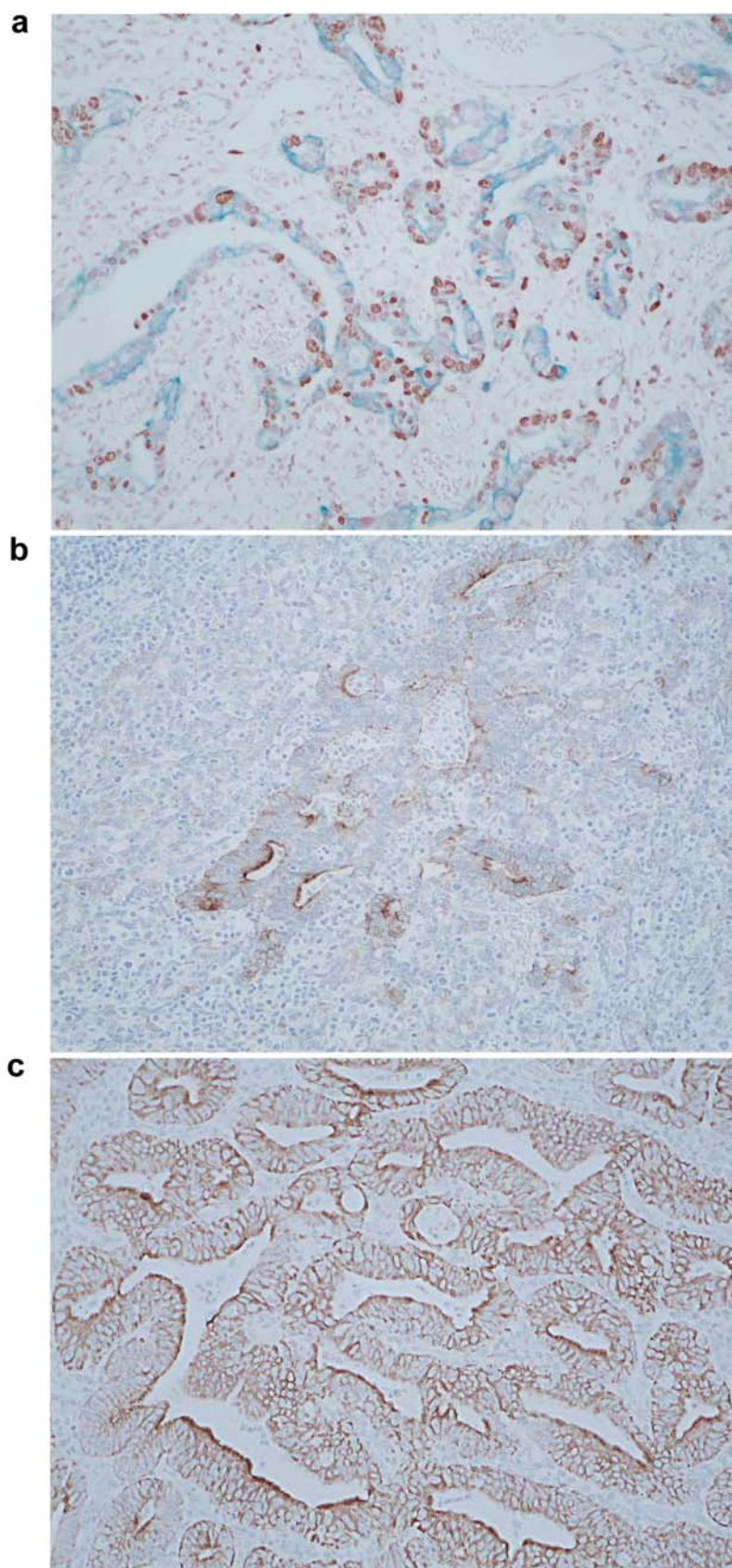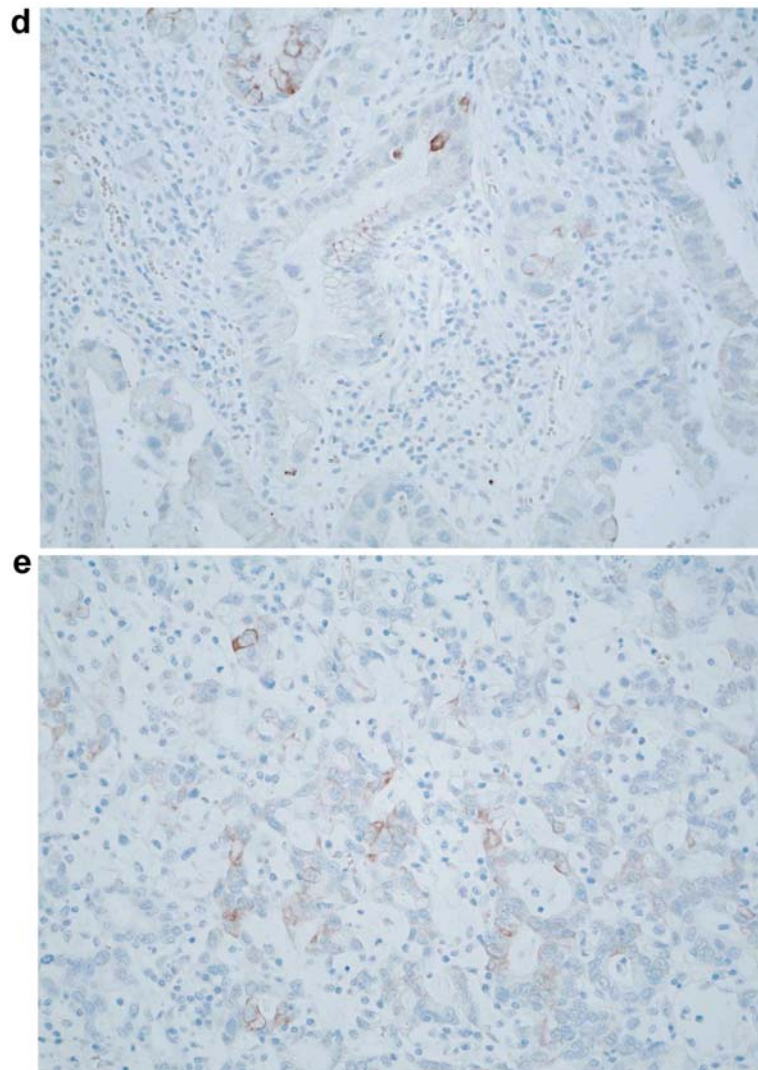
Figure 1. *CKs IHC stain and its grading. a. CK7 IHC stain showed moderate positivity (grading ++); b. more than 10% of tumor cells showed CK8 (grading +); c. almost all tumor cells showed strong positivity for CK18 (grading +++); d. weak positivity for CK19 (grading ±); e. weak positivity for CK20 (grading ±).*
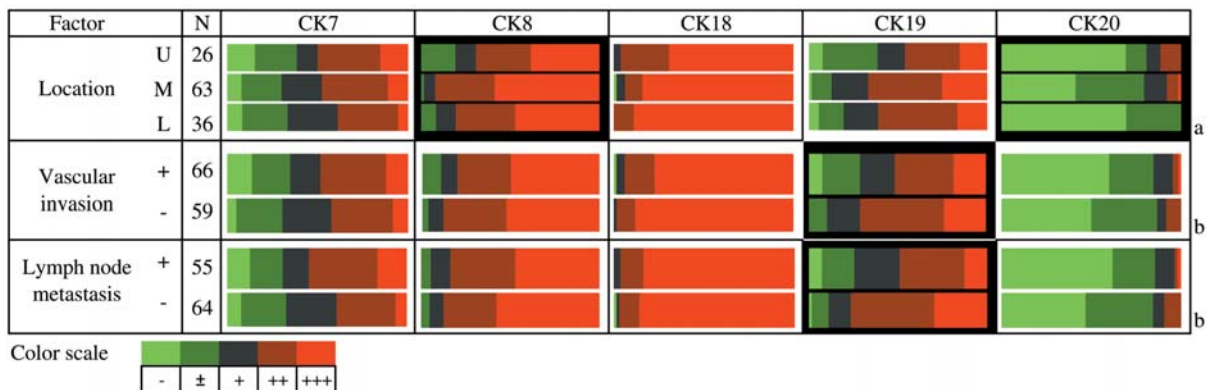

Figure 2. *CKs and other factors in EBVnGC. a: CK positivity of each location (U, M, L) was compared with the other locations using Kruskal-Wallis tests. b: Unpaired Wilcoxon tests were used for comparison of the IHC score of CKs. Black-filled boxes show statistically significant difference of CK expression by the factors. The upper location was correlated with decreased expressions of CK8, the middle location with increased expressions of CK8 and 20 and the lower location with decreased expression of CK20. A decreased level of CK19 was correlated with vascular invasion and lymph node metastasis.*

Table III. *Summary of estimated CK mRNA quantity and hHb1-ΔN expression in 31 GC tissues by qrt-RT-PCR and RT-PCR analysis.*

		EBVaGC	EBVnGC	
qrt-RT-PCR	CK7	57.6±166[a]	225±257	*p*=0.031[*,b]
	CK8	0.961±1.17	0.884±0.697	NS[c]
	CK18	621±591	1133±839	p=0.042[*]
	CK19	645±597	1904±1390	*p*=0.004[*]
	CK20	113±324	60.0±113	NS
RT-PCR	*hHb1-ΔN*	2(/11cases)	3(/20cases)	NS[d]

[a]molecules per 1000 molecules of *GAPDH*. Means±standard error of the mean values obtained from triplicate assays.
[b]*p*-values were calculated in *t*-tests.
[c]NS: no significant difference in *t*-tests.
[d]NS: no significant deviation in a Chi-square test.

Figure 3. *RT-PCR of hHb1. RT-PCR of GC tissues. Samples 1 and 2 were positive for the primer set for the 3'-side, hHB1-3, but negative for the primer set for the 5'-side, hHb1-5. These 2 cases are interpreted to express hHb1-ΔN. Samples 1 and 3 were EBVaGC and samples 2 and 4 were EBVnGC. P: positive control (total RNA of the scalp), N: negative control (water)*

significantly lower in EBVaGC than in EBVnGC, while mRNA expressions of *CK8* and *CK20* were not different between the GC groups. Sixteen tumors were examined by both IHC stain and qrt-RT-PCR. When the cases were divided into a low expression group, scored – or ±, and a high expression group, scored +, ++, or +++, for each CK in the IHC stain, there was no significant difference in mRNA quantity of each CK between the groups.

RT-PCR of hHb1: Regarding the expression of human basic hair keratin 1 (hHb1), neither *hHb1* nor *hHb1-ΔN* was detected by RT-PCR assays in any samples of non-tumorous mucosa. On the other hand, *hHb1-ΔN* mRNA expression was observed in 3 out of 11 tumor tissue specimens of EBVaGC, and in 2 out of 20 EBVnGC (Figure 3, Table III). The 5 cases expressing *hHb1-ΔN* showed statistically lower *CK18* mRNA expression than the other cases and no significant difference in the other *CK* mRNAs expression. The *hHb1-ΔN*-expressing group and non-expressing group expressed 436±154 and 1028±818 copies of *CK18* mRNA per 1000 copies of *GAPDH* mRNA, respectively (*p*=0.001).

Keratin expression in GC cell lines with or without infection of EBV. The results of qrt-RT-PCR of GC cell lines are summarized in Table IV. The amount of *CK8* mRNA in the GC cell lines was low, as observed for the tissue specimens. The estimated mRNA quantity by qrt-RT-PCR was compared in each pair of the original cell line and recombinant EBV-infected cells. In 2 cell lines, MKN1 and MKN7, the amount of *CK7* expression was less than half in the EBV-infected cell lines compared to the original cell lines. In comparison with the corresponding original GC cell lines, a lower level of mRNA expression was also observed in *CK18* and *19* of the EBV-infected MKN7, and in *CK18* of EBV-infected MKN74.
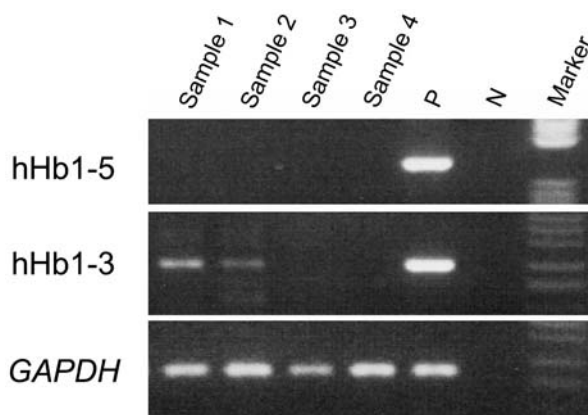
All 5 sets of GC cell lines, without or with EBV infection, showed *hHb1-ΔN* mRNA expression in RT-PCR.

Discussion

The cytokeratin profile of the carcinoma, especially its expression pattern of CK7 and 20, is strongly related to its primary organ. In the present study, when positivity was defined as stronger than ±, the positivities of CK8, 18 and 19 were 96, 99 and 97% of total GC, respectively, whereas the positivities of CK7 and 20 were 77 and 39%, respectively. These frequencies were compatible with the previous study by Kim *et al.* (8), and such variable frequencies suggest that different mechanisms are at work in the regulation of CK proteins in the stomach. In this context, it is of interest that the location of the carcinoma correlated with CK expression of EBVnGC. A lower level of CK8 and 19 expressions in the upper portion might reiterate the CK profile of pyloric/cardiac glands, and higher levels of CK8 and 20 may reflect the phenotype of an incomplete type of intestinal metaplasia.

In the present study, a strong correlation was observed between a decreased level of CK7 and EBVaGC. This correlation was still significant even after stratification by the histological GC types, and there were no clinico-pathological factors affecting CK expression in EBVaGC. Furthermore, in the qrt-RT-PCR study, the mRNA expressions of CK7, 18 and 19 were lower in EBVaGC, compared to EBVnGC. Qrt-RT-PCR is a more sensitive method than IHC staining, meanwhile mRNA quantity is affected by the interstitium and infiltrating inflammatory

Table IV. *Keratin expression in qrt-RT-PCR analysis of gastric cancer cell lines and EBV-infected cell lines (per GAPDH 1000 molecules).*

Cell Line	CK7	CK8	CK18	CK19	CK20
MKN1	504±131	0.72±0.28	1162±179	734±166	0.00212±0.00103
MKN1+EBV[a]	219±49 ↓	1.30±0.46	1476±113	1309±529	0.00268±0.00069
MKN7	337±52	0.39±0.06	525±16	446±223	0.00143±0.00051
MKN7+EBV	109±50 ↓	0.18±0.03 ↓	215±22 ↓	160±51 ↓	0.00194±0.00037
MKN74	0±0	0.13±0.06	297±45	22±12	0.00947±0.00045
MKN74+EBV	0±0	0.06±0.04 ↓	173±19	26±9	0.01504±0.00124
NU-GC-3	185±23	0.10±0.02	372±1	79±10	0.00064±0.00013
NU-GC-3+EBV	218±32	0.36±0.04 ↑	498±36	191±40 ↑	0.00079±0.00038
TMK-1	630±49	0.09±0.02	512±57	292±58	0.00027±0.00017
TMK-1+EBV	848±64	0.16±0.02	480±11	303±12	0.00000±0.00000 ↓

[a]+EBV: EBV-infected subclone.

↓ shows CK expression decreased less than half of the expression of original cells by EBV infection, and ↑ shows an increase of more than double.

cells of cancer tissue. These differences might cause the somewhat different results between IHC staining and qrt-RT-PCR. In the case of EBVa nasopharyngeal carcinoma (NPC), Sriuranpong *et al.* have recently demonstrated that CK7 was underexpressed in NPC in a genome-wide transcriptome analysis of laser capture microdissected cells (15). Thus, EBV infection leads to the down-regulation of CK7 proteins in gastric epithelial cells as well as the nasopharynx. As for the CK7 in the stomach, CK7 is expressed transiently in the fetal stomach from the 14th to 16th gestational week, at the start of gastric pit development, and disappears thereafter (6). CK7 is expressed in the epithelium just below the esophago-gastric junction in the adult stomach (16). In the pathological state, 80% of intestinal metaplasia (6) and 52% of GC express CK7 (17). In spite of these facts, common down-regulation of CK7 expression in glandular and squamous epithelia, both of which are infected with EBV, strongly indicates that viral infection directly causes the down-regulation, suggesting an important role of CK regulation in EBVa epithelial malignancies.

It is worth noting that a lower level of CK19 expression correlated with vascular invasion and lymph node metastasis in EBVnGC. Hepatocellular carcinoma (HCC) usually expresses CK8 and CK (18), but in CK19-positive HCC, the incidence of extrahepatic disease, especially lymph node metastasis, was significantly higher, suggesting that CK19 expression is a predictor of early postoperative recurrence (18). Thus, although the precise mechanism has not been clarified yet, decreased CK19 expression may cause abnormality of the intermediate filament network, resulting in the metastatic potential of cancer cells.

Nishikawa *et al. (*9) demonstrated that an EBV-infected gastric cancer cell line, NU-GC-3, up-regulated *hHb1-ΔN* and down-regulated CK8. In the present study, evaluating mRNA by RT-PCR, however, up-regulated *hHb1-ΔN* was not specific to EBVaGC, but was observed in 16% of GC (5/31 cases), 3/11 tumor tissue specimens of EBVaGC and 2/20 EBVnGC. Since we also observed that *hHb1-ΔN* was expressed in 5/5 GC cell lines, aberrant expression of *hHb1-ΔN* might serve as a tumor marker, and its significance in cancer biology should be further studied.

In the present study, the expression profiles of soft and hard keratins were also evaluated in GC cell lines with and without EBV infection. Using recombinant Neo^r-integrated EBV and a Burkitt lymphoma-derived cell line, Imai *et al.* demonstrated that efficient EBV infection is achieved by direct contact of EBV-producing lymphocytes and gastric cancer cell lines (12). In spite of the inherent limitation using cancer cell lines, this system is useful for the investigation of cell biology of EBV-infected epithelial cells. Although the changes of CK-expression were not uniform, 2/5 EBV-infected GC cell lines, MKN1 and MKN7, down-regulated CK7 expression. MKN7 showed decreased expression of *CK7*, *8*, *18* and *19* after EBV infection. MKN7 is a cell line originated from well-differentiated tubular adenocarcinoma and MKN1 from adenosquamous carcinoma (19). Meanwhile, MKN74 is derived from moderately-differentiated tubular adenocarcinoma (19), while NU-GC-3 and TMK-1 are from poorly-differentiated adenocarcinoma (20, 21). The origin and other characteristics of MKN7 and MKN1 might cause the decrease of CK by EBV infection. There have been few attempts to investigate the significance of CK molecules in epithelial cells infected with human oncogenic viruses. In the case of human papilloma virus (HPV), *in vitro* data suggest an HPV16 *E7* mRNA/CK7 interaction (22) or association between HPV16 E7 protein level and CK19 in squamous cell carcinoma cell lines (23).

The recombinant EBV-infected GC cell line, thus, may be useful for further evaluation of the cellular mechanisms of carcinogenesis of EBVaGC.

In conclusion, CK7 expression was down-regulated in protein and mRNA in EBVaGC. CK18 and 19 were also down-regulated in mRNA level in EBVaGC. Up-regulation of truncated hHb1 was observed in a subset of GC, but was not specific to EBVaGC. These abnormalities of CK expression may be important in the association between human oncogenic viruses and carcinogenesis.

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