American Journal of Pathology, Vol. 161, No. 4, October 2002
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Different Pattern of Allelic Loss in Epstein-Barr Virus-Positive Gastric Cancer with Emphasis on the p53 Tumor Suppressor Pathway

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Both *Helicobacter pylori* (HP) and Epstein-Barr virus (EBV) have been implicated in carcinogenesis of the stomach. Fifty-seven gastric carcinomas were tested for microsatellite instability and allelic loss at several tumor suppressor loci using 21 polymorphic microsatellite markers. Furthermore, immunohistochemistry for p53 and DPC4/SMAD4 was performed. Results were analyzed according to HP and EBV status of the tumors, as assessed by immunohistochemistry and RNA *in situ* hybridization, respectively. Fractional allelic loss was lower in EBV-positive carcinomas (n = 15) when compared to EBV-negative carcinomas (P < 0.001). EBV positivity was inversely associated with allelic loss at specific markers on chromosomal arms 5q (*APC*), 17p (*TP53*), and 18q (*DPC4/SMAD4*). Allelic loss at the *TP53* locus was not encountered in EBV-positive carcinomas, but occurred in 51% of EBV-negative carcinomas (P < 0.005). Moreover, none of the EBV-positive carcinomas showed unequivocal p53 immunopositivity in contrast to 39% of the EBV-negative carcinomas (P < 0.01). EBV-status was not related to microsatellite instability. There was no correlation between HP-status and any of the molecular alterations tested. In conclusion, EBV-positive gastric carcinomas follow a distinct pathogenesis at the molecular level, in which p53 is not, or differently inactivated. *(Am J Pathol 2002, 161:1207–1213)*

Despite its declining incidence in the western world, gastric cancer remains one of the most frequent and lethal malignancies worldwide.[1] The natural history of gastric cancer is complex and incompletely understood but diet, infections, and genetic factors are involved. More than 90% of gastric cancers are adenocarcinomas, which can be divided into two major histological types (intestinal and diffuse) by the Laurén classification.[2]

Of these two types, the tumorigenesis of the intestinal type of gastric cancer is best understood. It is thought to be governed by environmental factors and is characterized by precursor lesions of the gastric mucosa.[1,3] These precursor lesions are the morphological substrates of a stepwise neoplastic process in which genetic changes have accumulated gradually with tumor progression, similar to the adenoma-carcinoma sequence in colorectal cancer.[4,5]

Infectious agents are important factors in carcinogenesis of the stomach. *Helicobacter pylori* (HP) is a well-known risk factor and it is now considered a first class carcinogen for stomach cancer.[6,7] Epstein-Barr virus (EBV) is encountered in a subset of tumors but its role in gastric carcinogenesis is less well understood.[8] The latency type in gastric carcinomas is different from the known EBV latency types as described for Burkitt's lymphoma and nasopharyngeal carcinoma.[9,10] For example, the latent membrane protein-1 (LMP-1) is not expressed in EBV-positive stomach cancers.[10] Recent *in vitro* work by Subramanian and colleagues[11] suggests that the EBV nuclear protein EBNA-3C may functionally inactivate the human metastatic suppressor protein Nm23-H1. Hypermethylation of CpG islands as a mechanism of tumor suppressor gene silencing in EBV carrying gastric cancers has also been mentioned,[12] and expression of *RUNX3*, a gene causally related to stomach cancer is induced by the EBV transcription factor EBNA-2.[13,14]

In the present study, using a variety of molecular markers, we investigated gastric cancers for loss of heterozygosity (LOH) at tumor suppressor loci known to be involved in carcinogenesis of the gastrointestinal tract[15–17] and near tumor suppressor genes involved in syndromes that include gastric cancer in their phenotype. Furthermore, the presence of microsatellite instability (MSI), a hallmark of a defective DNA mismatch repair system, was assessed. The results of these analyses were evaluated with respect to the HP and EBV status of the tumors to evaluate the possible role of these infectious agents in carcinogenesis of the stomach.

This work was supported by the Netherlands Organization for Scientific Research (grant 950-10-643).

Accepted for publication June 20, 2002.

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Table 1. Markers for LOH and MSI Analysis

Marker	Chromosomal location	Repeat type	Putative tumor suppressor gene(s)/remarks
D2S123	2p16-21	Dinucleotide	*hMSH2*; MSI consensus marker
D3S1478	3p21	Dinucleotide	*FHIT*; *hMLH1*
D3S2456	3p	Tetranucleotide	*FHIT*; *hMLH1*
D5S346	5q21	Dinucleotide	*APC*; MSI consensus marker
D5S107	5q11.2-q13.3	Dinucleotide	*APC*
D9S171	9p21	Dinucleotide	$p14^{ARF}$; $p16^{INK4A}$; $p15^{INK4B}$
D9S932	9p	Tetranucleotide	$p14^{ARF}$; $p16^{INK4A}$; $p15^{INK4B}$
D10S2491	10q23	Dinucleotide	*PTEN* (intragenic marker)
D14S68	14q24.3-q	Dinucleotide	frequently deleted region in Barrett carcinomas
D16S2624	16q22.1	Tetranucleotide	*E-cadherin*
P53 Alu	17p	Alu repeat	*p53* (intragenic marker)
TP53	17p13.1	Dinucleotide	*p53*
D17S250	17q11.2-q12	Dinucleotide	*BRCA1*; MSI consensus marker
D18S64	18q21.32	Dinucleotide	*DCC*; *DPC4/SMAD4*; *SMAD2*
D18S474	18q	Dinucleotide	*DCC*; *DPC4/SMAD4*; *SMAD2*
D19S565	19p13.3	Dinucleotide	*STK11/LKB1*
D19S886	19p13.3	Dinucleotide	*STK11/LKB1*
D21S49	21q22.3	Dinucleotide	*TFF1*
BAT25	4q12	Mononucleotide	MSI consensus marker
BAT26	2p16	Mononucleotide	MSI consensus marker
BAT40	1p13.1	Mononucleotide	MSI consensus marker

Materials and Methods

Patient Material

Formalin-fixed, paraffin-embedded tissue of 57 gastric carcinomas was retrieved from the archives of the pathology departments of the Academic Medical Center (Amsterdam, The Netherlands), the Lublin Medical Academy (Lublin, Poland), and the Johns Hopkins Hospital (Baltimore, MD). DNA was isolated from these tumors and polymerase chain reaction (PCR) was performed using several microsatellite primers as described below. Of these 57 gastric carcinomas the tumors consisted of 28 gastric stump carcinomas (GSCs) and 29 gastric carcinomas of the intact stomach. The tumors were classified according to the Laurén classification by an experienced gastrointestinal pathologist (GJAO). None of the tumors had a lymphoepithelioma-like histology. Patient and tumor characteristics of the GSC and gastric carcinoma of the intact stomach were comparable and not significantly different. GSCs were used in this study because remote partial gastrectomy is a premalignant condition that has our interest,[18] and EBV is relatively common in GSC.[19] The prevalence of HP and EBV positivity was not significantly different in GSCs and gastric carcinoma of the intact stomach in this series. Baseline characteristics according to EBV status are summarized in Table 2.

Detection of Epstein-Barr Virus and H. pylori

In situ hybridization for EBER1 nuclear RNA transcripts was performed as previously described.[19] HP status was assessed initially by histopathological examination of the hematoxylin and eosin-stained sections. Cases that were negative for HP were subsequently tested by immunohistochemistry using the B471 polyclonal rabbit anti-HP antibody (DAKO, Glostrup, Denmark), as described previously.[19]

Microdissection and DNA Isolation

Tumor tissue was carefully microdissected from deparaffinized hematoxylin-stained 5-μm tissue sections. The percentage of cancer cells had to be at least 50 to 60%. For each case, matching nontumorous tissue was obtained from either a tumor-free lymph node or, when this was not available, from duodenum or smooth muscle cells. The tissue was incubated overnight in 50 to 100 μl of PK1 buffer (10 mmol/L Tris, pH 8.3, 50 mmol/L KCl, 2.5 mmol/L $MgCl_2$, 0.45% Nonidet P-40, 0.45% Tween 20, 0.01% gelatin) containing 5% Chelex resin (Chelex 100; Bio-Rad Laboratories, Hercules, CA) and 3 to 5 μl of Proteinase-K (10 mg/ml) at 56°C followed by a 10-minute incubation at 95°C to inactivate Proteinase-K.

Microsatellite Analysis

Microsatellite analysis was performed by PCR with 21 microsatellite primer pairs. Markers were selected either because of their location at tumor suppressor loci known to be involved in gastric carcinogenesis, near (or in) genes involved in syndromes that contain gastric cancer in their phenotype or because of their inclusion in marker panels used for the determination of MSI. The markers used are listed in Table 1. The sequences and their corresponding locations on the chromosomes were obtained from the Genome Data Base (*http://www.gdb.org*), the Cooperative Human Linkage Center (C*http://lpg.nci.nih.gov/CHLC*), or Genéthon (*http://www.genethon.fr*). One of the primers of each marker was fluorescently labeled. Optimal $MgCl_2$ and dNTP concentrations were obtained for each primer pair at an annealing temperature of 55°C using control human DNA. PCR was performed in a PTC-100 thermal cycler (MJ Research, Inc., Waltham, MA) during 40 cycles in a total reaction volume of 20 μl, containing 40 ng of each primer, 0.1 mg/ml

bovine serum albumin, and 1.0 U of Platinum *Taq* (Life Technologies, Inc., Rockville, MD) in the buffer supplied by the manufacturer. The PCR products were analyzed using an automated ABI 377 sequencer and Genescan 2.1 software (PE Biosystems, Foster City, CA).

Scoring of LOH and MSI

Normal samples with two distinctly sized alleles at a particular marker were called "informative." For all informative markers the allelic imbalance factor was calculated essentially as described by Cawkwell and colleagues.[20] A tumor was considered to show LOH at a particular marker if the allelic imbalance factor was >1.6 or <0.63. A finding of LOH had to be confirmed at least once to ensure reproducibility. For each individual the fractional allelic loss (FAL) was calculated as the ratio of LOH-positive markers to the total number of informative markers of that case. The FAL value therefore served as an overall measure of genetic instability at the tested loci.

Cases with an additional peak in the tumor DNA compared with their respective normal sample were scored as "microsatellite instable" (MSI) for a given marker. Tumors that exhibited MSI or that showed inconsistent results in repeated experiments at a given locus were excluded for analysis of LOH at that locus. With respect to MSI, tumors were classified according to international criteria.[21] Tumors were scored as stable (MSS) when no shifts were observed, as MSI-low (MSI-L) when shifts were seen in <40% of the markers and as MSI-high (MSI-H) with instability in ≥40% of the markers. MSI had to be confirmed at least once, to ensure reproducibility.

Immunohistochemistry

Immunohistochemistry for p53 and DPC4 was performed using the monoclonal antibodies DO-7 (DAKO) and clone B8 (Santa Cruz Biotechnology Inc., Santa Cruz, CA), respectively. Briefly, paraffin-embedded specimens were sectioned (5 μm), deparaffinized, and heat treated in 0.01 mol/L of Na-citrate buffer (pH 6.0) for 10 minutes in a Prestige Medical Series 2100 clinical autoclave (Prestige Medical, Blackburn, UK). Subsequently the slides were immersed in 0.3% hydrogen peroxide in methanol for 30 minutes. Nonspecific binding sites were blocked in 5% normal goat serum/phosphate-buffered saline (PBS) for 1 hour at room temperature after which the slides were incubated with the respective primary antibody in 5% normal goat serum/PBS for 1 hour. The Ultravision anti-polyvalent HRP detection system (Lab Vision Corp., Fremont, CA) was used to visualize antibody-binding sites with 3,3′-diaminobenzidine as a chromogen. Sections were counterstained with hematoxylin.

p53 immunoreactivity was scored as negative, weak (with weak to moderate staining in <30% of the tumor cells), or positive (with moderate to strong staining in >30% of the tumor cells). DPC4/SMAD4 immunoreactivity was scored as either negative or positive.

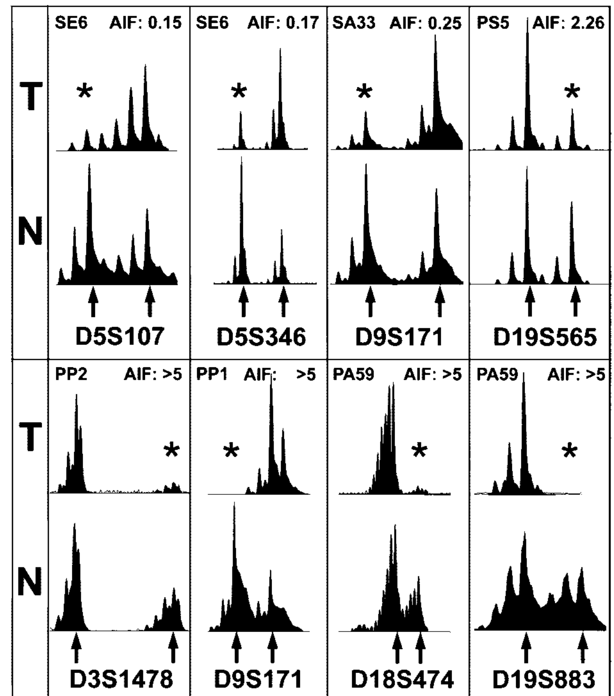

Figure 1. Representative examples of LOH. Electropherograms of labeled PCR products of paired tumor (T) and normal (N) DNA are shown. The tumor number and the allelic imbalance factor are shown at the **top** of each frame and the lost alleles are marked by **asterisks** (see Materials and Methods for calculation of the allelic imbalance factor and scoring of LOH). The tested marker is indicated at the **bottom** of each frame and individual alleles are marked by **arrows**.

Statistical Analysis

For statistical analysis the Fisher exact test, and the Mann-Whitney *U*-test were used where indicated. A two-sided *P* value <0.05 was considered statistically significant.

Results

A total of 57 tumors was studied for the presence of HP and EBV and molecular alterations using polymorphic microsatellite markers and immunohistochemistry. The overall frequency of LOH markers as indicated by the mean FAL value was 0.278 for the complete study group. Representative examples of LOH are shown in Figure 1. Markers that showed relatively frequent LOH (>30%) were on chromosomal arms 3p (31%), 9p (37%), 17p (40%), 18q (42%), and 19p (48%).

Of all 57 tumors, 15 tumors (26%) were positive for EBV as tested by EBER RNA *in situ* hybridization. Patients with EBV-positive tumors were predominantly male and on average 5.7 years younger when compared to patients with EBV-negative tumors. Other baseline characteristics were not significantly different between these two groups (Table 2). A frequency distribution of allelic loss according to EBV status at the chromosomal arms tested is depicted in Figure 2. There was a significant inverse relationship between positivity for EBV and the mean FAL value (Table 3). When stratifying LOH results according

Table 2. Patient and Tumor Characteristics

	EBV-positive (n = 15)	EBV-negative (n = 42)
Sex*		
Male	13	21
Female	2	21
Age†		
Mean	64.5	70.2
SD	7.37	9.49
Median	63	73
Origin		
Netherlands	7	28
Poland	6	9
U.S.A.	2	5
Stomach type		
Primary (GC-IS)	5	24
Stump (GSC)	10	18
Histology		
Intestinal	14	32
Mixed	0	5
Diffuse	1	5
Stage		
Early	4	11
Advanced	11	31
HP		
Positive	2	16
Negative	13	26

*P = 0.014; Fisher's exact test.
†P = 0.015; Mann-Whitney U-test.

to EBV status a significant inverse association was found between EBV-positivity and LOH at chromosomal arms 5q (*APC*), 17p (*TP53*), and 18q (*DPC4/SMAD4*) (Table 4; typical results are shown in Figure 3).

Using immunohistochemistry, we analyzed the tumors for positivity for p53 (17p) and DPC4/SMAD4 (18q). Immunohistochemistry for p53 was evaluated in 54 carcinomas and 33 cases were scored positive (61%). Unequivocal positivity, defined as moderate to strong positivity in more than 30% of the carcinoma cells, was observed in 16 cases (29%). None of the EBV-positive carcinomas were scored positive for p53 immunoreactivity in contrast to 39% of the EBV-negative carcinomas (Figure 3, Table 5). Loss of DPC4/SMAD4 expression was observed in eight cases (15%). No association was found between EBV positivity and loss of DPC4/SMAD4 expression. In addition, no association was found between EBV positivity and MSI (Table 3). MSI was found in 17 tumors (30%), 6 of which showed MSI-H and 11 of which were MSI-L.

Of the 57 tumors analyzed, 18 tumors (32%) were positive for HP, as tested by histopathological evaluation and immunohistochemistry of the tissue sections. There was no association between HP positivity and mean FAL value (Table 3). HP status was neither significantly associated with LOH at specific markers nor with MSI (Table 3).

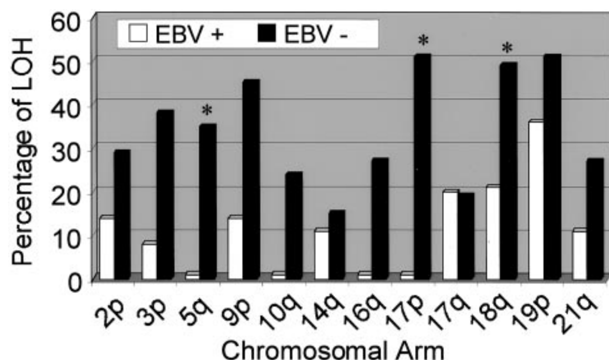

Figure 2. Frequency distribution of allelic loss of EBV-positive and EBV-negative gastric carcinomas at the chromosomal arms tested. **Asterisks** indicate statistically significant differences at specific chromosomal arms.

Table 3. Fractional Allelic Loss and MSI Relative to HP and EBV Status

	FAL	MSI-H	MSI-L
EBV-positive	0.097*	2/15 (13%)	3/15 (20%)
EBV-negative	0.341*	4/42 (10%)	8/42 (19%)
HP-positive	0.295	1/18 (6%)	2/18 (11%)
HP-negative	0.268	5/39 (13%)	9/39 (23%)

*EBV-positive *versus* EBV-negative; $P < 0.001$, Mann-Whitney U-test.

Discussion

In the present study we compared the prevalence of LOH at several tumor suppressor loci and MSI in 57 gastric carcinomas using 21 polymorphic microsatellite markers. The LOH data for the total number of carcinomas were comparable to those reported in other allelotype studies of gastric cancer.[15–17]

The overall prevalence of LOH at the tested loci, as measured by the mean FAL value, was not associated with HP status. In addition HP status was not significantly associated with any specific molecular changes, including MSI, which is in line with several previous reports on HP and molecular alterations.[22,23]

In contrast, a significantly lower FAL value appeared to be associated with the presence of EBV in the carcinoma cells, as tested by *in situ* hybridization for EBER1 nuclear RNA transcripts. The pattern of LOH was also different in the EBV-positive and EBV-negative tumors. These results strongly suggest that EBV-positive gastric carcinomas follow a different pathogenetic pathway, at least on the genetic level, a notion that is in line with recent reports on EBV-positive gastric carcinomas.[12,24,25]

LOH is thought to contribute to tumor suppressor gene inactivation. Methylation is currently regarded as an alternative mechanism for silencing tumor suppressor genes. In a recent publication more CpG islands were found to be methylated in EBV-positive gastric cancers when compared with cancers negative for EBV.[12] It would be conceivable that the lower FAL in the present study among the EBV-positive cancers might be compensated for by a higher frequency of gene inactivation through promotor hypermethylation. Frequent targets of hypermethylation are *p16* on chromosomal arm 9p, *STK11/LKB1* on 19p, and the mismatch repair genes.[26] However, LOH of 9p and 19p or MSI were not significantly different among the EBV-positive and EBV-negative tumors in the current investigation making it somewhat less likely that methylation, as an alternative

Table 4. Results of LOH Analysis: EBV-Positive Carcinomas *Versus* EBV-Negative Carcinomas

		EBV positive (n = 15)		EBV negative (n = 42)		
Chr. Arm	Marker	No. of LOH/informative cases	%LOH	No. of LOH/informative cases	%LOH	*P* value*
2p	D2S123	1/7	14%	7/24	29%	0.641
3p	D3S1478	1/8	13%	11/34	32%	0.402
	D3S2456	0/9	0%	6/25	24%	0.162
	Combined	1/12	8%	14/37	38%	0.075
5q	**D5S346**	**0/12**	**0%**	**11/32**	**34%**	**0.021**
	D5S107	0/12	0%	9/34	26%	0.086
	Combined	**0/15**	**0%**	**14/40**	**35%**	**0.006**
9p	D9S171	1/10	10%	12/29	41%	0.120
	D9S932	2/12	17%	11/29	38%	0.275
	Combined	2/14	14%	17/38	45%	0.055
10q	D10S2491	0/11	0%	8/33	24%	0.165
14q	D14S68	1/9	11%	4/27	15%	0.999
16q	D16S2624	0/9	0%	8/30	27%	0.160
17p	**p53 Alu**	**0/6**	**0%**	**14/24**	**58%**	**0.019**
	TP53	**0/10**	**0%**	**12/27**	**44%**	**0.016**
	Combined	**0/10**	**0%**	**19/37**	**51%**	**0.003**
17q	D17S250	2/10	20%	4/21	19%	0.999
18q	**D18S64**	**1/11**	**9%**	**12/23**	**52%**	**0.024**
	D18S474	2/11	18%	12/31	39%	0.282
	Combined	3/14	21%	19/39	49%	0.115
19p	D19S565	3/7	42%	15/27	56%	0.681
	D19S883	1/9	11%	7/30	23%	0.625
	Combined	4/11	36%	18/35	51%	0.497
21q	D21S49	1/9	11%	7/26	27%	0.647

*All, Fisher's exact test.
Statistical significant differences are highlighted in **bold**.

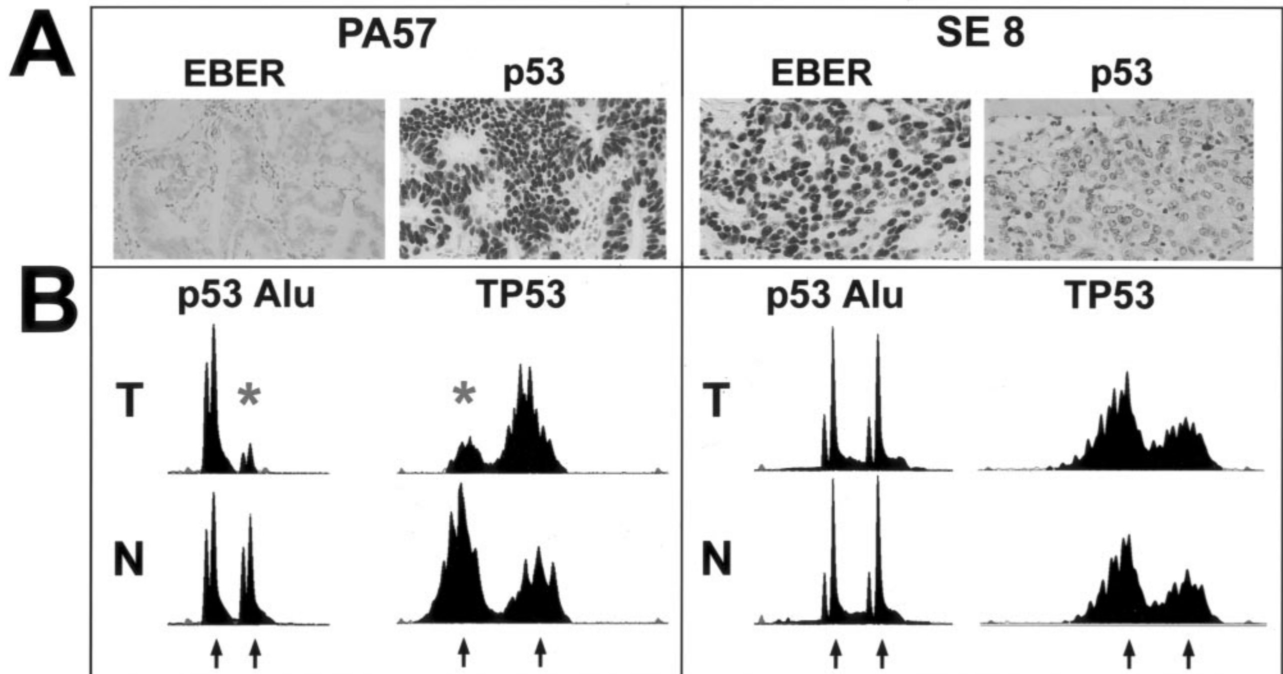

Figure 3. Relation of EBV and p53 alterations, illustrated by two representative tumors (PA57 and SE8). **A:** Epstein-Barr virus was detected by *in situ* hybridization for EBER1 nuclear RNA transcripts. PA57 is negative whereas SE8 shows strong nuclear positivity in the vast majority of tumor cells. Immunohistochemistry for p53 is strongly positive in PA57 but negative in SE8. **B:** Electropherograms of labeled PCR products of paired tumor (T) and normal (N) DNA. Two microsatellite markers at the TP53 locus are shown (p53 Alu and TP53). Both PA57 and SE8 are informative for each marker. The individual alleles are indicated by **arrows**. PA57 (EBV-negative, p53-immunopositive) shows LOH at both markers. The lost alleles are marked by **asterisks**. SE8 (EBV-positive, p53-immunonegative) has retained both alleles in the tumor.

Table 5. EBV and p53 Immunohistochemistry

	p53 immunohistochemistry negative OR weak	p53 immunohistochemistry positive
EBV-positive	14	0
EBV-negative	25	16

$P < 0.01$, Fisher's exact test.

mechanism for tumor suppressor gene inactivation, could explain the current observations.

Also, LOH was measured at a limited number of specific loci, and it may not be legitimate to generalize this finding and to conclude that EBV infection is accompanied by a genome-wide reduction in genetic instability. The microsatellite markers in this study were chosen based on the reported frequency of LOH at their respective loci in gastric cancer in general and EBV-positive carcinomas comprise only a minority (~8 to 10%) of conventional gastric adenocarcinomas. Therefore, these results may reflect the fact that the LOH markers were in some way selected for EBV negativity of the tumors.

When LOH at specific markers was assessed, a strong inverse correlation was seen between EBV positivity and LOH at specific markers at chromosomal arms 5q, 17p, and 18q. Particularly, the inverse relation between EBV and LOH at chromosomal arm 17p suggests a difference with regard to the p53 tumor suppressor pathway. For example, it has been reported that the EBV-encoded EBNA-5 protein (alternatively designed EBNA-LP) can form a molecular complex *in vitro* with both the p53 and retinoblastoma (RB) proteins.[27] It is conceivable that binding with EBNA-5 may lead to an accelerated degradation of either one or both of these tumor suppressor proteins. This would imply a mechanism sharing analogy to that reported for the E6 and E7 proteins of certain human papillomaviruses in the pathogenesis of squamous cell carcinoma of the cervix, resulting in an abrogated tumor suppressor pathway without the need of genetic alteration of the involved gene itself. In line with this, immunohistochemical analysis for p53 protein revealed an inverse correlation of EBV positivity and p53 positivity.

It is more difficult to speculate about the possible mechanisms that are involved in the observed negative association between EBV positivity and LOH at 5q and 18q. Putative targets of LOH on these chromosomal arms may be *APC* on 5q and *DCC, DPC4/SMAD4*, or *JV18* on 18q. However, there is little evidence for interaction of any of these tumor suppressor gene products and EBV.

LOH at chromosomal arm 18q21, the location of the *DPC4/SMAD4* tumor suppressor gene was observed frequently. Inactivation of this gene at the genetic level is strongly correlated to loss of expression of DPC4/SMAD4 protein in pancreatic cancer.[28] We observed loss of expression of DPC4/SMAD4 protein in only 15% of all gastric carcinomas examined and this was not correlated with LOH at 18q21. Furthermore, genetic inactivation of *DPC4/SMAD4* is rare in gastric carcinomas,[29] suggesting that *DPC4/SMAD4* is not the target of LOH at this locus. In view of the above, also hypermethylation as a potential phenomenon that could explain the observed differences in LOH at 5q, 17p, and 18 is unlikely.

In a previous study using comparative genomic hybridization, no association was found between DNA ploidy and the EBV status and also loss of chromosomal arm 17p was not different.[25] These somewhat contradictory results are not easily explained. Differences in study materials and technicalities because of different methodology provide the most likely explanation. In general, allelic loss measured by specific microsatellite markers will be considered more sensitive and provide more accurate results.

The role of EBV in carcinogenesis of the stomach is not completely understood. The latency type of EBV in gastric adenocarcinomas is distinct from the known EBV latency types, eg, in Burkitt's lymphomas and nasopharyngeal carcinomas.[9,10] This is mainly because of the expression of the latent membrane protein 2A (LMP2A) and the absence of LMP1 in gastric adenocarcinomas. The transforming *BARF1* gene is frequently expressed in EBV-positive gastric carcinomas.[10] Sharing homology with the cellular proto-oncogene *c-fms*, *BARF1* may provide an alternative way for the pathogenesis of EBV-associated epithelial cancers, ie, gastric adenocarcinoma and nasopharyngeal carcinoma, independent of LMP-1 expression. In this manner, EBV could provide a surrogate for further accumulation of genetic instability once the cells are infected and this may also explain our findings in EBV-positive tumors.

An alternative explanation for our results could be that susceptibility for EBV infection is determined by a specific molecular genetic route that involves other genetic changes than those reported frequently for conventional gastric carcinomas. This would be in line with our unpublished observations (Zur Hausen and colleagues, submitted) that EBV infection occurs most likely at a relatively late stage of carcinogenesis in the stomach, ie, at the transition of high-grade dysplasia into invasive carcinoma. EBV positivity would then rather be a consequence of the different molecular pathway.

In conclusion, EBV-positive carcinomas should be regarded as a separate entity with a distinct pathogenesis at the molecular level, when compared to EBV-negative carcinomas. Whether EBV positivity is a cause or merely a consequence of this difference remains to be elucidated. Likewise, the exact mechanism of a possible oncogenic role of EBV in gastric epithelial cells needs further study.

Acknowledgments

We thank F. H. Morsink, A. Musler, and M. J. Clement for excellent technical assistance; Dr. A. M. Cleton-Jansen for helpful advice regarding the allelotype analysis; and Dr. P. Sipponen (Jorvi Hospital, Espoo, Finland), Dr. W. Polkowski, Dr. D. Chibowski (Lublin Medical Academy, Lublin, Poland), and Dr. R. H. Hruban (The Johns Hopkins Hospital, Baltimore, MD) for kindly providing patient material.

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