

Comparative Genomic Hybridization of Esophageal Squamous Cell Carcinoma

Correlations between Chromosomal Aberrations and Disease Progression/Prognosis

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BACKGROUND. Esophageal carcinoma is a major cause of cancer-related deaths among males in Taiwan. However, to date, the genetic alterations that accompany this lethal disease are not understood.

METHODS. Chromosomal aberrations of 46 samples of esophageal squamous cell carcinoma (EC-SCC) were analyzed by comparative genomic hybridization (CGH), and their correlations with pathologic staging and prognosis were analyzed statistically.

RESULTS. In total, 321 gains and 252 losses were found in 46 tumor samples; thus, the average gains and losses per patient were 6.98 and 5.47, respectively. Frequent gain abnormalities were found on chromosome arms 1q, 2q, 3q, 5p, 7p, 7q, 8q, 11q, 12p, 12q, 14q, 17q, 20q, and Xq. Frequent deletions were found on chromosome arms 1p, 3p, 4p, 5q, 8p, 9p, 9q, 11q, 13q, 16p, 17p, 18q, 19p, and 19q. It was found that deletions of 4p and 13q12–q14 and gain of 5p were significantly correlated with pathologic staging. Losses of 8p22-pter and 9p also were found more frequently in patients with advanced disease. Gain of 8q24-qter was seen more frequently in patients with Grade 3 tumors. A univariate analysis found that pathologic staging; gains of 5p and 7q; and deletions of 4p, 9p, and 11q were significant prognostic factors. However, pathologic staging became the only significant factor in a multivariate analysis.

CONCLUSIONS. CGH not only revealed novel chromosomal aberrations in EC-SCC, but also found possible genotypic changes associated with disease progression. Despite all of the possible associations of chromosomal aberrations with disease progression, the most important prognostic factor for patients with EC-SCC was pathologic staging. *Cancer* 2001;92:2769–77. © 2001 American Cancer Society.

KEYWORDS: chromosomal aberrations, comparative genomic hybridization, disease progression, esophageal neoplasms, prognosis.

Esophageal carcinoma (EC) is among the ten most frequent malignancies in the world.¹ The incidence rate for EC varies widely, from over 130 per 100,000 population in endemic regions (Linxian, China and Kazakhstan) to around 4 per 100,000 population in Western countries.¹ In Taiwan, the EC mortality rate was ranked sixth among cancer-related deaths of males in the year 2000 (information from the official website of the Department of Health, Taiwan, Republic of China), and esophageal squamous cell carcinoma (EC-SCC) was the major histologic type. Traditionally, patients with EC who undergo surgery or who are treated with radiation alone have had 6–10% 5-year survival rates. More recently, in large randomized trials, the 5-year survival rates increased to 25% in patients with localized disease who received combined-modality therapy. Despite this im-

provement in prognosis, most patients, even those with clinically localized disease, will have a recurrence within the first few years of treatment.¹

Conventional cytogenetic analysis of EC-SCC was reported in several early studies.²⁻⁵ Structural changes were found frequently in chromosomes 1, 2, 3, 6, 7, 9, 11, and 12. The most frequent break points were on chromosome 3p14, 6q15, 6q23, 7p22, 7q22, 9p11, 9q12, 11p11.2, 11p14, and 11q12.²⁻⁵ Loss of heterozygosity also was used for the study of genetic changes in EC-SCC, and studies have indicated that potential tumor suppressor genes, such as *p53* (17p13),^{6,7} *RBI* (13q14),^{6,7} *APC* (5q21),^{6,7} *MCC* (5q21),^{6,7} *IRF-1* (5q31.1),⁸ *MTS1/CDKN2A* (9p21-22),^{9,10} *TOC* (17q25),¹¹ and *DCC* (18q23),⁶ may be involved in the pathogenesis of EC-SCC. Other potential tumor suppressor genes have been located on 2q, 3p, 3q, 4p, 4q, 6q, 8p, 9q, 10p, 14q, 15q, 19q, and 21q.¹²⁻¹⁴

Comparative genomic hybridization (CGH) is a powerful method that can survey the entire genome of tumor cells to detect DNA copy number changes. This method not only can reveal novel chromosomal aberrations in tumor cell that were not detected by conventional cytogenetics¹⁵⁻¹⁷ but also can provide a comprehensive understanding of possible phenotype and genotype correlation in tumor progression.¹⁸ In this study, we applied CGH to investigate the chromosomal aberrations of primary EC-SCC tumor samples from 46 ethnic Chinese patients in Taiwan and statistically analyzed the possible association of these chromosomal alterations with disease progression and prognosis.

MATERIALS AND METHODS

Patients and Samples

From 1995 to 1997, 46 ethnic Chinese patients with EC-SCC were enrolled. Informed consent was obtained from all patients. All but four patients underwent transthoracic esophagectomy, laparotomy, two-field (mediastinum and intraabdomen, which included the celiac and perigastric areas) lymph node (LN) dissection, and left side neck LN sampling. Four patients underwent transhiatal esophagectomy and intraabdominal LN dissection. The mean number of dissected LNs was 24.5 (range, 12-56 LNs). Tumor and LN samples were fixed in 4% buffered formalin, embedded in paraffin, and routinely stained for histologic diagnosis. Tumor stage and grade were defined according to the Cancer Staging Manual (5th edition; American Joint Committee on Cancer [AJCC]). Distant LN (neck or celiac trunks) metastasis was considered as metastatic disease. No preoperative chemotherapy or radiotherapy was given. Patients with T4 lesions or

LN metastasis received postoperative concurrent chemotherapy (5-fluorouracil [5-FU] 500 mg/m² and cisplatin 20 mg/m² given weekly during radiation) and radiotherapy (total dose, 4500-6000 centigrays). Three patients (Patients 4, 19, and 34) died shortly after surgery due to surgical complications. The remaining patients were followed as outpatients and received chest X-ray, liver sonography, and whole body bone scan examination every 3 months. Computed tomography scans of the chest and upper gastrointestinal endoscopy were done if local recurrence was suspected. Patients who survived for > 2 years received the above-mentioned follow-up examinations every 6 months, and patients who survived for > 5 years received yearly check-ups thereafter.

Thirty-four patients experienced recurrent disease, and most received only palliative radiotherapy for local recurrent disease. Five patients with distant recurrences received 5-FU and cisplatin-based chemotherapy. The median follow-up was 15.5 months, and 9 patients were still alive at last follow-up. The clinicopathologic data are summarized in Table 1.

DNA Extraction

The procedure of DNA extraction from formalin-fixed, paraffin-embedded samples was modified from a previously described procedure.¹⁹ Briefly, portions of tumor from the specimens were identified under the microscope by pathologist (J.-T.C.) and were cut into small pieces to include as little paraffin as possible, then incubated in xylene at 45 °C for 1 hour, and washed twice with 70% ethanol. After complete drying in air, 1 mL of lysis buffer (100 mM Tris-HCl, pH 7.8; 5 mM ethylenediamine tetraacetic acid; 0.2% sodium dodecyl sulfate; and 200 mM NaCl) supplemented with proteinase K at a final concentration of 0.3 mg/mL was added and incubated at 55 °C for 72 hours; additional proteinase K (10 μL of 20 mg/mL stock solution) was added at 24 hours and 48 hours. DNA was then extracted by using the standard phenol-chloroform-isoamyl alcohol method. DNA samples were quantified using a DyNA Quant 200 fluorometer (Hoefer, Pharmacia Biotech, San Francisco, CA).

CGH

CGH analyses were performed essentially according to previously described methods.^{15,20} Genomic DNA extracted from tumor tissues was labeled with Spectrum Green-dUTP (Vysis, Downers Grove, IL) by nick translation, and normal reference DNA prepared from peripheral lymphocytes of normal donors was labeled with Spectrum Red-dUTP. The labeled DNA (200 ng) was then mixed with 10 μg of unlabeled human Cot-1 DNA (Life Technologies, Gaithersburg, MD), then eth-

TABLE 1
Clinicopathologic Characteristics of Tumors

Patient	Age (yrs)	Gender	TNM	Stage	Grade	Gain	Loss
1	69	M	T3N0M0	IIA	G3	9	0
2	50	M	T3N1M0	III	G2	7	3
3	63	M	T3N0M0	IIA	G2	3	1
4	58	M	T3N0M0	IIA	G2	3	0
5	72	M	T3N0M0	IIA	G2	5	2
6	52	M	T2N0M0	IIA	G2	5	7
7	60	M	T4N0M0	III	G2	16	7
8	69	M	T3N1M0	III	G2	11	3
9	75	M	T3N1M0	III	G2	9	2
10	76	M	T3N0M1	IV	G2	12	1
11	71	M	T2N0M0	IIA	G2	1	0
12	68	M	T1N0M0	I	G2	13	2
13	63	M	T2N0M1	IV	G3	8	6
14	50	M	T4N1M0	III	G2	4	3
15	52	M	T3N1M0	III	G2	7	2
16	50	M	T3N0M0	IIA	G2	9	7
17	75	M	T3N0M0	IIA	G2	14	8
18	61	M	T3N1M0	III	G2	9	0
19	69	M	T3N0M0	IIA	G2	9	3
20	60	M	T4N0M0	III	G2	17	14
21	62	M	T3N1M0	III	G3	8	5
22	76	M	T3N1M0	III	G2	0	0
23	70	M	T3N0M1	IV	G2	7	9
24	71	F	T3N1M0	III	G3	9	10
25	52	M	T3N1M0	III	G2	8	10
26	58	M	T3N1M0	III	G2	3	8
27	37	M	T4N1M1	IV	G2	5	8
28	51	M	T3N1M0	III	G2	0	0
29	63	M	T3N0M0	IIA	G3	4	9
30	51	M	T2N1M0	IIB	G2	3	6
31	65	M	T3N1M0	III	G3	9	2
32	48	M	T3N0M0	IIA	G2	10	10
33	41	M	T3N1M1	IV	G3	13	13
34	63	M	T4N1M0	III	G2	11	9
35	58	M	T3N0M0	IIA	G2	9	8
36	45	M	T3N1M0	III	G2	10	18
37	63	M	T1N0M0	I	G2	1	1
38	39	M	T3N0M0	IIA	G2	6	11
39	68	M	T4N0M0	III	G2	5	8
40	73	M	T3N0M0	IIA	G2	3	3
41	68	M	T3N1M1	IV	G2	4	2
42	62	M	T1N0M0	I	G2	2	0
43	74	M	T2N1M0	IIB	G3	8	11
44	59	M	T4N0M1	IV	G2	3	7
45	65	M	T2N0M0	IIA	G3	2	4
46	59	M	T4N1M0	III	G2	7	9

M: male; F: female; Stage: American Joint Committee on Cancer staging; G3: poorly differentiated; G2: moderately differentiated; Gain: total number of gains; Loss: total number of losses.

anol precipitated before being redissolved in 10 μ L hybridization solution (70% formamide, 2 \times standard saline citrate, and 10% dextran sulfate). After denaturation, the probe mixture was dropped onto the metaphase spread, covered with a coverslip, sealed with rubber cement, and hybridized in a moist chamber at 37 $^{\circ}$ C for 3 days. After posthybridization washes in 50% formamide with 2 \times standard saline citrate and phos-

phate buffer at 43 $^{\circ}$ C, the slides were counterstained with diamidino-2-phenylindole and mounted in Vectashield mounting medium (Vector Laboratories, Burlingame, CA). The hybridized chromosomes were observed under a fluorescence microscope (Zeiss Axioskop, Oberkochen, Germany). Typically, 6–10 sets of metaphase chromosomes from one sample slide were captured and analyzed using the QUIPS XL genetics

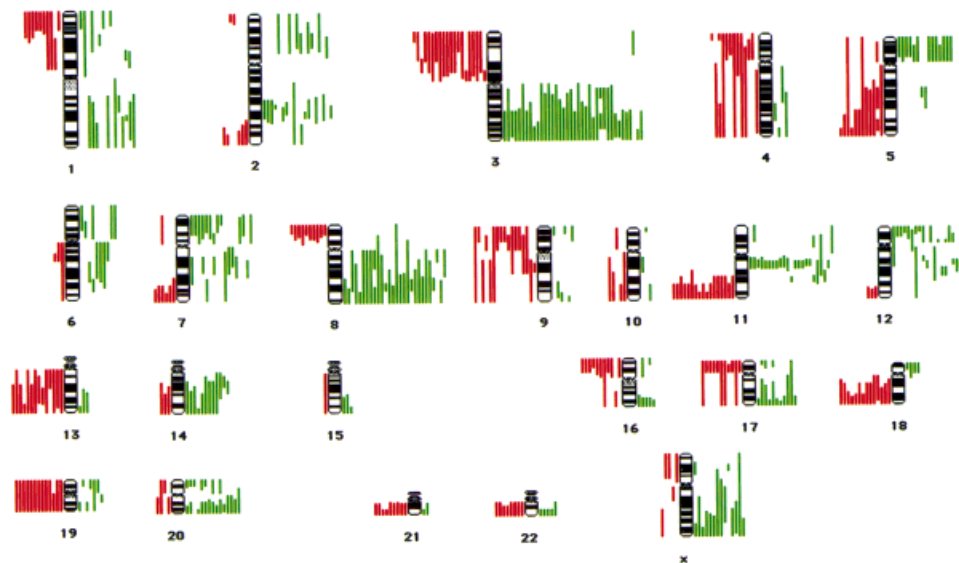


FIGURE 1. Summary of copy number alterations of 46 esophageal squamous cell carcinomas analyzed by comparative genomic hybridization. Chromosomal regions of gain are represented in green on the right side of the chromosome ideograms, and regions of loss are represented in red on the left side.

workstation system (Vysis). Gain or loss abnormalities were defined by setting the thresholds at 1.2 and 0.8, respectively.²⁰

Statistical Analysis

Chi-square tests or Fisher exact tests were used to evaluate the correlations between chromosomal aberrations and pathologic staging or tumor cell differentiation. Univariate survival analysis was done by using the Kaplan–Meier method with log-rank tests. A Cox regression analysis was applied to test for independent prognostic significance. A *P* value < 0.05 was considered statistically significant, and a *P* value between 0.1 and 0.05 was considered of borderline significance.

RESULTS

CGH Analysis

Chromosomal aberrations were found in all but two samples. The pooled CGH results are shown in Figure 1. There were 321 gains and 252 losses in total found in 46 primary EC-SCC samples; thus, the average gains and losses per patient were 6.98 and 5.47, respectively. Frequent ($\geq 20\%$ of samples) gain abnormalities occurred on chromosome arms 1q (24%), 2q (28%), 3q (80%), 5p (28%), 7p (26%), 7q (20%), 8q (61%), 11q (48%), 12p (33%), 12q (22%), 14q (26%), 17q (24%), 20q (30%), and Xq (28%). The minimal overlapping regions were at 1q32–q42, 2q31–32, 3q26–qter, 5p13–p14, 7p14–pter, 8q24–qter, 11q12.3–q13, 12p12, 14q23–qter, 17q24–qter, 20q13.1–qter, and Xq27–q28. Frequent losses were found on 1p (20%), 3p (44%), 4p (24%), 5q (26%), 8p (24%), 9p (28%), 9q (20%), 11q (37%), 13q (30%), 16p (22%), 17p (24%), 18q (30%), 19p (26%),

TABLE 2
Correlation of Chromosomal Aberrations with Pathologic Stage

Chromosomal change	Stage I and IIA (%)	Stage IIB and III (%)	Stage IV (%)	<i>P</i> value
Loss of 4p	2 of 18 (11)	4 of 21 (19)	5 of 7 (71)	0.005
Gain of 5p	1 of 18 (6)	8 of 21 (38)	4 of 7 (57)	0.015
Loss of 8p22–pter	2 of 18 (11)	5 of 21 (24)	4 of 7 (57)	0.053
Loss of 9p	2 of 18 (11)	7 of 21 (33)	4 of 7 (57)	0.056
Loss of 13q12–q14	2 of 18 (11)	3 of 21 (14)	4 of 7 (57)	0.024

and 19q (28%). The minimal overlapping regions were at 1p34.1–pter, 3p21.32–pter, 4p14–p15.3, 5q32–qter, 8p22–pter, 9p21, 9q21, 11q23–qter, 13q12–q14, 16p13.1–pter, 17cen–p12, 18q22–qter, 19p, and 19q.

Correlation of Chromosomal Aberrations with Pathologic Staging

We divided all patients into three pathologic staging groups: Group 1, with T1–T3 disease and no LN or distant metastasis (Stage I and IIA); Group 2, with regional LN metastasis or T4 disease with no distant metastasis (Stage IIB and III); and Group 3, with distant LN metastasis (Stage IV). Correlations between pathologic staging and chromosomal aberrations of primary EC-SCC tumors are summarized in Table 2. Losses of 4p and 13q12–q14 and gain of 5p all were correlated significantly with pathologic staging. Deletions of 8p22–pter and 9p were found more commonly in patients with advanced-stage disease; however, the statistical difference fell into borderline significance.

The search for a correlation between tumor differentiation and chromosomal aberration revealed

TABLE 3
Univariate and Multivariate Analyses of Prognostic Influence of Pathologic Staging and Chromosomal Aberrations on Overall Survival

Pathologic staging/chromosomal aberrations	Univariate analysis (P value)	Multivariate analysis	
		Relative risk (95% confidence interval)	P value
Pathologic staging	0.0004	2.2624 (1.2003–4.2643)	0.0116
Gain of 5p	0.0301	1.1340 (0.4809–2.6743)	0.7739
Gain of 7q	0.0108	1.2512 (0.5083–3.0798)	0.6259
Loss of 4p	0.0014	1.2281 (0.4722–3.1937)	0.6736
Loss of 9p	0.0007	1.6307 (0.6262–4.2464)	0.3166
Loss of 11q	0.0128	1.4727 (0.6367–3.4066)	0.3656

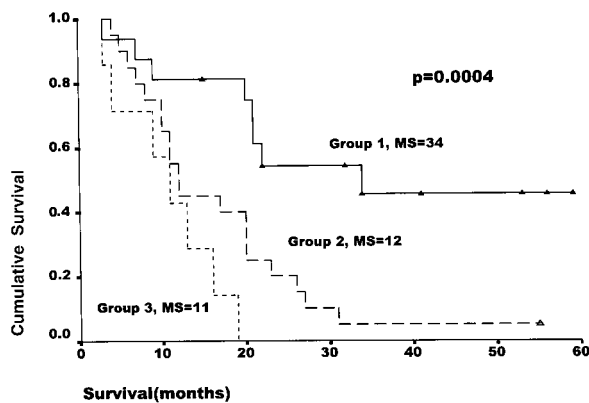


FIGURE 2. Kaplan–Meier analysis of cumulative survival (Cum Survival) among patients with esophageal squamous cell carcinoma in three different pathologic groups: Group 1, patients with Stage I and Stage IIA disease; Group 2, patients with Stage IIB and Stage III disease; Group 3, patients with Stage IV disease. MS: median survival.

that the frequency of DNA over-representation of 8q24-qter was significantly greater in patients with poorly differentiated tumors (7 of 9 patients; 78%) compared with that in patients with moderately differentiated tumors (14 of 37 patients; 38%; $P = 0.037$).

Prognostic Influence of Chromosomal Aberrations

Three patients died shortly after undergoing surgery due to surgical complications, and they were excluded from the survival analysis. The results of univariate and multivariate survival analyses are summarized in Table 3. The univariate analysis revealed that pathologic staging; gains of 5p and 7q; and deletions of 4p, 9p, and 11q were significant prognostic factors. In the multivariate analysis, pathologic staging was the only significant factor (Table 3, Fig. 2).

DISCUSSION

This is the first CGH analysis of primary EC-SCC in ethnic Chinese patients. Compared with previous re-

ports (Table 4), we found that gains of 3q, 5p, 8q, and 11q and losses of 3p, 5q, 11q, and 18q were common in the CGH study of patients with EC-SCC.^{21–25} Gains of 3q and 11q and deletions of 3p also were found easily in patients with squamous cell carcinoma (SCC) arising from other organs^{26–30} as well as in patients with nasopharyngeal carcinoma.³¹ These regions may harbor genes that can be altered in early-stage disease. Possible candidate genes included *PI3K* on 3q,²⁷ *FHIT* on 3p,¹⁷ and *BCL1* on 11q13.¹⁶

However, different chromosomal abnormalities have been reported by different authors. DNA copy number increases of 2p, 12p, 14q, 20p, and 20q and decreases of 9p and 13q were reported commonly in Asian studies but were not found uniformly in studies from South Africa or the Netherlands. Conversely, gains of 1q, 2q, and 7p and losses of 1p, 4p, 19p, and 19q were reported by our group as well as by Du Plessis et al.²⁴ from South Africa but were not seen in Japanese studies. Frequent deletion of 10p was seen only in the study from the Netherlands. Pack et al.²³ from the United States reported a more complex chromosomal aberration pattern in patients with EC-SCC compared with the patterns reported in other studies (Table 4).

One possible explanation for such differences is that there may be some ethnic or geographic factors that contribute to the various patterns of chromosomal change in patients with EC-SCC from different reports. For example, gain of 1q and loss of 19p in patients with EC-SCC were reported only by our group and by Du Plessis et al. from South Africa.²⁴ Both chromosome regions harbor genes related to the prostaglandin system,^{32,33} which may play a role in the pathogenesis of EC-SCC.^{32,34} Conversely, the betel nut, a common carcinogen found in both Taiwan and South Africa, has been identified recently as a possible factor in esophageal carcinogenesis,³⁵ and the carcinogenic effect of betel nuts has been related to the

TABLE 4
Comparison of Chromosomal Aberrations with Previous Comparative Genomic Hybridization Studies of Patients with Esophageal Squamous Cell Carcinoma

Aberration	Yen et al. (Taiwan) (n = 46 patients)	Shinomiya et al. (Japan) ²¹ (n = 29 patients)	Tada et al. (Japan) ²² (n = 36 patients)	Pack et al. (USA) ²³ (n = 17 patients)	Du Plessis et al. (South Africa) ²⁴ (n = 29 patients)	Weiss et al. (Netherlands) ²⁵ (n = 14 patients)
Gain (%)						
1q	24	—	—	—	41	64
2p	17	—	19	—	—	—
2q	28	—	—	—	52	—
3q	80	45	75	50	72	86
5p	28	38	25	—	31	29
5q	4	—	—	47	—	—
7p	26	—	—	—	48	—
7q	20	—	22	—	45	—
8q	61	41	50	53	55	71
9q	7	—	—	53	—	—
11q	48	28	44	70	—	79
12p	33	—	17	—	—	—
12q	22	—	—	59	—	—
14q	26	31	—	—	—	—
16p	4	—	—	65	—	—
17p	9	—	—	59	—	—
17q	24	—	—	59	—	—
19p	9	—	—	100	—	—
19q	9	—	—	100	—	—
20p	15	—	17	—	—	—
20q	30	28	25	94	—	—
22q	13	—	—	94	—	—
Xq	28	38	—	—	69	—
Loss (%)						
1p	20	—	—	—	52	—
1q	0	—	—	53	—	—
2q	4	—	—	100	21	—
3p	44	—	50	100	38	79
4p	24	—	—	82	52	—
4q	17	—	33	82	—	57
5q	26	—	39	82	28	—
6q	7	—	—	70	—	—
8p	24	—	—	—	—	—
9p	28	17.2	44	76	—	—
9q	20	—	—	—	—	—
10p	2	—	—	—	—	43
11q	37	—	19	59	—	71
12q	2	—	—	70	—	—
13q	30	—	22	100	—	—
14q	7	—	—	65	—	—
16p	22	—	—	—	—	—
17p	24	—	—	—	—	—
18q	30	—	58	76	48	—
19p	26	—	—	—	52	—
19q	28	—	—	—	55	—
22q	17	—	—	—	41	—
Xq	4	—	—	94	—	—

prostaglandin system.³⁶ Thus, the possible association between betel nuts and aberrations of chromosomal regions related to the prostaglandin system deserves further studies.

Another explanation is the *mutator phenotype*

theory.³⁷ Tumor cells initially gain their phenotype as a result of mutations in genes that function in the maintenance of genomic stability. The genetic instability of tumor cells then increases with tumor progression, which allows a rapid accumulation of errors

that favor their survival. Therefore, the different chromosomal changes in EC-SCC seen in different reports may be the results of repetitive rounds of selective expansion from different clones of tumor cells.³⁷

An association of chromosomal aberrations with disease progression in patients with EC-SCC had been reported.^{13,22,38-41} Chromosomal regions that have been linked to LN metastasis include deletions of 3p25,³⁹ 6q21,⁴⁰ 9q22.3-q31,⁴⁰ 11q22-qter,²² 13q12-13,³⁸ 18q23.3,⁴¹ and 19q¹³ and gains of 8q23-qter and 20q.²² Allelic loss of some chromosomal regions also has been related to tumor grading.^{40,41}

In this study, it was found that deletions of 4p and 13q12-q14 and gain of 5p were correlated significantly with pathologic staging. In addition, there was a trend toward an association between advanced disease and deletions of 8p22-pter and 9p. Gain of 5p and deletion of 9p were seen commonly in CGH studies of patients with EC-SCC,²¹⁻²⁵ and both have been linked to disease progression of in patients with SCC of the uterine cervix.^{28,29} Deletion of 9p also was related with metastasis in patients with other solid tumor types.⁴²⁻⁴⁴ *MST1/CDKN2A* on 9p21 was identified as an important tumor suppressor gene in patients with different tumor types^{10,45} and has been associated with metastatic and invasive phenotypes of EC-SCC.¹⁰ An association of 5p gain and disease progression in patients with EC-SCC has never been reported, and potential candidate genes in the region include *cyclin A*¹⁶ and a prostaglandin receptor (*PTGER2*).³²

An association of 13q12-14 deletion with LN metastasis was found in a previous report.³⁸ Possible candidate genes in this region include *BRCA2*, *RBI*, and *LATS2*.¹⁷ Deletion of 8p22-pter has not been reported in patients with EC-SCC²¹⁻²⁵ but has been found in patients with SCC of the lung.⁴⁶ Mutation of the *FEZ1* gene at 8p22 has been found in patients with EC-SCC,⁴⁷ and mutations of two other genes in this regions, tumor necrosis factor-related apoptosis-inducing ligand receptor 1 (TRAIL-R1) and receptor 2 (TRAIL-R2), have been found in patients with metastatic breast carcinoma.⁴⁸ There are few reports regarding deletion of 4p in association with EC-SCC.^{14,23,24} *ACTN4* is a recently found gene on 4p that encodes a nonmuscle α -actinin, which suppresses tumorigenicity of human neuroblastoma cells,⁴⁹ but its role in EC-SCC or other solid tumors is not clear. Gain of 8q24-qter was found more easily in patients with high-grade tumors, and the possible oncogene is *c-myc*.⁵⁰ Thus, the current results not only confirm the results from some previous reports but also point out several new chromosomal regions that may harbor genes involved in the disease progression of EC-SCC.

An association between chromosomal changes of tumor cells and patient survival has been reported in patients with EC-SCC¹² or with other types of SCC.^{51,52} In the current study, we found that pathologic staging; gains of 5p and 7q; and losses of 4p, 9p, and 11q were poor prognostic factors in univariate analysis (Table 3) and that deletions of 4p and 9p and gain of 5p were associated with advanced-stage disease. Deletion of 11q has been associated with LN metastasis in patients with EC-SCC.²² One potential protooncogene on 7q21 is *HGF*, which encodes the hepatocyte growth factor and has been associated with tumor cell differentiation in EC-SCC.⁵³ In the multivariate analysis, however, only pathologic staging retained a prognostic influence (Table 3).

In conclusion, CGH not only revealed many novel chromosomal aberrations in EC-SCC but also identified possible genotypic changes in tumor progression. Despite all of the possible associations of chromosomal aberrations with disease progression, the most important prognostic factor for patients with EC-SCC was pathologic staging. Various patterns of chromosomal change in patients with EC-SCC from different areas of the world may be explained either by the contribution of possible environmental factors, by ethnic factors, or by the *mutator phenotype* theory.

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