Genetic pathways of multiple esophageal squamous cell carcinomas

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Abstract. Whether multiple esophageal squamous cell carcinomas (SCCs) in a patient develop through an identical genetic pathway is still unclear. We examined multiple esophageal SCCs for alterations of p53, p16, IRF and mitochondrial DNA (mtDNA) and microsatellite instability (MSI). Thirty patients with multiple superficial esophageal SCCs, 23 with double lesions and 7 with triple lesions, were enrolled. Loss of heterozygosity (LOH) of p53 (TP53), p16 (D9S171), IRF (IRF) and other microsatellite loci including D1S191, D17S858, D18S58 and D18S61 of the tumors was examined by microsatellite assay. Mutations of p16 and mtDNA were examined with PCR single-strand conformation polymorphism (SSCP) analysis. LOH of p53, p16 and IRF were detected in 16 of 50 (32%), 5 of 38 (13%) and 5 of 48 (10%) tumors, respectively. Mutations of p16 were detected in 4 of 67 (6%) tumors. Six of 67 (9%) tumors had mtDNA alterations and none of the tumors showed high-frequency MSI. All 30 patients showed one or more gene alterations in one or more genetic loci. Discordant genetic patterns among individual lesions within a patient were observed in 28 of the 30 (93%) patients. The most discordant locus was TP53, present in 11 of 29 (38%) informative cases, followed by D18S61, present in 11 of 30 (37%) informative cases. These results suggest that the genetic pathways of multiple esophageal SCCs may differ even within the same patient.

Introduction

Multiple esophageal squamous cell carcinomas (SCCs) are observed in 20-30% of patients with esophageal SCC (1,2).

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We previously reported that the mean annual incidence of newly diagnosed tumors was 4.4% in patients with esophageal SCC (1). Interestingly, patients with synchronous esophageal SCCs were reported to have a significantly worse prognosis than those with metachronous esophageal SCCs because patients with metachronous SCCs have a higher rate of earlystage SCC than do those with synchronous SCCs (3). The number of patients with esophageal SCCs has increased because both the Lugol spraying method and narrow-band imaging facilitate the early detection of this carcinoma (1).

There are two theories regarding multiple carcinogenesis in an organ. One is the multicentric carcinogenesis theory, which postulates that cells from a single neoplastic cell may be developed in multiple cites independently (4). The other is the field carcinogenesis theory, which postulates that an area of tissue simultaneously becomes genetically unstable and is predisposed to neoplasia due to prolonged exposure to carcinogens, resulting in multiple tumors (5). Esophageal SCC is regarded as a representative example of field carcinogenesis (6). According to the theory, the entire esophageal epithelial surface or 'field' is exposed to repeated carcinogenic insults, and multiple epithelial tumors can arise from multifocal precancerous lesions, which may develop and progress at different rates. Whether multiple esophageal SCCs within a patient develop through an identical genetic pathway is of great interest. Although multiple genetic alterations, such as mutations of p53, p16, interferon-regulatory factor (IRF) and mitochondrial DNA (mtDNA), and microsatellite instability (MSI) are reported to be involved in the development of esophageal SCC (7-9), little information is available regarding the molecular alterations of multiple esophageal SCCs. Therefore, we examined multiple esophageal SCCs for alterations of p53, p16, IRF and mtDNA, and MSI to evaluate this issue.

Patients and methods

Patients. Thirty Japanese patients with multiple superficial esophageal SCCs, 23 with double cancers and 7 with triple cancers, who had been treated endoscopically at Hiroshima University Hospital from November 1994 to September 2006 without radiation or chemotherapy prior to the treatment, were enrolled in this study. Superficial esophageal SCC is

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Key words: multiple esophageal squamous cell carcinomas, *p53*, *p16*, *IRF*, mitochondrial DNA

Table I. Characteristics of patients with multiple esophageal SCCs.

1-A 75 M Li 0-lic 9 EP 1-B 78 M Li 0-lic 20 EP 2-A 68 M Mt 0-lic 10 EP 2-B 68 M Mt 0-lic 10 EP 3-A 64 M Li 0-lic 10 EP 3-A 64 M Li 0-lic 15 EP 3-A 64 M Mt 0-lic 10 EP 4-A 68 M Mt 0-lic 10 EP 5-A 63 M Mt 0-lic 10 EP 5-B 63 M Mt 0-lic 10 EP 6-A 76 M Mt 0-lic 20 EP 7-A 67 M Mt 0-lic 30 SMI 7-B 67 M Lt <t< th=""><th></th><th>Age (years)</th><th>Gender</th><th>Location^a</th><th>Morphologic type^b</th><th>Size (mm)</th><th>Depth^c</th></t<>		Age (years)	Gender	Location ^a	Morphologic type ^b	Size (mm)	Depth ^c
1.B. 78 M Li 0-lic 20 EP 2-A 68 M Mt 0-lic 10 EP 2-B 68 M Mt 0-lic 10 EP 3-A 64 M Mt 0-lic 10 EP 3-B 64 M Li 0-lic 10 EP 4-A 68 M Mt 0-lic 15 EP 5-A 63 M Li 0-lic 10 EP 5-B 63 M Mt 0-lic 15 EP 6-A 76 M Mt 0-lic 25 LPM 6-B 76 M Mt 0-lic 20 EP 7-A 67 M Mt 0-lic 20 MM 8-B 76 M Li 0-lic 30 MM 9-B 70 M Li 0-lic 30 SMI 10-B 74 M Cc 0-lic	1-A	75	М	Lt	0-IIc	9	EP
2.A 68 M Mt 0-IIc 8 FP 2.B 68 M Mt 0-IIc 10 FP 3.A 64 M L1 0-IIc 10 FP 3.B 64 M L1 0-IIc 10 FP 4.B 69 M L1 0-IIc 15 EP 5.A 63 M MI 0-IIc 10 EP 6.A 76 M MI 0-IIc 10 EP 6.A 76 M MI 0-IIc 10 EP 6.C 76 M MI 0-IIc 20 EP 7.A 67 M MI 0-IIc 20 EP 8.A 72 M L1 0-IIc 30 MM 9.A 67 M L1 0-IIc 30 SMI 0.A 67 M L1 0-IIc 30 SMI 0.B 70 M L1 0-IIc	1-B	78	М	Lt	0-IIc	20	EP
2.B68MMt0Ic10EP3-A64MLt0IIc10EP3-B64MLt0IIc10EP4-A68MMt0IIc15EP5-A63MLt0IIc15EP5-B63MLt0IIc10EP6-A76MMt0IIc15EP7-A67MMt0IIc25LPM6-C76MMt0IIc25LPM7-A67MMt0IIc20EP7-A67MLt0IIc30MM9-B70MLt0IIc30MM9-B70MLt0IIc30SMI9-B70MLt0IIc30SMI10-B74MCc0IIc50EP11-A50MLt0IIc15LPM12-A55MLt0IIc10LPM12-A56MLt0IIc10LPM13-A56MLt0IIc10LPM15-A75MLt0IIc10LPM15-A76MUt0IIc12LPM15-A56MLt0IIc12LPM15-A76M <t< td=""><td>2-A</td><td>68</td><td>Μ</td><td>Mt</td><td>0-IIc</td><td>8</td><td>EP</td></t<>	2-A	68	Μ	Mt	0-IIc	8	EP
3.A64MMt0-Ic25EP3-B64MLt0-Ic10EP4-A68MMt0-Ic45EP4-B69MLt0-Ic15EP5-A63MLt0-Ic10EP5-B63MLt0-Ic10EP6-A76MMt0-Ic15EP6-C76MMt0-Ic25LPM7-A67MMt0-Ic20EP8.A72MLt0-Ic30MM9-A69MLt0-Ic30MM9-A69MLt0-Ic30SM110-B74MCc0-Ic30SM110-B74MCc0-Ic15LPM11-A50MLt0-Ic15LPM12-B56MLt0-Ic15LPM13-B57MLt0-Ic10EP12-C56MLt0-Ic12LPM13-B74MUt0-Ic25EP12-C56MLt0-Ic15LPM13-B79MLt0-Ic15LPM13-B79MLt0-Ic15LPM15-A61MUt0-Ic15EP <td>2-B</td> <td>68</td> <td>Μ</td> <td>Mt</td> <td>0-IIc</td> <td>10</td> <td>EP</td>	2-B	68	Μ	Mt	0-IIc	10	EP
3-B64MLt0-Ic10EP4-A68MMt0-Ic45EP4-B69MLt0-Ic5EP5-A63MMt0-Ic10EP6-A76MMt0-Ic60LPM6-B76MMt0-Ic5EP7-A67MMt0-Ic25LPM7-B67MMt0-Ic25LPM7-B76MLt0-Ic30MM9-A69MLt0-Ic30MM9-B76MLt0-Ic30SMI10-B76MLt0-Ic30SMI10-B76MLt0-Ic30SMI10-B76MLt0-Ic30SMI11-B51MLt0-Ic15LPM11-A55MLt0-Ic15LPM12-B56MLt0-Ic10EP12-A57MUt0-Ic10LPM13-B57MUt0-Ic10LPM15-A57MUt0-Ic12LPM15-A57MUt0-Ic15MM15-B61MUt0-Ic12LPM15-A57MUt0-Ic15MM<	3-A	64	М	Mt	0-IIc	25	EP
4-A66MMt0-Ic45EP4-B69MLt0-Ic15EP5-A63MI.r0-Ic15EP5-B63MI.r0-Ic15EP6-A76MMt0-Ic15EP6-B76MMt0-Ic25UPM6-B76MMt0-Ic20EP6-C76MMt0-Ic20EP8-A72ML10-Ic30MM9-B76ML10-Ic10LPM9-B70ML10-Ic10EP9-B70ML10-Ic30SMI10-A67ML10-Ic30SMI10-B74MCc0-Ic30EP11-A50ML10-Ic15LPM11-B51ML10-Ic15LPM12-C56ML10-Ic15LPM13-A56ML10-Ic10LPM13-B57ML10-Ic10LPM13-B57ML10-Ic15LPM13-A56ML10-Ic15LPM13-B61MUt0-Ic25EP12-C56ML10-Ic15LP	3-B	64	М	Lt	0-IIc	10	EP
4-B69MLt0-llc5EP5-A63MMt0-llc15EP5-B63MMt0-llc10EP6-A76MMt0-llc15EP6-C76MMt0-llc25LPM6-R77MMt0-llc20EP7.A67MMt0-llc20EP8.A72MLt0-llc60MM8.B76MLt0-llc10LPM9.A69MLt0-llc30MM9.A69MLt0-llc30SMI10.B74MCc0-llc50EP11.A50MLt0-llc15LPM11.B51MLt0-llc10EP12.A55MLt0-llc10EP12.A56MUt0-llc10LPM13.A57MUt0-llc10LPM14.A78MUt0-llc15LPM15.B61MUt0-llc15LPM15.A57MUt0-llc15LPM15.A57MUt0-llc15LPM15.A57MUt0-llc15LPM15.B61MUt0-llc<	4-A	68	М	Mt	0-IIc	45	EP
5.A 63 M Mt 0-lic 15 EP $5.B$ 63 M L1 0-lic 10 EP $6.A$ 76 M Mt 0-lic 60 LPM $6.B$ 76 M Mt 0-lic 25 EP $7.A$ 67 M Mt 0-lic 25 LPM $7.B$ 67 M Mt 0-lic 20 EP $8.A$ 72 M L1 0-lic 30 MM $9.B$ 70 M L1 0-lic 30 MM $9.B$ 70 M L1 0-lic 30 SM $10.A$ 67 M L1 0-lic 15 LPM $11.A$ 50 M L1 0-lic 25 LPM $12.A$ 55 M L1 0-lic 10 EP $12.A$ 55 M L1 0-lic 10 LPM $12.A$ 57 <td>4-B</td> <td>69</td> <td>М</td> <td>Lt</td> <td>0-IIc</td> <td>5</td> <td>EP</td>	4-B	69	М	Lt	0-IIc	5	EP
S-B 63 M Lt 0-Hc 10 EP 6-A 76 M Mt 0-Hc 15 EP 6-C 76 M Mt 0-Hc 5 EP 7-A 67 M Mt 0-Hc 20 EP 7-B 67 M Mt 0-Hc 20 EP 8-A 72 M Lt 0-Hc 20 EP 8-A 72 M Lt 0-Hc 60 MM 8-B 76 M Lt 0-Hc 10 LPM 9-A 69 M Lt 0-Hc 10 EP 10-A 67 M Lt 0-Hc 50 EP 10-B 74 M Cc 0-Hc 50 EP 12-A 55 M Lt 0-Hc 10 EP 12-A 55 M Lt 0-Hc 10 EP 12-A 55 M Lt 0-Hc 10 <td>5-A</td> <td>63</td> <td>М</td> <td>Mt</td> <td>0-IIc</td> <td>15</td> <td>EP</td>	5-A	63	М	Mt	0-IIc	15	EP
6-A 76 M Mt 0-lic 60 LPM $6-B$ 76 M Mt 0-lic 15 EP $7-A$ 67 M Mt 0-lic 25 LPM $7-B$ 67 M Mt 0-lic 20 EP $8-A$ 72 M Lt 0-lic 30 MM $8-A$ 72 M Lt 0-lic 30 MM $8-B$ 76 M Lt 0-lic 30 MM $9-B$ 70 M Lt 0-lic 30 SMI $10-A$ 67 M Lt 0-lic 30 SMI $10-B$ 74 M Ce 0-lic 15 LPM $11-A$ 50 M Lt 0-lic 15 LPM $12-A$ 55 M Lt 0-lic 10 LPM $12-A$ 55 M Lt 0-lic 10 LPM $12-A$ 56 M	5-B	63	М	Lt	0-IIc	10	EP
6-B 76 M Mt 0-lic 15 EP $6-C$ 76 M Mt 0-lic 25 LPM $7-B$ 67 M Mt 0-lic 20 EP $8-A$ 72 M Lt 0-lic 30 MM $9-A$ 69 M Lt 0-lic 30 MM $9-B$ 70 M Lt 0-lic 30 SMI $0-A$ 67 M Lt 0-lic 30 SMI $0-A$ 67 M Lt 0-lic 30 EP $11-A$ 50 M Lt 0-lic 30 EP $11-A$ 50 M Lt 0-lic 10 EP $12-B$ 56 M Lt 0-lic 10 EP $12-C$ 56 M Lt 0-lic 10 LPM $13-A$ 77 M Ut 0-lic 12 LPM $13-B$ 57 M <	6-A	76	М	Mt	0-IIc	60	LPM
6-C 76 M Mt 0-IIc 5 EP 7-A 67 M Mt 0-IIc 20 EP 8-A 72 M Lt 0-IIc 20 EP 8-A 72 M Lt 0-IIc 30 MM 9-A 69 M Lt 0-IIc 30 MM 9-B 70 M Lt 0-IIc 8 EP 10-A 67 M Lt 0-IIc 30 SMI 10-B 74 M Cc 0-IIc 30 SMI 10-A 57 M Lt 0-IIc 50 EP 11-A 50 M Lt 0-IIc 15 LPM 12-B 56 M Ut 0-IIc 10 EP 12-C 56 M Ut 0-IIc 10 LPM 13-B 57 M Ut 0-IIc 10 LPM 13-A 56 M Ut 0-IIc </td <td>6-B</td> <td>76</td> <td>Μ</td> <td>Mt</td> <td>0-IIc</td> <td>15</td> <td>EP</td>	6-B	76	Μ	Mt	0-IIc	15	EP
7-A67MMt0-IIc25LPM7-B67MMt0-IIc20EP8-A72MLt0-IIc30MM8-B76MLt0-IIc30MM9-B70MLt0-IIc30SMI9-B70MLt0-IIc30SMI10-A67MLt0-IIc30SMI10-B74MCe0-IIc50EP11-A50MLt0-IIc25LPM12-A55MLt0-IIc10EP12-B56MUt0-IIc10EP12-C56MLt0-IIc10LPM13-B57MUt0-IIc10LPM14-A78MUt0-IIc12LPM15-B61MUt0-IIc12LPM15-B7MUt0-IIc12LPM15-B7MUt0-IIc15LPM15-B74FUt0-IIc15LPM15-B74MUt0-IIc15LPM15-B74FUt0-IIc15LPM15-B74FUt0-IIc15LPM15-B74FUt0-IIc15LPM15-B74FUt<	6-C	76	Μ	Mt	0-IIc	5	EP
7-B67MMt0-IIc20EP8-A72MLt0-IIc60MM9-B76MLt0-IIc30MM9-B70MLt0-IIc30SMI10-A67MLt0-IIc30SMI10-B74MCe0-IIc15LPM11-A50MLt0-IIc15LPM11-B51MLt0-IIc15LPM12-A55MLt0-IIc5EP12-A56MLt0-IIc5EP12-A56MLt0-IIc7EP13-A56MLt0-IIc10LPM13-B57MUt0-IIc10LPM14-A78MUt0-IIc25MM15-B61MUt0-IIc25EP16-A74FUt0-IIc15MM17-A54MUt0-IIc15MM17-B54MUt0-IIc10EP18-A71MLt0-IIc10EP19-A75MLt0-IIc10EP19-A75MLt0-IIc10EP19-A75MLt0-IIc10EP19-A75MLt0-IIc	7-A	67	Μ	Mt	0-IIc	25	LPM
8-A72MLt0-IIc60MM8-B76MLt0-IIc30MM9-A69MLt0-IIc30LPM9-B70MLt0-IIc8EP10-A67MLt0-IIc30SMI10-B74MCe0-IIc50EP11-A50MLt0-IIc25LPM11-B51MLt0-IIc25EP12-A55MLt0-IIc8EP12-B56MUt0-IIc8EP12-C56MLt0-IIc10LPM13-B57MUt0-IIc10LPM14-A78MUt0-IIc12LPM15-B61MUt0-IIc12LPM15-B61MUt0-IIc12LPM15-B74FUt0-IIc15MM15-B74FUt0-IIc20MM16-B74FUt0-IIc15LPM17-A54MUt0-IIc20EP17-C56MUt0-IIc10EP17-C56MUt0-IIc10EP18-A71MLt0-IIc10EP19-A75MLt0-I	7-B	67	Μ	Mt	0-IIc	20	EP
8-B76MLt0-IIc30MM9-A69MLt0-IIc10LPM9-B70MLt0-IIc8EP10-A67MLt0-IIc30SM110-B74MCe0-IIc50EP11-A50MLt0-IIc15LPM11-B51MLt0-IIc10EP12-A55MLt0-IIc5EP12-A56MLt0-IIc8EP13-A56MLt0-IIc10LPM13-B57MUt0-IIc10LPM14-B79MLt0-IIc12LPM15-A77MUt0-IIc12LPM15-B61MUt0-IIc25MM15-B61MUt0-IIc25EP16-A74FUt0-IIc15LPM17-A54MUt0-IIc25EP17-C56MLt0-IIc10EP19-A75MLt0-IIc10EP19-B75MLt0-IIc10EP19-A75MLt0-IIc10EP19-A75MLt0-IIc10EP19-B75MLt0-I	8-A	72	Μ	Lt	0-IIc	60	MM
9-A69MLt0-IIc10LPM9-B70MLt0-IIc8EP10-A67MLt0-IIc30SMI10-B74MCe0-IIc50EP11-A50MLt0-IIc15LPM11-B51MLt0-IIc25LPM12-A55MUt0-IIc5EP12-C56MUt0-IIc8EP13-A56MLt0-IIc10LPM13-B57MUt0-IIc10LPM14-A78MUt0-IIc12LPM15-A57MUt0-IIc12LPM15-A57MUt0-IIc25MM15-B61MUt0-IIc25PP16-A74FUt0-IIc25EP17-A54MUt0-IIc15LPM17-B54MUt0-IIc20SM219-A75MLt0-IIc10EP19-A75MLt0-IIc10EP19-C75MLt0-IIc10EP19-A75MLt0-IIc10EP19-A75MLt0-IIc10EP19-A75MLt	8-B	76	М	Lt	0-IIc	30	MM
9-B70MLt0-IIc8EP10-A67MLt0-IIc30SM110-B74MCe0-IIc50EP11-A50MLt0-IIc15LPM11-B51MLt0-IIc25LPM12-A55MLt0-IIc10EP12-B56MUt0-IIc10LPM13-A56MLt0-IIc10LPM13-A56MUt0-IIc7EP14-A78MUt0-IIc10LPM15-B61MUt0-IIc25EP16-A74FUt0-IIc25EP16-A74FUt0-IIc25EP16-A74FUt0-IIc15LPM17-A54MUt0-IIc15MM17-B54MUt0-IIc15EP17-C56MUt0-IIc15EP19-A75MLt0-IIc10EP19-A75MLt0-IIc10EP19-A75MLt0-IIc10EP19-A75MLt0-IIc10EP19-A75MLt0-IIc10EP19-A75MLt0	9-A	69	М	Lt	0-IIc	10	LPM
10-A67MLt0-IIc30SM110-B74MCe0-IIc50EP11-A50MLt0-IIc15LPM11-B51MLt0-IIc25LPM12-A55MLt0-IIc10EP12-B56MUt0-IIc10LPM13-A56MLt0-IIc10LPM13-A56MUt0-IIc10LPM13-A56MUt0-IIc10LPM14-A78MUt0-IIc10LPM15-B57MUt0-IIc12LPM15-A57MUt0-IIc25EP16-A74FUt0-IIc25EP16-A74FUt0-IIc15LPM17-A54MLt0-IIc20EP17-C56MUt0-IIc20EP18-A71MLt0-IIc10EP19-A75MLt0-IIc30SM219-A75MLt0-IIc10EP19-C75MLt0-IIc10EP20-A80MMt0-IIc20MM21-B78MLt0-IIc10EP22-A54MMt <td>9-B</td> <td>70</td> <td>М</td> <td>Lt</td> <td>0-IIc</td> <td>8</td> <td>EP</td>	9-B	70	М	Lt	0-IIc	8	EP
10-B74MCe0-IIc50EP11-A50MLt0-IIc15LPM11-B51MLt0-IIc25LPM12-A55MUt0-IIc5EP12-B56MUt0-IIc5EP12-C56MLt0-IIc8EP13-A56MUt0-IIc10LPM13-B57MUt0-IIc10LPM14-A78MUt0-IIc12LPM15-A57MUt0-IIc25MM15-B61MUt0-IIc25EP16-A74FUt0-IIc15LPM17-A54MMt0-IIc25EP17-C56MUt0-IIc20PM18-B74MLt0-IIc20EP17-C56MUt0-IIc20EP18-A71MLt0-IIc10EP19-A75MLt0-IIc10EP19-B75MLt0-IIc30SM219-A76MMt0-IIc30SM221-A76MMt0-IIc20EP22-A54MMt0-IIc20EP23-B50MMt	10-A	67	М	Lt	0-IIc	30	SM1
11-A50MLt0-IIc15LPM11-B51MLt0-IIc25LPM12-A55MUt0-IIc10EP12-B56MUt0-IIc5EP12-C56MLt0-IIc10LPM13-A56MLt0-IIc10LPM13-B57MUt0-IIc10LPM14-A78MUt0-IIc12LPM15-A57MUt0-IIc25MM15-B61MUt0-IIc25EP16-A74FUt0-IIc15LPM17-A54MUt0-IIc15MM17-B54MUt0-IIc20EP18-A71MLt0-IIc20EP19-A75MLt0-IIc10EP19-A75MLt0-IIc10EP19-A75MLt0-IIc10EP19-A75MLt0-IIc10EP19-B75MLt0-IIc10EP19-C75MLt0-IIc10EP19-B78MMt0-IIc20MM21-B78MMt0-IIc20EP22-A54MMt <td< td=""><td>10-B</td><td>74</td><td>М</td><td>Ce</td><td>0-IIc</td><td>50</td><td>EP</td></td<>	10-B	74	М	Ce	0-IIc	50	EP
11-B51MLt0-IIc25LPM12-A55MLt0-IIc10EP12-B56MUt0-IIc5EP12-C56MLt0-IIc10LPM13-A56MLt0-IIc10LPM13-B57MUt0-IIc10LPM14-A78MUt0-IIc10LPM14-B79MLt0-IIc12LPM15-B61MUt0-IIc25EP16-A74FUt0-IIc20MM16-B74FUt0-IIc15LPM17-A54MMt0-IIc25EP16-A71MLt0-IIc25EP17-C56MUt0-IIc20EP18-A71MLt0-IIc20EP18-A71MLt0-IIc10EP19-A75MLt0-IIc10EP19-A75MLt0-IIc10EP20-A80MMt0-IIc30SM221-A76MMt0-IIc20EP22-A54MMt0-IIc20EP23-A50MMt0-IIc15EP23-B50MMt <t< td=""><td>11-A</td><td>50</td><td>М</td><td>Lt</td><td>0-IIc</td><td>15</td><td>LPM</td></t<>	11-A	50	М	Lt	0-IIc	15	LPM
12-A55MLt0-IIc10EP12-B56MUt0-IIc5EP12-C56MLt0-IIc8EP13-A56MLt0-IIc10LPM13-B57MUt0-IIc10LPM13-B77MUt0-IIc10LPM14-A78MUt0-IIc10LPM14-B79MLt0-IIc25MM15-B61MUt0-IIc25EP16-A74FUt0-IIc20MM16-B74FUt0-IIc15LPM17-A54MLt0-IIc20EP18-A71MLt0-IIc20EP19-A75MLt0-IIc10EP19-B75MLt0-IIc10EP19-B75MLt0-IIc10EP19-B75MLt0-IIc10EP20-A80MMt0-IIc30SM221-A76MMt0-IIc20EP22-A54MMt0-IIc15EP23-A50MMt0-IIc15EP23-B50MMt0-IIc15EP	11-B	51	М	Lt	0-IIc	25	LPM
12-B56MUt0-IIc5EP12-C56MLt0-IIc8EP13-A56MLt0-IIc10LPM13-B57MUt0-IIc7EP14-A78MUt0-IIc10LPM14-B79MLt0-IIc12LPM15-A57MUt0-IIc25MM15-B61MUt0-IIc25EP16-A74FUt0-IIc15LPM17-B54MUt0-IIc15MM17-B54MUt0-IIc25EP17-C56MUt0-IIc20EP18-A71MLt0-IIc20SM219-A75MLt0-IIc10EP19-A75MLt0-IIc10EP19-A75MLt0-IIc10EP20-A80MMt0-IIc30SM221-A76MMt0-IIc20EP22-A54MLt0-IIc20EP23-A50MMt0-IIc15EP23-A50MMt0-IIc40EP23-B50MMt0-IIc15EP	12-A	55	М	Lt	0-IIc	10	EP
12-C56MLt0-IIc8EP13-A56MLt0-IIc10LPM13-B57MUt0-IIc7EP14-A78MUt0-IIc10LPM14-B79MLt0-IIc12LPM15-B51MUt0-IIc25EP16-A74FUt0-IIc20MM16-B74FUt0-IIc15LPM17-A54MMt0-IIc25EP17-C56MUt0-IIc20EP18-A71MLt0-IIc20EP19-A75MLt0-IIc20EP19-A75MLt0-IIc8MM19-B74MLt0-IIc10EP19-A75MLt0-IIc10EP19-A75MLt0-IIc10EP19-A75MLt0-IIc10EP20-A80MMt0-IIc30SM221-A76MMt0-IIc20EP22-A54MMt0-IIc20MM22-B54MMt0-IIc15EP23-A50MLt0-IIc15EP23-B50MLt0-I	12-B	56	М	Ut	0-IIc	5	EP
13-A56MLt0-IIc10LPM13-B57MUt0-IIc7EP14-A78MUt0-IIc10LPM14-B79MLt0-IIc12LPM15-A57MUt0-IIc25MM15-B61MUt0-IIc20MM16-A74FUt0-IIc15LPM16-A74FUt0-IIc15MM17-B54MMt0-IIc20EP17-C56MUt0-IIc20EP18-A71MLt0-IIc20EP18-B74MLt0-IIc20SM219-A75MLt0-IIc10EP19-B75MLt0-IIc10EP19-A75MLt0-IIc10EP19-B75MLt0-IIc10EP19-B75MLt0-IIc10EP20-A80MMt0-IIc30SM221-A76MMt0-IIc20EP22-A54MMt0-IIc20EP23-A50MMt0-IIc15EP23-A50MMt0-IIc15EP	12-C	56	М	Lt	0-IIc	8	EP
13-B57MUt0-IIc7EP14-A78MUt0-IIc10LPM14-B79MLt0-IIc12LPM15-A57MUt0-IIc25MM15-B61MUt0-IIc25EP16-A74FUt0-IIc20MM16-B74FUt0-IIc15LPM17-A54MMt0-IIc25EP17-C56MUt0-IIc20EP18-A71MLt0-IIc20EP18-A71MLt0-IIc20SM219-A75MLt0-IIc10EP19-B75MLt0-IIc10EP19-C75MLt0-IIc10EP20-A80MMt0-IIc30SM221-A76MLt0-IIc20EP22-A54MMt0-IIc20MM22-B54MMt0-IIc15EP23-A50MMt0-IIc40EP23-B50MMt0-IIc15EP	13-A	56	М	Lt	0-IIc	10	LPM
14-A78MUt0-IIc10LPM14-B79MLt0-IIc12LPM15-A57MUt0-IIc25MM15-B61MUt0-IIc25EP16-A74FUt0-IIc20MM16-B74FUt0-IIc15LPM17-A54MMt0-IIc25EP18-A71MLt0-IIc25EP18-A71MLt0-IIc20EP18-A71MLt0-IIc8MM18-B74MLt0-IIc10EP19-A75MLt0-IIc10EP19-A75MLt0-IIc10EP19-B75MLt0-IIc10EP20-A80MMt0-IIc30SM221-A76MMt0-IIc20EP22-A54MMt0-IIc20MM22-B54MMt0-IIc15EP23-B50MLt0-IIc40EP23-B50MLt0-IIc40EP	13-B	57	М	Ut	0-IIc	7	EP
14-B79MLt0-IIc12LPM15-A57MUt0-IIc25MM15-B61MUt0-IIc25EP16-A74FUt0-IIc20MM16-B74FUt0-IIc15LPM17-A54MMt0-IIc25EP17-C56MUt0-IIc20EP18-A71MLt0-IIc8MM18-B74MLt0-IIc8MM18-B74MLt0-IIc10EP19-A75MLt0-IIc10EP19-A75MLt0-IIc10EP20-A80MMt0-IIc30SM221-A76MMt0-IIc20EP22-A54MMt0-IIc20EP22-A54MMt0-IIc20EP22-A54MMt0-IIc20EP23-A50MMt0-IIc40EP23-B50MMt0-IIc40EP23-B50MMt0-IIc15EP	14-A	78	М	Ut	0-IIc	10	LPM
15-A57MUt0-IIc25MM15-B61MUt0-IIc25EP16-A74FUt0-IIc20MM16-B74FUt0-IIc15LPM17-A54MMt0-IIc15MM17-B54MLt0-IIc20EP17-C56MUt0-IIc20EP18-A71MLt0-IIc8MM18-B74MLt0-IIc10EP19-A75MLt0-IIc10EP19-A75MLt0-IIc10EP20-A80MMt0-IIc10EP21-A76MMt0-IIc30SM221-B78MLt0-IIc20EP22-A54MMt0-IIc20EP23-A50MMt0-IIc40EP23-B50MMt0-IIc40EP23-B50MMt0-IIc40EP23-B50MMt0-IIc40EP23-B50MMt0-IIc15EP	14-B	79	М	Lt	0-IIc	12	LPM
15-B61MUt0-IIc25EP16-A74FUt0-IIc20MM16-B74FUt0-IIc15LPM17-A54MMt0-IIc15MM17-B54MLt0-IIc25EP17-C56MUt0-IIc20EP18-A71MLt0-IIc8MM18-B74MLt0-IIc10EP19-A75MLt0-IIc10EP19-B75MLt0-IIc10EP20-A80MMt0-IIc30SM221-A76MMt0-IIc30SM221-B78MLt0-IIc20EP22-A54MMt0-IIc15EP23-A50MLt0-IIc15EP23-B50MMt0-IIc15EP	15-A	57	М	Ut	0-IIc	25	MM
16-A 74 FUt $0-IIc$ 20 MM $16-B$ 74 FUt $0-IIc$ 15 LPM $17-A$ 54 MMt $0-IIc$ 15 MM $17-B$ 54 MLt $0-IIc$ 25 EP $17-C$ 56 MUt $0-IIc$ 20 EP $18-A$ 71 MLt $0-IIc$ 8 MM $18-B$ 74 MLt $0-IIc$ 8 MM $18-B$ 74 MLt $0-IIc$ 10 EP $19-A$ 75 MLt $0-IIc$ 10 EP $19-A$ 75 MLt $0-IIc$ 10 EP $19-A$ 75 MLt $0-IIc$ 10 EP $20-A$ 80 MMt $0-IIc$ 140 MM $20-B$ 80 MMt $0-IIc$ 30 SM2 $21-A$ 76 MMt $0-IIc$ 20 EP $22-A$ 54 MMt $0-IIc$ 20 EP $22-A$ 54 MMt $0-IIc$ 15 EP $23-A$ 50 MMt $0-IIc$ 15 EP $23-B$ 50 MMt $0-IIc$ 15 EP	15-B	61	М	Ut	0-IIc	25	EP
16-B74FUt0-IIc15LPM17-A54MMt0-IIc15MM17-B54MLt0-IIc25EP17-C56MUt0-IIc20EP18-A71MLt0-IIc8MM18-B74MLt0-IIc10EP19-A75MLt0-IIc10EP19-B75MLt0-IIc10EP19-C75MLt0-IIc10EP20-A80MMt0-IIc30SM221-A76MMt0-IIc30SM221-A78MLt0-IIc20EP22-A54MMt0-IIc20EP23-A50MMt0-IIc15EP23-B50MMt0-IIc15EP	16-A	74	F	Ut	0-IIc	20	MM
17-A 54 MMt $0-IIc$ 15 MM $17-B$ 54 MLt $0-IIc$ 25 EP $17-C$ 56 MUt $0-IIc$ 20 EP $18-A$ 71 MLt $0-IIc$ 8 MM $18-B$ 74 MLt $0-IIc$ 10 EP $19-A$ 75 MLt $0-IIc$ 10 EP $19-B$ 75 MLt $0-IIc$ 10 EP $19-C$ 75 MLt $0-IIc$ 10 EP $20-A$ 80 MMt $0-IIc$ 140 MM $20-B$ 80 MMt $0-IIc$ 30 $SM2$ $21-A$ 76 MMt $0-IIc$ 20 EP $22-A$ 54 MMt $0-IIc$ 20 EP $22-B$ 54 MMt $0-IIc$ 15 EP $23-A$ 50 MMt $0-IIc$ 15 EP $23-B$ 50 MMt $0-IIc$ 15 EP	16-B	74	F	Ut	0-IIc	15	LPM
17-B 54 MLt $0-Ic$ 25 EP $17-C$ 56 MUt $0-Ic$ 20 EP $18-A$ 71 MLt $0-Ic$ 8 MM $18-B$ 74 MLt $0-Ic$ 10 EP $19-A$ 75 MLt $0-Ic$ 10 EP $19-B$ 75 MLt $0-Ic$ 10 EP $19-C$ 75 MLt $0-Ic$ 10 EP $20-A$ 80 MMt $0-Ic$ 30 SM2 $21-A$ 76 MMt $0-Ic$ 20 EP $22-A$ 54 MMt $0-Ic$ 20 MM $22-B$ 54 MMt $0-Ic$ 20 MM $22-B$ 50 MMt $0-Ic$ 15 EP $23-B$ 50 MLt $0-Ic$ 15 EP	17-A	54	Μ	Mt	0-IIc	15	MM
17-C 56 MUt $0-IIc$ 20 EP $18-A$ 71 MLt $0-IIc$ 8 MM $18-B$ 74 MLt $0-IIa$ 20 $SM2$ $19-A$ 75 MLt $0-IIc$ 10 EP $19-B$ 75 MLt $0-IIc$ 10 EP $19-C$ 75 MLt $0-IIc$ 10 EP $20-A$ 80 MMt $0-IIc$ 140 MM $20-B$ 80 MMt $0-IIc$ 30 $SM2$ $21-A$ 76 MLt $0-IIc$ 20 EP $22-A$ 54 MMt $0-IIc$ 20 EP $22-B$ 54 MMt $0-IIc$ 15 EP $23-A$ 50 MLt $0-IIc$ 15 EP	17-B	54	М	Lt	0-IIc	25	EP
18-A 71 MLt $0-IIc$ 8 MM $18-B$ 74 MLt $0-IIa$ 20 $SM2$ $19-A$ 75 MLt $0-IIc$ 10 EP $19-B$ 75 MLt $0-IIc$ 10 EP $19-C$ 75 MLt $0-IIc$ 10 EP $20-A$ 80 MMt $0-IIc$ 140 MM $20-B$ 80 MMt $0-IIc$ 30 $SM2$ $21-A$ 76 MMt $0-IIc$ 50 MM $21-B$ 78 MLt $0-IIc$ 20 EP $22-A$ 54 MMt $0-IIc$ 15 EP $23-A$ 50 MMt $0-IIc$ 15 EP $23-B$ 50 MLt $0-IIc$ 15 EP	17-C	56	М	Ut	0-IIc	20	EP
18-B74MLt0-IIa20SM219-A75MLt0-IIc10EP19-B75MLt0-IIc10EP19-C75MLt0-IIc10EP20-A80MMt0-IIc140MM20-B80MMt0-IIc30SM221-A76MMt0-IIc50MM21-B78MLt0-IIc20EP22-A54MMt0-IIc15EP23-A50MMt0-IIc40EP23-B50MLt0-IIc15EP	18-A	71	Μ	Lt	0-IIc	8	MM
19-A75MLt0-IIc10EP19-B75MLt0-IIc10EP19-C75MLt0-IIc10EP20-A80MMt0-IIc140MM20-B80MMt0-IIc30SM221-A76MMt0-IIc50MM21-B78MLt0-IIc20EP22-A54MMt0-IIc15EP23-A50MMt0-IIc15EP23-B50MLt0-IIc15EP	18-B	74	М	Lt	0-IIa	20	SM2
19-B75MLt0-IIc10EP19-C75MLt0-IIc10EP20-A80MMt0-IIc140MM20-B80MMt0-IIc30SM221-A76MMt0-IIc50MM21-B78MLt0-IIc20EP22-A54MMt0-IIc15EP23-A50MMt0-IIc15EP23-B50MLt0-IIc15EP	19-A	75	М	Lt	0-IIc	10	EP
19-C75MLt0-IIc10EP20-A80MMt0-IIc140MM20-B80MMt0-IIc30SM221-A76MMt0-IIc50MM21-B78MLt0-IIc20EP22-A54MMt0-IIc20MM22-B54MMt0-IIc15EP23-A50MMt0-IIc15EP23-B50MLt0-IIc15EP	19-B	75	М	Lt	0-IIc	10	EP
20-A 80 M Mt 0-IIc 140 MM 20-B 80 M Mt 0-IIc 30 SM2 21-A 76 M Mt 0-IIc 50 MM 21-B 78 M Lt 0-IIc 20 EP 22-A 54 M Mt 0-IIc 20 MM 22-B 54 M Mt 0-IIc 15 EP 23-A 50 M Mt 0-IIc 40 EP 23-B 50 M Lt 0-IIc 15 EP	19-C	75	М	Lt	0-IIc	10	EP
20-B 80 M Mt 0-IIc 30 SM2 21-A 76 M Mt 0-IIc 50 MM 21-B 78 M Lt 0-IIc 20 EP 22-A 54 M Mt 0-IIc 20 MM 22-B 54 M Mt 0-IIc 15 EP 23-A 50 M Mt 0-IIc 40 EP 23-B 50 M Lt 0-IIc 15 EP	20-A	80	М	Mt	0-IIc	140	MM
21-A76MMt0-IIc50MM21-B78MLt0-IIc20EP22-A54MMt0-IIc20MM22-B54MMt0-IIc15EP23-A50MMt0-IIc40EP23-B50MLt0-IIc15EP	20-В	80	М	Mt	0-IIc	30	SM2
21-B78MLt0-IIc20EP22-A54MMt0-IIc20MM22-B54MMt0-IIc15EP23-A50MMt0-IIc40EP23-B50MLt0-IIc15EP	21-A	76	М	Mt	0-IIc	50	MM
22-A 54 M Mt 0-IIc 20 MM 22-B 54 M Mt 0-IIc 15 EP 23-A 50 M Mt 0-IIc 40 EP 23-B 50 M Lt 0-IIc 15 EP	21-B	78	М	Lt	0-IIc	20	EP
22-B 54 M Mt 0-IIc 15 EP 23-A 50 M Mt 0-IIc 40 EP 23-B 50 M Lt 0-IIc 15 EP	22-A	54	М	Mt	0-IIc	20	MM
23-A 50 M Mt 0-IIc 40 EP 23-B 50 M Lt 0-IIc 15 EP	22-B	54	М	Mt	0-IIc	15	EP
23-B 50 M Lt 0-IIc 15 EP	23-A	50	М	Mt	0-IIc	40	EP
	23-В	50	Μ	Lt	0-IIc	15	EP

	Age (years)	Gender	Location ^a	Morphologic type ^b	Size (mm)	Depth ^c
24-A	62	М	Ut	0-IIc	20	EP
24-B	62	М	Lt	0-IIc	50	MM
25-A	63	М	Mt	0-IIc	30	SM2
25-В	63	М	Lt	0-IIc	40	EP
26-A	60	Μ	Lt	0-IIc	10	MM
26-B	60	Μ	Lt	0-IIc	15	LPM
27-A	66	Μ	Mt	0-IIc	10	MM
27-В	66	Μ	Mt	0-IIc	10	LPM
27-С	66	Μ	Mt	0-IIc	30	MM
28-A	68	Μ	Mt	0-IIc	10	EP
28-B	69	Μ	Mt	0-IIc	5	EP
28-C	69	Μ	Mt	0-IIc	10	EP
29-A	74	Μ	Mt	0-IIc	20	MM
29-В	74	Μ	Lt	0-IIc	12	MM
29-С	74	Μ	Ut	0-IIc	10	LPM
30-A	58	Μ	Mt	0-IIc	25	MM
30-В	58	М	Lt	0-IIc	20	LPM

Table I.	Continued.
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^aCe, cervical esophagus; Lt, lower thoracic esophagus; Mt, middle thoracic esophagus; Ut, upper thoracic esophagus. ^bO-IIa, slightly elevated type; O-IIc, slightly depressed type. ^cEP, epithelium; LPM, lamina propria mucosae; MM, muscularis mucosae; SM, submucosal layer. SCC, squamous cell carcinoma.

defined as a lesion that is confined to the esophageal mucosa or submucosa, regardless of the presence or absence of regional lymph node metastasis (10). The description of the esophageal SCCs and histologic evaluation of the resected specimens were in accordance with the Japanese Classification of Esophageal Carcinoma (10). Tumor locations were defined as the cervical esophagus (Ce), upper thoracic esophagus (Ut), middle thoracic esophagus (Mt), lower thoracic esophagus (Lt) and the abdominal esophagus (Ae). The depth of invasion of superficial esophageal SCC was classified as intramucosal or submucosal. The depth of intramucosal invasion was subclassified as epithelium (EP), lamina propria mucosae (LPM) and muscularis mucosae (MM), and the depth of submucosal invasion was subclassified as SM1 to SM3 according to the grade of invasion (1, mild; 2, moderate; or 3, remarkable).

Clinicopathologic features of the 30 patients are shown in Table I. Mean age of the patients was 66.3±8.6 years (range, 50-80 years) and male/female ratio was 29/1. Morphologically, of the 67 tumors, 66 were classified as O-IIc or slightly depressed type and 1 was classified as O-IIa or slightly elevated type. Tumor depth was EP in 34 tumors, LPM in 13, MM in 16, SM2 in 3 and SM1 in 1.

The following criteria were used for the diagnosis of primary multiple esophageal SCCs: i) each cancerous lesion histologically showed definite malignant features and was located individually with no continuity and ii) concomitant carcinomas accompanied the areas of intraepithelial carcinomas. The purpose of the latter criterion was to exclude intramural metastatic lesions, which frequently occur in patients with esophageal SCC (6). Lesions detected within 1 year of the initial endoscopic treatment were regarded as synchronous multiple lesions because the presence of microcarcinoma might have been missed at the time of endoscopic treatment. Metachronous multiple lesions were defined as new esophageal SCCs occurring in different areas from the initial cancer and at least 1 year after the initial endoscopic treatment (1).

DNA extraction. Tissue sections (10- μ m thick) were stained with hematoxylin and eosin, dehydrated in a graded ethanol series, and then dried without a cover glass. Tissue samples from tumors and corresponding normal tissues were cut with sterile needles, and the DNA was extracted with 20 μ l of extraction buffer (100 mM Tris-HCl, pH 8.0, 2 mM EDTA, 400 μ g/ml proteinase K) at 55°C overnight. The tubes were boiled for 5 min to inactivate proteinase K, and 1-2 μ l of each extract was used for polymerase chain reaction (PCR) amplification.

Loss of heterozygosity analysis of p53, p16, IRF and other microsatellite loci. Loss of heterozygosity (LOH) analysis of p53 (TP53), p16 (D9S171), IRF (IRF) and other microsatellite loci including D1S191, D17S858, D18S58 and D18S61 was performed with microsatellite assay as described previously (11). In brief, each 15- μ l reaction mixture contained 10-20 ng genomic DNA, 6.7 mM Tris-HCl, pH 8.8, 6.7 mM EDTA, 6.7 mM MgCl₂, 0.175 μ M primer set, 1.5 mM [α^{32} -P]-dCTP,

	<i>p53</i> (TP53)	<i>p53</i> (D9S171)	<i>p16</i> (Ex 1-3)	IRF (IRF)	D1S191	D17S858	D18S58	D18S61	BAT26	mtDNA
1-A	-	Hom	-	Hom	MSI	Hom	-	-	-	-
1-B	-	Hom	Mut (Ex 3)	MSI	LOH	Hom	-	-	-	-
2-A	-	Hom	-	LOH	-	Hom	-	Hom	-	-
2-B	LOH	Hom	-	-	-	Hom	-	Hom	-	-
3-A	LOH	Hom	-	-	-	NI	Hom	-	-	-
3-B	-	Hom	-	-	-	NI	Hom	-	-	-
4-A	-	-	-	-	-	Hom	Hom	-	-	-
4-B	LOH	-	-	-	-	Hom	Hom	LOH	-	-
5-A	-	-	-	-	Hom	Hom	-	-	-	-
5-B	-	-	-	-	Hom	Hom	-	-	-	-
6-A	-	Hom	-	-	Hom	NI	Hom	-	-	-
6-B	LOH	Hom	-	-	Hom	NI	Hom	-	-	-
6-C	-	Hom	-	-	Hom	NI	Hom	LOH	-	-
7-A	NI	Hom	-	-	-	Hom	-	-	-	-
7-B	NI	Hom	-	LOH	LOH	Hom	LOH	-	-	-
8-A	-	Hom	Mut (Ex 2)	-	MSI	Hom	Hom	-	-	-
8-B	-	Hom	Mut (Ex 1)	-	Hom	Hom	Hom	-	-	-
9-A	-	-	-	NI	Hom	MSI	Hom	NI	-	-
9-B	-	MSI	-	NI	Hom	Hom	Hom	NI	-	-
10-A	LOH	-	-	LOH	LOH	-	Hom	-	-	-
10-B	-	-	-	-	-	-	Hom	LOH	-	-
11-A	-	-	-	MSI	LOH	Hom	Hom	-	-	-
11 - B	-	-	-	-	LOH	Hom	Hom	-	-	-
12-A	LOH	-	-	-	MSI	-	-	-	-	-
12-B	-	-	-	-	Hom	LOH	-	LOH	-	-
12-C	-	-	-	-	Hom	-	-	LOH	-	-
13-A	Hom	-	Mut (Ex 1)	LOH	MSI	Hom	Hom	-	-	-
13-B	Hom	-	-	NI	-	Hom	Hom	-	-	-
14-A	Hom	Hom	-	NI	-	Hom	Hom	NI	-	-
14-B	Hom	Hom	-	NI	-	Hom	Hom	NI	-	-
15-A	Hom	LOH	-	-	MSI	Hom	Hom	-	-	-
15-B	Hom	-	-	-	Hom	Hom	Hom	-	-	-
16-A	-	LOH	-	-	LOH	LOH	MSI	NI	-	Mut
16-B	NI	-	-	MSI	LOH	LOH	Hom	NI	-	-
17-A	-	-	-	-	-	Hom	-	-	-	-
17 - B	-	-	-	-	LOH	Hom	-	LOH	-	Mut
17-C	-	LOH	-	-	LOH	Hom	LOH	LOH	-	-
18-A	NI	Hom	-	-	Hom	NI	-	-	-	-
18-B	LOH	Hom	-	-	MSI	LOH	-	-	-	-
19-A	LOH	LOH	-	Hom	Hom	LOH	-	-	-	Mut
19-B	-	-	-	Hom	Hom	-	LOH	-	-	Mut
19-C	-	-	-	Hom	Hom	-	-	LOH	-	Mut
20-A	LOH	-	-	Hom	-	-	LOH	-	-	Mut
20-В	LOH	-	-	Hom	-	-	-	-	-	-
21-A	LOH	-	-	Hom	-	-	LOH	-	-	-
21-B	LOH	LOH	-	Hom	-	-	LOH	LOH	-	-
22-A	LOH	-	-	Hom	Hom	-	-	Hom	-	-
22-В	-	-	-	Hom	Hom	-	-	Hom	-	-
23-A	LOH	Hom	-	-	Hom	-	MSI	-	-	-
23-В	-	Hom	-	-	Hom	-	LOH	-	-	-

Table II. Results of genetic analysis in each patient with multiple esophageal SCCs.

	<i>p53</i> (TP53)	<i>p53</i> (D9S171)	<i>p16</i> (Ex 1-3)	IRF (IRF)	D1S191	D17S858	D18S58	D18S61	BAT26	mtDNA
24-A	_	Hom	-	Hom	MSI	_	Hom	LOH	-	-
24-B	LOH	Hom	-	Hom	Hom	-	Hom	-	-	-
25-A	Hom	-	-	Hom	LOH	-	Hom	-	-	-
25-B	Hom	-	-	Hom	-	-	Hom	-	-	-
26-A	MSI	NI	-	-	Hom	NI	NI	LOH	-	-
26-B	-	NI	-	-	Hom	NI	NI	-	-	-
27-A	Hom	-	-	-	Hom	NI	NI	-	-	-
27-B	Hom	-	-	-	Hom	NI	NI	LOH	-	-
27-C	Hom	-	-	-	Hom	NI	LOH	LOH	-	-
28-A	-	NI	-	-	Hom	NI	-	Hom	-	-
28-B	-	NI	-	-	Hom	NI	LOH	Hom	-	-
28-C	-	NI	-	-	Hom	NI	-	Hom	-	-
29-A	LOH	Hom	-	-	Hom	NI	Hom	-	-	-
29-B	NI	Hom	-	-	Hom	NI	Hom	-	-	-
29-C	NI	Hom	-	MSI	Hom	NI	Hom	-	-	-
30-A	-	LOH	-	-	Hom	NI	-	Hom	-	-
30-B	-	LOH	-	LOH	Hom	NI	-	Hom	-	-

Table II. Continued.

SCC, squamous cell carcinoma; Hom, homozygous allele; LOH, loss of heterozygosity (+); MSI, microsatellite instability (+); Mut, mutation (+); -, no alteration; NI, not informative.

1.5 mM of each deoxynucleotide triphosphate and 0.75 units AmpliTaq Gold DNA polymerase (Perkin-Elmer, Branchburg, NJ). The reaction mixtures were heated to 95°C for 10 min, followed by 45 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 2 min and strand elongation at 72°C for 2 min. PCR products were separated by electrophoresis on 6% polyacrylamide-8 M urea-32% formamide gels and subjected to autoradiography overnight at -80°C on Fuji RX film. LOH was identified when only one major band was detected in DNA isolated from cancerous tissue; two major bands were present in the normal tissue specimen from the same sample. MSI was identified when additional bands not present in the normal tissue DNA were detected. Microsatellite assay with BAT26 was used to evaluate high-frequency MSI (MSI-H) (12).

Mutational analysis of p16 and mtDNA. Mutations of *p16* and the D310 region of mtDNA were examined with PCRsingle-strand conformation polymorphism (SSCP) analysis as described previously (13). In brief, In brief, each 15- μ l reaction mixture contained 10-20 ng genomic DNA, 6.7 mM Tris-HCl, pH 8.8, 6.7 mM EDTA, 6.7 mM MgCl₂, 0.175 μ M primer set, 1.5 mM [α^{32} -P]-dCTP, 1.5 mM of each deoxynucleotide triphosphate and 0.75 units AmpliTaq Gold DNA polymerase. The reaction mixtures were heated to 95°C for 10 min, followed by 45 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 2 min, and strand elongation at 72°C for 2 min. After PCR, the samples were electrophoresed on 6% polyacrylamide gels (acrylamide:bis-acrylamide ratio, 19:1) with 10% glycerol at 4°C. The gels were then subjected to autoradiography overnight at -80°C.



Figure 1. Representative examples of loss of heterozygosity analysis of *p16* and *IRF*. N, normal tissue; T, tumor. Microsatellite loci are indicated in parentheses. Arrows indicate allelic loss.

Results

Genetic analyses of the multiple esophageal SCCs in the 30 patients are summarized in Table II. In microsatellite assay, if the lengths of microsatellite loci in alleles are identical, LOH status cannot be determined. Therefore, uninformative cases are inevitable. In LOH analysis of p53, 50 of the 67 tumors were informative, and LOH was detected in 16 (32%) of the tumors (Fig. 1). For p16, 38 tumors were informative, and LOH was detected in 5 (13%). For *IRF*, 48 tumors were informative, and LOH was detected in 5 (10%). Mutation of

p16 was detected in 4 of the 67 (6%) tumors. Alteration of mtDNA was detected in 6 of the 67 (9%) tumors and none of the tumors showed MSI-H. There were no significant relations between the mutations and clinicopathologic features (data not shown).

All 30 patients showed one or more gene alterations in one or more genetic loci. Discordant genetic patterns among multiple esophageal SCCs were observed in 28 of the 30 (93%) patients. Only in the remaining 2 (7%) patients was the genetic pattern among the tumors identical. The number of discordant genetic loci observed included 1 in 9 patients, 2 in 11 patients, 3 in 3 patients, 4 in 3 patients and 5 in 2 patients. The most discordant locus observed was that of TP53, occurring in 11 of 29 (38%) informative cases, followed by D18S61, occurring in 11 of 30 (37%) cases.

Discussion

In the present study, we showed that in most patients, the genetic pathways of multiple esophageal SCCs can differ within the same patient. To our knowledge, this is the first study to report such a finding.

The p53 tumor suppressor gene has an open reading frame of 393 amino acids in length, and is located at the short arm of human chromosome 17 (14). Normal p53 functions in cell cycle regulation, in the maintenance of genomic stability and in controlled cell death (apoptosis). By preventing continued cell proliferation in the face of damaged DNA, p53 limits the likelihood of mutations becoming fixed, thereby acting as a 'guardian of the genome' (15). Frequent p53 mutations and p53 accumulation have been found in cases of esophageal SCC (16,17). It has been reported that p53 mutations occur as early events in 30-80% of esophageal SCCs. The results of the present study are consistent with those of previous investigations.

The tumor suppressor gene p16 is located on chromosome 9p21, a frequent site of LOH in many human malignancies. Giroux *et al* (18) reported that LOH of p16 was found in 14.7% of the SCCs investigated. In the present study, LOH of p16 was detected in 5 of 38 (13%) informative cases. These results are consistent with those of Giroux *et al. IRF*, another tumor suppressor gene, is located on chromosome 5q31.1 (19). LOH of this gene was reported to occur in 44-57% of esophageal tumors. In addition to alterations of these tumor suppressor genes, somatic mutations of mtDNA have previously been reported in esophageal SCCs (8). The reported frequencies varied from 9 to 34%. Few esophageal SCCs show MSI-H.

In the present study, the frequency of LOH of p53 and p16, mutations of mtDNA, and MSI-H was consistent with those of previous studies, but the frequency of LOH of *IRF* was relatively lower than those of the previous reports. This may be due to the difference of genetic markers examined or to tumor stage differences.

Esophageal SCC is regarded as one of the representative examples of field carcinogenesis because the incidence of multiple occurrences of esophageal SCC is quite high in heavy smokers and heavy drinkers (20). Both tobacco smoking and alcohol drinking can induce genetic/epigenetic alterations in esophageal mucosal cells. Kammori *et al* (21) reported that telomere shortening in the esophageal epithelium is linked to carcinogenesis in esophageal SCC and field carcinogenesis in the esophagus.

It is of great interest to know whether multiple esophageal SCCs within a patient develop through identical or different genetic pathways. In the present study, discordant genetic patterns among individual lesions within a patient were observed in 28 of the 30 (93%) patients, and this finding suggests that multiple esophageal SCCs in the same patient may develop via different genetic pathways. Discordant *p53* mutations have been identified in esophageal SCC and preinvasive lesions from the same patient (22). These results can be explained by the theories of both field and multicentric carcinogenesis. Our results suggest further evidence of field or multicentric carcinogenesis of the human esophagus.

We previously reported the incidence of a speckled pattern of Lugol-voiding lesions (LVLs) of the background esophageal mucosa to be 83% in patients with metachronous multiple SCCs, 50% in those with synchronous SCCs and 27% in those with solitary SCC (1). LVLs are detectable in dysplastic lesions and in esophagitis, and the esophagus with a speckled pattern of LVLs may be at high risk for development of esophageal SCC. This finding may support the field carcinogenesis theory, although the genetic analysis in the present study did not show any definitive evidence of the field carcinogenesis.

Our results may explain the difficulty seen in treating esophageal SCCs with chemotherapy. Few esophageal tumors regress completely with chemotherapy. The genetic background of esophageal SCCs may vary considerably, whereas reagents used in chemotherapy generally target certain proteins or genes. Thus, target conditions may vary from tumor to tumor. In the future, the effect of chemotherapeutic agents may be predicted by detailed genetic analysis of the tumor.

In conclusion, our results suggest that genetic pathways of multiple esophageal SCCs may differ even within the same patient. Further investigation to clarify the genetic pathways of multiple esophageal SCCs may be needed. In addition, investigation into the genetic differences between solitary and multiple esophageal SCCs may also be necessary.

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