Deletion of 17p and Amplification of the int-2 Gene in Esophageal Carcinomas

Takashi Waga,
Tanji Ishizaki,\textsuperscript{2} Masayuki Imamura, Yutaka Shimada, Mituo Ikenaga, and Takayoshi Tobe

Radiation Biology Center (T. W., K. I., M. I.) and First Department of Surgery, Faculty of Medicine (T. W., M. I., Y. S., T. T.), Kyoto University, Yoshida-Konoecho, Sakyo-ku, Kyoto 606, Japan

ABSTRACT

We have analyzed allelic deletion at 23 loci on 18 different chromosomes in 35 esophageal squamous cell carcinoma tissues by using restriction fragment length polymorphism markers. Loss of heterozygosity was detected on chromosomes 2, 3, 6, 7, 11-14, 16-18, 21, and 22, while no loss was detected on chromosomes 1, 4, and 8-10. Only the loss of chromosome 17p was detected with high frequency (45%), and losses on other chromosomes had frequencies of <22%. These losses with low frequencies might be random losses caused by chromosomal rearrangement during the course of tumor development and progression. On the contrary, the loss of 17p might play an important role in the development of esophageal squamous cell carcinoma, such as inactivation of a tumor suppressor gene. Amplification of the int-2 gene was observed in 39% of the tumors. However, no significant relationship between int-2 amplification and the deletion of any chromosome was detected.

INTRODUCTION

Cancer is thought to appear as a result of multiple genetic alterations (1–3). These include mutational activation of protooncogenes, such as ras or myc (4, 5), which are dominantly acting oncogenes; loss-of-function mutations, which inactivate tumor suppressor genes, are also believed to play important roles in various types of tumors (6–8). This inactivation of tumor suppressor genes, resulting from chromosomal deletion or point mutation, allows a cell to escape from normal growth controls into tumorigenesis with uncontrolled cell growth. Some of these inactivations of tumor suppressor genes can be detected by analysis of RFLPs\textsuperscript{3} as loss of heterozygosity at neighboring loci (9). Loss of heterozygosity on specific chromosomes or chromosomal regions has been reported in several human cancers (10–17).

Although esophageal carcinomas are common throughout the world, some areas, such as France, China, and South Africa, have very high incidences (18). Environmental factors such as trace element deficiencies, nutritional deficiencies, and some chemical substances in food, especially those such as nitrosamine and their precursors, have been suggested as possible risk factors (19). There have been a few reports of the genetic alterations in esophageal carcinomas which mention the amplification of epidermal growth factor receptor and c-myc genes (20, 21) or the coamplification of hst-1 and int-2 genes (22, 23). The hst-1 and int-2 genes encode for proteins homologous to fibroblast growth factors (24). These two genes are very closely located in the human genome, namely, the hst-1 gene is located about 35 kilobase pairs downstream of the int-2 gene in the same transcriptional orientation on chromosome 11q13 (25–28). Coamplification of these genes has been reported in human melanoma (28), urinary bladder carcinoma (22), and esophageal carcinoma (22, 23). The coamplification of the hst-1 and int-2 genes in esophageal carcinomas was reported in 52% of Japanese patients (23). On the contrary, it has been reported that there is no evidence of mutational activation of the ras gene family in esophageal carcinomas (21, 29). Very recently, point mutations of the p53 gene in 36% of esophageal squamous cell carcinomas have been discovered and loss-of-function mutation of the p53 gene which is one of tumor suppressor genes should be important in development of these tumors (30). However, since it is not clear whether inactivation of other tumor suppressor genes is also involved in the development of esophageal carcinomas, we studied the possibility of involvement of inactivation of other tumor suppressor genes.

We examined 35 primary esophageal squamous cell carcinomas to detect the loss of constitutional heterozygosity in tumor tissues by RFLP analysis using 23 polymorphic DNA markers on 18 different chromosomes. We also studied the amplification of the int-2 gene in esophageal tumors to determine whether any relationship could be observed between chromosomal deletions and int-2 amplification. Furthermore, we analyzed the relationship between the loss of heterozygosity and the clinicopathological characteristics of each tumor.

MATERIALS AND METHODS

Thirty-five patients with esophageal squamous cell carcinoma who were operated on at Kyoto University Hospital were studied (Table 1). In each case, samples of tumor and normal mucosal tissue were frozen immediately after surgical removal and stored at \(-80^\circ\text{C}\) until isolation of DNA. Histological studies were also performed by members of the Clinical Pathology Department of the hospital.

High molecular weight DNA was isolated from the tumors and normal esophageal mucosal tissues according to standard procedures with phenol extraction (31). Restriction endonuclease digestion of the DNA samples, agarose gel electrophoresis, labeling the probes by nick-translation, Southern hybridization, and autoradiography were performed as described previously (31).

The 23 polymorphic DNA probes used in this study are listed in Table 2 with the restriction enzymes used to detect RFLPs. The loci detected by these probes are described in Human Gene Mapping 10 (32). The probe pHRVNR was previously described (33). The probe p68RS2.0 was obtained from Dr. T. P. Dryja and probes 6929 and SS6 were obtained from Japanese Resources for Cancer Study (Gene Bank). All other RFLP probes were obtained from Dr. Y. Nakamura.

To determine the degree of amplification of the int-2 and hst-1 genes, DNA samples were digested with EcoRI, and Southern hybridizations were performed with the int-2 gene probe obtained from Japanese Resources for Cancer Study (Gene Bank) or with the hst-1 gene probe (p0RF1) obtained from Dr. M. Terada. The densities of the bands were determined by scanning of the autoradiographs with the Gelman densitometer (ACD-8).

To correct the amount of DNA loaded on each lane, the filters were stripped and rehybridized with the probe of the \(\beta\)-actin gene which is located on chromosome 7 (35), and the densities of the bands were determined. The degree of amplification of int-2 and hst-1 genes was expressed as a ratio of the density of the tumor tissue to that of the corresponding normal tissue.

RESULTS

We analyzed 23 loci on 18 chromosomes to detect loss of heterozygosity in 35 esophageal squamous cell carcinomas. Fig.

\textsuperscript{1}Received 10/4/90; accepted 2/1/91.
\textsuperscript{2}To whom requests for reprints should be addressed.
\textsuperscript{3}The abbreviation used is: RFLP(s), restriction fragment length polymorphism(s).
Table 1: Clinicopathological characteristics* of patients with esophageal squamous cell carcinoma, the degree of amplification of int-2 and hst-1 genes, and chromosomal loss

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Location of lesion*</th>
<th>Lymph node</th>
<th>Depth of invasion#</th>
<th>Stage</th>
<th>Histology'</th>
<th>Amplification of int-2</th>
<th>Amplification of hst-1</th>
<th>Chromosomes on which loss was detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>69</td>
<td>F</td>
<td>E</td>
<td>n4</td>
<td>a2</td>
<td>IV</td>
<td>Poor</td>
<td>×6</td>
<td>×6</td>
<td>17p, 18p</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>M</td>
<td>Im</td>
<td>n0</td>
<td>sm</td>
<td>0</td>
<td>Well</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>76</td>
<td>M</td>
<td>Im</td>
<td>n2</td>
<td>a3</td>
<td>IV</td>
<td>Poor</td>
<td>×3</td>
<td>×3</td>
<td>17p</td>
</tr>
<tr>
<td>4</td>
<td>66</td>
<td>F</td>
<td>Ce</td>
<td>n2</td>
<td>a3</td>
<td>IV</td>
<td>Poor</td>
<td>×3</td>
<td>×3</td>
<td>3p, 17p, 21q, 22</td>
</tr>
<tr>
<td>5</td>
<td>58</td>
<td>M</td>
<td>Im</td>
<td>n3</td>
<td>a1</td>
<td>IV</td>
<td>Poor</td>
<td>×1</td>
<td>×1</td>
<td>17p, 22</td>
</tr>
<tr>
<td>6</td>
<td>59</td>
<td>M</td>
<td>Im</td>
<td>n3</td>
<td>a1</td>
<td>IV</td>
<td>Poor</td>
<td>×1</td>
<td>×1</td>
<td>17p, 22</td>
</tr>
<tr>
<td>7</td>
<td>58</td>
<td>F</td>
<td>E</td>
<td>n0</td>
<td>a1</td>
<td>II</td>
<td>Mod</td>
<td>×1</td>
<td>×1</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>71</td>
<td>M</td>
<td>E</td>
<td>n4</td>
<td>a2</td>
<td>IV</td>
<td>Mod</td>
<td>×1</td>
<td>×1</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>55</td>
<td>M</td>
<td>Im</td>
<td>n3</td>
<td>mp</td>
<td>IV</td>
<td>Well</td>
<td>×1</td>
<td>×1</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>51</td>
<td>M</td>
<td>Ce</td>
<td>n2</td>
<td>a2</td>
<td>III</td>
<td>Well</td>
<td>×3</td>
<td>×3</td>
<td>2q, 14q</td>
</tr>
<tr>
<td>11</td>
<td>75</td>
<td>M</td>
<td>Im</td>
<td>n0</td>
<td>a3</td>
<td>IV</td>
<td>Mod</td>
<td>×9</td>
<td>×9</td>
<td>13q, 14q, 17p</td>
</tr>
<tr>
<td>12</td>
<td>59</td>
<td>M</td>
<td>Im</td>
<td>n0</td>
<td>sm</td>
<td>0</td>
<td>Well</td>
<td>×9</td>
<td>×9</td>
<td>11q</td>
</tr>
<tr>
<td>13</td>
<td>63</td>
<td>M</td>
<td>Im</td>
<td>n0</td>
<td>a1</td>
<td>II</td>
<td>Poor</td>
<td>×1</td>
<td>×1</td>
<td>12q</td>
</tr>
<tr>
<td>14</td>
<td>55</td>
<td>M</td>
<td>E</td>
<td>n1</td>
<td>a3</td>
<td>II</td>
<td>Mod</td>
<td>×1</td>
<td>×1</td>
<td>7p, 16q, 17p</td>
</tr>
<tr>
<td>15</td>
<td>60</td>
<td>F</td>
<td>Im</td>
<td>n3</td>
<td>mp</td>
<td>IV</td>
<td>Mod</td>
<td>×1</td>
<td>×1</td>
<td>17p</td>
</tr>
<tr>
<td>16</td>
<td>66</td>
<td>M</td>
<td>Im</td>
<td>n2</td>
<td>a2</td>
<td>III</td>
<td>Mod</td>
<td>×1</td>
<td>×1</td>
<td>17p</td>
</tr>
<tr>
<td>17</td>
<td>66</td>
<td>M</td>
<td>Im</td>
<td>n2</td>
<td>a2</td>
<td>III</td>
<td>Well</td>
<td>×3</td>
<td>×3</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>55</td>
<td>M</td>
<td>Im</td>
<td>n0</td>
<td>a3</td>
<td>IV</td>
<td>Mod</td>
<td>×6</td>
<td>×6</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>60</td>
<td>M</td>
<td>E</td>
<td>n4</td>
<td>a2</td>
<td>IV</td>
<td>Mod</td>
<td>×1</td>
<td>×1</td>
<td>6q</td>
</tr>
<tr>
<td>20</td>
<td>78</td>
<td>M</td>
<td>Im</td>
<td>n4</td>
<td>mp</td>
<td>IV</td>
<td>Poor</td>
<td>×9</td>
<td>×9</td>
<td>13q, 21q</td>
</tr>
<tr>
<td>21</td>
<td>58</td>
<td>M</td>
<td>Im</td>
<td>n2</td>
<td>a2</td>
<td>III</td>
<td>Mod</td>
<td>×1</td>
<td>×1</td>
<td>13q</td>
</tr>
<tr>
<td>22</td>
<td>62</td>
<td>M</td>
<td>Lu</td>
<td>n0</td>
<td>mp</td>
<td>I</td>
<td>Mod</td>
<td>×1</td>
<td>×1</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>78</td>
<td>F</td>
<td>Im</td>
<td>n2</td>
<td>a1</td>
<td>II</td>
<td>Mod</td>
<td>×3</td>
<td>×3</td>
<td>14q</td>
</tr>
<tr>
<td>24</td>
<td>75</td>
<td>M</td>
<td>Lu</td>
<td>n1</td>
<td>a1</td>
<td>II</td>
<td>Mod</td>
<td>×3</td>
<td>×3</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>57</td>
<td>F</td>
<td>E</td>
<td>n4</td>
<td>a2</td>
<td>III</td>
<td>Poor</td>
<td>×3</td>
<td>×3</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>72</td>
<td>M</td>
<td>Im</td>
<td>n3</td>
<td>a2</td>
<td>IV</td>
<td>Poor</td>
<td>×1</td>
<td>×1</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>46</td>
<td>M</td>
<td>Ce</td>
<td>n3</td>
<td>a2</td>
<td>IV</td>
<td>Mod</td>
<td>×1</td>
<td>×1</td>
<td>17p</td>
</tr>
<tr>
<td>28</td>
<td>65</td>
<td>M</td>
<td>—</td>
<td>n2</td>
<td>a1</td>
<td>IV</td>
<td>Well</td>
<td>×1</td>
<td>×1</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>73</td>
<td>F</td>
<td>E</td>
<td>n1</td>
<td>mp</td>
<td>II</td>
<td>Poor</td>
<td>×1</td>
<td>×1</td>
<td>13q, 14q, 17p, 17q</td>
</tr>
<tr>
<td>30</td>
<td>51</td>
<td>M</td>
<td>Lu</td>
<td>n1</td>
<td>a1</td>
<td>II</td>
<td>Poor</td>
<td>×1</td>
<td>×1</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>59</td>
<td>M</td>
<td>Ce</td>
<td>n2</td>
<td>sm</td>
<td>IV</td>
<td>—</td>
<td>×1</td>
<td>×1</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>62</td>
<td>M</td>
<td>Lu</td>
<td>n0</td>
<td>mp</td>
<td>I</td>
<td>Mod</td>
<td>×1</td>
<td>×1</td>
<td>17p, 22</td>
</tr>
<tr>
<td>33</td>
<td>60</td>
<td>M</td>
<td>E</td>
<td>n3</td>
<td>sm</td>
<td>IV</td>
<td>Poor</td>
<td>×3</td>
<td>×3</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>84</td>
<td>F</td>
<td>E</td>
<td>n0</td>
<td>a2</td>
<td>II</td>
<td>Mod</td>
<td>×1</td>
<td>×1</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>62</td>
<td>M</td>
<td>Lu</td>
<td>n4</td>
<td>a2</td>
<td>IV</td>
<td>Poor</td>
<td>×3</td>
<td>×3</td>
<td></td>
</tr>
</tbody>
</table>

* Ce, cervical esophagus; lu, upper intrathoracic esophagus; Im, middle intrathoracic esophagus; E, lower intrathoracic and abdominal esophagus.
* Degree of metastasis to the lymph nodes.
* mp, invasion to muscularis propria; a1, invasion reaching the adventitia; a2, definite invasion to the adventitia; a3, invasion to neighboring structures.

I shows examples of Southern hybridization with the pYNZ22 probe which is specific for chromosome 17p. Cases 11, 14, 15, and 32 showed loss of constitutional heterozygosity in tumor tissues, while cases 18 and 23 retained heterozygosity. Residual faint bands observed in tumor samples might be caused by contamination of normal cells. When it was difficult to determine whether there was loss, we performed densitometric analysis and only the cases in which the density of a fainter band was <50% of that of another one were determined as allele losses. The results of the analysis of 23 loci on 18 chromosomes are summarized in Table 2. Allele loss was found on chromosomes 2, 3, 6, 7, 11–14, 16–18, 21, and 22, while no loss was found on chromosomes 1, 4, and 8–10. Among the loci on which allele loss was observed, only the loss of the chromosome 17p...
Fig. 1. Examples of Southern blot analysis with the probe pYNZ22, which is homologous to the polymorphic locus on 17p13 (D17S5). Each DNA sample was digested with Taq. Cases 18 and 23 retain constitutional heterozygosity and cases 11, 14, 15, and 32 show loss of heterozygosity. T, tumor; N, normal esophageal mucosal tissue.

The examples of amplification of the hst-1 and int-2 genes and degree of amplification in each sample is shown in Fig. 2 and Table 1. Because of limited amounts of DNA samples, the degree of amplification was not determined in all cases. The amplification of the int-2 gene was detected in 39% (12 of 31 cases) with 3- to 9-fold amplifications. Coamplification of the hst-1 gene was detected in all cases with the int-2 amplification except one, case 35. In case 35 only the amplification of the int-2 gene was detected. No significant relationship was observed between the amplification of the int-2/hst-1 genes and the loss of heterozygosity on 17p or any other chromosomes.

The clinicopathological data for each case are also summarized in Table 1. Since only 17p showed significant frequency of loss of heterozygosity, we correlated clinicopathological data with the loss or retention of constitutional heterozygosity on 17p and also the frequencies of the cases with the int-2 gene amplification (Table 3). No statistically significant correlation was observed among them, but the loss of heterozygosity on 17p showed a slight correlation with the histological type of the tumor.

DISCUSSION

In this report we have shown that loss of heterozygosity was observed at loci on 13 of 18 chromosomes in esophageal squamous cell carcinomas. Among them, only the frequency of allele loss at the locus of D17S5 on chromosome 17p was significantly high (45%, 10 of 22), while the frequencies of loss at other loci were relatively low (<22%). We surmise that losses at low frequencies might be caused by random chromosomal rearrangement during development of the tumors. It has been reported that cytogenetic analysis of a cell line established from esophageal carcinoma detected a number of abnormalities and structural rearrangements of chromosomes such as 1, 9, 14, X, and Y (36). Although this was not consistent with our present data, this cytogenetic study was performed with only one cell line and its results might not represent general phenomenon in esophageal carcinoma cells. Allelic deletions seem to be rather common in human tumors, and deletions of multiple chromosomes with substantial frequencies, which might be caused by random chromosomal rearrangement, have been noted in colorectal cancers (37). Loss of heterozygosity on 17p has been found in a variety of human cancers, e.g., lung cancers (10), colorectal cancers (11, 12), breast cancers (13), osteosarcomas (14, 15), bladder cancers (16), and malignant astrocytomas (17).

It has also been reported that the majority of the deletions represent subchromosomal events such as interstitial deletion, recombination, or gene conversion rather than loss of a whole chromosome (36). Consistent with this notion, the present data show that the frequency of allele loss on chromosome 17q is as low as that of loci on other chromosomes and that loss of chromosome 17 in esophageal carcinomas appears to be restricted to the short arm. By using other polymorphic DNA markers spanning chromosome 17, we may be able to determine more precisely the region on 17p which is commonly deleted in esophageal carcinomas. In any case, the present data suggest that deletion of 17p in esophageal carcinoma is specific and that a tumor suppressor gene which might be inactivated in the development of this tumor may be located on 17p. In this regard, it is intriguing that the p53 gene has been mapped on 17p13 (38). Although the p53 gene was previously considered to be one of the dominantly acting transforming genes (39-41), a recent experiment has shown that the wild type of p53 gene has properties of a tumor suppressor gene (42). It has been reported that in colorectal cancers with deletion of one copy of 17p, including the region where the p53 gene is located, point...
mutations are present in the remaining alleles of the p53 gene (43). Furthermore, introduction of the wild type of p53 gene into colorectal cancer cell lines suppressed their growth (44). It has also been reported that in several tumors point mutations were detected in conservative domains of the p53 gene, suggesting that inactivation of the p53 gene might be involved in the development of many types of tumors, such as glioblastomas, breast cancers, and colon cancers (45). Very recently, point mutations have also been discovered in esophageal squamous cell carcinoma (30), which is very consistent with our findings of the chromosome 17p loss. Our tumor samples with loss of 17p might possess mutations on the remaining alleles of the p53 locus. However, we have not yet analyzed DNA sequences of the p53 alleles in these cases. Mutations in the p53 alleles in both cases, with or without loss of the chromosome 17p, should be analyzed.

A high incidence of coamplification of hst-1 and int-2 genes in esophageal carcinomas (52%) has been reported (22, 23). In agreement with these reports, amplification of the int-2 gene was observed in 39% of our cases and the degrees of amplification were 3- to 9-fold compared to the corresponding normal tissues. Although these results suggest that amplification of the int-2 and hst-1 genes may play an important role in tumorigenesis and/or tumor progression in esophageal carcinomas, its actual role still remains to be elucidated at the molecular level. Since no correlation was observed between the amplification of int-2 gene and allele loss on chromosome 17p, these two genetic alterations are probably independent in the development of esophageal carcinoma.

In this study, the relationship among chromosomal deletions, amplification of the int-2 gene, and clinicopathological findings was analyzed. We could not detect any statistically significant relationship between any combination of characteristics described in Table 1 except that when there was a loss of heterozygosity on 17p the carcinoma was histologically more malignant (Table 3). Recently, Vogelstein et al. (37) studied the loss of heterozygosity on all chromosomes in colorectal cancers and found that patients with increased rates of chromosomal deletions had a considerably worse prognosis than those with low rates of chromosomal loss. Further studies of a larger number of patients with polymorphic markers on all chromosomes may reveal relationships between chromosomal deletions and clinicopathological features in esophageal carcinomas and may provide useful information for the diagnosis and treatment of patients.

ACKNOWLEDGMENTS

We are grateful to Dr. Yusuke Nakamura for his generous gifts of many RFLP probes, to Dr. Thaddeus P. Dryja for generously providing the p68RS2.0 probe and to Dr. Masaaki Terada for the p0RF1 probe. We also thank Miki Wada for her excellent help in preparation of the manuscript.

REFERENCES


Deletion of 17p and Amplification of the int-2 Gene in Esophageal Carcinomas
Takashi Wagata, Kanji Ishizaki, Masayuki Imamura, et al.

Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/51/8/2113

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.