Genetic evolution of α fetoprotein producing gastric cancer

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ORIGINAL ARTICLE

Background: α Fetoprotein (AFP) producing gastric cancer is an unusual form of aggressive adenocarcinoma with a complex histological picture, including enteroblastic and hepatoid differentiation.

Aims: To investigate the genetic events underlying the phenotypic diversity in AFP producing gastric cancer and the ability of these tumours to produce AFP ectopically.

Methods: Multiple foci from 19 AFP producing gastric adenocarcinomas were microdissected and loss of heterozygosity (LOH) analysis was performed with a panel of microsatellite markers on nine chromosomal arms.

Results: For informative cases, LOH was most frequently detected on 17p (100%), followed by 13q (88%), 3p (87%), 5q and 9p (80%), 11q (70%), 18q (58%), 16q (53%), and 8p (50%). The average fractional allelic loss was 0.72. LOH was detected either homogeneously throughout the microdissected foci, or only in some parts of the neoplastic foci for each case. Heterogeneous patterns of LOH indicated genetic progression and/or divergence in clonal evolution. Furthermore, in six cases with heterogeneous LOH of 13q, 13q LOH was restricted to immunohistochemically AFP positive neoplastic foci.

Conclusion: AFP-GC arises as an aggressive clone with extensive LOH and high fractional allelic loss. The presence of heterogeneous patterns of LOH suggested that the AFP producing carcinoma foci might evolve through genetic progression and/or genetic divergence. Silencing of the crucial gene on 13q may be involved in the acquisition of the AFP producing phenotype.

Alpha fetoprotein (AFP) is a normal fetal serum glycoprotein, which is synthesised and secreted by fetal hepatocytes, yolk sac cells, and fetal gastrointestinal cells.1 2 Synthesis usually stops at birth, and hence its presence in the serum after 1 year of age is associated with pathological conditions.1 2 The presence of serum AFP has been well documented in some human cancers, including hepatocellular carcinoma (HCC) and yolk sac tumour. Serum AFP is raised in up to 80–90% of patients with HCC, and is a useful marker for the detection of HCC, in addition to monitoring tumour recurrence after surgery and adjuvant treatment.2

AFP producing gastric cancer (AFP-GC) was first described by Bourreille in 1970.3 Ishikura et al proposed the term “hepatoid adenocarcinoma of the stomach” for a gastric cancer with the histological features of hepaticytic differentiation and AFP production.3 Although rare, reported cases of AFP-GC have been accumulating, and this entity is now well recognised.3-12 Most AFP-GC cases show an aggressive clinical course, with frequent metastasis to the liver, lymph nodes, and other organs, and more than 90% of patients succumb within five years.3 6 10 11 13 Morphologically, in addition to the well documented hepatoid appearance of the tumour cells, these tumours often contain cells with clear cytoplasm and tubule formation, which are indicative of fetal enteroblastic differentiation.4 In fact, many of these tumours contain a combination of areas with ordinary tubular histology, tubulopapillary adenocarcinoma foci, poorly differentiated medullary carcinoma, and enteroblastic and hepatoid foci.4-7 9 11

“Inactivation of tumour suppressor genes through deletion or mutation is thought to be crucial in the development of various human tumours”

To our knowledge, no genetic studies have been conducted on AFP-GC. The genetic mechanism behind its unusually complex histology, capacity for heterotopic AFP production, and its aggressiveness still remains unknown. Inactivation of tumour suppressor genes through deletion or mutation is thought to be crucial in the development of various human tumours. Loss of heterozygosity (LOH) is the deletion of the normal allele of a chromosome region. LOH at a specific chromosomal locus in a tumour could indicate the presence of a tumour suppressor gene in the region of loss. Using microdissected tumour samples and subsequent LOH analysis, we previously revealed that genetic progression and heterogeneity accompany the phenotypic or histological diversity of tumours such as gynaecological carcinosarcomas and combined hepatocellular–cholangiocarcinomas.4 14 Thus, these genetic approaches would be useful methods for the genetic analysis of AFP-GCs showing complex histology. In our present study, in an attempt to reveal the genetic basis of the clonal evolution of AFP-GC, we microdissected multiple foci from 19 such cases and LOH patterns were analysed with a panel of microsatellite markers on nine chromosomal arms.

MATERIALS AND METHODS

Tissue samples

Nineteen cases of AFP-GC were identified in the archival pathology files of the pathology division, National Cancer Centre Research Institute and Hospital, Tokyo, Japan, and the department of pathology at Jikei University, School of Medicine, Tokyo, Japan. All the cases selected had been tested for the quality of DNA preservation for adequate and reproducible polymerase chain reaction (PCR) amplification. The samples came from 16 men and three women, whose ages ranged from 40 to 80 years, with a mean age of 61. All

Abbreviations: AFP, α fetoprotein; AFP-GC, α fetoprotein producing gastric cancer; FAL, Fractional allelic loss; LOH, loss of heterozygosity; HCC, hepatocellular carcinoma; H&E, haematoxylin and eosin; HI, heterogeneity index; PCR, polymerase chain reaction
the patients showed raised serum AFP concentrations, ranging from 42 to 2177 000 ng/ml. All samples were formalin fixed, paraffin wax embedded tissue blocks from surgically resected tumours. The maximum diameter of the tumours ranged from 18 mm to 120 mm. The depth of tumour invasion was to the submucosa in one patient, muscularis propria in three, subserosa in five, and exposure to the serosal surface in 10. Lymph node metastases were detected in 14 patients. Histology of all the cases showed a complex mixture of ordinary tubular, tubulopapillary adenocarcinoma, poorly differentiated adenocarcinoma with medullary growth pattern, enteroblastic foci with tubular formation and clear cytoplasm, and areas of hepatoid differentiation with large polygonal cells with eosinophilic cytoplasm in solid or trabecular patterns. Periodic acid Schiff positive hyaline globules were frequently seen. In 11 cases, in addition to invasive foci, preinvasive intramucosal neoplastic foci were identified. Table 1 summarises the clinicopathological profiles.

**AFP immunohistochemistry**

Paraffin wax block sections of 3 μm were immunohistochemically stained for AFP using anti-AFP monoclonal antibody (A0008; 1/5000 dilution; Dakopatts, Glostrup, Denmark). Except for cases HP24 and HP36 (AFP negative by stains but showed serum AFP of 300 ng/ml and 125 ng/ml, respectively), the AFP stains were positive for at least some of the neoplastic foci. The percentage of AFP positive foci ranged from less than 1% (case HP27) to 70% (cases HP6 and HP15) of the tumour area. Table 1 summarises the staining results.

**Tissue microdissection and DNA extraction**

Multiple tumour foci with different histological profiles, in addition to AFP positive and negative foci, were individually selected for microdissection. Serial 8 μm sections were cut, dewaxed, stained with haematoxylin and eosin (H&E), visualised with an inverted microscope, and microdissected using a 27 gauge needle. At least 90% of the microdissected cells were visually estimated to be tumour cells. For each case, four to 10 foci were microdissected. Stromal and inflammatory cells were collected separately and used as a normal control. The microdissected tissue was digested overnight at 50°C in buffer containing 0.5% NP40, 50mM Tris/HCl, pH 8.0, 1mM EDTA, and 200 μg/ml proteinase K. The lysate was heated at 95°C for 10 minutes, and stored at −20°C until used directly in the PCR reaction.

**Detection of LOH**

The PCR reactions contained 1 μl of DNA lysate, 0.4μM [γ-32P] ATP radiolabelled microsatellite primers, 0.2mM dNTP, 10mM Tris/HCl, pH 8.3, 1.5mM MgCl2, 50nM KCl, and 0.4 U Taq polymerase in a total reaction volume of 10 μl. Taq was added to the reactions, which were prewarmed to 94°C (hot start PCR), and the samples were amplified with 35 cycles of PCR amplification. On average, each PCR reaction contained 100–200 cells. The PCR products were separated on a 5% denaturing polyacrylamide/urea/formamide gel and visualised using a phosphorimager (Bas 2500 Bio Imaging System; Fuji Film, Tokyo, Japan). The presence of LOH was defined as more than a 75% reduction of the relative intensity in one of the two alleles compared with the normal control. When only a proportion of the microdissected foci showed LOH, the PCR reactions were repeated at least three times in duplicate to confirm the LOH and to exclude spurious PCR reactions. Other informative microsatellite markers located on the same chromosomal arm also confirmed LOH. If necessary, the microdissection was repeated. Fractional allelic loss (FAL) was calculated for each case as the number of chromosomal arms with at least one of the microdissected foci showing LOH divided by the number of chromosomal arms with informative markers. Because homogeneous LOH throughout the microdissected tumour foci is presumed to have occurred early in the neoplastic evolution, and heterogeneous LOH later during clonal progression and divergence, we calculated the heterogeneity index (HI) of each chromosomal arm as follows: HI is expressed as the number of cases with heterogeneous patterns of allelic loss divided by the total number of cases with one or more microdissected focus showing LOH.

**Microsatellite markers**

All of the PCR primers for the microsatellite markers except for D13S171, D13S260, D13S168, and D13S154 were pur-

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**Table 1** Summary of clinicopathological profiles

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Serum AFP (ng/ml)</th>
<th>Maximum diameter (mm)</th>
<th>Mucosal lesion</th>
<th>Depth</th>
<th>Presence of Ent and/or Hep foci</th>
<th>AFP immunostain</th>
<th>LN metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP1</td>
<td>M</td>
<td>54</td>
<td>125</td>
<td>70</td>
<td>+</td>
<td>ss</td>
<td>Ent</td>
<td>40–50%</td>
<td>+</td>
</tr>
<tr>
<td>HP2</td>
<td>F</td>
<td>51</td>
<td>3342</td>
<td>37</td>
<td>+</td>
<td>mp</td>
<td>Ent</td>
<td>20–30%</td>
<td>+</td>
</tr>
<tr>
<td>HP4</td>
<td>F</td>
<td>60</td>
<td>42</td>
<td>70</td>
<td>–</td>
<td>se</td>
<td>Ent</td>
<td>&lt;5%</td>
<td>–</td>
</tr>
<tr>
<td>HP5</td>
<td>M</td>
<td>55</td>
<td>1092</td>
<td>60</td>
<td>–</td>
<td>ss</td>
<td>Ent/Hep</td>
<td>50%</td>
<td>–</td>
</tr>
<tr>
<td>HP6</td>
<td>M</td>
<td>66</td>
<td>25 400</td>
<td>42</td>
<td>–</td>
<td>ss</td>
<td>Ent/Hep</td>
<td>60–70%</td>
<td>–</td>
</tr>
<tr>
<td>HP7</td>
<td>M</td>
<td>42</td>
<td>15 551</td>
<td>25</td>
<td>+</td>
<td>ss</td>
<td>Ent/Hep</td>
<td>50–60%</td>
<td>–</td>
</tr>
<tr>
<td>HP8</td>
<td>M</td>
<td>74</td>
<td>130</td>
<td>40</td>
<td>+</td>
<td>sm</td>
<td>Ent</td>
<td>50%</td>
<td>–</td>
</tr>
<tr>
<td>HP9</td>
<td>M</td>
<td>53</td>
<td>179 500</td>
<td>75</td>
<td>+</td>
<td>se</td>
<td>Ent/Hep</td>
<td>&lt;5%</td>
<td>–</td>
</tr>
<tr>
<td>HP12</td>
<td>M</td>
<td>53</td>
<td>39 980</td>
<td>90</td>
<td>+</td>
<td>se</td>
<td>Ent/Hep</td>
<td>5–10%</td>
<td>+</td>
</tr>
<tr>
<td>HP14</td>
<td>M</td>
<td>51</td>
<td>4103</td>
<td>74</td>
<td>+</td>
<td>se</td>
<td>Ent/Hep</td>
<td>10%</td>
<td>–</td>
</tr>
<tr>
<td>HP15</td>
<td>M</td>
<td>40</td>
<td>2.177×10^4</td>
<td>40</td>
<td>+</td>
<td>se</td>
<td>Ent/Hep</td>
<td>70%, faint/</td>
<td>–</td>
</tr>
</tbody>
</table>

| HP23 | M   | 66  | 1040             | 95                   | –             | se    | Ent                           | <10%            | –            |
| HP24 | M   | 65  | 300              | 85                   | +             | ss    | Hep                           | Negative        | –            |
| HP26 | M   | 77  | 5300             | 100                  | +             | se    | Ent/Hep                       | <5%            | +            |
| HP27 | F   | 80  | 11 000           | 90                   | +             | se    | Hep                           | <1%            | +            |
| HP36 | M   | 67  | 125              | 60                   | –             | mp    | Hep                           | Negative        | –            |
| HP38 | M   | 54  | 2100             | 120                  | –             | se    | Ent/Hep                       | <3%            | +            |
| HP40 | M   | 75  | 537              | 75                   | Equivocal     | se    | Ent/Hep                       | <10%            | –            |
| HP41 | M   | 72  | 511              | 18                   | +             | mp    | Hep                           | <3%            | –            |

AFP, α fetoprotein; Ent, focal enteroblastic foci are present; Ent/Hep, both enteroblastic and hepatoid foci are present; Hep, focal hepatoid foci are present; LN, lymph node; mp, muscularis propria; se, exposure to the serosal surface; sm, submucosa; ss, subserosa.
chromosomes were homogeneously deleted in all of the foci microdissected for each case, indicating a clonal neoplastic process. FAL ranged from 0.25 to 1.00, with an average of 0.72. Table 2 summarises the results of the genetic analysis.

### Heterogeneity index

Figure 1 shows the cumulative frequencies of homogeneous/heterogeneous LOH and HI. The lowest HI was seen for 18q (0.14), followed by 3p (0.15) and 17p (0.17). Therefore, these chromosomal deletions must occur early in the development of neoplasia. The highest HI was seen for 13q (0.60), followed by 9p and 16q (0.38). These genetic alterations tend to occur later during clonal genetic progression and/or divergence.

### Clonal evolution of AFP-GC

When patterns of homogeneous and heterogeneous LOH within the tumour are reconstituted, the relation between clonal evolution and tumour histology/AFP production was evident. Figures 2 and 3 show the neoplastic clone derived from the mucosal lesion with early LOH of 3p, 5q, 9p, 17p, and 18q in case HP1. Histologically, these mucosal and invasive foci showed an increase in the genetic diversity of the tumour clone progressing with LOH of 13q in microdissected T2 and T3 foci, showing enteroblastic histology and acquiring AFP producing ability. In case HP15 (fig 4), homogeneous and extensive LOH of 3p, 5q, 8p, 9p, and 11q was detected throughout the microdissected mucosal and invasive foci. Additional LOH of 13q was detected in the invasive foci with enteroblastic, poorly differentiated, solid and hepatoid foci, but not in the invasive foci.

### Table 2 Summary of genetic analysis

<table>
<thead>
<tr>
<th>Case</th>
<th>Homogeneous LOH</th>
<th>Heterogeneous LOH</th>
<th>Genetic pattern</th>
<th>FAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP1</td>
<td>3p (D3S1293), 5q (D5S644), 9p (D9S1748, D9S1752), 17p (TP53, CHRNB1), 18q (D18S487, D18S55)</td>
<td>13q (D13S171, D13S154)</td>
<td>Progression</td>
<td>0.75</td>
</tr>
<tr>
<td>HP2</td>
<td>3p (D3S1293), 5q (D5S644), 9p (D9S1748, D9S1752)</td>
<td>13q (D13S171, D13S154)</td>
<td>Progression</td>
<td>0.57</td>
</tr>
<tr>
<td>HP4</td>
<td>3p (D3S1293), 9q (D5S644), 9p (D9S1748, D9S1752)</td>
<td>13q (D13S171, D13S154)</td>
<td>Progression</td>
<td>0.57</td>
</tr>
<tr>
<td>HP5</td>
<td>3p (D3S1293), 5q (D5S644), 9q (D9S1748, D9S1752), 17p (TP53, CHRNB1)</td>
<td>13q (D13S171, D13S154)</td>
<td>Progression</td>
<td>0.57</td>
</tr>
<tr>
<td>HP6</td>
<td>3p (D3S1293), 11q (D11S29), 13q (D13S171)</td>
<td>13q (D13S171, D13S154)</td>
<td>Progression</td>
<td>0.57</td>
</tr>
<tr>
<td>HP7</td>
<td>3p (D3S1293), 5q (D5S644), 9q (D9S1748, D9S1752)</td>
<td>13q (D13S171, D13S154)</td>
<td>Progression</td>
<td>0.57</td>
</tr>
<tr>
<td>HP8</td>
<td>3p (D3S1293), 5q (D5S644), 11q (D11S29), 16q (D16S265, D16S261)</td>
<td>13q (D13S171, D13S154)</td>
<td>Progression</td>
<td>0.57</td>
</tr>
<tr>
<td>HP9</td>
<td>3p (D3S1293), 5q (D5S644), 7p (TP53, D17S786)</td>
<td>13q (D13S171, D13S154)</td>
<td>Progression</td>
<td>0.57</td>
</tr>
<tr>
<td>HP10</td>
<td>3p (D3S1293), 5q (D5S644), 9q (D9S1748, D9S1752)</td>
<td>13q (D13S171, D13S154)</td>
<td>Progression</td>
<td>0.57</td>
</tr>
<tr>
<td>HP11</td>
<td>3p (D3S1293), 5q (D5S644), 9q (D9S1748, D9S1752)</td>
<td>13q (D13S171, D13S154)</td>
<td>Progression</td>
<td>0.57</td>
</tr>
<tr>
<td>HP12</td>
<td>3p (D3S1293), 5q (D5S644), 9q (D9S1748, D9S1752)</td>
<td>13q (D13S171, D13S154)</td>
<td>Progression</td>
<td>0.57</td>
</tr>
<tr>
<td>HP13</td>
<td>3p (D3S1293), 5q (D5S644), 9q (D9S1748, D9S1752)</td>
<td>13q (D13S171, D13S154)</td>
<td>Progression</td>
<td>0.57</td>
</tr>
<tr>
<td>HP14</td>
<td>3p (D3S1293), 5q (D5S644), 9q (D9S1748, D9S1752)</td>
<td>13q (D13S171, D13S154)</td>
<td>Progression</td>
<td>0.57</td>
</tr>
<tr>
<td>HP15</td>
<td>3p (D3S1293), 5q (D5S644), 9q (D9S1748, D9S1752)</td>
<td>13q (D13S171, D13S154)</td>
<td>Progression</td>
<td>0.57</td>
</tr>
<tr>
<td>HP16</td>
<td>3p (D3S1293), 5q (D5S644), 9q (D9S1748, D9S1752)</td>
<td>13q (D13S171, D13S154)</td>
<td>Progression</td>
<td>0.57</td>
</tr>
<tr>
<td>HP17</td>
<td>3p (D3S1293), 5q (D5S644), 9q (D9S1748, D9S1752)</td>
<td>13q (D13S171, D13S154)</td>
<td>Progression</td>
<td>0.57</td>
</tr>
<tr>
<td>HP18</td>
<td>3p (D3S1293), 5q (D5S644), 9q (D9S1748, D9S1752)</td>
<td>13q (D13S171, D13S154)</td>
<td>Progression</td>
<td>0.57</td>
</tr>
<tr>
<td>HP19</td>
<td>3p (D3S1293), 5q (D5S644), 9q (D9S1748, D9S1752)</td>
<td>13q (D13S171, D13S154)</td>
<td>Progression</td>
<td>0.57</td>
</tr>
<tr>
<td>HP20</td>
<td>3p (D3S1293), 5q (D5S644), 9q (D9S1748, D9S1752)</td>
<td>13q (D13S171, D13S154)</td>
<td>Progression</td>
<td>0.57</td>
</tr>
<tr>
<td>HP21</td>
<td>3p (D3S1293), 5q (D5S644), 9q (D9S1748, D9S1752)</td>
<td>13q (D13S171, D13S154)</td>
<td>Progression</td>
<td>0.57</td>
</tr>
<tr>
<td>HP22</td>
<td>3p (D3S1293), 5q (D5S644), 9q (D9S1748, D9S1752)</td>
<td>13q (D13S171, D13S154)</td>
<td>Progression</td>
<td>0.57</td>
</tr>
<tr>
<td>HP23</td>
<td>3p (D3S1293), 5q (D5S644), 9q (D9S1748, D9S1752)</td>
<td>13q (D13S171, D13S154)</td>
<td>Progression</td>
<td>0.57</td>
</tr>
<tr>
<td>HP24</td>
<td>3p (D3S1293), 5q (D5S644), 9q (D9S1748, D9S1752)</td>
<td>13q (D13S171, D13S154)</td>
<td>Progression</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Microsatellite markers listed showed LOH for each case. *a, b* allelic losses of the opposite chromosomal arms; complex, single clonal process cannot be confirmed because of the complexity of the LOH pattern; divergence, single clonal process with subsequent genetic divergence; homogeneous, all the foci show the same genetic alteration; progression, linear genetic progression of a clone. FAL, fractional allelic loss; LOH, loss of heterozygosity.

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Figure 1  Frequency of homogeneous and heterogeneous loss of heterozygosity (LOH). Solid bars indicate number of cases with homogenous LOH, hatched bars indicate number of cases with heterogeneous LOH, open bars indicate cases with retention, and shaded bars indicate heterogeneity index.

Figure 2  Representative histology for case HP1. (A) Low power view of the section showing the microdissected foci (T1–T4). T1, microdissected mucosal focus; T2–T4, microdissected invasive foci in the muscularis propria. (B, C) Histology of the microdissected foci (haematoxylin and eosin stain). (B) T4, invasive foci with tubulopapillary carcinoma. T4 is negative for the α fetoprotein (AFP) stain. (C) T2, invasive foci with enteroblastic histology. T2 is immunoreactive for the AFP stain. (D, E) Representative AFP immunohistochemical stains. (D) Negative AFP stain for mucosal focus T1. (E) Positive AFP stain for invasive enteroblastic focus T2.
mucosal foci that were microdissected. AFP was strongly positive in the invasive foci, but unstained to faintly stained in the mucosal foci. Thus, enteroblastic and hepatoid foci genetically progressed from the early mucosal tumour, acquiring an AFP producing phenotype. However, the possibility of additional undetected genetic alterations between enteroblastic and hepatoid foci cannot be excluded. In case HP40 (figs 5 and 6), the lesion started with early LOH of 3p, 5q, 9p, 17p, and 18q, which was detected in the moderately/poorly differentiated adenocarcinoma foci (T1) and enteroblastic foci (T2). Further genetic progression and divergence was detected in hepatoid foci (AFP positive) with 13q LOH (T3) or 11q LOH (T4).

Furthermore, in six cases (cases HP1, HP2, HP9, HP12, HP13, HP15), the lesion started with early LOH of 3p, 5q, 9p, 17p, and 18q, which was detected in the moderately/poorly differentiated adenocarcinoma foci (T1) and enteroblastic foci (T2). Further genetic progression and divergence was detected in hepatoid foci (AFP positive) with 13q LOH (T3) or 11q LOH (T4). Furthermore, in six cases (cases HP1, HP2, HP9, HP12, HP13, HP15), the lesion started with early LOH of 3p, 5q, 9p, 17p, and 18q, which was detected in the moderately/poorly differentiated adenocarcinoma foci (T1) and enteroblastic foci (T2). Further genetic progression and divergence was detected in hepatoid foci (AFP positive) with 13q LOH (T3) or 11q LOH (T4).
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HP15, and HP40) with heterogeneous patterns of 13q LOH, 13q LOH was preferentially detected in AFP immunoreactive foci with solid, poorly differentiated adenocarcinoma foci and enteroblastic and/or hepatoid differentiation.

DISCUSSION

AFP is a glycoprotein that is produced by the fetal liver and fetal intestine. Embryologically, the liver arises as a ventral outgrowth of the distal end of the fetal foregut. Because of the embryological proximity, it has been speculated that the emergence of AFP producing hepatoid foci, or enteroblastic foci in gastric cancer, results from the dedifferentiation of the tumour cells to these progenitor cell types. Our present genetic study has revealed the genetic process of so called dedifferentiation of gastric carcinoma cells in the evolution of AFP-GC for the first time. Extensive LOH with high FAL (average of 0.72) seen in AFP-GC, as compared with the reported FAL of 0.288–0.472 in gastric cancer, may reflect the very aggressive biological behaviour and grave prognosis known for these tumours. Because extensive LOH was detected homogenously throughout the microdissected foci in many cases, AFP-GC may initially arise as a very aggressive clone with high FAL, and may clonally expand very rapidly. Thus, phenotypic diversity seems to be conferred by extensive genetic alterations of these neoplastic clones. Alternatively, AFP-GC may evolve through genetic progression and/or divergence with the acquisition of the AFP producing phenotype. We saw cases in which ordinary tubular or tubulopapillary carcinoma foci genetically progressed or diverged in some of the invasive foci. Only these progressed foci acquired the capacity to produce AFP. These foci showed various histological features, including poorly differentiated medullary carcinoma, enteroblastic carcinoma, and hepatoid carcinoma. In fact, in most of the cases with recognisable mucinous lesions, genetic progression, and/or divergence occur in the invasive foci, indicating that the AFP-GC phenotype is acquired in the invasive foci. Previous studies have described the presence of hepatoid foci in deep invasive foci, which can be clearly explained by our clonal genetic patterns. There are case reports of only metastatic gastric cancer foci showing a hepatoid pattern and AFP production. These cases may also be examples of clonal genetic progression and divergence in the metastatic foci with the acquisition of the AFP producing phenotype.

“‘It is interesting that the loci of 13q that are commonly deleted in α fetoprotein (AFP) producing gastric carcinoma are also frequently deleted in hepatocellular carcinoma, which often presents with raised serum AFP values’”

In ordinary gastric cancers, LOH has been frequently detected on 1p, 1q, 2q, 3p, 4p, 5p, 6p, 7p, 8p, 9p, 11q, 13q, 14q, 17p, 18q, and 21q, In hepatocellular carcinomas, 1q, 2q, 4q, 6q, 8p, 8q, 9q, 10q, 13q, 14q, 16q, 17p, and 19p have been shown to be frequently deleted, Thus, most of the regions of LOH detected in AFP-GC are also commonly deleted loci in both GC and HCC. However, there may be several crucial LOH regions that are particularly important for ectopic AFP production and the unique histological features of AFP-GC.

In our present study, 13q LOH was often associated with AFP immunoreactive tumour foci. One possibility is that a certain gene on 13q may function as a negative regulator of AFP production in normal cells. LOH of the gene locus and additional alteration of the remaining allele may silence or downregulate the gene. These possibilities remain to be investigated in future work. It is interesting that the loci of 13q that are commonly deleted in AFP-GC are also frequently deleted in HCC, which often presents with raised serum AFP values.

Other than the stomach, rare cases of AFP producing or hepatoid carcinomas have been reported in the extrahaepatic organs, including the lung, ovary, endometrium, colon-rectum, gall bladder, urinary bladder, kidney, and pancreas. Most showed aggressive clinical behaviour, with metastasis at the time of diagnosis. Histologically, many of them show a complex combination of various histological patterns, including poorly differentiated adenocarcinoma, hepatoid foci, enteroblastic foci with clear cytoplasm, and

Take home messages

- α Fetoprotein producing gastric carcinoma (AFP-GC) arises as an aggressive clone with extensive loss of heterozygosity (LOH) and high fractional allelic loss
- Because heterogeneous patterns of LOH are seen, the AFP producing carcinoma foci might evolve through genetic progression and/or genetic divergence
- Silencing of a crucial tumour suppressor gene on 13q may be involved in the acquisition of the AFP producing phenotype

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yolk sac tumour-like foci. None of these cases has been genetically studied, but we speculate that similar and unique clonal genetic alterations may be involved in the abnormal differentiation and ectopic induction of the AFP gene in these tumours also.

In conclusion, AFP-GC arises as an aggressive clone with extensive LOH. Further genetic progression and divergence may confer subclones with the capacity to produce AFP and unique histological features.

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REFERENCES
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Genetic evolution of α fetoprotein producing gastric cancer

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