ORIGINAL ARTICLE

Genetic evolution of α fetoprotein producing gastric cancer

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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 hepatocytic differentiation and AFP production.⁴ Although rare, reported cases of AFP-GC have been accumulating, and this entity is now well recognised.^{5–12} 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.^{5,6,10,11,13} 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.^{8,9} 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.^{4,5,7–9,11}

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 chromosomal 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.^{14,15} 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

“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

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 μ m 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 patterns, in addition to AFP positive and negative foci, were individually selected for microdissection. Serial 8 μ m 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 μ g/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 μ l of DNA lysate, 0.4 μ M [γ -³²p] ATP radiolabelled microsatellite primers, 0.2mM dNTP, 10mM Tris/HCl, pH 8.3, 1.5mM MgCl₂, 50mM KCl, and 0.4 U Taq polymerase in a total reaction volume of 10 μ l. 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 phosphoimager (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-

Table 1 Summary of clinicopathological profiles

Case	Sex	Age	Serum AFP (ng/ml)	Maximum diameter (mm)	Mucosal lesion	Depth	Presence of Ent and/or Hep foci	AFP immunostain	LN metastasis
HP1	M	54	125	70	+	ss	Ent	40–50%	+
HP2	F	51	3342	37	+	mp	Ent	20–30%	+
HP4	F	60	42	70	–	se	Ent	<5%	+
HP5	M	55	1092	60	–	ss	Ent/Hep	50%	–
HP6	M	66	25 400	42	–	ss	Ent/Hep	60–70%	–
HP7	M	42	15 551	25	+	ss	Ent/Hep	50–60%	–
HP8	M	74	130	40	+	sm	Ent	50%	–
HP9	M	53	179 500	75	–	se	Ent/Hep	<5%	+
HP12	M	53	39880	90	+	se	Ent/Hep	5–10%	+
HP14	M	51	4103	74	+	se	Ent/Hep	10%	+
HP15	M	40	2.177 $\times$ 10 ⁶	40	+	se	Ent/Hep	70%, faint/negative in the mucosa	+
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	1100	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	<5%	+

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.

chased from Research Genetics (Huntsville, Alabama, USA). The microsatellite markers were selected to cover chromosomal regions commonly deleted in gastric cancer, hepatocellular carcinoma, and many other types of epithelial cancer. The following primers were used: 3p (D3S1234, D3S1286, and D3S1293), 5q (D5S1956, D5S2072, D5S647, D5S644, and D5S346), 8p (D8S264, D8S261, D8S258, and D8S133), 9p (D9S1749, D9S1748, and D9S1752), 11q (Int2 and D11S29), 13q (D13S263 and D13S166), 16q (D16S265 and D16S261), 17p (TP53, D17S786, and CHRN1), and 18q (D18S474, D18S487, D18S46, and D18S55).

Primers for the following markers on 13q were custom made with the following sequences: D13S260 (13q12–13), forward, 5'-tgtctccgttcacgaaaa-3', reverse, 5'-cccagatataag gacctggcta-3'; D13S171 (13q12.3–13), forward, 5'-tagcaaca caagaaaaggatgc-3', reverse 5'-tctcccttcacatcctc-3'; D13S168 (13q14.3), forward, 5'-catgatgctggactggacat-3', reverse, 5'-tgcttgcttgctcctatgtt-3'; D13S154 (13q31–32), forward, 5'-agcctgcctgtctcattc-3', reverse, 5'-gccttggtcttgactggtt-3'.

RESULTS

Loss of heterozygosity

Overall, of nine chromosomal arms, an average of 4.6 chromosomal arms were deleted for each case. 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%). LOH was detected either homogeneously throughout the microdissected foci or only in some of the foci, as described below. Except for two cases (HP24 and HP26), one to six chromosomal alleles 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 cumulated 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 ordinal papillary and moderately differentiated tubular adenocarcinoma without AFP immunoreactivity (T1 and T4). The tumour clone progressed 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

Table 2 Summary of genetic analysis

Case	Homogeneous LOH	Heterogeneous LOH	Genetic pattern	FAL
HP1	3p (D3S1293), 5q (D5S644), 9p (D9S1748, D9S1752), 17p (CHRN1), 18q (D18S474, D18S487, D18S55)	13q (D13S171, D13S154)	Progression	0.75
HP2	3p (D3S1293), 5q (D5S644), 9p (D9S1748, D9S1752)	13q (α , β) (D13S171, D13S263, D13S154)	Divergence	0.57
HP4	3p (D3S1234), 11q (Int2), 13q (D13S171), 18q (D18S474, D18S55)		Homogeneous	0.67
HP5	13q (D13S260, D13S263, D13S168, D13S166, D13S154), 16q (D16S261)		Homogeneous	0.40
HP6	3p (D3S1234, D3S1293), 5q (D5S647, D5S644), 8p (D8S261, D8S133), 13q (D13S260, D13S171, D13S168, D13S166), 16q (D16S261), 17p (TP53, D17S786)		Homogeneous	0.67
HP7	3p (D3S1234, D3S1293), 16q (D16S265, D16S261)	9p (D9S1748, D9S1752), 17p (TP53, CHRN1)	Progression	0.50
HP8	13q (D13S154), 17p (TP53, D17S786, CHRN1)	8p (D8S133)	Progression	1.00
HP9	5q (D5S647, D5S644), 8p (D8S261, D8S133), 17p (TP53, CHRN1), 18q (D18S474, D18S55)	9p (D9S1748), 11q (Int 2), 13q (D13S260, D13S171, D13S168, D13S166)	Divergence	1.00
HP12	3p (D3S1293), 5q (D5S644), 11q (D11S29), 16q (D16S265, D16S261), 17p (TP53, CHRN1), 18q (D18S474)	13q (D13S168, D13S260)	Progression	0.88
HP14	3p (D3S1234, D3S1293), 17p (D17S786, CHRN1)	5q (D5S644), 9p (D9S1748), 16q (D16S265)	Divergence	0.65
HP15	3p (D3S1293), 5q (D5S647, D5S644), 8p (D8S133), 9p (D9S1748, D9S1752), 11q (Int 2)	13q (D13S171, D13S166, D13S154)	Progression	0.86
HP23	9p (D9S1748, D9S1752), 11q (Int2), 16q (D16S265), 17p, (CHRN1, TP53), 18q (D18S487)	13q (D13S171, D13S154)	Progression	0.86
HP24		3p (D3S1234), 5q (D5S647, D5S644), 8p (D8S133), 13q (D13S171, D13S166), 16q (D16S265)	Complex	1.00
HP26	3p (D3S1234), 5q (D5S647), 13q (D13S171, D13S166), 16q (D16S265, D16S261) (only in invasive foci)	3p (D3S1234), 5q (D5S647), 13q (D13S171, D13S166), 16q (D16S265, D16S261), 18q (D18S474, D18S55) (mucosal foci)	Complex (mucosa)	1.00
HP27	3p (D3S1234)	17p (α , β) (D17S786, CHRN1)	Homogeneous (invasion)	0.25
HP36	13q (D13S171, D13S168, D13S166, D13S154), 17p (TP53)		Divergence	0.50
HP38	5q (D5S644), 11q (D11S29, Int 2), 13q (D13S260, D13S171, D13S168), 17p (TP53)		Homogeneous	0.67
HP40	3p (D3S1293), 5q (D5S647), 8p (D8S261, D8S133), 17p (CHRN1)	11q (Int 2, D11S29), 13q (D13S171, D13S168, D13S166, D13S154)	Homogeneous	0.88
HP41	3p (D3S1293), 5q (D5S647, D5S644), 9p (D9S1749, D9S1748), 18q (D18S474, D18S487)		Divergence	0.57

Microsatellite markers listed showed LOH for each case.
 α , β , 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.

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.

Figure 3 Representative gels and proposed genetic pathway for case HP1. Gels for D18S474 and D5S644 show homogeneous loss of heterozygosity (LOH) for all of the foci microdissected. Arrowheads in the gels indicate LOH at all microdissected foci (T1–T4). Gels for D13S171 and D13S154 show 13q LOH only in invasive enteroblastic and α fetoprotein (AFP) positive foci, T2 and T3, but retention of mucosal (T1) and invasive tubulopapillary carcinoma foci (T4). Arrows indicate LOH of lower alleles for T2 and T3. Dot, normal alleles; N, normal control DNA; T, microdissected foci. The diagram summarises early LOH of 3p, 5q, 9p, 17p, and 18q, followed by the progressive LOH of 13q in α fetoprotein positive enteroblastic foci.

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, 8p, and 17p, 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,

Figure 4 Representative gels and proposed genetic pathway for case HP15. Arrowhead in the gel for D9S1752 indicates homogeneous loss of heterozygosity (LOH) of all T1–T4 foci. Arrows in the gel for D13S171 indicate LOH of T1, T2, and T4, but not T3. Dot, normal alleles; N, normal control DNA; T1, T2, and T4, microdissected submucosal invasive foci with enteroblastic and hepatoid features; T3, intramucosal tumour focus microdissected. α Fetoprotein (AFP) is strongly positive in invasive foci but faint/negative in the mucosal T3 focus. The diagram shows early and homogeneous LOH of 3p, 5q, 8p, 9p, and 11q, including the mucosal focus. Subsequent LOH of 13q is identified in invasive enteroblastic and hepatoid foci (T1, T2, and T4).

Figure 5 Representative histology for case HP40. (A) Ordinary adenocarcinoma focus in the submucosa, T1 (haematoxylin and eosin (H&E) stain). T1 and T2 foci are negative for α fetoprotein (AFP) immunostaining. (B) Hepatoid carcinoma foci in the muscularis propria, T3 (H&E stain). Hepatoid foci, T3 and T4, are positive for AFP immunostaining.

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.² 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.^{1,2} 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,¹⁶ may reflect the very aggressive biological behaviour and grave prognosis known for these tumours. Because extensive LOH was detected homogeneously 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 mucosal 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.^{7,17} 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, 5q, 6p, 7p, 8p, 9p, 11q, 13q, 14q, 17p, 18q, and 21q.^{16,18–21} In hepatocellular carcinomas, 1q, 2q, 4q, 6q, 8p, 8q, 9p, 9q, 10q, 13q, 14q, 16q, 17p, and 19p have been shown to be frequently deleted.^{22–25} 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.^{23,25,26}

Other than the stomach, rare cases of AFP producing or hepatoid carcinomas have been reported in the extrahepatic organs, including the lung,²⁷ ovary,²⁸ endometrium,²⁹ colorectum,³⁰ gall bladder,³¹ urinary bladder,³² kidney,³³ and pancreas.³⁴ 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

Figure 6 Gels and deduced genetic pathways for HP40. Arrowheads in gels for D3S1293 and D5S647 indicate loss of heterozygosity (LOH) of all foci T1–T4. Arrows in the gels for Int 2 (11q) and D11S29 indicate 11q LOH of only T4. Arrows in the gels for D13S171 and D13S154 indicate 13q LOH of only T3. Dot, normal alleles; N, normal control DNA; T, microdissected foci. T1 is a microdissected submucosal focus, and T2–T4 are microdissected foci in the muscularis propria. The diagram summarises early and homogeneous LOH of 3p, 5q, 8p, 17p, followed by divergent LOH of 13q in α fetoprotein (AFP) positive hepatoid foci (T3), and 11q LOH in another hepatoid focus (T4).

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

- Gitlin D, Perricelli A, Gitlin GM. Synthesis of α -fetoprotein by liver, yolk sac, and gastrointestinal tract of the human conceptus. *Cancer Res* 1972;**32**:979–82.
- Alpert E. Human alpha1-fetoprotein (AFP): developmental biology and clinical significance. *Prog Liver Dis* 1976;**5**:337–49.

- Bourrelle J, Metayer P, Sauger F, et al. Existence d'alpha foetoprotéine au cours d'un cancer secondaire du foie d'origine gastrique. *Presse Med* 1970;**78**:1277–8.
- Ishikura H, Fukasawa Y, Ogasawara K, et al. An AFP-producing gastric carcinoma with features of hepatic differentiation. A case report. *Cancer* 1985;**56**:840–8.
- deLorimier A, Park F, Aranha GV, et al. Hepatoid carcinoma of the stomach. *Cancer* 1993;**71**:293–6.
- Giustozzi G, Goracci G, Bufalari A, et al. Hepatoid carcinoma of the stomach: is it still an unusual anatomo-clinical entity? Six case-reports. *J Exp Clin Cancer Res* 1999;**18**:571–3.
- Kodama T, Kameya T, Hirota T, et al. Production of alpha-fetoprotein, normal serum proteins, and human chorionic gonadotropin in stomach cancer: histologic and immunohistochemical analyses of 35 cases. *Cancer* 1981;**48**:1647–55.
- Matsunou H, Konishi F, Jalal RE, et al. Alpha-fetoprotein-producing gastric carcinoma with enteroblastic differentiation. *Cancer* 1994;**73**:534–40.
- Motoyama T, Aizawa K, Watanabe H, et al. Alpha-fetoprotein producing gastric carcinomas: a comparative study of three different subtypes. *Acta Pathol Jpn* 1993;**43**:654–61.
- Nagai E, Ueyama T, Yao T, et al. Hepatoid adenocarcinoma of the stomach. A clinicopathologic and immunohistochemical analysis. *Cancer* 1993;**72**:1827–35.
- Ishikura H, Kimoto K, Shamoto M, et al. Hepatoid adenocarcinomas of the stomach. An analysis of seven cases. *Cancer* 1986;**58**:119–26.
- Ritter JH, Mills SE, Gaffey MJ, et al. Clear cell tumors of the alimentary tract and abdominal cavity. *Semin Diagn Pathol* 1997;**14**:213–19.
- Chang YC, Nagasue N, Kohno H, et al. Clinicopathologic features and long-term results of alpha-fetoprotein-producing gastric cancer. *Am J Gastroenterol* 1990;**85**:1480–5.
- Fujii H, Zhu XG, Matsumoto T, et al. Genetic classification of combined hepatocellular-cholangiocarcinoma. *Hum Pathol* 2000;**31**:1011–17.
- Fujii H, Yoshida M, Gong ZX, et al. Frequent genetic heterogeneity in the clonal evolution of gynecological carcinomas and its influence on phenotypic diversity. *Cancer Res* 2000;**60**:114–20.
- Choi SW, Park SW, Lee KY, et al. Fractional allelic loss in gastric carcinoma correlates with growth patterns. *Oncogene* 1998;**17**:2655–9.

- 17 Kang GH, Kim YI. Alpha-fetoprotein-producing gastric carcinoma presenting focal hepatoid differentiation in metastatic lymph nodes. *Virchows Arch* 1998;**432**:85–7.
- 18 Cho JH, Noguchi M, Ochiai A, et al. Loss of heterozygosity of multiple tumor suppressor genes in human gastric cancers by polymerase chain reaction. *Lab Invest* 1996;**74**:835–41.
- 19 Nishizuka S, Tamura G, Terashima M, et al. Loss of heterozygosity during the development and progression of differentiated adenocarcinoma of the stomach. *J Pathol* 1998;**185**:38–43.
- 20 Tahara E. Molecular mechanism of stomach carcinogenesis. *J Cancer Res Clin Oncol* 1993;**119**:265–72.
- 21 Yustein AS, Harper JC, Petroni GR, et al. Allelotype of gastric adenocarcinoma. *Cancer Res* 1999;**59**:1437–41.
- 22 Nagai H, Pineau P, Tiollais P, et al. Comprehensive allelotyping of human hepatocellular carcinoma. *Oncogene* 1997;**14**:2927–33.
- 23 Okabe H, Ikai I, Matsuo K, et al. Comprehensive allelotype study of hepatocellular carcinoma: potential differences in pathways to hepatocellular carcinoma between hepatitis B virus-positive and -negative tumors. *Hepatology* 2000;**31**:1073–9.
- 24 Piao Z, Park C, Park JH, et al. Allelotype analysis of hepatocellular carcinoma. *Int J Cancer* 1998;**75**:29–33.
- 25 Lin YW, Sheu JC, Liu LY, et al. Loss of heterozygosity at chromosome 13q in hepatocellular carcinoma: identification of three independent regions. *Eur J Cancer* 1999;**35**:1730–4.
- 26 Kuroki T, Fujiwara Y, Nakamori S, et al. Evidence for the presence of two tumour-suppressor genes for hepatocellular carcinoma on chromosome 13q. *Br J Cancer* 1995;**72**:383–5.
- 27 Ishikura H, Kanda M, Ito M, et al. Hepatoid adenocarcinoma: a distinctive histological subtype of alpha-fetoprotein-producing lung carcinoma. *Virchows Arch A Pathol Anat Histopathol* 1990;**417**:73–80.
- 28 Ishikura H, Scully RE. Hepatoid carcinoma of the ovary. A newly described tumor. *Cancer* 1987;**60**:2775–84.
- 29 Hoshida Y, Nagakawa T, Mano S, et al. Hepatoid adenocarcinoma of the endometrium associated with alpha-fetoprotein production. *Int J Gynecol Pathol* 1996;**15**:266–9.
- 30 Hocking GR, Shembrey M, Hay D, et al. Alpha-fetoprotein-producing adenocarcinoma of the sigmoid colon with possible hepatoid differentiation. *Pathology* 1995;**27**:277–9.
- 31 Vardaman C, Albores-Saavedra J. Clear cell carcinomas of the gallbladder and extrahepatic bile ducts. *Am J Surg Pathol* 1995;**19**:91–9.
- 32 Sinard J, Macleay L, Jr, Melamed J. Hepatoid adenocarcinoma in the urinary bladder. Unusual localization of a newly recognized tumor type. *Cancer* 1994;**73**:1919–25.
- 33 Ishikura H, Ishiguro T, Enatsu C, et al. Hepatoid adenocarcinoma of the renal pelvis producing alpha-fetoprotein of hepatic type and bile pigment. *Cancer* 1991;**67**:3051–6.
- 34 Yano T, Ishikura H, Wada T, et al. Hepatoid adenocarcinoma of the pancreas. *Histopathology* 1999;**35**:90–2.

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