

Figure 1. Multiple genetic and epigenetic alterations during human stomach carcinogenesis (intestinal type). \* Epigenetic alterations. LOH, loss of heterozygosity; HP, *Helicobacter pylori*. From Tahara (2002)

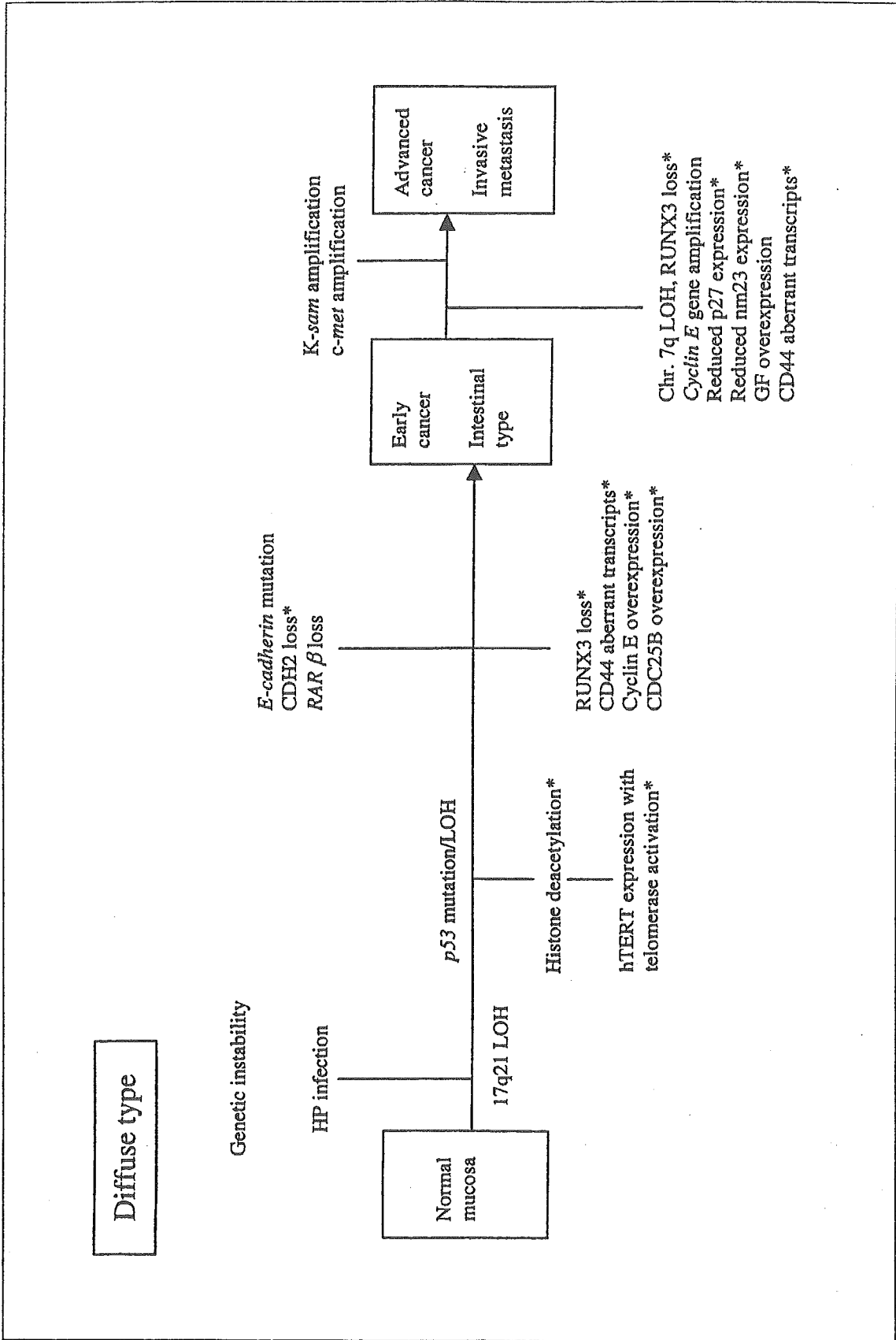


Figure 2. Multiple genetic and epigenetic alterations during human stomach carcinogenesis (diffuse type). \* Epigenetic alterations. LOH, loss of heterozygosity; HP, *Helicobacter pylori*; GF, growth factor. From Tahara (2002)

### Tumour suppressor genes

Alterations in the structure and function of tumour-suppressor genes, including *p53*, *p73*, *APC*, *DCC* and *FHIT*, are involved in stomach carcinogenesis. Among them, inactivation of the *p53* tumour-suppressor gene by LOH and mutation is the most frequent genetic event in gastric cancer, occurring in over 60% of gastric carcinomas regardless of histological type (Sano *et al.*, 1991; Tamura *et al.*, 1991; Yokozaki *et al.*, 1992). Alterations in the *p53* gene are also found in 13–37% of intestinal metaplasias and 33–58% of gastric adenomas or dysplasias (Tohdo *et al.*, 1993; Sakurai *et al.*, 1995; Ochiai *et al.*, 1996), indicating that *p53* gene mutation is an early event in stomach carcinogenesis. The mutation spectrum of this gene can serve as a marker of the effect of putative carcinogens (Harris, 1991). The mutation spectrum of the *p53* gene in gastric cancers in Hiroshima displays an intermediate pattern between those of colonic cancer and esophageal cancer (Uchino *et al.*, 1993; Maesawa *et al.*, 1995; Poremba *et al.*, 1995). *p53* Mutations at A:T sites are common in intestinal-type carcinomas; GC→AT transitions are predominant in diffuse-type carcinomas (Yokozaki *et al.*, 1992). Carcinogenic *N*-nitrosamines, which cause mainly GC→AT base substitutions, are found in many foods and can also be produced from amines and nitrates in the acidic environment of the stomach (Sugimura *et al.*, 1970; Mirvish, 1971).

LOH of the *p73* gene, a newly discovered tumour-suppressor gene related to *p53*, is detected in 38% of gastric cancers, especially intestinal adenocarcinomas that exhibit papillary structure similar to foveolar epithelium and express the pS2 trefoil factor (Yokozaki *et al.*, 1999a).

This type of gastric cancer with *p73* LOH shows allele-specific expression of *p73* but no gene mutation in the remaining allele. In addition, the incidence of *p53* abnormalities is low (25%). These observations indicate that LOH and abnormal expression of the *p73* gene may play a large role in the genesis of foveolar-type gastric adenocarcinoma, although this is not in line with Knudson's classic 'two-hit' model of carcinogenesis. We have already reported that 25% of intestinal gastric cancers show LOH on chromo-

some 1p by restriction fragment length polymorphism analysis using the MS1 (1p33–p35) probe (Sano *et al.*, 1991). However, these loci are rather centromeric when compared with the mapped region of the *p73* gene (1p36–33).

*APC* is a tumour-suppressor gene that is a susceptibility factor for familial polyposis coli (Kinzler *et al.*, 1991). Mutations in the *APC* gene also take place in gastric cancers and sporadic colorectal cancers. Interestingly, more than 50% of intestinal-type gastric cancers harbour *APC* mutations, whereas diffuse-type gastric cancers have none. Moreover, there is a distinct difference in the nature of *APC* mutations between gastric and colorectal cancers: missense mutation is dominant in gastric cancer whereas nonsense and frameshift mutations are common in colorectal cancers (Nakatsuru *et al.*, 1992). Somatic mutations of the *APC* gene are also seen in 20–40% of gastric adenomas and 6% of incomplete intestinal metaplasias (Nakatsuru *et al.*, 1993; Nishimura *et al.*, 1995). *APC* alteration is viewed as an early genetic event in the pathogenesis of intestinal-type gastric cancers (Yokozaki *et al.*, 1997). LOH at the *DCC* locus also is one of the characteristics of intestinal-type gastric cancer and is seen in 50–60% of primary gastric cancers (Sano *et al.*, 1991; Uchino *et al.*, 1992).

The hypothesis that *FHIT* gene alterations are involved in the development of primary gastric cancer remains controversial. Huebner's group reported the rearrangement of the *FHIT* gene, aberrant transcripts or both in 53% of primary gastric cancers and loss of *FHIT* protein in 67% (Ohta *et al.*, 1996; Baffa *et al.*, 1998). Chen *et al.* (1997a) demonstrated that aberrant transcripts were found not only in 46% of gastric cancers but also in 30% of non-cancerous gastric mucosae. Other studies showed that 13–16% of primary gastric cancers shared LOH of the *FHIT* gene and no abnormal transcripts (Tamura *et al.*, 1997; Noguchi *et al.*, 1999), although four of seven gastric cancer cell lines exhibited LOH of the *FHIT* gene (Tamura *et al.*, 1997). *FHIT* gene alterations and loss of *FHIT* protein should be evaluated in series involving many cases of gastric cancer and precancerous lesions to determine whether environmental factors or putative carci-

nogens are associated with differences between countries in frequency of FHIT abnormalities.

Several distinct chromosomal loci are deleted in gastric cancers. LOH at 1q and 7q are frequently associated with intestinal gastric cancer, whereas loss of 1p is relatively common in advanced diffuse gastric cancer (Sano *et al.*, 1991). Moreover, LOH at the *bcl-2* gene locus is seen in many intestinal gastric cancers and colorectal cancers (Ayhan *et al.*, 1994). Our deletion mapping study on 7q shows that LOH at the D7S95 locus correlates well with peritoneal dissemination (Kuniyasu *et al.*, 1994a). Recently, investigators in a study on allelic loss in xenografted human gastric carcinomas reported a high degree of allelic loss on several chromosomal arms in 18 xenografted gastric adenocarcinomas: 3p (81%), 4p (64%), 5q (69%), 8p (57%), 13q (59%), 17p (80%) and 18q (61%) (Yustein *et al.*, 1999). From these assigned loci, candidates for the tumour-suppressor gene responsible for stomach carcinogenesis may be identified in the future.

pS2, a gastric-specific trefoil factor normally expressed in the gastric foveolar epithelial cells, may function as a gastric-specific tumour suppressor, since the inactivation of the *pS2* gene by gene targeting causes dysplasia, adenoma and adenocarcinoma of the glandular stomach in mice (Masiakowski *et al.*, 1982; Lefebvre *et al.*, 1996). Recently, we found that the reduction or loss of the *pS2* gene by DNA methylation at the promoter region occurs in intestinal metaplasias and gastric adenomas. Conversely, 32% of gastric cancers display strong expression of the *pS2* gene and 40% of gastric cancers, especially the intestinal type, show no expression (Fujimoto *et al.*, 2000). Reduced expression or loss of the *pS2* gene by promoter methylation may play a role in the early stages of carcinogenesis of intestinal stomach carcinoma.

Recent in-vivo and in-vitro studies suggest that the nuclear retinoic acid receptor  $\beta$  (RAR $\beta$ ) functions as a tumour suppressor and that loss of RAR $\beta$  by CpG promoter hypermethylation is associated with tumorigenesis (Lotan *et al.*, 1995; Seewaldt *et al.*, 1995; Hayashi *et al.*, 2001a). More recently, we found that hypermethylation of

the RAR $\beta$  gene promoter is preferentially observed in 64% of intestinal gastric cancers associated with reduced expression (Hayashi *et al.*, 2001b), but not in the diffuse type. Promoter hypermethylation is also detected in gastric intestinal metaplasia. Three gastric cancer cell lines (MKN-28, -45 and -74), all of which are derived from intestinal-type adenocarcinomas, exhibit a loss of RAR $\beta$  expression by promoter methylation. RAR $\beta$  expression is restored in these cell lines by 5-azacytidine or the histone deacetylase inhibitor trichostatin A. Overexpression of the RAR $\beta$  in MKN-28 cells induces G<sub>0</sub>-G<sub>1</sub> arrest, followed by down-regulation of the DNA methyltransferase 3 $\alpha$  and DNA demethylase, and up-regulation of the acetylated histone H4. These results suggest that inactivation of RAR $\beta$  as well as pS2 is implicated in gastric carcinogenesis of the intestinal type.

### Cell-cycle regulators

Genetic and epigenetic abnormalities in cell-cycle regulators are involved in the development and progression of gastric cancer by causing unbridled proliferation. Most gastric cancers are associated with overexpression of positive regulators and reduction or loss of negative regulators, both of which co-operate to drive normal cells into malignancy.

The *cyclin E* gene is amplified in 15–20% of gastric carcinomas that are associated with its overexpression. The gene amplification or overexpression of cyclin E, or both cause aggressiveness and lymph node metastasis (Akama *et al.*, 1995). *Cyclin D1* gene amplification, on the other hand, is exceptional in gastric carcinomas but frequently occurs in esophageal carcinoma (Yoshida *et al.*, 1996).

CDC25 phosphatases dephosphorylate threonine and tyrosine residues at positions 14 and 15 in the cyclin-dependent kinases (CDKs) and then activate them (Honda *et al.*, 1993). Three types of CDC25 have been identified: CDC25A, -B and -C (Nagata *et al.*, 1991). CDC25A is expressed early in the G<sub>1</sub> phase of the cell cycle; CDC25B is expressed in both the G<sub>1</sub>/S and G<sub>2</sub> phases (Jinno *et al.*, 1994) and CDC25C is predominantly expressed in the G<sub>2</sub> phase. CDC25B is over-

expressed in more than 70% of gastric cancers regardless of histological type and is closely correlated with tumour invasion and nodal metastasis (Kudo *et al.*, 1997). On the other hand, only 2% of gastric adenomas overexpress CDC25B. However, no gene amplification of *CDC25B* has been found in any gastric cancer. In 38% of gastric cancers, CDC25A is overexpressed but CDC25C is at very low or undetectable levels. Thus, the overexpression of CDC25B in tumour cells may stimulate progression of gastric cancer.

With regard to negative-cell cycle regulators, the p53-inducible CDK inhibitor p21 is associated with the senescence of non-neoplastic gastric epithelial cells (Harper *et al.*, 1993). In neoplastic lesions, the expression of p21 is seen in 78% of gastric adenomas and 76% of gastric adenocarcinomas regardless of p53 gene mutation, suggesting that a p53-independent pathway is substantially involved in the induction of p21 in gastric tumours (Yasui *et al.*, 1996a). In fact, the growth inhibition of transforming growth factor (TGF)- $\beta$  or retinoic acid is associated with p53-independent induction of p21 in a gastric cancer cell line (Akagi *et al.*, 1996). Moreover, the strong expression of p21 in cancer cells is frequently observed in advanced cancers and nodal metastasis, whereas there is no inverse correlation between p21 expression and proliferative activity measured by Ki-67. These findings indicate overall that the proliferative activity of gastric cancer cells is not solely dependent on control of the cell cycle by p21. In addition, mutation of the p21 gene is exceptional in gastric cancer (Akama *et al.*, 1996a) and a codon 31 polymorphism does not affect the expression levels of p21 (Akama *et al.*, 1996b).

p27, a member of the cip/kip family of CDK inhibitors, binds to a wide variety of cyclin/CDK complexes and inhibits kinase activity. We have found that growth suppression of interferon- $\beta$  is associated with the induction of p27 in a gastric cancer cell, TMK-1 (Kuniyasu *et al.*, 1997a). More importantly, reduction in p27 expression is frequently seen in advanced gastric cancers, whereas p27 is well preserved in 90% of gastric adenomas and 85% of early cancers (Yasui *et al.*, 1997). Gastric adenomas with reduction or loss of

p27 are capable of developing into malignancies. Reduced expression of p27 significantly correlates with depth of tumour invasion and nodal metastasis. Moreover, metastatic tumour cells in lymph nodes express p27 at lower levels than do cells in primary tumours, suggesting that tumour cells with reduction or loss of p27 may selectively metastasize to lymph nodes or distant organs (Yasui *et al.*, 1999a). The expression of p27 in gastric cancer is inversely correlated with the expression of cyclin E (Igaki *et al.*, 1995). Loss of p27 function and gain of cyclin E evidently stimulate progression and metastasis of gastric carcinomas. Reduction in p27 expression occurs at post-translational levels, resulting from ubiquitin-mediated proteosomal degradation rather than genetic abnormalities (Yasui *et al.*, 1999a).

Deletion or mutations of the *p16* gene are uncommon in primary gastric carcinomas (Igaki *et al.*, 1995; Lee *et al.*, 1997; Gunther *et al.*, 1998), but homozygous deletion of this gene has been found in two of eight gastric cancer cell lines and lack of p16 protein expression in five of eight gastric cancer cell lines (Akama *et al.*, 1996b). Another mechanism of *p16* gene silencing is hypermethylation of the 5'CpG island (Merlo *et al.*, 1995). Reduced expression of p16 protein, probably by gene methylation, is found in about 20% of primary gastric cancers regardless of their histological type (Yasui *et al.*, 1996b). In particular, loss of p16 protein is often seen in advanced cancers with nodal metastasis. Loss of p16 and p27 proteins may be associated with the progression of gastric carcinoma. Chen *et al.* (1997b) reported that aberrant RNA transcripts of the *p16* gene is noted in 30–45% of primary gastric cancers.

Iida *et al.* (2000) reported that the *p14* (*ARF*) gene is more frequently inactivated by LOH or DNA methylation in diffuse-type gastric cancer than in those of the intestinal type, suggesting that alterations of *p14* (*ARF*) may be involved in diffuse-type gastric carcinogenesis.

Major alterations in the *Rb* gene are also infrequent in primary gastric cancers (Constancia *et al.*, 1994). All primary tumours and all gastric cancer cell lines express pRb (Akama *et al.*, 1996b).

An important downstream target of cyclin/CDKs at the G1/S transition is a family of E2F transcription factors. Gene amplification of *E2F-1* is seen in 4% of gastric cancers and 25% of colorectal cancers. Overexpression of E2F is found in 40% of primary gastric carcinomas (Suzuki *et al.*, 1999). Moreover, E2F and cyclin E tend to be co-expressed in gastric cancer. In contrast, 70% of gastric cancers exhibit lower levels of E2F-3 expression than corresponding non-neoplastic mucosas. These results suggest that gene amplification and anomalous expression of the *E2F* gene may permit the development of gastric cancer.

### Cell-adhesion molecules and metastasis-related genes

Cell-adhesion molecules may also work as tumour suppressors. Mutations in the *E-cadherin* gene have been reported to occur preferentially in 50% of diffuse gastric carcinomas (Becker *et al.*, 1994). *E-cadherin* gene mutation is found in the diffuse component of mixed gastric carcinomas composed of both intestinal and diffuse types (Machado *et al.*, 1999). The results of Handschuh *et al.* (1999) indicate that *E-cadherin* mutations affecting exons 8 or 9 induce the scattered morphology, decrease cellular adhesion and increase cellular motility of diffuse gastric cancers. The mutations are even detected in intramucosal carcinoma (Muta *et al.*, 1996). *E-cadherin* germline mutations in familial gastric cancer have been reported since 1998, but their frequency is extremely rare (Guilford *et al.*, 1998; Iida *et al.*, 1999; Keller *et al.*, 1999; Yoon *et al.*, 1999). Kawanishi *et al.* (1995) found that a diffuse gastric carcinoma cell line, HSC-39, contained a mutation of the  $\beta$ -catenin gene. Moreover, Caca *et al.* (1999) reported that  $\beta$ - and  $\gamma$ -catenin mutations but not *E-cadherin* inactivation brought about constitutive Tcf transcriptional activity in gastric and pancreatic cancer cells. In addition to genetic alterations in *E-cadherin* and  $\beta$ -catenin, crosstalk between  $\beta$ -catenin and receptor tyrosine kinases including *c-met*, epidermal growth factor (EGF) receptor and *c-erbB2* takes place in gastric cancer cells *in vitro* and *in vivo*, leading to diffuse spreading or scattering of gastric cancer cells

(Ochiai *et al.*, 1994; Shibata *et al.*, 1996). These results indicate that genetic and epigenetic alterations in *E-cadherin* and catenins are involved in the development and progression of diffuse and scirrhus-type gastric cancers.

The *CD44* gene contains at least 20 exons, 12 of which can be alternatively spliced to make up a wide variety of molecular variants (Cooper *et al.*, 1992; Matsumura & Tarin, 1992). We have found that expression of abnormal CD44 transcripts, including exon 11, is frequently associated with primary gastric carcinomas and metastatic tumours (Yokozaki *et al.*, 1994). Moreover, the pattern of abnormal CD44 transcripts in the tumours differs between intestinal and diffuse gastric cancers. More importantly, all gastric cancer tissues and gastric cancer cell lines show overexpression of abnormal CD44 transcripts containing the intron 9 sequence (Higashikawa *et al.*, 1996), suggesting that the abnormal CD44 transcript containing the intron 9 sequence is presumably an effective biomarker for early detection of gastric cancers. Sixty per cent of gastric intestinal metaplasias express CD44 variants containing an intron 9 sequence; normal gastric mucosa does not express these variants (Yoshida *et al.*, 1995).

Osteopontin (OPN), also termed Eta-1 (early T-lymphocyte activation-1), which is a reported protein ligand of CD44, is overexpressed in 73% of gastric carcinomas (Weber *et al.*, 1996). The co-expression of OPN and CD44v9 in tumour cells correlates with the degree of invasion of lymphatic vessels or distant lymph node metastasis in diffuse gastric cancer (Ue *et al.*, 1998). In particular, clustering of the tumour cells in lymphatic vessels shows strong co-expression of OPN and Cd44v9. Therefore, mutual interactions between OPN and CD44v9 on the tumour cells may be used by CD44-bearing diffuse gastric carcinomas to promote lymphogenous metastasis.

A candidate suppressor gene related to metastasis, *nm23*, encodes nucleoside diphosphate kinase which may activate *c-myc* transcription factor. Although LOH of the *nm23* gene in gastric cancer is rare, the reduced expression of *nm23*, presumably as a result of epigenetic mechanisms, is frequently associated with metastasis of gastric

cancer (Nakayama *et al.*, 1993). In addition to *nm23*, galectin-3 (known as lactoside-binding lectin L-31), which belongs to a family of galactoside-binding proteins, is frequently over-expressed in primary tumours and liver metastases of gastric cancer of the intestinal type (Lotan *et al.*, 1994). This higher expression of galectin-3 in gastric cancers and metastases implicates this lectin in the metastatic phenotype.

Amplification of *c-met* or *K-sam* in gastric cancer evidently contributes to progression and peritoneal invasion of diffuse gastric carcinoma. In addition, peritoneal dissemination requires LOH of 7q. Our study on deletion mapping of 7q has already demonstrated that LOH at the D7S95 locus is frequently associated with peritoneal dissemination (Kuniyasu *et al.*, 1994a). The D7S95 locus may contain a candidate suppressor gene for the progression and metastasis of gastric cancer.

### Genetic instability

Two types of genetic instability involved are microsatellite instability (MSI) and chromosomal instability. MSI is caused by altered DNA mismatch repair. MSI has been found in 15–39% of sporadic gastric carcinomas worldwide (Semba *et al.*, 1996; Yokozaki *et al.*, 1999b). Gastric carcinomas with a high frequency of MSI (MSI-H) can be divided into two subtypes, intestinal and diffuse carcinomas, each of which has specific clinicopathological characteristics. Intestinal-type gastric cancers with MSI-H are often seen in patients over 73 years of age and often occur in the *antrum pylori*. They are frequently associated with abundant lymphoid infiltration, a putative favourable prognosis, and multiple tumours (Wu *et al.*, 1998; Leung *et al.*, 1999). Hypermethylation of the *hMLH1* gene promoter occurs in over 70% of cases with this type of gastric cancer and is often associated with down-regulation or loss of *hMLH1* (Fleisher *et al.*, 1999; Leung *et al.*, 1999). This evidence indicates that MSI-H in intestinal-type gastric cancer is mostly due to epigenetic inactivation of the *hMLH1* gene.

On the other hand, diffuse-type gastric cancers with MSI-H occur mostly in patients under 35 years of age, and are often accompanied by scirrhous-type carcinoma with diffusely produc-

tive fibrosis (Semba *et al.*, 1998). However, diffuse-type gastric cancers harbour no germline mutation of *hMLH1* and *hMSH2* and no alteration at *BAT-RII*. This type of gastric cancer is frequently associated with LOH on chromosome 17q21, including the *BRCA1* gene. However, we have found no mutation of the *BRCA1* gene. This raises two possibilities: (1) chromosome 17q12–21, including the *BRCA1* locus, may contain a candidate tumour suppressor gene; (2) allelic loss of the *BRCA1* gene may be linked to frequent genetic instability in young patients with gastric cancer.

Microsatellite instability at the locus D1S191 (chromosome 1q) is found in 46% of intestinal gastric cancers but not in diffuse-type gastric carcinomas. Microsatellite alteration at the same locus is also seen in 26% of incomplete-type intestinal metaplasias adjacent to primary gastric cancers. Moreover, an identical pattern of microsatellite alteration at the locus D1S191 is detected in both intestinal-type adenocarcinoma and the adjacent intestinal metaplasia, suggesting the sequential development of intestinal adenocarcinoma from incomplete intestinal metaplasia (Hamamoto *et al.*, 1997). The results described above indicate that MSI at the D1S191 locus is one of the early events in the multistep process of stomach carcinogenesis.

Chromosomal instability leading to DNA aneuploidy is also an underlying factor in stomach carcinogenesis. Telomere length is necessary for maintaining chromosomal stability. Recent evidence indicates that in the absence of telomerase, telomere shortening can produce telomere dysfunction that causes both DNA breaks and chromosome gain or loss (Chin *et al.*, 1999; Hackett *et al.*, 2001). Therefore, telomere dysfunction may initiate chromosomal instability in tumorigenesis. Conversely, telomerase can inhibit chromosomal instability (Hackett *et al.*, 2001). Most intestinal carcinomas have remarkably shortened telomere length, associated with high levels of telomerase activity and significant expression of human telomerase reverse transcriptase (hTERT) (Tahara *et al.*, 1995; Yasui *et al.*, 1998). More importantly, over 50% of intestinal metaplasias, as well as adenomas, express low levels of telo-

merase activity equivalent to about one-tenth of the activity in gastric carcinomas (Yasui *et al.*, 1999b).

Immunohistochemistry shows that the hTERT protein is strongly expressed in the nuclei of the tumour cells of all carcinomas but weakly expressed in the nuclei of epithelial cells of intestinal metaplasia and gastric adenoma and in normal fundic mucosa (Yasui *et al.*, 1998). Thus, hTERT-positive epithelial cells in the above precancerous lesions and normal gastric mucosa may be viewed as epithelial 'stem cells'. Moreover, the prevalence of *H. pylori* infection in gastric mucosa correlates well with the grade of intestinal metaplasia and the levels of hTERT and of telomerase activity; the latter is frequently associated with hyperplasia of hTERT-positive epithelial cells (Kuniyasu *et al.*, 1997b; Yasui *et al.*, 1999b). These observations indicate that *H. pylori* infection may be a strong trigger for hyperplasia of hTERT-positive cells in intestinal metaplasia, followed by increased telomerase activity and telomere reduction. Hyperplasia of hTERT-positive cells caused by *H. pylori* may induce 'chronic mitogenesis' which can facilitate increased mutagenesis. In fact, DNA hypermethylation at the D17S5 locus, *p52* loss, abnormal CD44 transcripts, CA repeat instability at the D1S19 locus, and *APC* and *p53* mutations, all of which are commonly seen in intestinal gastric cancer, occur in over 30% of incomplete intestinal metaplasias (Tahara, 1998).

These data all indicate that telomere reduction and hTERT overexpression due to stem-cell hyperplasia are very early events in the multistep development of intestinal-type gastric cancer, followed by the above-mentioned epigenetic and genetic alterations. The frequent development of intestinal-type gastric cancer in elderly patients with *H. pylori* infection suggests that this type of gastric cancer is a disease of a 'chronically afflicted genome' rather than a genetic disease.

Telomerase-negative gastric carcinomas are only of the diffuse type, not the intestinal type, although their incidence is 13–15% (Tahara *et al.*, 1995). Diffuse-type gastric cancers occasionally harbour extremely long telomere length and have genetic alterations that are different from those of

carcinomas of the intestinal type. Hence, a telomerase-independent or alternative mechanism may be involved in neoplastic transformation and immortalization of the cells of some diffuse-type gastric cancers.

Mutations in the *p53* gene are also implicated in chromosomal instability. Recently Kaplan and co-workers found that mutation in *APC* may be responsible for chromosomal instability in colon cancer (Kaplan *et al.*, 2001). *APC* protein directly binds to a kinetochore protein and is an avid in-vitro substrate of the mitotic check-point protein Bub1 (Pellman, 2001). It remains to examine whether gastric cancer cells carrying a truncated *APC* gene are defective in chromosome segregation. Mutations of the *hBub1* gene have been reported in colon cancers (Cahill *et al.*, 1998). However, there is no mutation in the *hBub1* gene in gastric carcinomas (Shigeishi *et al.*, 2001).

### Growth factors and cytokines

Gastric cancer cells express a broad spectrum of growth factors, cytokines or both, including TGF- $\alpha$ , TGF- $\beta$ 1, EGF, amphiregulin (AR), cripto, heparin binding (HB)-EGF, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF) II, basic fibroblast growth factor (bFGF), interleukin (IL)-1 $\alpha$ , IL-6, IL-8 and OPN (Tahara, 1993; Tahara *et al.*, 1994, 1996b; Tahara, 1997). These growth factors and cytokines function as autocrine, paracrine and juxtacrine modulators of the growth of cancer cells, and they organize the complex interaction between cancer cells and stromal cells which plays a key role in morphogenesis, invasion, neovascularization and metastasis. Interestingly, the expression of these growth factors, cytokines or both by cancer cells differs in the two histological types of gastric carcinoma. The EGF family, including EGF, TGF- $\alpha$  and cripto, is commonly overexpressed in intestinal-type gastric carcinoma, whereas TGF- $\beta$ , IGF-II and bFGF are predominantly overexpressed in the diffuse type (Tahara *et al.*, 1999). Co-expression of EGF/TGF- $\alpha$ , EGF receptor and cripto correlates well with the biological malignancy of gastric cancer, because these factors induce metalloproteinases (Yasui *et al.*, 1988; Yoshida *et al.*, 1990; Kuniyasu



*et al.*, 1994b). Overexpression of *cripto* is frequently associated with intestinal metaplasia and gastric adenoma (Kuniyasu *et al.*, 1991).

AR, a member of the EGF family which is overexpressed in more than 60% of gastric carcinomas regardless of histological type (Kitadai *et al.*, 1993a), works as an autocrine growth factor and induces the expression of AR itself, TGF- $\alpha$  and EGF receptors by gastric cancer cells (Akagi *et al.*, 1995). Overexpression of the EGF family in gastric cancer usually does not accompany gene amplifications. The relative expression levels of positive transcription factor, Sp-1, and negative transcription factor, GC factor, may regulate gene expression of these growth factors and receptors (Kitadai *et al.*, 1993b).

IL-1 $\alpha$  is a cytokine mainly produced by activated macrophages and mediates many of the local and systemic responses to infection and inflammation (Dinarello, 1992). It is also produced by gastric cancer cells. We have found that IL-1 $\alpha$  evidently acts as an autocrine growth factor for gastric carcinoma cells and plays a pivotal role as a trigger for induction of EGF and EGF receptor expression (Ito *et al.*, 1993). The expression of IL-1 $\alpha$  by tumour cells is induced by either IL-1 $\alpha$ , EGF or TGF- $\alpha$ , while IL-1 $\alpha$  up-regulates the expression of TGF- $\alpha$  and EGF receptor by tumour cells themselves, indicating that an intimate interplay between IL-1 $\alpha$  and the EGF/receptor system stimulates the growth of gastric cancer.

In addition to IL-1 $\alpha$ , IL-6 is also an autocrine growth stimulator for gastric cancer cells. The expression of IL-1 $\alpha$  by tumour cells is induced by IL-6, while IL-1 $\alpha$  increases the expression of IL-6 by tumour cells themselves (Ito *et al.*, 1997).

Stromal cells, especially fibroblasts stimulated by growth factors or cytokines such as IL- $\alpha$ , TGF- $\alpha$  and TGF- $\beta$ , secrete HGF/SF (hepatocyte growth factor/scatter factor), which can function in a paracrine manner as a morphogen or motogen of tumour cells. For example, in the case of a clone maintaining expression of cell-adhesion molecules, HGF/SF promotes tubular formation of tumour cells, resulting in intestinal-type gastric cancer. Conversely, in the case of a clone with reduced expression of cell-adhesion molecules, HGF/SF can act as a motogen and induce scatter-

ring of tumour cells, resulting in diffuse gastric cancer (Tahara, 1993; Yokozaki *et al.*, 1997). Our recent findings suggest that interaction between *c-met* overexpressed in tumour cells and HGF/SF from stromal cells is related to the morphogenesis and progression of gastric cancer *in vivo*.

The negative growth factor TGF- $\beta$ 1 is commonly overexpressed in gastric carcinoma, particularly in diffuse-type carcinoma with diffusely productive fibrosis (Yoshida *et al.*, 1989). However, most human gastric cancer cells have escaped from TGF- $\beta$ -induced growth inhibition at the receptor or post-receptor levels. TGF- $\beta$  inhibited the growth of only one (TMK-1) of seven gastric carcinoma cell lines; this inhibition is associated with p53-independent induction of p21 which induces suppression of cyclin-dependent kinase activity, reduced phosphorylation of Rb and a decrease in cyclin A (Ito *et al.*, 1992a; Akagi *et al.*, 1996). Various mutations in the TGF- $\beta$  receptor type II (RII) gene have been reported in gastric cancer. One type of mutation in the TGF- $\beta$ RII gene is mutation in the polyA tract (i.e. deletion or insertion of 1–2 bases) that frequently occurs in the hereditary non-polyposis colon cancer syndrome (Markowitz *et al.*, 1995) and in gastric carcinoma with MSI-H (Yokozaki *et al.*, 1999b). Another type of mutation in the TGF- $\beta$ RII gene involves abnormal amplification and truncation of the gene (Yang *et al.*, 1999). However, we have not seen genetic alterations of the TGF- $\beta$ RII gene in any gastric carcinoma cell lines. Moreover, results of a study on expression of TGF- $\beta$ RI in TGF- $\beta$ -resistant gastric cancer cell lines that contain no discernible alteration in the TGF- $\beta$ RII gene suggest that hypermethylation of a CpG island in the 5' region of the TGF- $\beta$ RI gene is involved in another potentially important mechanism of escape from negative growth control by TGF- $\beta$  (Kim *et al.*, 1999). We have already found that most gastric carcinomas show reduced levels of TGF- $\beta$ RI and that this correlates well with the depth of tumour invasion (Ito *et al.*, 1992b).

A large number of angiogenic factors have been identified in human malignancy. Among them are vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and IL-8, which

are derived from tumour cells and participate mainly in neovascularization within gastric carcinoma tissues. We have shown that all eight gastric cancer cell lines secrete VEGF into conditioned media (Yamamoto *et al.*, 1998). EGF or IL-1 $\alpha$  up-regulates VEGF expression by tumour cells, whereas interferon- $\gamma$  down-regulates it. Moreover, VEGF promotes angiogenesis and the progression of gastric carcinomas, especially carcinomas of the intestinal type (Takahashi *et al.*, 1996). On the other hand, bFGF produced by tumour cells is frequently associated with angiogenesis and extensive fibrosis in diffuse gastric carcinomas, particularly those of the scirrhous type (Tanimoto *et al.*, 1991).

IL-8, a member of the CXC chemokine family, induces haptotactic migration and proliferation of melanoma cells and angiogenesis. More importantly, gastric carcinoma cell lines express mRNA and protein for IL-8 and IL-8 receptors (IL-8RA and IL-8RB) (Kitadai *et al.*, 1998, 2000). More than 80% of gastric carcinomas co-express IL-8 and IL-8 receptors; this co-expression correlates directly with tumour vascularity and disease progression. IL-8 enhances the expression of EGF receptor, type IV collagenase (metalloproteinase (MMP)-9), VEGF and IL-8 mRNA itself by gastric cancer cells, whereas IL-8 decreases expression of E-cadherin mRNA. In addition, IL-8 also increases MMP-9 activity and the ability of gastric cancer cells to invade through Matrigel. Altogether, IL-8 may play an important role in the growth and progression of gastric carcinoma by autocrine and paracrine mechanisms.

#### Factors associated with increased incidence of gastric cancer

Three major factors, including environmental factors, host factors and genetic factors, cooperatively affect the genesis of gastric cancer (Table 2). Of these, environmental factors are the most important, as diet and cigarette smoking are primary offenders; in particular, the presence of carcinogens such as *N*-nitroso compounds and benzo[*a*]pyrene is directly linked to carcinogenesis. As already described, the mutation spectrum of the *p53* gene differs between intestinal-type and diffuse-type gastric cancers,

**Table 2. Factors associated with increased incidence of gastric carcinoma**

Environmental factors	Diet (nitrites derived from nitrates, smoked and salted food, pickled vegetables, lack of fresh fruit and vegetables) Cigarette smoking
Host factors	<i>H. pylori</i> infection (chronic gastric and intestinal metaplasia) Partial gastrectomy Barrett esophagus
Genetic factors	Hereditary diffuse gastric cancer Hereditary non-polyposis colon cancer DNA polymorphism Genetic instability

suggesting that different carcinogens may be implicated in the two types of gastric carcinogenesis (Yokozaki *et al.*, 1997). Palli *et al.* (2001) found that the risk of MSI-H gastric cancer was positively associated with high consumption of red meat and meat sauce, and negatively associated with consumption of white meat.

With regard to host factors, meta-analysis of the relationship between *H. pylori* infection and gastric cancer has indicated that *H. pylori* infection is associated with a twofold increased risk of gastric cancer (Huang *et al.*, 1998; Eslick *et al.*, 1999). Younger *H. pylori*-infected patients have a higher relative risk for gastric cancer than older patients. *H. pylori* infection is equally associated with intestinal-type and diffuse-type gastric cancers (Huang *et al.*, 1998). In fact, the findings in a Mongolian gerbil model of stomach carcinogenesis indicate that *H. pylori* infection promotes stomach carcinogenesis induced by chemical carcinogens, and that histological types of gastric carcinoma may depend on the concentration of chemical carcinogens rather than on *H. pylori* infection (Shimizu *et al.*, 1999). Eradication of the bacteria evidently decreases the incidence of gastric carcinomas in the Mongolian gerbil model (Shimizu *et al.*, 2000).

*H. pylori* infection produces reactive oxygen and nitrogen species that cause DNA damage, followed by chronic gastric and intestinal meta-

plasia (Correa *et al.*, 1997). Goto *et al.* (1999) reported that the expression of inducible nitric oxide synthase (iNOS) and nitrotyrosine in the gastric mucosa was significantly high in *H. pylori*-infected patients who developed gastric cancer at least 2 years after the initial biopsies. These findings suggest that high production of iNOS and nitrotyrosine in the gastric mucosa by *H. pylori* may contribute to gastric carcinogenesis.

Cyclooxygenase-2 (Cox-2) expression is also induced by *H. pylori* infection (Sung *et al.*, 2000). Successful eradication of *H. pylori* leads to down-regulation of Cox-2 in the epithelial and stromal cells. High expression of Cox-2 mRNA, protein and enzymatic activity is detected preferentially in the tumour cells of intestinal-type gastric cancer (Saukkonen *et al.*, 2001). Loss of Cox-2 promoter methylation may enhance Cox-2 expression and promote gastric carcinogenesis associated with *H. pylori* infection (Akhtar *et al.*, 2001).

It should not be forgotten that in Japan the annual incidence of gastric cancer is about 100 000, accounting for 0.16% of 60 million individuals with *H. pylori* infection. Moreover, analysis of chromosomal aberrations in gastric cancer shows that they do not differ between *H. pylori*-related and non-related gastric cancers (van Grieken *et al.*, 2000). Genetic factors play a critical role in susceptibility to stomach carcinogenesis (Table 3).

Prinz *et al.* (2001) reported that *cagA*+/*vacAs1*+ strains of *H. pylori* that are blood-group antigen-binding adhesion (BabA2)-positive are associated with activity or chronicity of gastritis. Adherence of *H. pylori* via BabA2 may play a key role for efficient delivery of VacA and CagA.

In addition to *H. pylori* strains, DNA polymorphism including HLA, MUC1 (Carvalho *et al.*, 1997), T-cell helper 1 and IL-1 $\beta$  has been reported to be associated with an increased risk of both atrophic gastritis induced by *H. pylori* and gastric cancer (El-Omar *et al.*, 2000). More excitingly, Magnusson *et al.* (2001) found that distinct HLA class II DQ and DR alleles are associated with the development of gastric cancer and infection with *H. pylori*. The DQA1\*0102 is associated with protection from *H. pylori* infection, whereas the DRB\*1601 is associated with cancer deve-

**Table 3. Susceptibility to disease caused by *H. pylori***

Strain of <i>H. pylori</i>	<i>CagA</i> +/ <i>VacAs1</i> +strains that are BabA2-positive (Printz <i>et al.</i> , 2001)
Genetic factors	<i>HLA</i> polymorphism: <i>HLA</i> -DQA1 genetic typing <i>MUC1</i> polymorphism T-cell helper 1 phenotype IL-1 $\beta$ polymorphism <i>HLA</i> DR and DQ alleles: DQA1*0102 is associated with protection from infection by <i>H. pylori</i> , whereas DRB*1601 is associated with cancer development, particularly <i>H. pylori</i> -negative diffuse type (Magnusson <i>et al.</i> , 2001).

lopment, particularly *H. pylori*-negative diffuse gastric cancer. These host genetic factors may determine why some individuals infected with *H. pylori* develop gastric cancer while others do not. However, these studies need confirmation by a large number of prospective investigations in each of the countries concerned.

### Conclusion

Overall, the observations on the molecular events of gastric cancer may provide supporting evidence for our working hypothesis that there are two distinct major genetic pathways for stomach carcinogenesis (Figure 1). Genetic and epigenetic alterations found in two types of gastric cancer are summarized in Table 1. Among them, genetic instability including MIS and telomere reduction and immortality (activation of telomerase and expression of hTERT) are implicated in an initial step of stomach carcinogenesis. In the multistep process of intestinal-type gastric carcinogenesis, infection with *H. pylori* may be a strong trigger for hyperplasia of hTERT-positive stem cells in intestinal metaplasia. Genetic instability and hyperplasia of hTERT-positive stem cells may precede replication error at the D1S191 locus, DNA hypermethylation at the D17S5 locus, *pS2* loss, *RAR $\beta$*  loss, CD44 abnormal transcripts and *p53* mutation, all of which accumulate in at least 30% of incomplete intestinal metaplasias. All of these epigenetic and genetic alterations are

common events in intestinal-type gastric cancer. Incomplete intestinal metaplasia that contains an accumulation of the above multiple molecular events – that is, ‘metaplastic dysplasia’ – may be viewed as a bud of intestinal-type gastric cancer at genetic and epigenetic levels. An adenoma→carcinoma sequence is found in about 20% of gastric adenomas with *APC* mutations. In addition to these events, *p53* mutation and LOH, reduced p27 expression, cyclin E expression and presence of *c-met* 6.0-kb transcripts allow malignant transformation from the precancerous lesions to intestinal-type gastric cancer. *DCC* loss, *APC* mutations, 1q LOH, *p27* loss, reduced TGF- $\beta$ RI expression, reduced nm23 expression and *c-erbB* gene amplification are implicated in the progression and metastasis of intestinal-type gastric cancer. Another pathway for carcinogenesis of intestinal-type gastric cancer involves LOH and abnormal expression of the *p73* gene that may be responsible for the development of foveolar-type gastric cancers with pS2 expression.

On the other hand, LOH at chromosome 17p, mutation or LOH of *p53* and mutation or loss of *E-cadherin* are preferentially involved in the development of diffuse gastric cancers. In addition to these changes, gene amplification of *K-sam* and *c-met* and *p27* loss as well as reduced nm23 obviously confer progression, metastasis and diffusely productive fibrosis.

Mixed gastric carcinomas composed of intestinal and diffuse components exhibit some but not all of the molecular events described for each of the two types of gastric cancer.

## References

- Akagi, M., Yokozaki, H., Kitadai, Y., Ito, R., Yasui, W., Haruma, K., Kajiyama, G. & Tahara, E. (1995) Expression of amphiregulin in human gastric cancer cell lines. *Cancer*, **75**, 1460–1466
- Akagi, M., Yasui, W., Akama, Y., Yokozaki, H., Tahara, H., Haruma, K., Kajiyama, G. & Tahara, E. (1996) Inhibition of cell growth by transforming growth factor beta-1 is associated with p53-independent induction of p21 in gastric cells. *Jpn. J. Cancer Res.*, **87**, 377–384
- Akama, Y., Yasui, W., Yokozaki, H., Kuniyasu, H., Kitahara, K., Ishikawa, T. & Tahara, E. (1995) Frequent amplification of the cyclin E gene in human gastric carcinomas. *Jpn. J. Cancer Res.*, **86**, 617–621
- Akama, Y., Yasui, W., Kuniyasu, H., Yokozaki, H., Akagi, M., Tahara, H., Ishikawa, T. & Tahara, E. (1996a) Genetic status and expression of the cyclin-dependent kinase inhibitors in human gastric carcinoma cell lines. *Jpn. J. Cancer Res.*, **87**, 824–830
- Akama, Y., Yasui, W., Kuniyasu, H., Yokozaki, H., Akagi, M., Tahara, H., Ishikawa, T. & Tahara, E. (1996b) No point mutations but a codon 31 polymorphism and decreased expression of the p21 SDI1/WAF1/CIP1/MDA6 gene in human gastric carcinomas. *Mol. Cell Differ.*, **4**, 187–198
- Akhtar, M., Cheng, Y., Magno, R.M., Ashktorab, H., Smoot, D.T., Meltszer, S. J. & Wilson, K.T. (2001) Promoter methylation regulates *Helicobacter pylori*-stimulated cyclooxygenase-2 expression in gastric epithelial cells. *Cancer Res.*, **61**, 2399–2403
- Ayhan, A., Yasui, W., Yokozaki, H., Seto, M., Ueda, R. & Tahara, E. (1994) Loss of heterozygosity at the *bcl-2* gene locus and expression of *bcl-2* in human gastric and colorectal carcinomas. *Jpn. J. Cancer Res.*, **85**, 584–591
- Baffa, R., Veronese, M.L., Santoro, R., Mandes, B., Palazzo, J.P., Rugge, M., Santoro, E., Croce, C.M. & Huebner, K. (1998) Loss of FHIT expression in gastric carcinoma. *Cancer Res.*, **58**, 4708–4714
- Becker, K.F., Atkinson, M.J., Reich, U., Becker, I., Nekarda, H., Siewert, J.R. & Hofler, H. (1994) E-cadherin gene mutations provide clues to diffuse type gastric carcinomas. *Cancer Res.*, **54**, 3845–3852
- Caca, K., Kolligs, F.T., Ji, X., Hayes, M., Qian, J., Yahanda, A., Rimm, D.L., Costa, J. & Fearon, E.R. (1999) Beta- and gamma-catenin mutations, but not E-cadherin inactivation, underlie T-cell factor/lymphoid enhancer factor transcriptional deregulation in gastric and pancreatic cancer. *Cell Growth Differ.*, **10**, 369–376
- Cahill, D.P., Lengauer, C., Yu, J., Riggins, G.J., Willson, J.K.V., Markowitz, S.D., Kinzler, K.W. & Vogelstein, B. (1998) Mutations of mitotic checkpoint genes in human cancers. *Nature*, **392**, 300–303
- Carvalho, F., Seruca, R., David, L., Amorim, A., Seixas, M., Bennett, E., Clausen, H. & Sobrinho-

- Simoës, M. (1997) MUC1 gene polymorphism and gastric cancer – an epidemiological study. *Glycoconj. J.*, **14**, 107–111
- Chen, Y.J., Chen, P.H., Lee, M.D. & Chang, J.G. (1997a) Aberrant FHIT transcripts in cancerous and corresponding non-cancerous lesions of the digestive tract. *Int. J. Cancer*, **17**, 955–958
- Chen, Y.J., Chang, J.G., Shih, L.S., Chen, P.H., Endo, M., Whang-Peng, J. & Chen, Y.M. (1997b) Frequent detection of aberrant RNA transcripts of the CDKN2 gene in human gastric adenocarcinoma. *Int. J. Cancer*, **71**, 350–354
- Chin, L., Artandi, S., Shen Q., Tam, A., Lee, S.-L., Gottlieb, G.J., Greider, C.W. & DePinho, R.A. (1999) p53 Deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. *Cell*, **97**, 527–538
- Constancia, M., Seruca, R., Carneiro, F., Silva, F. & Castedo, S. (1994) Retinoblastoma gene structure and product expression in human gastric carcinomas. *Br. J. Cancer*, **70**, 1018–1024
- Cooper, D.L., Dougherty, G., Harn, H.J., Jackson, S., Baptist, E.W., Byers, J., Datta, A., Phillips, G. & Isola, N.R. (1992) The complex CD44 transcriptional unit; alternative splicing of three internal exons generates the epithelial form of CD44. *Biochem. biophys. Res. Commun.*, **182**, 569–578
- Correa, P., Miller, M. & Mannick, E.E. (1997) Oxidative damage during the gastric precancerous process. In: Tahara, E., ed., *Molecular Pathology of Gastroenterological Cancer: Application to Clinical Practice*, Tokyo, Springer-Verlag, pp. 23–29
- Dinareello, C.A (1992) The biology of interleukin-1. *Chem. Immunol.*, **51**, 1–32
- El-Omar, E.M., Carrington, M., Chow, W.H., McColl, K.E., Bream, J.H., Young, H.A., Herrera, J., Lissowska, J., Yuan, C.C., Rothman, N., Lanyon, G., Martin, M., Fraumeni, J.F., Jr & Rabkin, C.S. (2000) Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature*, **404**, 398–402
- Eslick, G.D., Lim, L.L., Byles, J.E., Xia, H.H. & Talley, N.J. (1999) Association of *Helicobacter pylori* infection with gastric carcinoma: a meta-analysis. *Am. J. Gastroenterol.*, **94**, 2373–2379
- Fleisher, A.S., Esteller, M., Wang, S., Tamura, G., Suzuki, H., Yin, J., Zou, T.-T., Abraham, J.M., Kong, D., Smolinski, K.N., Shi, Y.-Q., Rhyu, M.-G., Powell, S.M., James, S.P., Wilson, K.T., Herman, J.G. & Meltzer, S.J. (1999) Hypermethylation of the hMLH1 gene promoter in human gastric cancers with microsatellite instability. *Cancer Res.*, **59**, 1090–1095
- Fujimoto, J., Yasui, W., Tahara, H., Tahara, E., Kudo, Y., Yokozaki, H. & Tahara, E. (2000) DNA hypermethylation at the pS2 promoter region is associated with early stage of stomach carcinogenesis. *Cancer Lett.*, **149**, 125–134
- Goto, T., Haruma, K., Kitadai, Y., Ito, M., Yoshimura, M., Sumii, K., Hayakawa, N. & Kajiyama, G. (1999) Enhanced expression of inducible nitric oxide synthase and nitrotyrosine in gastric mucosa of gastric cancer patients. *Clin. Cancer Res.*, **5**, 1411–1415
- van Grieken, N.C.T., Weiss, M.M., Meijer, G.A., Hermsen, M.A.J.A., Scholte, G.H.A. Lindeman, J., Craanen, M.E., Bloemena, E., Meuwissen, S.G.M., Baak, J.P.A. & Kuipers, E.J. (2000) *Helicobacter pylori*-related and -non-related gastric cancers do not differ with respect to chromosomal aberrations. *J. Pathol.*, **192**, 301–306
- Guilford, P., Hopkins, J., Harraway, J., McLeod, M., McLeod, N., Harawira, P., Taite, H., Scoular, R. & Miller, A. & Reeve, A.E. (1998) E-Cadherin germline mutations in familial gastric cancer. *Nature*, **392**, 402–405
- Gunther, T., Schneider-Stock, R., Pross, M., Manger, T., Malgertheiner, P., Lippert, H. & Roessner, A. (1998) Alterations of the p16/MTS1-tumor suppressor gene in gastric cancer. *Pathol. Res. Pract.*, **194**, 809–813
- Hackett, J.A., Feldser, D.M. & Greider, C.W. (2001) Telomere dysfunction increases mutation rate and genomic instability. *Cell*, **106**, 275–286
- Hamamoto, T., Yokozaki, H., Semba, S., Yasui, W., Yunotani, S., Miyazaki, K. & Tahara, E. (1997) Altered microsatellites in incomplete-type intestinal metaplasia adjacent to primary gastric cancers. *J. clin. Pathol.*, **50**, 841–846
- Hamilton, S.R. & Aaltonen, L.A., eds (2000) *Pathology and Genetics of Tumours of the Digestive System* (World Health Organization Classification of Tumours), Lyon, IARC Press, p. 38
- Handschuh, G., Candidus, S., Lubert, B., Reich, U., Schott, C., Oswald, S., Becke, H., Hutzler, P.,

- Birchmeier, W., Hofler, H. & Becker, K.F. (1999) Tumour-associated E-cadherin mutations alter cellular morphology, decrease cellular adhesion and increase cellular motility. *Oncogene*, **18**, 4301–4312
- Harper, J.W., Adami, G.R., Wei, N., Keyomarsi, K. & Elledge, S.J. (1993) The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell*, **75**, 805–816
- Harris, C.C. (1991) Chemical and physical carcinogenesis: advances and perspectives for the 1990s. *Cancer Res.*, **51** (Suppl. 18), 5023s–5044s
- Hattori, Y., Odagiri, H., Nakatani, H., Miyagawa, K., Naito, K., Sakamoto, H., Katoh, O., Yoshida, T., Sugimura, T. & Terada, M. (1990) K-sam, an amplified gene in stomach cancer, is a member of the heparin-binding growth factor receptor genes. *Proc. natl Acad. Sci. USA*, **87**, 5983–5987
- Hayashi, K., Yokozaki, H., Naka, K., Yasui, W., Lotan, R. & Tahara, E. (2001a) Overexpression of retinoic acid receptor induces growth arrest and apoptosis in oral cancer cell lines. *Jpn. J. Cancer Res.*, **92**, 42–50
- Hayashi, K., Yokozaki, H., Goodison, S., Oue, N., Suzuki, T., Lotan, R., Yasui, W. & Tahara, E. (2001b) Inactivation of retinoic acid receptor  $\beta$  by promoter CpG hypermethylation in gastric cancer. *Differentiation*, **68**, 13–21
- Higashikawa, K., Yokozaki, H., Ue, T., Taniyama, K., Ishikawa, T., Tarin, D. & Tahara, E. (1996) Evaluation of CD44 transcription variants in human digestive tract carcinomas and normal tissues. *Int. J. Cancer*, **66**, 11–17
- Honda, R., Ohba, Y., Nagata, A., Okayama, H. & Yasuda, H. (1993) Dephosphorylation of human p34cdc2 kinase on both Thr-14 and Tyr-15 by human cdc25B phosphatase. *FEBS Lett.*, **318**, 331–334
- Huang, J.Q., Sridhar, S., Chen, Y. & Hunt, R.H. (1998) Meta-analysis of the relationship between *Helicobacter pylori* seropositivity and gastric cancer. *Gastroenterology*, **114**, 1169–1179
- Igaki, H., Sasaki, H., Tachimori, Y., Kato, H., Watanabe, H., Kitamura, T., Harada, Y., Sugimura, T. & Terada, M. (1995) Mutation frequency of the p16/CDKN2 gene in primary cancers in the upper digestive tract. *Cancer Res.*, **55**, 3421–3423
- Iida, S., Akiyama, Y., Ichikawa, W., Yamashita, T., Nomizu, T., Nihei, Z., Sugihara, K. & Yuasa, Y. (1999) Infrequent germ-line mutation of the E-cadherin gene in Japanese familial gastric cancer kindreds. *Clin. Cancer Res.*, **5**, 1445–1447
- Iida, S., Akiyama, Y., Nakajima, T., Ichikawa, W., Nihei, Z., Sugihara, K. & Yuasa, Y. (2000) Alterations and hypermethylation of the p14(ARF) gene in gastric cancer. *Int. J. Cancer*, **87**, 654–658
- Isogaki, J., Shinmura, K., Yin, W., Arai, T., Kodo, K., Kimura, T., Kino, I. & Sugimura, H. (1999) Microsatellite instability and K-ras mutations in gastric adenomas, with reference to associated gastric cancers. *Cancer Detect. Prev.*, **23**, 204–214
- Ito, M., Yasui, W., Kyo, E., Yokozaki, H., Nakayama, H., Ito, H. & Tahara, E. (1992a) Growth inhibition of transforming growth factor-beta on human gastric carcinoma cells: receptor and postreceptor signaling. *Cancer Res.*, **52**, 295–300
- Ito, M., Yasui, W., Nakayama, H., Yokozaki, H., Ito, H. & Tahara, E. (1992b) Reduced levels of transforming growth factor-beta type I receptor in human gastric carcinomas. *Jpn. J. Cancer Res.*, **83**, 86–92
- Ito, R., Kitadai, Y., Kyo, E., Yokozaki, H., Yasui, W., Yamashita, U., Nikai, H. & Tahara, E. (1993) Interleukin 1 $\alpha$  acts as autocrine growth stimulator for human gastric carcinoma cells. *Cancer Res.*, **53**, 4102–4106
- Ito, R., Yasui, W., Kuniyasu, H., Yokozaki, H. & Tahara, E. (1997) Expression of interleukin-6 and its effect on the cell growth of gastric carcinoma cell lines. *Jpn. J. Cancer Res.*, **88**, 953–958
- JRSGC (Japanese Research Society for Gastric Cancer) (1999) *Japanese Classification of Gastric Carcinoma*, 13th Ed., Tokyo, Kanehara
- Jinno, S., Suto, K., Nagata, A., Igarashi, M., Kanaoka, Y., Nojima, H. & Okayama, H. (1994) Cdc25A is a novel phosphatase functioning early in the cell cycle. *EMBO J.*, **13**, 1549–1556
- Kameda, T., Yasui, W., Yoshida, K., Tsujino, T., Nakayama, H., Ito, M., Ito, H. & Tahara, E. (1990) Expression of ERBB2 in human gastric carcinomas: relationship between p185 ERBB2 expression and the gene amplification. *Cancer Res.*, **50**, 8002–8009
- Kaplan, K.B., Burds, A.A., Swedlow, J.R., Bekir, S.S., Sorger, P.K. & Näthke, I.S. (2001) A role for the adenomatous polyposis coli protein in chromosome segregation. *Nature Cell Biol.*, **3**, 429–432

- Katoh, M. & Terada, M. (1993) Oncogenes and tumor suppressor genes. In: Nishi, M., Ichikawa, H., Nakajima, T., Maruyama, K., Tahara, E., eds, *Gastric Cancer*, Tokyo, Springer-Verlag, pp. 196–208
- Katoh, M., Hattori, Y., Sasaki, H., Tanaka, M., Sugano, K., Yazaki, Y., Sugimura, T. & Terada, M. (1992) K-sam Gene encodes secreted as well as transmembrane receptor tyrosine kinase. *Proc. natl Acad. Sci. USA*, **89**, 2960–2964
- Kawanishi, J., Kato, J., Sasaki, K., Fujii, S., Watanabe, N. & Niitsu, Y. (1995) Loss of E-cadherin-dependent cell-cell adhesion due to mutation of the beta-catenin gene in a human cancer cell line, HSC-39. *Mol. cell. Biol.*, **15**, 1175–1181
- Keller, G., Vogelsang, H., Becker, I., Hutter, J., Ott, K., Candidus, S., Grundei, T., Becker, K.F., Mueller, J., Siewert, J.R. & Hofler, H. (1999) Diffuse type gastric and lobular breast carcinoma in a familial gastric cancer patient with an E-cadherin germline mutation. *Am. J. Pathol.*, **155**, 337–342
- Kim, S.-J., Yang, H.K., Im, Y.-H., Bang, Y.J. & Yang, H.K. (1999) Mechanisms of TGF- $\beta$  receptor inactivation and development of resistance to TGF- $\beta$  in human gastric cancer. In: *Proceedings of the Third International Gastric Cancer Congress, Seoul*, Bologna, Monduzzi Editore, pp. 81–90
- Kinzler, K.W., Nilbert, M.C., Su, L.K., Vogelstein, B., Bryan, T.M., Levy, D.B., Smith, K.J., Preisinger, A.C., Hedge, P., McKechnie, D., Finniear, R., Markham, A., Groffen, J., Boguski, M.S., Altschul, S.F., Horii, A., Miyoshi, Y., Miki, Y., Nishisho, I. & Nakamura, Y. (1991) Identification of FAP locus genes from chromosome 5q21. *Science*, **253**, 661–665
- Kitadai, Y., Yasui, W., Yokozaki, H., Kuniyasu, H., Ayhan, A., Haruma, K., Kajiyama, G., Johnson, G.R. & Tahara, E. (1993a) Expression of amphiregulin, a novel gene of the epidermal growth factor family, in human gastric carcinomas. *Jpn. J. Cancer Res.*, **84**, 879–884
- Kitadai, Y., Yamazaki, H., Yasui, W., Kyo, E., Yokozaki, H., Kajiyama, G., Johnson, A.C., Pastan, I. & Tahara, E. (1993b) GC factor represses transcription of several growth factor/receptor genes and causes growth inhibition of human gastric carcinoma cell lines. *Cell Growth Differ.*, **4**, 291–296
- Kitadai, Y., Haruma, K., Sumii, K., Yamamoto, S., Ue, T., Yokozaki, H., Yasui, W., Ohmoto, Y., Kajiyama, G., Fidler, I.J. & Tahara, E. (1998) Expression of interleukin-8 correlates with vascularity in human gastric carcinomas. *Am. J. Pathol.*, **152**, 93–100
- Kitadai, Y., Haruma, K., Mukaida, N., Ohmoto, Y., Matsutani, N., Yasui, W., Yamamoto, S., Sumii, K., Kajiyama, G., Fidler, I.J. & Tahara, E. (2000) Regulation of disease-progression genes in human gastric carcinoma cells by interleukin-8. *Clin. Cancer Res.*, **6**, 2735–2740
- Kudo, Y., Yasui, W., Ue, T., Yamamoto, S., Yokozaki, H., Nikai, H. & Tahara, E. (1997) Overexpression of cyclin-dependent kinase-activating CDC25B phosphatase in human gastric carcinomas. *Jpn. J. Cancer Res.*, **88**, 947–952
- Kuniyasu, H., Yoshida, K., Yokozaki, H., Yasui, W., Ito, H., Toge, T., Ciardiello, F., Persico, M.G., Saeki, T., Salomon, D.S. & Tahara, E. (1991) Expression of cripto, a novel gene of the epidermal growth factor family, in human gastrointestinal carcinomas. *Jpn. J. Cancer Res.*, **82**, 969–973
- Kuniyasu, H., Yasui, W., Kitadai, Y., Yokozaki, H., Ito, H. & Tahara, E. (1992) Frequent amplification of the c-met gene in scirrhous type stomach cancer. *Biochem. biophys. Res. Commun.*, **189**, 227–232
- Kuniyasu, H., Yasui, W., Yokozaki, H., Kitadai, Y. & Tahara, E. (1993) Aberrant expression of c-met mRNA in human gastric carcinomas. *Int. J. Cancer*, **55**, 72–75
- Kuniyasu, H., Yasui, W., Yokozaki, H., Akagi, M., Akama, Y., Kitahara, K., Fujii, K. & Tahara, E. (1994a) Frequent loss of heterozygosity of the long arm of chromosome 7 is closely associated with progression of human gastric carcinomas. *Int. J. Cancer*, **59**, 597–600
- Kuniyasu, H., Yasui, W., Akama, Y., Akagi, H., Tohdo, H., Ji, Z.-Q., Kitadai, Y., Yokozaki, H. & Tahara, E. (1994b) Expression of cripto in human gastric carcinomas: an association with tumor stage and prognosis. *J. exp. clin. Cancer Res.*, **13**, 151–157
- Kuniyasu, H., Yasui, W., Kitahara, K., Naka, K., Yokozaki, H., Akama, Y., Hamamoto, T., Tahara, H. & Tahara, E. (1997a) Growth inhibitory effect of interferon- $\beta$  is associated with the induction of cyclin-dependent kinase inhibitor p27 Kip1 in a human gastric carcinoma cell line. *Cell Growth Differ.*, **8**, 47–52

- Kuniyasu, H., Domen, T., Hamamoto, T., Yokozaki, H., Yasui, W., Tahara, H. & Tahara, E. (1997b) Expression of human telomerase RNA is an early event of stomach carcinogenesis. *Jpn. J. Cancer Res.*, **88**, 103–107
- Lauren, P. (1965) The two histological main types of gastric carcinoma. Diffuse and so-called intestinal type carcinoma. An attempt at histochemical classification. *Acta pathol. microbiol. Scand.*, **64**, 31–49
- Lee, K.H., Lee, J.S., Suh, C., Kim, S.W., Kim, S.B., Lee, J.H., Lee, M.S., Park, M.Y., Sun, H.S. & Kim, S.H. (1995) Clinicopathologic significance of the K-ras gene codon 12 point mutation in stomach cancer. An analysis of 140 cases. *Cancer*, **75**, 2794–2801
- Lee, Y.Y., Kang, S.H., Seo, J.Y., Jung, C.W., Lee, K.U., Choe, K.J., Kim, B.K., Kim, N.K., Koeffler, H.P. & Bang, Y.J. (1997) Alterations of p16INK4A and p15INK4B genes in gastric carcinomas. *Cancer*, **80**, 1889–1896
- Lefebvre, O., Chenard, M.P., Masson, R., Linares, J., Dierich, A., LeMeur, M., Wendling, C., Tomasetto, C., Chambon, P. & Rio, M.C. (1996) Gastric mucosa abnormalities and tumorigenesis in mice lacking the pS2 trefoil protein. *Science*, **274**, 259–262
- Leung, S.Y., Yuen, S.T., Chung, L.P., Chu, K.M., Chan, A.S.Y. & Ho, J.C.I. (1999) hMLH1 promoter methylation and lack of hMLH1 expression in sporadic gastric carcinomas with high-frequency microsatellite instability. *Cancer Res.*, **59**, 159–164
- Lotan, R., Ito, H., Yasui, W., Yokozaki, H., Lotan, D. & Tahara, E. (1994) Expression of a 31-kDa lacto-side-binding lectin in normal human gastric mucosa and in primary and metastatic gastric carcinomas. *Int. J. Cancer*, **56**, 474–480
- Lotan, R., Xu, C., Lippman, S.M., Ro, J.Y., Lee, J.S., Lee, J.J. & Hong, W.K. (1995) Suppression of retinoic acid receptor  $\beta$  in premalignant oral lesions and its upregulation by isotretinoin. *New Engl. J. Med.*, **332**, 1405–1410
- Machado, J.C., Soares, P., Carneiro, F., Rocha, A., Beck, S., Blin, N., Berx, G. & Sobrinho-Simoes, M. (1999) E-Cadherin gene mutations provide a genetic basis for the phenotypic divergence of mixed gastric carcinomas. *Lab. Invest.*, **79**, 459–465
- Maesawa, C., Tamura, G., Suzuki, Y., Ogasawara, S., Sakata, K., Kashiwaba, M. & Satodate, R. (1995) The sequential accumulation of genetic alterations characteristic of the colorectal adenoma–carcinoma sequence does not occur between gastric adenoma and adenocarcinoma. *J. Pathol.*, **176**, 249–258
- Magnusson, P.K.E., Enroth, H., Eriksson, I., Held, M., Nyren, O., Engstrand, L., Hansson, L.E. & Gyllensten, U.B. (2001) Gastric cancer and human leukocyte antigen. *Cancer Res.*, **61**, 2684–2689
- Markowitz, S., Wang, J., Myeroff, L., Parsons, R., Sun, L., Lutterbaugh, J., Fan, R.S., Zborowska, E., Kinzler, K.W., Vogelstein, B., Brattain, M. & Willson, J.K.V. (1995) Inactivation of the type II TGF- $\beta$  receptor in colon cancer cells with microsatellite instability. *Science*, **268**, 1336–1338
- Masiakowski, P., Breathnach, R., Bloch, J., Gannon, F., Krust, A. & Chambon, P. (1982) Cloning of cDNA sequences of hormone-regulated genes from MCF-7 human breast cancer cell lines. *Nucleic Acids Res.*, **10**, 7895–7903
- Matsumura, Y. & Tarin, D. (1992) Significance of CD44 gene products for cancer diagnosis and disease evaluation. *Lancet*, **340**, 1053–1058
- Merlo, A., Herman, J.G., Mao, L., Lee, D.J., Gabrielson, E., Burger, P.C., Baylin, S.B. & Sidransky, D. (1995) 5' CpG island methylation is associated with transcriptional silencing of the tumor suppressor p16/CDKN2/MTS1 in human cancers. *Nature Med.*, **1**, 686–692
- Mirvish, S.S. (1971) Kinetics of nitrosamide formation from alkylureas, N-alkylurethanes, and alkylguanidines: possible implications for the etiology of human gastric cancer. *J. natl. Cancer Inst.*, **46**, 1183–1193
- Muta, H., Noguchi, M., Kanai, Y., Ochiai, A., Nawata, H. & Hirohashi, S. (1996) E-Cadherin gene mutations in signet ring cell carcinoma of the stomach. *Jpn. J. Cancer Res.*, **87**, 843–848
- Nagata, A., Igarashi, M., Jinno, S., Suto, K. & Okayama, H. (1991) An additional homolog of the fission yeast cdc25+ gene occurs in humans and is highly expressed in some cancer cells. *New Biol.*, **3**, 959–968
- Nakatsuru, S., Yanagisawa, A., Ichii, S., Tahara, E., Kato, Y., Nakamura, Y. & Horii, A. (1992) Somatic mutation of the APC gene in gastric cancer: frequent mutations in very well differentiated adenocarcinoma and signet-ring cell carcinoma. *Hum. mol. Genet.*, **1**, 559–563
- Nakatsuru, S., Yanagisawa, A., Furukawa, Y., Ichii, S., Kato, Y., Nakamura, Y. & Horii, A. (1993) Somatic



- mutations of the APC gene in precancerous lesion of the stomach. *Hum. mol. Genet.*, **2**, 1463–1465
- Nakayama, H., Yasui, W., Yokozaki, H. & Tahara, E. (1993) Reduced expression of nm23 is associated with metastasis of human gastric carcinomas. *Jpn. J. Cancer Res.*, **84**, 184–190
- Nishimura, K., Yokozaki, H., Haruma, K., Kajiyama, G. & Tahara, E. (1995) Alterations of the APC gene in carcinoma cell lines and precancerous lesions of the stomach. *Int. J. Oncol.*, **7**, 587–592
- Noguchi, T., Muller, W., Wirtz, H.C., Willers, R. & Gabbert, H.E. (1999) FHIT gene in gastric cancer: association with tumor progression and prognosis. *J. Pathol.*, **188**, 378–381
- Ochiai, A., Akimoto, S., Kanai, Y., Shibata, T., Oyama, T. & Hirohashi, S. (1994) c-erbB-2 Gene product associates with catenins in human cancer cells. *Biochem. biophys. Res. Commun.*, **205**, 73–78
- Ochiai, A., Yamauchi, Y. & Hirohashi, S. (1996) p53 Mutations in the non-neoplastic mucosa of the human stomach showing intestinal metaplasia. *Int. J. Cancer*, **69**, 28–33
- Oda, N., Tsujino, T., Tsuda, T., Yoshida, K., Nakamura, H., Yasui, W. & Tahara, E. (1990) DNA ploidy pattern and amplification of ERBB and ERBB2 genes in human gastric carcinomas. *Virchows Arch. B. Cell Pathol. mol. Pathol.*, **58**, 273–277
- Ohta, M., Inoue, H., Cotticelli, M.G., Kastury, K., Baffa, R., Palazzo, J., Sibrashvili, Z., Mori, M., McCue, P., Druck, T., Croce, C.M. & Huebner, K. (1996) The FHIT gene, spanning the chromosome 3p14.2 fragile site and renal carcinoma-associated t(3;8) breakpoint, is abnormal in digestive tract cancers. *Cell*, **84**, 587–597
- Palli, D., Russo, A., Ottini, L., Masala, G., Saieva, C., Amorosi, A., Cama, A., D'Amico, C., Falchetti, M., Palmirota, R., Decarli, A., Costantini, R.M. & Fraumeni, J.F., Jr (2001) Red meat, family history, and increased risk of gastric cancer with microsatellite instability. *Cancer Res.*, **61**, 5415–5419
- Pellman, D. (2001) Cancer. A CINtillating new job for the APC tumor suppressor. *Science*, **291**, 2555–2556
- Poremba, C., Yandell, D.W., Huang, Q., Little, J.B., Mellin, W., Schmid, K.W., Bocker, W. & Dockhorn-Dworniczak, B. (1995) Frequency and spectrum of p53 mutations in gastric – a molecular genetic and immunohistochemical study. *Virchows Arch.*, **426**, 447–455
- Prinz, C., Schoniger, M., Rad, R., Becker, I., Keiditsch, E., Wagenpfeil, S., Classen, M., Rosch, T., Schepp, W. & Gerhard, M. (2001) Key importance of the *Helicobacter pylori* adherence factor blood group antigen binding adhesin during chronic gastric inflammation. *Cancer Res.*, **61**, 1903–1909
- Sakurai, S., Sano, T. & Nakajima, T. (1995) Clinico-pathological and molecular biological studies of gastric adenomas with special reference to p53 abnormality. *Pathol. int.*, **45**, 51–57
- Sano, T., Tsujino, T., Yoshida, K., Nakayama, H., Haruma, K., Ito, H., Nakamura, Y., Kajiyama, G. & Tahara, E. (1991) Frequent loss of heterozygosity on chromosomes 1q, 5q, and 17p in human gastric carcinomas. *Cancer Res.*, **51**, 2926–2931
- Saukkonen, K., Nieminen, O., Van Rees, B., Vilkkki, S., Harkonen, M., Juhola, M., Mecklin, J.-P., Sipponen, P. & Ristimaki, A. (2001) Expression of cyclooxygenase-2 in dysplasia of the stomach and in intestinal-type gastric adenocarcinoma. *Clin. Cancer Res.*, **7**, 1923–1931
- Seewaldt, V.L., Johnson, B.S., Parker, M.B., Collins, S.J. & Swisshelm, K. (1995) Expression of retinoic acid receptor- $\beta$  mediates retinoic acid-induced growth arrest and apoptosis in breast cancer cells. *Cell Growth Differ.*, **6**, 1077–1088
- Semba, S., Yokozaki, H., Yamamoto, S., Yasui, W. & Tahara, E. (1996) Microsatellite instability in precancerous lesions and adenocarcinomas of the stomach. *Cancer (Suppl. 8)*, **77**, 1620–1627
- Semba, S., Yokozaki, H., Yasui, W. & Tahara, E. (1998) Frequent microsatellite instability and loss of heterozygosity in the region including BRCA1 (17q21) in young patients with gastric cancer. *Int. J. Oncol.*, **12**, 1245–1251
- Shibata, T., Ochiai, A., Kanai, Y., Akimoto, S., Gotoh, M., Yasui, N., Machinami, R. & Hirohashi, S. (1996) Dominant negative inhibition of the association between beta-catenin and c-erbB-2 by N-terminally deleted beta-catenin suppresses the invasion and metastasis of cancer cells. *Oncogene*, **13**, 883–889
- Shigeishi, H., Yokozaki, H., Kuniyasu, H., Nakagawa, H., Ishikawa, T., Tahara, E. & Yasui, W. (2001) No mutations of the *Bub1* gene in human gastric carcinomas. *Oncol. Rep.*, **8**, 791–794

- Shimizu, N., Inada, K., Nakanishi, H., Tsukamoto, T., Ikehara, Y., Kaminishi, M., Kuramoto, S., Sugiyama, A., Katsuyama, T. & Tatematsu, M. (1999) *Helicobacter pylori* infection enhances glandular stomach carcinogenesis in Mongolian gerbils treated with a chemical carcinogen. *Carcinogenesis*, **20**, 669–676
- Shimizu, N., Ikehara, Y., Inada, K., Nakanishi, H., Tsukamoto, T., Nozaki, K., Kaminishi, M., Kuramoto, S., Sugiyama, A., Katsuyama, T. & Tatematsu, M. (2000) Eradication diminishes enhancing effects of *Helicobacter pylori* infection on glandular stomach carcinogenesis in Mongolian gerbils. *Cancer Res.*, **60**, 1512–1514
- Soman, N.R., Correa, P., Ruiz, B.A. & Wogan, G.N. (1991) The TPR-MET oncogenic rearrangement is present and expressed in human gastric carcinoma and precursor lesions. *Proc. natl Acad. Sci. USA*, **88**, 4892–4896
- Sugimura, T., Fujimura S. & Baba, T. (1970) Tumor production in the glandular stomach and alimentary tract of the rat by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. *Cancer Res.*, **30**, 455–465
- Sung, J.J., Leung, W.K., Go, M.Y., To, K.F., Cheng, A.S., Ng, E.K. & Chan, F.K. (2000) Cyclooxygenase-2 expression in *Helicobacter pylori*-associated premalignant and malignant gastric lesions. *Am. J. Pathol.*, **157**, 729–735
- Suzuki, T., Yasui, W., Yokozaki, H., Naka, K., Ishikawa, T. & Tahara, E. (1999) Expression of the E2F family in human gastrointestinal carcinomas. *Int. J. Cancer*, **81**, 535–538
- Tahara, E. (1993) Molecular mechanism of stomach carcinogenesis. *J. Cancer Res. clin. Oncol.*, **119**, 265–272
- Tahara, E. (1997) Cell growth regulation and cancer: stromal interaction. In: Sugimura, T. & Sasako, M., eds, *Gastric Cancer*, New York, Oxford University Press, pp. 100–108
- Tahara, E. (1998) Molecular mechanism of human stomach carcinogenesis implicated in *Helicobacter pylori* infection. *Exp. Toxicol. Pathol.*, **50**, 375–378
- Tahara, E. (2002) Histone acetylation and retinoic acid receptor beta DNA methylation as novel targets for gastric cancer therapy. *Drug News Perspect.*, **15**, 581–585
- Tahara, E., Yokozaki, H. & Yasui, W. (1993) Growth factors in gastric cancer. In: Nishi, M., Ichikawa, H., Nakajima, T., Maruyama, K. & Tahara, E., eds, *Gastric Cancer*, Berlin, Springer-Verlag, pp. 209–217
- Tahara, E., Kuniyasu, H., Yasui, W. & Yokozaki, H. (1994) Abnormal expression of growth factors and their receptors in stomach cancer. In: Nakamura, T. & Matsumoto, K., eds, *Gann Monograph on Cancer Research Growth Factors: Cell Growth, Morphogenesis and Transformation*, Tokyo, Japan Scientific Society Press, pp. 163–173
- Tahara, H., Kuniyasu, H., Yokozaki, H., Yasui, W., Shey, J.W., Ide, T. & Tahara, E. (1995) Telomerase activity in preneoplastic and neoplastic gastric and colorectal lesions. *Clin. Cancer Res.*, **1**, 1245–1251
- Tahara, E., Semba, S. & Tahara, H. (1996a) Molecular biological observations in gastric cancer. *Semin. Oncol.*, **23**, 307–315
- Tahara, E., Yasui, W. & Yokozaki, H. (1996b) Abnormal growth factor networks in neoplasia. In: Puzstai, L., Lewis, C.E. & Yap, E., eds, *Cell Proliferation in Cancer*, Oxford, Oxford University Press, pp.131–153
- Tahara, E., Yokozaki, H. & Yasui, W. (1999) Stomach-genetic and epigenetic alterations of preneoplastic and neoplastic lesions. In: Srivastava, S., Henson, D.E. & Gazdar, A., eds, *Molecular Pathology of Early Cancer*, Amsterdam, IOS Press, pp. 341–361
- Takahashi, Y., Cleary, K.R., Mai, M., Kitadai, Y., Bucana, C.D. & Ellis, L.M. (1996) Significance of vessel count and vascular endothelial growth factor and its receptor (KDR) in intestinal-type gastric cancer. *Clin. Cancer Res.*, **2**, 1679–1684
- Tamura, G., Kihana, T., Nomura, K., Terada, M., Sugimura, T. & Hirohashi, S. (1991) Detection of frequent p53 gene mutations in primary gastric cancer by cell sorting and polymerase chain reaction single-strand conformation polymorphism analysis. *Cancer Res.*, **51**, 2056–3058
- Tamura, G., Sakata, K., Nishizuka, S., Maesawa, C., Suzuki, Y., Iwaya, T., Terashima, M., Saito, K. & Satodate, R. (1997) Analysis of the fragile histidine triad gene in primary gastric carcinomas and gastric carcinoma cell lines. *Genes Chromosomes Cancer*, **20**, 98–102
- Tanimoto, H., Yoshida, K., Yokozaki, H., Yasui, W., Nakayama, H., Ito, H., Ohama, K. & Tahara, E. (1991) Expression of basic fibroblast growth factor

- in human gastric carcinomas. *Virchows Arch. B. Cell Pathol. mol. Pathol.*, **61**, 263–267
- Tohdo, H., Yokozaki, H., Haruma, K., Kajiyama, G. & Tahara, E. (1993) p53 Gene mutations in gastric adenomas. *Virchows Arch. B. Cell Pathol. mol. Pathol.*, **63**, 191–195
- Uchino, S., Tsuda, H., Noguchi, M., Yokota, J., Terada, M., Saito, T., Kobayashi, M., Sugimura, T. & Hirohashi, S. (1992) Frequent loss of heterozygosity at the DCC locus in gastric cancer. *Cancer Res.*, **52**, 3099–3102
- Uchino, S., Noguchi, M., Ochiai, A., Saito, T., Kobayashi, M. & Hirohashi, S. (1993) p53 Mutation in gastric cancer: a genetic model for carcinogenesis is common to gastric and colorectal cancer. *Int. J. Cancer*, **54** 759–764
- Ue, T., Yokozaki, H., Kitadai, Y., Yamamoto, S., Yasui, W., Ishikawa, T. & Tahara, E. (1998) Co-expression of osteopontin and CD44V9 in gastric cancer. *Int. J. Cancer*, **79**, 127–132
- Weber, G.F., Ashkar, S., Glimcher, M.J. & Cantor, H. (1996) Receptor-ligand interaction between CD44 and osteopontin (Eta-1). *Science*, **271**, 509–512
- Wu, M.S., Lee, C.W., Shun, C.T., Wang, H.P., Lee, W.J., Sheu J.C. & Lin, J.T. (1998) Clinicopathological significance of altered loci of replication error and microsatellite instability-associated mutations in gastric cancer. *Cancer Res.*, **58**, 1494–1497
- Yamamoto, S., Yasui, W., Kitadai, Y., Yokozaki, H., Haruma, K., Kajiyama, G. & Tahara, E. (1998) Expression of vascular endothelial growth factor in human gastric carcinomas. *Pathol. int.*, **48**, 499–506
- Yang, H.-K., Kang, S.H., Kim, Y.-S., Won, K., Bang, Y.-J. & Kim, S.-J. (1999) Truncation of the TGF-beta type II receptor gene results in insensitivity to TGF-beta in human gastric cancer cells. *Oncogene*, **18**, 2213–2219
- Yasui, W., Hata, J., Yokozaki, H., Nakatani, H., Ochiai, A., Ito, H. & Tahara, E. (1988) Interaction between epidermal growth factor and its receptor in progression of human gastric carcinoma. *Int. J. Cancer*, **41**, 211–217
- Yasui, W., Akama, Y., Kuniyasu, H., Yokozaki, H., Semba, S., Shimamoto, F. & Tahara, E. (1996a) Expression of cyclin-dependent kinase inhibitor p21WAF1/CIP1 in non-neoplastic mucosa and neoplasia of the stomach: relation with p53 status and proliferative activity. *J. Pathol.*, **180**, 122–128
- Yasui, W., Yokozaki, H., Kuniyasu, H., Shimamoto, F. & Tahara, E. (1996b) Expression of CDK inhibitor p16CDKN2/MTS1/INK4A in human gastric carcinomas. In: Tahara, E., Sugimachi, K. & Oohara, T., eds, *Recent Advances in Gastroenterological Carcinogenesis I*, Bologna, Monduzzi Editore, pp. 765–769
- Yasui, W., Kudo, Y., Semba, S., Yokozaki, H. & Tahara, E. (1997) Reduced expression of cyclin-dependent kinase inhibitor p27Kip1 is associated with advanced stage and invasiveness of gastric carcinomas. *Jpn. J. Cancer Res.*, **88**, 625–629
- Yasui, W., Tahara, H., Tahara, E., Fujimoto, J., Nakayama, J., Ishikawa, F., Ide, T. & Tahara, E. (1998) Expression of telomerase catalytic component, telomerase reverse transcriptase, in human gastric carcinomas. *Jpn. J. Cancer Res.*, **89**, 1099–1103
- Yasui, W., Naka, K., Suzuki, T., Fujimoto, J., Hayahi, K., Matsutani, N., Yokozaki, H. & Tahara, E. (1999a) Expression of p27Kip1, cyclin E, and E2F-1 in primary and metastatic tumors of gastric carcinoma. *Oncol. Rep.*, **6**, 983–987
- Yasui, W., Tahara, E., Tahara, H., Fujimoto, J., Naka, K., Nakayama J., Ishikawa, F., Ide, T. & Tahara, E. (1999b) Immunohistochemical detection of human telomerase reverse transcriptase in normal mucosa and precancerous lesions of the stomach. *Jpn. J. Cancer Res.*, **90**, 589–595
- Yokota, J., Yamamoto, T., Miyajima, N., Toyoshima, K., Nomura, N., Sakamoto, H., Yoshida, T., Terada, M. & Sugimura T. (1988) Genetic alterations of the c-erbB-2 oncogene occur frequently in tubular adenocarcinoma of the stomach and are often accompanied by amplification of the v-erbA homologue. *Oncogene*, **2**, 283–287
- Yokozaki, H., Kuniyasu, H., Kitadai, Y., Nishimura, K., Todo, H., Ayhan, A., Yasui, W., Ito, H. & Tahara, E. (1992) p53 Point mutations in primary human gastric carcinomas. *J. Cancer. Res. clin. Oncol.*, **119**, 67–70
- Yokozaki, H., Ito, R., Nakayama, H., Kuniyasu, H., Taniyama, K. & Tahara, E. (1994) Expression of CD44 abnormal transcripts in human gastric carcinomas. *Cancer Lett.*, **83**, 229–234
- Yokozaki, H., Kuniyasu, H., Semba, S., Yasui, W. & Tahara, E. (1997) Molecular bases of human stomach carcinogenesis. In: Tahara, E., ed., *Mole-*

- cular Pathology of Gastroenterological Cancer: Application to Clinical Practice*, Tokyo, Springer-Verlag, pp. 55–70
- Yokozaki, H., Shitara, Y., Fujimoto, J., Hiyama, T., Yasui, W. & Tahara, E. (1999a) Alterations of p73 preferentially occur in gastric adenocarcinomas with foveolar epithelial phenotype. *Int. J. Cancer*, **83**, 192–196
- Yokozaki, H., Semba, S., Fujimoto, J. & Tahara, E. (1999b) Microsatellite instabilities in gastric cancer patients with multiple primary cancers. *Int. J. Oncol.*, **14**, 151–155
- Yonemura, Y., Ninomiya, I., Ohoyama, S., Kimura, H., Yamaguchi, A., Fushida, S., Kosaka, T., Miwa, K., Miyazaki, I., Endou, Y., Tanaka, M. & Sasaki, T. (1991) Expression of c-erbB-2 oncoprotein in gastric carcinoma: immunoreactivity for c-erbB-2 protein is an independent indicator of poor short-term prognosis in patients with gastric carcinoma. *Cancer*, **67**, 2914–2918
- Yoon, K.A., Ku, J.L., Yang, H.K., Kim, W.H., Park, S.Y. & Park, J.G. (1999) Germline mutations of E-cadherin gene in Korean familial gastric cancer patients. *J. hum. Genet.*, **44**, 177–180
- Yoshida, M.C., Wada, M., Satoh, H., Yoshida, T., Sakamoto, H., Miyagawa, K., Yokota, J., Koda, T., Kakinuma, K., Sugimura, T. & Terada, M. (1988) Human *HST1* (*HSTF1*) gene maps to chromosome band 11q13 and co-amplifies with the *INT2* gene in human cancer. *Proc. natl Acad. Sci. USA*, **85**, 4861–4864
- Yoshida, K., Yokozaki, H., Niimoto, M., Ito, H., Ito, M. & Tahara, E. (1989) Expression of TGF- $\alpha$  and pro-collagen type I and type III in human gastric carcinomas. *Int. J. Cancer*, **44**, 394–398
- Yoshida, K., Tsujino, T., Yasui, W., Kameda, T., Sano, T., Nakayama, H., Toge, T. & Tahara, E. (1990) Induction of growth factor-receptor and metalloproteinase genes by epidermal growth factor and/or transforming growth factor- $\beta$  in human gastric carcinoma cell lines MKN-28. *Jpn. J. Cancer Res.*, **81**, 793–798
- Yoshida, K., Bolodeoku, J., Sugino, T., Goodison, S., Matsumura, Y., Warren, B.F., Toge, T., Tahara, E. & Tarin, D. (1995) Abnormal retention of intron 9 in CD44 gene transcripts in human gastrointestinal tumors. *Cancer Res.*, **55**, 4273–4277
- Yoshida, K., Yasui, W., Kagawa, Y. & Tahara, E. (1996) Multiple genetic alterations and abnormal growth factor network in human esophageal carcinomas. In: Tahara, E., ed., *Molecular Pathology of Gastroenterological Cancer: Application to Clinical Practice*, Tokyo, Springer-Verlag, pp. 31–41
- Yustein, A.S., Harper, J.C., Petroni, G.R., Cummings, O.W., Moskaluk, C.A. & Powell, S.M. (1999) Allelotype of gastric adenocarcinoma. *Cancer Res.*, **59**, 1437–1441

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