Tumour suppressor genes in gastric carcinogenesis: an up-to-date review of genetic and epigenetic changes in the development of gastric adenocarcinoma

Lucy K. Satherley[1], Lin Ye[2], Wen G. Jiang[2], Rachel Hargest[1]

[1]Department of Colorectal Surgery, University Hospital of Wales, Cardiff, CF14 4XN, UK; [2]Cardiff University-Peking University Cancer Institute, Cardiff University School of Medicine, Heath Park, Cardiff, CF14 4XN, UK
Contributions: (I) Conception and design: LK Satherley, R Hargest; (II) Administrative support: None; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: LK Satherley; (V) Data analysis and interpretation: LK Satherley, R Hargest; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.
Correspondence to: Lucy K. Satherley, Research Registrar in General Surgery and PhD student at Cardiff University, c/o Secretary to Miss R Hargest, University Hospital of Wales, Heath Park, Cardiff CF14 4XN, UK. Email: SatherleyLK2@cardiff.ac.uk.

Abstract: Gastric cancer is the fifth commonest cancer worldwide and is the third leading cause of cancer related death. The 5-year survival rate is just 20%, partly as a consequence of our limited understanding of the pathogenesis of gastric cancer. Tumour suppressor genes (TSGs) play an important protective role in preventing the malignant transformation of cells by repairing DNA, inhibiting cell proliferation and initiating programmed cell death. Over recent decades there has been a rapid expansion in the number of TSGs that have been identified in relation to gastric adenocarcinoma and it is hoped that these may act as biomarkers of disease or provide potential therapeutic targets. Mutations in TSGs, oncogene activation and epigenetic change have all been shown to participate in gastric carcinogenesis. Here we discuss some of the key TSGs implicated in the development of gastric adenocarcinoma and present an up-to-date review of what is currently understood about the genetic and epigenetic alterations of tumour suppressors that underpin gastric carcinogenesis.

Keywords: Tumour suppressor genes (TSGs); gastric cancer; gene therapy

Introduction

Gastric cancer is the fifth commonest cancer worldwide, with approximately 1 million new cases diagnosed in 2012 (1). More than 70% of cases occur in developing countries, mainly in Eastern Asia (1). It is the third leading cause of cancer-related death, with a 5-year survival of just 20%, which is partly attributable to our relatively poor understanding of the pathogenesis of gastric cancer (1-3). The commonest type of gastric cancer is adenocarcinoma, which accounts for over 95% of diagnoses (4).

Gastric adenocarcinoma is a heterogeneous, multifactorial disease (5). Risk factors include male gender, increasing age, a diet high in salty food, pickled food, red meat or nitrosamines, infection with *Helicobacter pylori* (*H. pylori*), smoking, previous gastric ulcer or gastric polyps, pernicious anaemia, previous vagotomy or partial gastrectomy, family history, history of other cancers, radiation exposure, immunosuppression, occupational exposure and blood group A (4,6). Of these, *H. pylori* infection, particularly cytotoxin-associated gene A-positive [CagA (+)] *H. pylori*, is the most important risk factor for gastric cancer and is responsible for at least 95% of cases (7,8).

The Lauren classification divides gastric adenocarcinoma into two main histological types: intestinal or diffuse (9,10). The pathophysiology of intestinal type gastric cancer is relatively well understood and involves the transition

through chronic gastritis, atrophy, intestinal metaplasia, dysplasia, intramucosal carcinoma to invasive cancer (9). By comparison, the development of diffuse gastric cancer is less well understood (9). Solcia *et al*. suggested that diffuse cancer develops from hyperplastic, non-metaplastic gastric glands and involves genes involved in cell-cell and cell-matrix interactions (11). A study by Nakayama *et al*. found that there were frequent differences in the genetic lineages between early [invasive gastric cancer that invades no more deeply than the submucosa, irrespective of lymph node metastases (T1, any N)] and advanced tubular gastric adenocarcinomas suggesting that some early cancers may not necessarily develop into advanced cancers and may instead represent a distinct variant of cancer (12).

Tumour suppressor genes (TSGs) in cancer

TSGs are genes that normally play a protective role in preventing the malignant transformation of cells by repairing DNA, inhibiting cell proliferation and initiating programmed cell death (apoptosis). They are involved in the regulation of a range of cell functions including cell adhesion, cell-cell interaction, cytoplasmic signal transduction and nuclear transcription (13).

The first identified TSG was retinoblastoma 1 (*RB1*) gene, which was discovered by Cavenee *et al*. in 1983 (14). This confirmed the "two-hit" hypothesis proposed by Knudson a decade earlier, based on observation of retinoblastoma cases and published reports, that retinoblastoma arises as a result of two mutational events (15). Several authors have since established that *RB1* is lost or inactivated in many other more common human cancers including bladder, breast and lung carcinoma and that it is also a target for oncogenic proteins of DNA tumour viruses which may bind to RB family proteins inhibiting their activity at protein, rather than at gene, level (16,17).

Over recent decades, there has been a rapid expansion in the number of TSGs that have been identified in relation to a broad range of inherited and non-inherited human cancers and it has been suggested that alterations in TSGs account for some of the most common molecular changes in human carcinogenesis (18). It is hoped that greater understanding of the pattern of TSG expression in gastric cancer may enable the identification of specific biomarkers that could be used for early diagnosis and the development of targeted treatments.

Mutations in TSGs may result in their loss or inactivation, enabling cells to undergo uncontrolled cell division or malignant change resulting in the development of many types of human cancer. Most TSG mutations are acquired, although they may be inherited.

TSGs in gastric cancer

A number of TSGs have been implicated in gastric carcinogenesis (see *Table 1*).

A study by Lee *et al*. found that TSGs accounted for the majority of proteins significantly associated with survival in gastric carcinomas (39). Specifically, the overexpression of p53 and loss of expression of PTEN, E-cadherin, smad4, FHIT, MGMT and CD82 were all significantly associated with poor prognosis in gastric carcinomas (39). Loss of smad4 expression was more common in intestinal type, whilst loss of FHIT and E-cadherin expression was more common in diffuse type gastric carcinoma (39). Loss of p53 expression was more common in poorly differentiated tumours (39). Loss of E-cadherin, smad4, CD82, MGMT and PTEN expression was associated with advanced stage of disease whilst loss of FHIT and p16 expression was seen in both early and advanced stages of disease (39). Moreover, loss of TSG expression accumulates in a stepwise fashion during gastric cancer tumour progression and there is a significant correlation between this accumulation and patient survival (39). Lee *et al*. suggest that TSG expression status is one of the most important factors in determining the prognosis of gastric cancer (39).

In addition to mutations in TSG, genetic instability, activation of oncogenes and aberrant growth factor expression/receptor activation all play a role in gastric carcinogenesis (40). For example, in Epstein-Barr virus-associated gastric carcinoma (EBVaGC), which accounts for 10% of all gastric carcinomas, global CpG island hypermethylation occurs via viral proteins or microRNAs (miRNAs) resulting in epigenetic silencing of TSGs (41,42).

Here we discuss some of the main TSGs that have been implicated in the development of gastric adenocarcinoma and present an up-to-date review of what is currently understood about the genetic and epigenetic mediated changes that may underpin gastric carcinogenesis.

TP53

Abnormalities in TP53 have been found in over half of human cancers including leukaemia, breast, colon and lung carcinoma, and *TP53* mutations are the most common genetic alteration seen in human cancer (18). They are

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Table 1 TSGs implicated in gastric carcinogenesis

Official full name	Official symbol	Genomic location	Role (references)
Tumour protein p53	TP53	17p13.1	Regulates target genes to induce cell cycle arrest, apoptosis, senescence and DNA repair (19,20)
Phosphatase and tensin homolog	PTEN	10q23.3	Downregulates Akt/PKB signalling pathways that promote cell survival (21-23)
Cadherin 1, type 1, E-cadherin (epithelial)	CDH1	16q22.1	Involved in cell-cell adhesion (24)
SMAD family member 4	SMAD4	18q21.1	Regulates gene transcription (25)
Fragile histidine triad	FHIT	3p14.2	Involved in purine metabolism (26)
O-6-methylguanine-DNA methyltransferase	MGMT	10q26	DNA repair enzyme (27,28)
CD82 molecule/KAI1	CD82	11p11.2	Tumour metastasis suppressor that can be activated by p53 (29)
Cyclin-dependent kinase inhibitor 2A/p16	CDKN2A	9p21	Cell cycle G1 control (30)
WW domain containing oxidoreductase	WWOX	16q23	Induces apoptosis (31)
Gastrokine 1	GKN1	2p13.3	Induces tumour senescence (32)
Adenomatous polyposis coli	APC	5q21-q22	Antagonist of Wnt signalling pathway (33). Involved in cell migration, adhesion, transcriptional activation and apoptosis (33)
DCC netrin 1 receptor	DCC	18q21.3	A member of the immunoglobulin superfamily of cell adhesion molecules (34). Induces apoptosis in the absence of ligand (34)
Retinoblastoma 1	RB1	13q14.2	Negative regulator of cell cycle, maintains chromatin structure (35)
Promyelocytic leukaemia	PML	15q22	Functions as a transcription factor and as a tumour suppressor, regulating p53 response to oncogenic signals (36)
KIAA1324	KIAA1324	1p13.3	Transmembrane protein thought to be involved in the cellular response to stress (37). Associated with survival in certain carcinomas (37)
CCAAT/enhancer binding protein (C/EBP), alpha	CEBPA	19q13.1	Transcription factor (38). Modulates expression of genes involved in cell cycle regulation (38)

TSGs, tumour suppressor genes.

identified in over 60% of human gastric carcinomas, regardless of histological type (43,44). The p53 protein plays a key role in cell cycle progression, preventing G1/S phase transition after DNA damage has occurred, allowing DNA repair or cell apoptosis (19). Downstream targets of p53 have also been shown to play a role in gastric cancer invasion (45).

Some studies have shown that abnormalities in *TP53* can occur in non-neoplastic gastric mucosa with intestinal metaplasia suggesting that *TP53* mutations may occur early in gastric carcinogenesis, whilst others have suggested that *TP53* gene mutations occur relatively late in the sequence of events underlying the development

of gastric carcinoma (46,47). There is some variation depending on the histological type of gastric cancer, with *TP53* mutations seen early in the development of intestinal type gastric cancer but late in the development of diffuse gastric cancer (11,39).

Hamada *et al.* analyzed the surgical specimens of patients with gastric cancer and found that there were significantly fewer apoptotic cells following pre-operative chemotherapy or radiotherapy in tumours with mutant *TP53* expression compared to those expressing wild-type *TP53*, suggesting that *TP53* mutations are associated with poorer gastric cancer response to chemotherapy and radiotherapy (48).

Moreover, mutant *TP53* is associated with shorter life expectancy, not only in gastric cancer, but also in breast, lung and colorectal cancer (19).

Recent studies have suggested that *H. pylori* CagA (+) contributes to the inactivation of *TP53* (49). The CagA protein is encoded by the cag pathogenicity island of *H. pylori* and interferes with the p53 tumour suppressor pathway resulting in an anti-apoptotic effect (50). CagA also targets the tumour suppressor *ASPP2* (50).

PTEN

PTEN is a lipid phosphatase that dephosphorylates phosphatidylinositol-3,4,5-triphosphate (PIP$_3$), reducing the activation of phosphoinositide 3-kinase (PI3K) and the serine/threonine-specific protein kinase Akt, both of which promote cell survival (21). Loss of PTEN increases levels of PIP$_3$, increases Akt activation and consequently inhibits apoptosis (22).

Inactivation of PTEN may occur as a result of gene mutation, loss of heterozygosity (LOH), promoter hypermethylation or miRNA-mediated alteration in gene expression or post-translational modification and has been shown to occur in many cases of gastric cancer (51).

In gastric adenocarcinoma, mutation of *PTEN* is associated with more advanced stage of disease, poorer tumour differentiation, lymphatic vessel invasion and tumour chemoresistance (39,51,52). Wen *et al.* found that PTEN and E-cadherin protein expression are significantly downregulated in gastric cancer tissue compared with normal gastric tissue whilst PI3K, Akt, MMP-2, MMP-9 and NF-κBp65 protein are overexpressed in gastric cancer (52).

CDH1

E-cadherin has been implicated in early gastric carcinogenesis, tumour progression, invasion and metastasis (7,53). Deregulation of E-cadherin may occur as a consequence of *CDH1* gene mutation, epigenetic promoter hypermethylation, LOH, transcriptional modification and regulation by miRNAs (53). Somatic alterations in *CDH1* are found in approximately 30% of all patients with gastric cancer of both histological types with approximately one third of these having structural alterations (7.5% LOH, 1.7% mutation) with the remainder having epigenetic modifications (18.4% hypermethylation) (54). Epigenetic modifications are more common than structural in diffuse

gastric cancer whilst intestinal type cancer has roughly similar rates of structural and epigenetic changes (54). Structural alterations, rather than epigenetic modifications, of *CDH1* are associated with the poorest prognosis (54).

Loss of E-cadherin expression resulting from *CDH1* gene alteration is associated with increased cancer cell proliferation, invasion and/or metastasis and is thought to be the primary carcinogenic event in hereditary diffuse gastric cancer (53,55,56). The cumulative lifetime risk of developing gastric cancer in *CDH1* mutation carriers is quoted by Chun and Ford [2012] as being up to 80% (3). The level of *CDH1* hypermethylation is also higher in gastric cancer and adjacent gastric mucosa than in normal gastric tissue (55). Furthermore, hypermethylation of *CDH1* correlates with *H. pylori* status in gastric cancer (55). E-cadherin interacts with β-catenin, a proto-oncogene, at the cell-membrane and aberrant expression of this complex is also seen in gastric cancer (7).

A recent study by Wei *et al.* found that mRNA and protein expression levels of T-cadherin (another member of the cadherin family) are significantly lower in gastric cancer tissue compared with corresponding adjacent normal gastric tissue (57). Moreover, decreased T-cadherin protein expression is correlated with larger tumour size, lymph node metastases and higher TNM stage (57). Multivariate analysis reveals that T-cadherin expression is an independent prognostic factor for overall survival (57).

SMAD4

Mutations in *SMAD4* are associated with gastric cancer (39,58). Loss of expression of smad4 is associated with advanced pT stage and poorer outcome in gastric carcinoma patients and is more common in intestinal compared with diffuse type gastric adenocarcinomas (39).

FHIT/FRA3B locus

The *FHIT* gene is located at the most fragile human chromosome site, a location which predisposes genes to chromosomal rearrangements that may result in neoplastic growth (39,59). LOH for alleles in this region is seen in around 50% of uncultured stomach and colon carcinomas and it is also lost or absent in lung, kidney and cervical carcinomas (59,60). Loss of FHIT protein expression is a significant and independent predictor of survival in gastric adenocarcinoma and is significantly correlated with diffuse type, poor differentiation and

advanced stage of disease (39,61).

MGMT

MGMT plays an important role in the repair of DNA damage and inactivation of *MGMT* by promoter methylation is implicated in gastric carcinogenesis (62,63). A study of gastric cancer patients by Park *et al*. found that *MGMT* promoter methylation was significantly associated with *KRAS* mutation, lymph node invasion, tumour stage and disease free survival (64). The *MGMT* promoter is more frequently methylated in advanced gastric cancer but has also been found in early gastric cancer and precancerous lesions (65,66).

CD82 molecule (CD82)

The CD82 molecule is a membrane glycoprotein that belongs to the transmembrane 4 superfamily, which act as inhibitors of tumour cell motility and metastasis (67). Reduced expression of CD82 is found in gastric cancer and loss of CD82 protein expression is associated with disease progression, metastasis and poor clinical outcome (68,69).

CDKN2A/p16

CDKN2A is more commonly methylated in gastric cancer compared to normal gastric tissue (70).

WW domain containing oxidoreductase (WWOX)

WWOX is a TSG, involved in the negative regulation of cell growth, which is frequently mutated in gastric cancer (71,72). It is thought to play a role in tumour progression by regulating the TGFβ/SMAD and Wnt signaling pathways and loss of WWOX expression is associated with more aggressive disease and poorer prognosis in a range of cancers including gastric carcinoma (71,73,74). Increased hypermethylation of *WWOX* is found in EBVaGC and in the presence of *H. pylori* infection (75,76).

Gastrokine 1 (GKN1)

GKN1 is a TSG, highly expressed in normal stomach (77). GKN1 expression is frequently lost in the presence of *H. pylori* infection and gastric cancer, particularly the diffuse subtype, with conflicting evidence on its expression in metaplasia (77-79). Reduced or lost GKN1 expression occurs with similar frequency in gastric adenomas and carcinomas, suggesting it may play a role in the early stages of gastric carcinogenesis (80). GKN1 plays a role in the inflammatory response of gastric mucosa via the regulation of cytokine production, the NF-κB signaling pathway and cyclooxygenase-2 expression, inhibits the carcinogenic effect of *H. pylori* CagA (+) and has a pro-antioxidant effect (78). GKN1 negatively regulates cell survival, proliferation, colony formation, epithelial-to-mesenchymal transition (EMT), cancer cell migration, invasion and metastases, and promotes apoptotic cell death (78,81). Moreover, expression of GKN1 is associated with increased tumour sensitivity to fluorouracil treatment (82). Loss of GKN1 is associated with significantly shorter survival in intestinal type gastric cancer (79).

Adenomatous polyposis coli (APC)

The *APC* gene is located on the long arm of chromosome 5 close to other TSGs involved in gastric adenocarcinoma (83). Inactivation of *APC* as a consequence of germline mutations is seen in familial adenomatous polyposis (FAP) and as a result of somatic mutations in the development of a range of human cancers (84). Unlike colorectal cancer, changes in genes such as *APC*, though present in gastric carcinoma, are rare in gastric adenomas, suggesting that the adenoma-carcinoma sequence is less common in gastric carcinogenesis and indeed Tahara [2004] suggest that the sequence is found in only 20% of gastric adenomas with *APC* mutations (85,86). *APC* can also be inactivated by promoter hypermethylation (87).

Allele loss on chromosome 5q is often seen in gastric adenocarcinoma, particularly in well-differentiated early cancer (44). LOH at the *APC* genetic locus is found in over 25% of cases of gastric cancer, including early cancers, suggesting that abnormalities in the *APC* gene may occur early in tumourigenesis (88). In advanced gastric cancer, LOH at the *APC* locus is found in 30% of informative cases (89).

DCC netrin 1 receptor (DCC)

LOH at the *DCC* locus is associated with well differentiated and advanced gastric cancer (89,90).

RB1

RB1 is involved in the negative regulation of the cell cycle at the G1/S transition (16,91). LOH at the *RB1* locus is found in 30% of informative cases of advanced gastric cancer (89). *RB1* has also been shown to be a target of miRNA-

Table 2 Putative TSGs in gastric carcinogenesis

Official full name	Official symbol	Genomic location	Role (references)
Mutated in colorectal cancers	MCC	5q21	Negatively regulates cell cycle progression (96)
Cytoplasmic polyadenylation element binding protein 1	CPEB1	15q25.2	Regulates mRNA translation and processing of 3' untranslated region (97). May play a role in cell proliferation and tumourigenesis (97)
ADAM metallopeptidase with thrombospondin type 1 motif, 9	ADAMTS9	3p14.1	Inhibits angiogenesis (98)
Paired box 5	PAX5	9p13	Transcription factor (99)
ZFP82 zinc finger protein	ZFP82	19q13.12	(100)
B-cell CLL/lymphoma 6, member B	BCL6B	17p13.1	(101)
FAT atypical cadherin 4	FAT4	4q28.1	Regulates planar cell polarity (102)
Zinc finger, MYND-type containing 10	ZMYND10	3p21.3	(103)
Checkpoint with forkhead and ring finger domains, E3 ubiquitin protein ligase	CHFR	12q24.33	Cell cycle progression and tumourigenesis (104)
STIP1 homology and U-box containing protein 1, E3 ubiquitin protein ligase	STUB1	16p13.3	Functions as a ubiquitin ligase/cochaperone (105)

TSGs, tumour suppressor genes.

106b~25, which promotes cell proliferation, EMT, cell-cycle progression and exerts an anti-apoptotic effect (92).

Promyelocytic leukaemia (PML)

PML protein is a tumour suppressor, involved in the control of apoptosis and cell proliferation via p73 and the yes-associated protein (YAP 1), which is downregulated in gastric cancer (93).

KIAA1324

KIAA1324 is downregulated in gastric cancer and plays a role in the control of cell proliferation, invasion and apoptosis as well as inhibiting GRP78 oncoprotein (94).

CCAAT/enhancer binding protein (C/EBP), alpha (CEBPA)

CEBPA encodes a transcription factor involved in inducing terminal differentiation and in the control of cell proliferation (95). It has been identified as a TSG in several types of cancer and its expression is lost in 30% of cases of gastric cancer (95).

Candidate TSGs

Over recent years, several putative TSG have been identified.

These are shown in *Table 2* and discussed below.

Mutated in colorectal cancers (MCC)

The *MCC* gene, like *APC*, is mapped to chromosome 5. A study of 24 surgical specimens of primary gastric cancer analysed by Tamura *et al.* found that LOH at the *MCC* locus occurred in all cases though other studies have found much lower incidences (88,106-108). Sanz-Ortega *et al.* subsequently reported the loss of *MCC* heterozygosity in intestinal metaplasia and in dysplastic lesions, in addition to gastric cancer (109). LOH at the *MCC* locus is frequently accompanied by LOH at the *APC* locus and both are found in differentiated and undifferentiated, early and advanced gastric carcinoma (106). Interestingly, a study by Rhyu *et al.* found that allelic deletion at *MCC/APC* was not detected in tumours that did not have allelic deletion of *TP53*, suggesting that *MCC* allelic deletions do not occur independently of *TP53* alterations in gastric carcinogenesis (110).

FAT atypical cadherin 4 (FAT4)

FAT4 suppresses tumour growth via activation of Hippo signalling (111,112). In a recent study by Jung *et al.*, loss of FAT4 protein expression was found in 24% of gastric cancer cases and was associated with increased invasiveness, high

TNM stage and reduced disease-free survival time (111).

Zinc finger, MYND-type containing 10 (ZMYND10)

ZMYND10 is involved in the regulation of gastric cancer cell proliferation and colony formation (113). Methylation of *ZMYND10* is significantly higher in gastric cancer compared with normal tissues with subsequent reduction in ZMYND10 protein expression levels (114).

Ubiquitin protein ligases

The *CHFR* gene codes for E3 ubiquitin-protein ligase and abnormal promoter methylation of this gene is thought to play a role in gastric carcinogenesis (115). The carboxyl terminus of Hsc-70-interacting protein (CHIP) is a U-box-type ubiquitin ligase, the overexpression of which is associated with reduced gastric cancer growth (116).

Controversial TSGs in gastric cancer

Runt-related transcription factor 3 (RUNX3), a member of the RUNX transcription factor family, is a downstream effector of the TGFβ signaling pathway which regulates cell proliferation, apoptosis, angiogenesis, adhesion and invasion (117). Several studies have found that loss of function of RUNX3 is associated with gastric carcinogenesis and this effect appears to be mediated via the Akt1, TGFβ and Wnt signaling pathways (7,117-119). Loss of RUNX3 is also significantly associated with the presence of *H. pylori* Cag (+), spontaneous EMT and is associated with the degree of gastric mucosal inflammation, atrophy and intestinal metaplasia (7,117). The expression of RUNX3 also correlates with that of E-cadherin (7).

Lu *et al*. found that the proportion of gastric lesions with *RUNX3* promoter methylation increased with increasing stage of gastric cancer progression, with promoter methylation evident in 16% of chronic atrophic gastritis, 37% of intestinal metaplasia, 42% in gastric adenoma, 55% in dysplasia and 75% in gastric cancer (120). These effects were more pronounced in *H. pylori* positive patients compared to those who were *H. pylori* negative (120).

Despite these features, however, Lotem *et al*. have recently re-appraised the evidence regarding the role of *RUNX3* and assert that it is not a bone fide cell-autonomous TSG although it may have an indirect influence on tumour development by virtue of its important roles in immunity and inflammation (121).

Epigenetic changes in gastric cancer

Altered gene expression can occur not only as a consequence of genetic or environmental factors but as a result of epigenetic factors resulting in gastric carcinogenesis (122,123). These inheritable, but non-genetic chromosomal modifications, which include DNA methylation and histone modification, occur as a result of chromatin modifiers and non-coding RNA (ncRNA) and result in altered gene expression (123). Inactivation of TSGs in gastric cancer is more commonly due to epigenetic change by promoter methylation than it is due to mutation (124).

For example, epigenetic inactivation of hepatocyte growth factor (HGF) activator inhibitor type 2 (*HAI-2*) is found in gastric cancer (125). Loss or reduction in expression of the iroquois homeobox 1 (*IRX1*) *TSG* on chromosome 5p15.33 is also found in gastric cancer and work by Guo *et al*. has shown that this is not due to mutations in *IRX1*, but rather, it is due to hypermethylation suppressing the transcription of *IRX1* (126). Another study by Ling *et al*. found that N-myc downstream regulated gene 2 (*Ndrg2*), a candidate metastasis suppressor gene, is frequently downregulated in gastric cancer cell lines and tissues (127). Moreover, this effect, which occurs secondary to hypermethylation of the *Ndrg2* promoter is associated with *H. pylori* infection and it is thought that *H. pylori* induces this effect by NF-κB activation and up-regulating DNMT3b (127).

Non-coding RNAs (ncRNAs)

ncRNAs regulate gene expression and are essential for the control of important cell functions including cell proliferation and survival (128). Deregulation of ncRNAs contributes to gastric cancer development by altering the expression of oncogenes or TSGs and by their regulatory effect on factors such as PTEN, E-cadherin, Akt and p53 (128,129). They have been shown to play a major role in gastric cancer development, invasion and metastasis (130). They can be sub-divided into long non-coding (lncRNAs) and miRNAs (128).

miRNAs are small, endogenous single stranded RNA molecules that negatively regulate gene expression by cleaving target mRNA or inhibiting translation (131). They play a significant role in the development of gastric cancer via the regulation of cell cycle progression, invasion, motility, metastasis and apoptotic cell death (129,132). Altered expression of miRNAs has been associated with gastric tumour size, degree of differentiation, disease stage and the

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presence of metastasis (130). Several previous studies have suggested that several miRNAs act as tumour suppressors in gastric carcinogenesis via a number of mechanisms including deregulation of E-cadherin (133-142).

lncRNAs are non-coding transcripts over 200 nucleotides long that have also been shown to act as oncogenes and tumour suppressors and may play an important role in the development of gastrointestinal carcinomas (143,144). They regulate gene expression by chromosome remodeling, transcriptional or post-transcriptional gene processing, resulting in a range of effects including imprinting, gene activation, gene repression and cell proliferation (145-147). In gastric cancer, lncRNAs have been implicated in the regulation of cell proliferation, cell-cell adhesion, EMT, extracellular matrix degradation, cell migration, invasion, metastasis, angiogenesis and tumour cell apoptosis (128,148,149).

Piwi-interacting RNAs (piRNAs) are a recently discovered class of small ncRNAs that bind specifically to Piwi protein family members to form piRNA compounds (piRC) that regulate gene-silencing pathways (130). They are implicated in gastric carcinogenesis and have shown promise as a possible peripheral blood-based diagnostic biomarker (150).

H. pylori and TSGs in gastric cancer

H. pylori promotes gastric carcinogenesis by a variety of means: it stimulates oxygen-derived free radical release from activated neutrophils, increases cytidine deaminase activity in infected gastric tissues resulting in mutations, CagA is secreted into gastric epithelial cells causing inflammation and oncogene activation, it induces epigenetic transformations such as promoter methylation in TSGs, and it induces aberrant expression of miRNAs (151).

Cumulative loss of TSGs in gastric cancer

There is a wealth of evidence to suggest that many TSGs may play a role in gastric cancer individually but also evidence to suggest that cumulative loss or abnormalities in these genes may also be important. A study by Cho *et al*. investigating simultaneous LOH in advanced gastric cancer, found that 33% of tumours informative at *APC*, *DCC* and *RB1* loci had LOH at all three of these loci (89).

Gene therapy and TSG in gastric cancer

Improved understanding of the TSGs involved in gastric

carcinogenesis has opened up potential opportunities for novel therapies involving the reintroduction of TSGs by gene therapy using viral or synthetic vectors (152). In particular, adenoviruses provide an attractive vehicle for gene therapy and have been used in phase III clinical trials to deliver wild-type *TP53* to patients with head and neck and ovarian cancer (153). The reintroduction of TSGs by gene therapy is an attractive treatment strategy since it offers the prospect of cancer treatment without toxicity to nearby normal cells (154). However, there are currently a number of issues limiting the clinical application of gene therapy including concerns regarding toxicity, ethical and regulatory issues, practical limitations such as efficiency of gene transfer as well as limitations in disease response, which need to be overcome (155).

Reintroduction of TSGs by gene therapy aims to reinstate the cell's natural regulatory mechanism for suppressing tumour growth but may have additional therapeutic effects, such as inducing apoptosis via the activation of other genes and increasing the sensitivity of cancer cells to chemotherapy (156,157).

Previous studies have shown that infection of human gastric carcinoma cell lines with recombinant *TP53* adenovirus vector results in inhibition of growth and apoptotic cell death (157,158). Meanwhile, a review by Ishii *et al*. found that reintroduction of *FHIT* inhibited *in vitro* tumour cell growth or tumourigenicity in 57% of experiments analysed (159). Moreover, experimental work by Ishii *et al*. has shown that in *FHIT*-deficient mice, orally introduced viral vector-mediated *FHIT* was associated with reduced tumour development and reversal of established tumours by apoptosis (160). Reintroduction of *CDKN2A* using an expression vector resulted in reduced gastric cancer cell growth rate and enhanced sensitivity to chemotherapeutic drugs (161).

Conclusions

Gastric cancer is a complex, multifactorial disease in which TSGs play an essential role. Genetic and epigenetic changes in these genes contribute to gastric carcinogenesis regardless of histological type at all stages of disease progression and show promise as potential biomarkers of disease or as therapeutic targets.

Acknowledgements

collaborative research partners in Peking University, Capital Medical University and Yiling Medical Research Institute.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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