Genomic and genetic alterations influence the progression of gastric cancer

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Abstract

Gastric cancer is one of the leading causes of cancer-related deaths worldwide, although the incidence has gradually decreased in many Western countries. Two main gastric cancer histotypes, intestinal and diffuse, are recognized. Although most of the described genetic alterations have been observed in both types, different genetic pathways have been hypothesized. Genetic and epigenetic events, including 1q loss of heterozygosity (LOH), microsatellite instability and hypermethylation, have mostly been reported in intestinal-type gastric carcinoma and its precursor lesions, whereas 17p LOH, mutation or loss of E-cadherin are more often implicated in the development of diffuse-type gastric cancer.  

In this review, we summarize the sometimes contradictory findings regarding those markers which influence the progression of gastric adenocarcinoma.  

Key words: Gastric cancer; Gene alterations; Prognosis; Molecular pathology  

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INTRODUCTION

Gastric cancer is one of the leading causes of cancer-related deaths worldwide, although the incidence has gradually decreased in many Western countries. Several attempts to classify gastric cancer have been made over the past decades. Most successful, and widely used, is the classification by Lauren, which, by microscopic morphology alone, distinguishes two main cancer pathogeneses, diffuse and intestinal subtypes, which clearly appear as dissimilar clinical and epidemiological entities. Although most of the genetic alterations that have been reported are observed in both intestinal and diffuse gastric cancers, it has become apparent that these two tumor types result from different genetic pathways (Table 1).

Microsatellite instability and p53 mutation, reduced...
p27 expression, cyclin E overexpression and 6.0-kb transcripts of the c-met gene are involved in malignant transformation from precancerous lesions to intestinal-type gastric cancer. In addition, DCC loss, APC mutations, 1q loss of heterozygosity (LOH), p27 loss, reduced expression of tumor growth factor (TGF)-β1 type 1 receptor and HER2 gene amplification are frequently associated with an advanced stage of intestinal-type gastric carcinoma. In contrast, LOH at chromosome 17p (p53) and mutation or loss of E-cadherin are more often implicated in the development of diffuse-type gastric cancer, while loss of p27 and gene amplification of K-ras and c-met lead to disease progression and metastatic spread.

The two types of gastric carcinoma organize different patterns of interplay between neoplastic and stromal cells through the growth factor/cytokine receptor system, which has a critical role in cell growth, apoptosis, morphogenesis, angiogenesis and metastasis. Other genetic factors, such as DNA polymorphism and genetic instability, may also be implicated in the two distinct major genetic pathways of gastric carcinogenesis.

**GENOMIC INSTABILITY**

Two phenotypes of genomic instability are generally recognized in gastric cancer: the phenotype associated with microsatellite instability (MSI) and that which is associated with chromosomal instability (CIN). These phenotypes are not necessarily independent and may even overlap in some cases[8].

**MSI**

MSI is a common feature of gastric cancer due to a deficit in the DNA mismatch repair system and derives from the presence of spontaneous DNA replication errors in simple repetitive sequences[9]. A standard panel of microsatellite markers, including mononucleotide (BAT26 and BAT25) and dinucleotide (D2S123, D5S346 and D17S250) repeats, has been recommended and guidelines for MSI testing (Bethesda Guidelines) have been drawn up[9]. Using the reference panel, three levels of MSI can be identified: high-level MSI (MSI-H), low-level MSI (MSI-L) and microsatellite stable (MSS). Recently, it has been established that mononucleotide repeats are instrumental in detecting MSI-H tumors because of their high sensitivity and specificity, and MSI-L has been defined as instability limited to dinucleotide loci[10]. After the adoption of the Bethesda panel, MSI-H phenotype was reported in a range of 5%-50% of all gastric carcinomas with significant differences in various ethnic groups. MSI-H appears to be a phenotypical marker of an underlying cellular defect involving DNA mismatch repair (MMR). Functional inactivation by mutations or epigenetic mechanisms of MMR genes, including hMLH1 and hMSH2, is responsible for the MSI-H phenotype in gastric cancer. Abnormal loss of protein expression of either hMLH1 or hMSH2 has been observed in MSI-H gastric carcinomas[11]. In particular, altered expression of hMLH1 has been associated with gene inactivation by promoter hypermethylation.

MSI-H gastric carcinomas follow a molecular pathway of tumor progression, characterized by the presence of multiple frameshift mutations affecting mononucleotide tracts within genes involved in cancer-related molecular networks which control cellular homeostasis at different levels. MSI-related mutations occur in many genes at variable frequencies[8]. Genes regulating cell-cycle and apoptotic signaling are frequently targeted in MSI-H gastric carcinomas and include TGFβRI, IGFBIR, TGF4, RIZ, BAX, CASPASE3, FANX, BCL10 and APAF[10]. Moreover, genes involved in genomic integrity maintenance, i.e. hMSH6, hMSH3, MED1, RAD50, BLM, ATR and MRE11, are also frequently altered in MSI-H tumors[7]. Several studies indicate that, in most MSI-H gastric cancers, multiple target genes are simultaneously mutated and multiple hits impact on different genes in the same pathway[11]. In contrast, gastric carcinomas with MSS and MSI-L exhibit predominant p53 mutations[12].

As compared with MSS or MSI-L, gastric carcinomas with MSI-H show a significantly higher frequency of antral location, intestinal subtype, a lower incidence of lymph node metastasis and improved survival[12,13].

**CIN**

CIN is a feature of various tumors, including gastric cancer, commonly associated with chromosomal aberrations responsible for major modifications of DNA content, i.e. changes in chromosome copy number, and also high-level LOH, gene deletions and/or amplifications[14,15]. All these alterations may lead to oncogene activation and/or tumor suppressor gene inactivation. As with other tumors, aneuploidy is generally considered an unfavorable prognostic factor[16,17], though contrasting results have been reported[18,19].

High CIN levels have also been associated with a shorter survival in gastric cancer patients[18] and high LOH frequencies have been identified at several chromosome arms, including 1p, 3p, 4p, 5q, 7p, 8p, 8q, 9p, 12p, 13q, 17p, 18q, 20q and 22q[17-20].

The allelotype of gastric carcinoma is similar to that of colorectal and esophageal cancers, suggesting the presence of a common genetic pathway for tumor development. Some of these chromosomal segments include genes which are strongly implicated in carcinogenesis, such as the p53 gene on chromosome 17, DCC, DPC4 and SMAD2 genes on chromosome 18, and APC and MCC genes on chromosome 5. Several studies have found that tumors with LOH at chromosome 5q, 18q or 17p had a poorer prognosis than tumors that did not show LOH at these sites[20].

**EPIGENETIC INSTABILITY**

Epigenetic changes, such as aberrant methylation of CpG islands in promoter regions, are commonly detected in human cancers and can permanently inactivate tumor-suppressor genes and affect important pathways of cell cycle regulation and proliferation. The methylation of CpG islands may be considered a third molecular phenotype of
gastric cancer and the tumor-related genes more commonly methylated are APC, CDH1, MHL1, CDKN2A, CDKN2B and RUNX3. It has also been widely reported that CDKN2A, CDH1 and MHL1 are more frequently inactivated by promoter methylation rather than by mutations[32].

A series of individual methylated genes has been related to prognosis in gastric cancer. Methylation of tumor-suppressor genes, such as CDH1[33], DKK3[34], Pten[35] and MGMT[36], of putative tumor-suppressor genes, such as TFPI2[37] and CACNA2D3[38], and of other tumor-related genes, such as PCDH10[39] and SOX2[40], has been associated with shorter disease-free and/or overall survival.

The combined use of APC and CDH1 methylation markers has identified a subgroup of patients with worse prognosis[32]. Conversely, methylation of single genes has been associated with a better prognosis in some cases. Patients showing methylation of APC[41], the M1 region of MAL promoter[42] and cyclooxygenase-2 (COX2)[43] showed prolonged survival, compared to patients without methylation of these genes.

As with colorectal cancer, the CpG island methylator phenotype (CIMP), characterized by concurrent promoter hypermethylation of multiple genes, has also been described in gastric cancer[44,46] and it has been shown to correlate with hypermethylation of other known cancer-related genes, such as p16, bMLH1 and THBS-3[47,48]. Furthermore, the CIMP status is associated with clinically useful information and patients with negative CIMP methylation have significantly shorter survival than those with high CIMP methylation[40,41].

### ALTERATIONS OF GENES INVOLVED IN MOLECULAR PATHWAYS

Genetic and genomic variations occurring in genes and molecules that participate in proliferation, invasion and metastasis (e.g. growth factors and their receptors, signal transducers, cell-cycle and apoptosis regulators, cell adhesion molecules, DNA repair genes and matrix metallo-
proteinases) may influence the prognosis of patients with gastric cancer.

**Tyrosine kinases**
Amplification of some tyrosine kinases (c-met, K-sam and HER2/neu) is associated with human gastric cancer progression. Alternatively, spliced transcripts and enhanced protein expression levels for some of these tyrosine kinases are correlated with the clinical outcome of gastric cancer patients.[69]

The oncogene c-met, encoding for the hepatocyte growth factor receptor, is preferentially amplified in diffuse-type tumors and has been described to be well correlated with stage and prognosis.[50,53]. Overexpression of c-met has also been shown to be associated with lower survival probability.[62,63].

*K-sam* oncogene, a member of the fibroblast growth factor receptor family, is more frequently activated in diffuse-type tumors[9]. Overexpression of K-sam occurs in approximately 32% of diffuse-type gastric cancers, and the prognosis of K-sam-positive patients is poorer than that of K-sam-negative patients.[14].

The HER2 protein (HER2/neu or ErbB-2) is a glycoprotein with tyrosine kinase activity, homologous to the epidermal growth factor receptor. HER2 is codified by a gene located on chromosome 17q21 and does not bind to any known ligand. Some studies demonstrated that overexpression of c-erbB2 is selectively found in intestinal tumors and may serve as a prognostic marker for tumor invasion and lymph node metastasis. Overexpression of HER2 protein in gastric cancer has been reported to range from 7.4% to 38%.[64-67]. The prognostic value of HER2 expression and/or amplification has been widely investigated with controversial findings. Although most available studies indicate that the overexpression of HER2 is an independent prognostic factor associated with a shorter disease-free[68] and overall survival[67,69], some studies failed to confirm its prognostic role on multivariate analysis[90] or to find a correlation between HER2 overexpression and survival parameters.[66,60]. Also associated with poor survival is the presence of HER2 amplification.[61].

**RUNX3**
RUNX3, a gene that codifies for a member of the runt domain-containing family of transcription factors, frequently shows loss of expression due to hemizygous deletion and hypermethylation in gastric cancer. This gene, generally expressed in only 45%-50% of gastric cancer patients,[62,66], positively regulates the expression of BIM and p21, and negatively regulates vascular endothelial growth factor (VEGF), thus affecting apoptosis, cell growth arrest and angiogenesis. The loss or substantial decrease of RUNX3 protein expression in gastric cancer has been significantly associated with shorter survival[62-64].

**FHIT**
The fragile histidine triad gene (*FHIT*) encodes a diadenosine 5',5''-P1, P3-triphosphate hydrolase and is generally inactivated by deletion or methylation in several tumors, including gastric cancer. The absence of FHIT protein has been shown to correlate with higher tumor stage and histological grade[64], as well as with poor overall survival[53,66].

**NM23**
The NM23 gene maps to chromosome 17q21 and encodes the nucleoside diphosphate kinase A, a member of the NDP kinase family. NM23 expression is reduced in metastatic melanoma and breast cancer cell lines[67]. Transfection into cell lines affects invasion, motility, colonization, differentiation and liver metastasis[68]. Decreased expression of NM23-H1, the human homologue, is found in advanced stages of human cancer.[69].

The expression of the putative metastasis-suppressor gene NM23 in gastric carcinoma is controversial. In several studies, expression of NM23 has been shown to be inversely correlated with the metastatic potential of gastric cancer[71,72] and with prolonged overall survival[73]. The results of other studies, however, suggest that NM23 is not a metastasis suppressor gene and does not show correlation with metastasis[74,75].

**VEGF**
VEGF is a pro-angiogenic factor, frequently overexpressed in tumors. Mutations of p53, which under physiological conditions downregulates VEGF, may be responsible for its overexpression[70].

A correlation of the expression of VEGF with lymph node and liver metastasis has been described[71] and patients with VEGF-positive tumors have a rather worse prognosis than those with VEGF-negative tumors[76,77].

**HIF-1α**
The hypoxia inducible factor, HIF-1α, is a transcription factor that plays an essential role in cellular and systemic homeostatic responses to hypoxia. The prognostic role of HIF-1α expression in gastric cancer patients is controversial: high levels have been associated with a shorter overall survival[68], but also with no difference in survival parameters[81]. However, its upregulation (high HIF-1α mRNA or protein levels) has been found to be positively correlated with VEGF[82] or p53[80] protein expression in gastric cancer patients, and overall survival of patients with high mRNA levels of HIF-1α and VEGF, as well as of HIF-1α and p53, was shorter compared to patients with different features.

**COX2**
COX2 is one of the key isoenzymes in the production of prostaglandins, and is thought to be involved in carcinogenesis. Some studies indicate that COX2 may play a role in the development of gastric cancer, and its overexpression is associated with nodal metastasis, tumor invasion and differentiation, implicating a poor prognosis[77,83,84].

**SPARC**
The secreted protein acidic and rich in cysteine (SPARC or osteonectin) is a member of a family of matricellular
proteins that modulates cell-matrix interactions and cell function without participating in the structural scaffolding of the extracellular matrix. Since SPARC alters membrane permeability, cell shape, proliferation, migration and attachment, it may play a role in angiogenesis. It has been reported that its overexpression correlates with distant metastasis and poor prognosis. It is not clear whether SPARC overexpression is a useful marker in the prediction of lymph node metastasis development.

**p53**

The p53 protein plays a fundamental role in cell growth and division. The function of the p53 gene is more frequently altered due to LOH and mutation than to DNA methylation. Mutations of p53 are present in about 40% of early and advanced, well-differentiated gastric cancers. A lower incidence of p53 mutations has been shown in young patients compared to older patients.

p53 can be investigated by immunohistochemical techniques, bearing in mind that the half-life of the p53 mutant protein is prolonged. Cells carrying the p53 mutant protein can be stained with antibodies against p53, whereas cells carrying normal p53 are negative. Sequencing of the gene after screening can also be performed in order to determine the mutation location within the gene.

Overexpression of p53 often occurs in the early stages of intestinal-type tumors, and there is no significant difference between early and advanced cancers. In contrast, p53 abnormalities are not often seen in the early stages of diffuse-type tumors, but tend to occur as the disease progresses.

**p21**

p53 cell cycle regulatory function is mediated by different effectors. One of these is a cyclin-dependent kinase inhibitor (CDK I), the p21 protein. The cell cycle check points are controlled by a cascade of phosphorylation. Protein kinases such as cyclin-dependent kinases are activated by cyclins and inhibited by CDK I, although p21 is up-regulated not only through a p53 pathway, but also through a TGFβRII pathway.

Levels of p21 expression could indicate the absence of a functional p53 protein in neoplastic cells. It has been reported that the survival of gastric cancer patients with p21-positive tumors is significantly longer than that of patients with p21-negative tumors. The expression of p21 is usually assessed in combination with p53 status and contributes to predicting the clinical outcome of gastric cancer patients.

**p27**

It has been suggested that the cyclin-dependent inhibitor p27, which controls the transition from G1 to S in the cell cycle, has prognostic relevance in gastric cancer. Reduced p27 expression is detected in approximately 40%-50% of gastric cancers. Some studies have shown that tumors with a low expression of p27 protein are poorly differentiated and at an advanced stage. However, some authors have found no difference in overall survival of gastric cancer patients whether with high or low p27 expression, p53, p21 and p27 have also been analyzed in combination, confirming their role as prognostic markers.

**BCL2**

BCL2 and p53 are closely linked in the regulation of apoptosis. LOH at the BCL2 locus is frequently observed in gastric cancer. The overexpression of BCL2 may have a role in the development of gastric cancers. It has been shown that BCL2 overexpression reduces cellular proliferative activity and correlates with a less aggressive behavioral of the tumor. The prognostic role of BCL2 on its own or in association with p53 has not yet been elucidated.

**BAX**

BAX gene encodes a protein belonging to the BCL family members. Negative BAX protein expression has been associated with de-differentiation, lymph node metastasis and shorter survival, suggesting that BAX status may play a role in the development and differentiation of gastric cancer and tumor progression.

**pRb**

pRb encodes a protein that is a negative regulator of the cell cycle. Poor prognosis of gastric cancer patients with low levels of pRb expression has been reported.

**c-myc**

c-myc gene encodes a multifunctional, nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis and cellular transformation. It functions as a transcription factor that regulates transcription of specific target genes. The c-myc protein has been shown to be significantly enhanced in well-differentiated gastric cancer and associated with a poor prognosis. Although c-myc is a short-lived protein in normal cells, its stability is increased in transformed cells through several mechanisms. One of these has recently been identified in the overexpression of a human oncprotein, the cancerous inhibitor of protein phosphate 2A (CIP2A) that stabilizes c-myc. Interestingly, the expression of CIP2A has been associated with reduced overall survival in gastric cancer patients.

**Cyclin E**

Cyclin E overexpression correlates with invasiveness and proliferation and may be a marker of tumor aggressiveness. Although somatic mutations of the cell cycle inhibitor p16 are rare, its reduced expression is associated with depth of invasion and metastatic potential in both diffuse- and intestinal-type gastric carcinomas. However, recent data show that the survival of gastric cancer patients with cyclin E-positive tumors is not significantly shorter than that of negative patients.

**E-cadherin**

Cell adhesion molecules are implicated in human carcinogenesis. Cadherin is a superfamily of calcium-mediated membrane glycoproteins, forming one of the four classes of adhesion molecules. E-cadherin, one of the members...
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of the trasmembrane glycoprotein family expressed by epithelial tissues, not only acts as a cell adhesion molecule, but also plays an important role in growth development and carcinogenesis. The intact function of E-cadherin is crucial for the establishment and maintenance of epithelial tissue polarity and structural integrity. Around 25%-40% of hereditary diffuse gastric cancers are caused by heterozygous E-cadherin. The inactivation of the second allele occurs by mutation and methylation events, and this results in the complete inactivation of the protein[105]. Reduced expression of E-cadherin correlates with infiltrative and metastatic ability in gastric cancer[29] and the gene encoding E-cadherin, CDH1, was among the first to be considered as an invasion suppressor gene. Patients with E-cadherin-positive gastric cancers showed statistically significant prolonged 3- and 5-year survival rates, compared to patients with E-cadherin-negative tumors[103,104].

It has been shown that serum soluble E-cadherin is increased in several non-neoplastic diseases and also in various cancers, including gastric tumors. E-cadherin may be a potentially useful prognostic marker and high levels of soluble E-cadherin correlate with the depth of tumor invasion, as well as inoperability[107]. In addition, levels higher than 10000 ng/mL predict a survival of less than 3 years in more than 90% of patients[106].

The Wnt-frizzled-β-catenin signaling pathway is frequently activated in gastric carcinoma (e.g. upregulation of Wnt gene expression or of genes for Wnt ligand receptors, upregulation of RAC1 and inactivation of APC), leading to poor differentiation and increased tumor invasiveness[108].

MUC1
Mucins are high-molecular weight glycoproteins containing oligosaccharides. These glycoproteins constitute the major components of the mucus that protects the gastric epithelium. Overexpression of mucin 1 (MUC1) has been linked to poor prognosis in gastric cancer patients[65,109].

It has been reported that MUC1 may accelerate tumor invasion by the impairment of E-cadherin[111]. The combined expression of MUC1 and E-cadherin shows that survival for gastric cancer patients with abnormal E-cadherin/MUC-positive expression was shorter than for patients with other expression patterns[106].

PRL-3
The phosphatase regenerating liver 3 (PRL-3) gene encodes a protein belonging to a class of prenylated protein tyrosine phosphatases. These proteins are cell signaling molecules with a regulatory role in several cellular processes. The prognostic role of PRL-3 in solid tumors, including gastric cancer, has been recently reviewed by Bessette et al.[22]. High expression of PRL-3 has been associated with several unfavorable clinical parameters, such as tumor size, depth of invasion, lymphatic invasion, advanced stage and shorter overall survival. Successive studies have confirmed these findings[113,114].

Tumor-associated proteases
Tumor-associated proteases and their inhibitors play a central role in tumor invasion and metastasis. The positive correlation of histological data with the urokinase-type plasminogen activator (uPA) and the plasminogen activator inhibitor type I (PAI-1) has been reported. Moreover, the independent prognostic impact of both uPA and PAI-1 on the survival of gastric cancer patients has been demonstrated. Elevated uPA and PAI-1 levels have been shown to be associated with shorter survival[115,116]. A trend towards poor prognosis has also been observed in patients with high expression of the u-PA receptor (uPAR)[113] and the uPA system may therefore be a target for novel therapeutic agents.

The prognostic role of some uPA genotypes has recently been investigated and an association was demonstrated between the exon 6 C/T polymorphism with invasive phenotype, but not with susceptibility or survival[117].

CONCLUSION
Gastric carcinomas are histologically and genetically heterogeneous and are influenced by gene-environment interactions resulting in the activation of multiple molecular pathways. The molecular subtypes of gastric cancer include three main groups of tumors characterized by either the CIN, the MSI or the CIMP pathways. Currently, it is not clear whether or in what way knowledge of these subtypes of gastric carcinomas is of use in clinical practice, with regard to predicting specific pathways with mutational and regulatory alterations that may interfere with targeted therapies.

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