Gastric Cancer Pathology and Underlying Molecular Mechanisms

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Key Words

Gastric cancer · Genetic alterations · Pathology

Abstract

The development of gastric adenocarcinoma is a complex multistep process involving multiple genetic alterations. Based on pathology, four different macroscopic types and at least two major histological types, intestinal and diffuse, have been described. Most gastric cancer (GC) show genetic instability, either microsatellite instability or chromosomal instability, which is considered an early event in gastric carcinogenesis. Molecular studies of alterations of single genes have provided evidence that intestinal and diffuse type GC evolve via different genetic pathways. Recent results from high-throughput whole-genome expression or copy number studies have demonstrated extensive genetic diversity between cases and within individual GC. Sets of commonly up- or downregulated microRNAs have been identified in GC and might be useful in the near future to identify pathways of GC progression. Results from detailed molecular and/or pathological GC studies, although promising, still have limited clinical utility in predicting survival and stratifying GC patients for appropriate treatment.

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Introduction

Gastric cancer (GC) remains one of the most common cancers and second leading cause of cancer death in the world, accounting for 1,000,000 new cases and 738,000 deaths per year. Although the incidence and mortality rates have been declining steadily, the absolute number of new cases is actually increasing due to the aging population.

Approximately 95% of GC are adenocarcinomas which have been classified by anatomic site as cardia/proximal cancers or noncardia/distal cancers in epidemiological studies [1] and by histological phenotype as intestinal type, diffuse type and mixed/unclassifiable according to Lauren's classification [2]. Patients with proximal GC have a poorer survival independent of TNM stage [3]. Cancers in the distal stomach are more frequently seen in the elderly male population, are related to *Helicobacter pylori* infection, and are usually of intestinal type histology. More recently, several molecular classifications of GC have been proposed based on the analysis of whole-genome gene expression studies and/or gene copy number studies [4–8].

The decline in GC incidence has been attributed to a decrease in the number of intestinal type GC and the number of distal GC, and correlated with a parallel decrease in the prevalence of *H. pylori* infection [9]. Never-

theless, intestinal type GC still predominates in high-risk areas such as Asia, Eastern Europe, and South America, whereas diffuse type GC has a much more uniform geographic distribution and increasing incidence [10]. Diffuse type GC is more common in females and younger patients, but studies have failed to demonstrate that the decline in intestinal type cancers is related to a shift in the male:female incidence ratio or related to changes in the age of the population (for review, see [11]).

Pathology of Gastric Cancer

Macroscopy

In order to optimize communication between surgeons, endoscopists, radiologists, and pathologists, and facilitate the appropriate treatment strategy, the macroscopic appearance of GC has been described using the Borrmann classification for advanced GC (type I: polypoid with broad base and no ulceration; type II: ulcerating with sharp margin and elevated borders; type III: ulcerating and diffusely infiltrating into surrounding wall, and type IV: diffusely infiltrating mostly without ulceration [12]) and the Paris classification for early GC (type 0-I: polypoid growth (subcategorized into 0-Ip for pedunculated growth and 0-Is for sessile growth); type 0-II: non-polypoid growth (subcategorized into type 0-IIa for slightly elevated growth, type 0-IIb for flat growth, and type 0-IIc for slightly depressed growth), and type 0-III: for excavated growth [13]).

A relationship between the macroscopic growth pattern (Borrmann type), tumor location within the stomach, gender, age at diagnosis, histological subtype, and survival has been described. Polypoid and ulcerated tumors with elevated edges (Borrmann types I and II) are most commonly of intestinal type and located in the antrum of the elderly male, whereas diffusely infiltrating tumors (Borrmann types III and IV) are most commonly diffuse type GC in the proximal stomach in middle-aged females [14]. The Borrmann type has been shown to be an independent prognostic factor in GC, with patients with type IV GC having the poorest survival [15].

Microscopy

GC is histologically very heterogeneous: more than 50% of GC are pluriform and the variability of the histological appearance has been shown to increase with in-

creasing depth of infiltration into the wall [14]. As a result of this morphological diversity, a number of different classification systems have been advocated: the classifications according to Lauren [2], Ming [16], the World Health Organization (WHO) [17], Nakamura et al. [18], Mulligan [19], Goseki et al. [20], and Carneiro [21], as well as the Japanese classification [22].

The most commonly used classifications are those of Lauren [2] and the WHO [17]. Lauren's intestinal type GC shows a predominance of glandular epithelium with cells similar to intestinal columnar cells, good cellular cohesion and a pushing margin at the invasive edge. Lauren's diffuse type GC is composed of scattered poorly cohesive cells or small clusters of cells with little or no gland formation and a diffuse infiltrative margin. Tumor cells may contain mucus and can have a signet ring cell appearance. GC that consists of 50% diffuse and 50% intestinal type, solid type cancers, and others that cannot be classified as diffuse or intestinal are called indeterminate, unclassifiable, or mixed. Diffuse and intestinal type GC have different clinicopathological characteristics (reviewed in [14]): intestinal type GC grows in a more shallow fashion, is significantly larger in size before breaching the serosal surface, and has a higher incidence of blood vessel invasion and liver and lung metastases, whereas diffuse type GC spreads more commonly via the lymphatics to the pleura and peritoneum. The male:female ratio is 2.3 for intestinal type GC and 1.5 for diffuse type GC. Patients with intestinal type GC are at a median 7 years older at diagnosis and the background stomach shows multifocal atrophic gastritis and intestinal metaplasia.

Apart from the classification based on tumor morphology, GC can be classified on the basis of mucin stainings into G type (gastric phenotype; positive for antibodies against MUC5AC, MUC6, HGM, and TFF1), I type (intestinal phenotype; positive for MUC2, CDX2, and CD10), GI type (mixed profile), and N (null type) [23].

More recently, three subtypes of GC have been proposed based on tumor location, histological features, and clinical course [6]: (1) proximal nondiffuse GC which is located in the gastric cardia and shows evidence of glandular dysplasia and chronic inflammation without atrophy; (2) diffuse GC which can be located anywhere in the stomach, has no glandular component, and no evidence of inflammation or atrophy, and (3) distal nondiffuse GC which is an intestinal type GC with chronic gastritis, atrophy, and intestinal metaplasia in the background.

Fig. 1. The Yasui/Tahara multistep model of molecular pathogenesis of gastric cancer (reproduced with permission from [94]).

Molecular Mechanisms of Gastric Carcinogenesis

GC is thought to result from a combination of environmental factors such as *H. pylori* infection and diet and the accumulation of generalized as well as specific genetic alterations. A model summarizing the sequence of molecular events for intestinal type and diffuse type GC has been proposed by Tahara and Yasui (fig. 1) [24]. This model incorporates the previously proposed 'Correa model' of GC development via an intestinal metaplasia-adenoma-carcinoma sequence which was based on epidemiological, pathological, and clinical observations as one possible strand in the development of intestinal type GC [25]. As one can see from this model, there are certain alterations which are common to both major histological subtypes of GC, such as p53 mutation, cyclin E overexpression/amplification, or aberrant CD44 transcripts. Others like *KRAS* mutations, *CDH1* mutations, and amplifications of *HER2*, *FGFR2*, and *MET* appear to be more 'specific' for one of the histological subtypes.

Genetic Predisposition

Ten to 15% of GC show familial clustering [26], but only 1–3% of GC are related to identified inherited GC predisposition syndromes [27] such as hereditary diffuse GC, hereditary nonpolyposis colon cancer (Lynch syndrome), familial adenomatous polyposis, Peutz-Jeghers syndrome, Li-Fraumeni syndrome, or familial breast and ovarian cancer.

One of the defining criteria of hereditary diffuse GC is the presence of a *CDH1* (E-cadherin) germline mutation [28]. *CDH1* mutations have only been found in hereditary and sporadic diffuse type GC, but not in intestinal type GC. In contrast, GC in patients with germline mutations in one of the DNA mismatch repair genes (hereditary nonpolyposis colon cancer patients) show intestinal type morphology in 79% of patients [29].

Genomic Instability

One of the hallmarks of cancer development is destabilization of the genome, also referred to as 'genetic or genomic instability' [30], which can be found in all dif-

ferent histological subtypes of GC and is believed to be one of the initial steps of gastric carcinogenesis [31]. Three phenotypes of instability have been identified in GC: (1) microsatellite instability (MSI) due to a defect in the DNA mismatch repair pathway [32]; (2) chromosomal instability (CIN) which is characterized by an increased rate of loss or gain of whole chromosomes or parts of chromosomes during cell division due to mutations in genes controlling the segregation of genetic material during mitosis [30], and (3) the cytosine and guanine (CpG) island methylator phenotype (CIMP) [33, 34].

Consistent with the hypothesis that genomic instability is an initial step in gastric carcinogenesis, DNA aneuploidy has been observed in intramucosal GC less than 5 mm in diameter [59] as well as in early GC [60]. Similarly, copy number alterations have been found in GC precursor lesions [61, 62]; MSI has been identified in intestinal metaplasia [63, 64], gastric adenoma [64], and early GC [65], and CIMP is present in 15% of intestinal metaplasia and 50% of adenomas [34].

Microsatellite Instability

Patients with a deficiency or inactivation of one of the DNA mismatch repair proteins MLH1, MSH2, MSH6, or PMS2 in their cancer are unable to repair naturally occurring DNA replication errors due to slippage of the DNA polymerase during DNA synthesis, leading to the appearance of new alleles not present in the normal DNA – the so-called ‘MSI phenotype’ or ‘replication error phenotype’. MSI can lead to subsequent genetic changes, usually frameshift mutations, in hundreds to thousands of genes which have also been demonstrated in GC [35].

The reported frequency of MSI in GC varies between 15 and 38%, strongly depending on the number of loci investigated [36, 37]. Overall, the frequency of MSI was higher in intestinal type GC, older age females, and distal GC [38, 39]. MSI GC were more often classified as Borrmann type I or II and showed microscopically a high number of tumor-infiltrating lymphocytes. Patients with MSI GC were usually diagnosed at an earlier disease stage and some but not all studies showed a relationship of MSI and improved survival [40]. In most GC, MSI was due to hypermethylation of the MLH1 promoter [41].

Chromosomal Instability

Chromosomal instability (CIN), defined as an increased rate of loss or gain of whole chromosomes or large portions of chromosomes, can lead to oncogene activation or tumor suppressor gene inactivation. Although CIN is defined as a rate, e.g. a dynamic process, CIN can

only be assessed with surrogate (static) markers such as DNA cytometry to determine changes in nuclear DNA content (DNA ploidy), comparative genomic hybridization (CGH), or other methods such as fluorescence in situ hybridization to determine gene copy number or loss of heterozygosity (LOH) studies.

DNA aneuploidy in GC has been reported in 27–100% of cases with prominent heterogeneity within the same cancer. Conflicting results have been reported regarding the relationship between DNA ploidy status, clinicopathological variables (depth of invasion, lymph node status, histological subtypes), and patient survival (for review see [42]).

Whole genome array CGH studies have identified complex recurrent patterns of copy number gains and losses in GC (for review see [43]). Specific DNA copy number changes have been found to be related to histological subtypes [44–46], lymph node status [46–48], depth of tumor invasion [47], tumor location [44], age [49], ethnicity [50], and patient survival [51, 52]. Most of the published studies have used CGH as a method to identify candidate genes which may play a role in gastric carcinogenesis or can potentially be used for predicting patient prognosis [46, 52, 53]. It needs to be recognized that CGH can only measure DNA copy number aberrations, thus balanced structural aberrations, translocations, inversions, insertions, and fusions cannot be detected with this method.

LOH is also a marker of CIN and the presence of LOH at specific chromosomal regions can facilitate the identification of tumor suppressor genes. Several LOH studies have been performed in GC demonstrating that the extent of LOH on certain chromosomes, such as chromosome 6q, 8p, 16q, and 22q, appears to be related to patient prognosis [54, 55].

CpG Island Methylator Phenotype

Aberrant methylation of CpG-rich regions results in silencing of genes and is a common phenomenon in cancer. CpG island methylation is increased in the normal mucosa of patients with chronic inflammatory conditions such as *H. pylori* and Epstein-Barr virus infection and has been considered a precursor lesion for the development of GC [56].

Inactivation of tumor suppressor genes by methylation may result in uncontrolled cellular growth, vascular invasion, and metastasis. CIMP is characterized by concordant methylation of multiple genes and has been described in up to 50% of GC [33, 57]. Data analyzing the relationship between CIMP and clinicopathological data

is still limited in GC. It has been suggested that CIMP is more frequently seen in proximal and diffuse type GC [58], whereas the relationship between CIMP and prognosis is still controversial [33, 58].

Selected Single Gene Alterations

Many genes have been analyzed in an attempt to better understand gastric carcinogenesis as well as GC progression and to discover biomarkers for diagnosis, prognosis prediction and potential drug targets. A summary of selected genes is presented below.

The oncogene *MET* encodes a transmembrane tyrosine kinase receptor that binds hepatocyte growth factor and is amplified at higher frequency in diffuse type GC compared to intestinal type GC (39 vs. 19% [66]). Overexpression of *MET* has been related to tumor stage and clinical outcome [67]. With the advent of *MET* inhibitors, the interest in this molecule had a revival, and a very recent large study conducted in Korea demonstrated that *MET* was amplified in 21% of GC and related to poor patient survival [68].

Abnormalities of the fibroblast growth factor system tend to be more commonly associated with diffuse type GC, whereas intestinal type GC harbor more frequently abnormalities in the genes/proteins related to the epidermal growth factor system.

K-SAM [now called fibroblast growth factor receptor 2 (*FGFR2*)] amplification has been detected in diffuse type GC and only rarely in intestinal type GC. *FGFR2* overexpression proved to be an independent predictive marker of poor survival in one GC study [69], but was not related to prognosis in another GC study [70]. *HST-1* (now called fibroblast growth factor 4) was the first oncogene identified in GC in 1986 [71] and was found to be amplified exclusively in metastatic GC [72]. The expression of basic fibroblast growth factor and *FGFR* was increased more commonly in undifferentiated and scirrhous GC and was associated with larger tumor size and higher stage [73].

Human epidermal growth factor receptor 2 (*HER2*), also known as c-erbB2 or *HER2/neu*, is a tyrosine kinase which does not have any known ligands. *HER2* amplification and overexpression has been reported in up to 27% of intestinal type GC and only rarely in diffuse type GC [74]. The relationship between *HER2* expression/*HER2* amplification and GC patient survival remains controversial (for recent studies see [75, 76]). *HER2* positivity predicted response to trastuzumab in the recent TOGA trial [77].

KRAS mutations on codons 12 and 13 have been found on average in 5% of GC and were preferentially present in well-differentiated intestinal type GC [78, 79]. In contrast to colorectal cancer, *KRAS* mutations in GC are more frequently seen in GC with MSI [29, 79, 80].

p53 is a nuclear protein involved in cell cycle control, DNA repair, and programmed cell death. *p53* is frequently inactivated in GC by LOH or mutations. *p53* mutations have been identified in 60% of GC with approximately equal frequency in different histological subtypes, which makes it the most frequently mutated gene in GC [81]. *p53* mutations have also been identified in GC adenomas [82] and intestinal metaplasia [83]. The prognostic impact of *p53* mutations, LOH, and *p53* expression in GC is still controversial.

APC is a multidomain protein with binding sites for numerous proteins including Wnt signaling pathway components β -catenin and axin and cytoskeletal regulators EB1. *APC* plays a major role in cell adhesion, cell migration, spindle formation, and chromosome segregation. *APC* mutations are the second most frequent mutations in GC and have been observed in 30–40% of well- and moderately differentiated intestinal type GC and in less than 2% of diffuse type GC [84]. LOH at the *APC* locus was associated with intestinal type GC [29]. *APC* mutations have also been described in adenomas of the stomach [85] and intestinal metaplasia [85], indicating that they occur during early stages of GC development.

RUNX3 is a member of the runt domain-containing family of transcription factors regulating apoptosis, cell growth, and angiogenesis. *RUNX3* is expressed in up to 50% of GC and has been associated with better patient prognosis [86]. Inactivation of *RUNX3* in GC is most frequently due to hypermethylation of the promoter region [87].

Whole Genome Studies

Major efforts have been made recently using modern high-throughput molecular methods with the aim to complement traditional histopathological diagnosis and prognosis prediction in GC and to contribute to a better understanding of the biology of GC at a molecular level.

Zang et al. [88] sequenced the exomes of 15 gastric adenocarcinomas and found on average 50 mutations/case, mostly involving genes involved in cell adhesion and chromatin remodeling. Furthermore, mutations in

two new putative tumor suppressor genes, FAT4 and ARID1A, were described in GC for the first time.

Whole genome expression profiling in GC can be challenging due to the relative large number of inflammatory cells and presence of stromal cells. To eliminate the interference of 'contaminating' cell populations with the profiling of cancer cells, Tan et al. [7] investigated the gene expression profile in a large number of GC cell lines and identified two major genomic subtypes which were then applied in primary GC tissue samples. Interestingly, concordance of the genotypic classification with the phenotypic classification according to Lauren was only seen in 64% of GC, suggesting that unsupervised gene expression studies can lead to the identification of distinct subtypes of GC which are currently not recognizable using classic morphological methods. Furthermore, the study showed that it is the genotype-based classification which is of prognostic value and can predict response to certain types of chemotherapy in GC cell lines.

On the other hand, a whole genome expression study using RNA extracted from GC tissue sections demonstrated that a high level of expression of stroma-related genes is significantly related to poor patient prognosis [89]. Interestingly, in contrast to the study which identified two genotypes which were only partly concordant with the phenotype, this study showed a good correlation between the genotype, e.g. high expression of stroma genes, and the phenotype, e.g. high amount of stroma measured morphometrically on routine histology slides, and prognostic value of both.

Two very recent genome-wide copy number profiling studies using high-resolution SNP arrays have been able to demonstrate the power of modern molecular technology in identifying new clinically relevant subtypes of GC [8, 90]. Both studies identified independently that up to 37% of GC show high amplifications of genes encoding druggable tyrosine kinase receptor proteins such as *FGFR2*, *HER2*, *EGFR*, and *MET*. Furthermore, these gene amplifications were almost always exclusive emphasizing the molecular heterogeneity of GC and the need to develop new treatment strategies based on molecular profiles.

MicroRNA

MicroRNAs (miRNAs) are small non-coding RNAs which regulate gene expression at the posttranscriptional level. Recent evidence indicates that miRNAs are involved in many important biological processes such as

proliferation, differentiation, angiogenesis, and immune response. It is therefore not surprising that more and more reports are published emphasizing the involvement of miRNAs in malignancy (for review see [91]). Several miRNAs have been shown to be related to certain GC subtypes, GC progression, and potential treatment targets, albeit with inconsistent results probably related to small sample sizes [92, 93].

Conclusion

GC is a heterogeneous and complex disease which has traditionally been subdivided based on epidemiological, macroscopical, and histological classifications to understand cancer biology and determine patient prognosis.

Alterations in multiple single genes and complex copy number and gene expression profiles have been identified in GC over the last two decades. However, their significance in gastric carcinogenesis, tumor progression, and patient survival remains to be determined. Definitive clinical utility has only been shown for two genes in GC so far: *CDH1* germline mutations to identify persons at high risk of developing hereditary GC who need to be entered into an appropriate surveillance program, and *HER2* amplification/overexpression as a predictor of response to trastuzumab.

Molecular classifications have enabled us to detect multiple parallel occurring molecular alterations in GC, and further studies are needed to unravel their significance for gastric carcinogenesis. It is likely that the response to chemotherapy and patient prognosis will depend on the molecular tumor type; therefore, the identification of a robust and clinically relevant genotype-based histopathological classification of GC is an essential strategy to individualize and guide treatment decisions for GC patients in the near future.

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