Overview on Gastric Cancer

Chapter 2

Genetics and Molecular Basis of Gastric Cancer

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Abstract

Even though there is a decline in worldwide incidence of Gastric Cancer (GC), the fact that its mortality rate is still high among other cancer types means we need more and advanced tools for prevention, early detection and effective treatment. The path that goes to all of these starts from better understanding the etiology at the molecular level, and finding new targets for the genetic alterations driving or accumulating during the progress of tumor evolution.

The genome-wide analysis of germ line and somatic genetic and epigenetic events has been facilitating understanding the pathogenesis and molecular classification of GCs as well as the identification of novel diagnostic biomarkers and therapeutic targets for cancer.

By adopting the early detection methods and treatment strategies in light of the new findings made possible by functional genomic approaches like genome-wide association studies, whole-genome and whole-exome sequencing, global DNA methylome mapping, and gene or noncoding RNA expression profiling, we will have better chances to prevent or treat more GC patients. Building the bridge from the laboratory studying the molecular level to the patient side at the clinic via Translational Medicine approach is the key factor for improving the success rate for this deadly cancer.

This chapter summarizes recent advances in our understanding of the genetics and molecular basis of GC (mostly gastric adenocarcinoma) and the incorporation of these advances into clinical practice.

1. Introduction

Gastric cancers exhibit a wide diversity in terms of their histopathology, etiology, and clinical course. However, there are other different types of cancer arising from the stomach, such as mucosa-associated lymphoid tissue (MALT) lymphomas, which originate from the lymphoid tissue, and leiomyosarcomas, which arise from the muscles surrounding the mucosa, the large majority (approximately 90%) of GCs are adenocarcinomas, which arise from the glands of the most superficial layer, or the mucosa, of the stomach. Therefore, in this chapter we will concentrate on gastric adenocarcinomas.

2. Epidemiology & Etiology

A number of variables have been implicated in the development of GC, including inflammation and infectious agents, environmental factors and genetics.

Age and gender are both non-modifiable risk factors for the development of GC. The incidence of GC varies enormously globally and between men and women. Globally, men are almost two times more likely to develop GC than women.

According to Globo can, there are about 952 000 new cases of stomach cancer in the world (7% of total cancer incidence) making it the fifth most common malignancy globally [1]. This represents a substantial change since 1975 when stomach cancer was the most common malignancy. More than 70% of gastric cancer occurs in developing countries and cases from China, Japan and Korea account for 60% of total cases. Age-standardised incidence rates of GC are about twice as high in men as in women, at 35.4 per 100 000 in Eastern Asia males and 13.8 in Eastern Asia females. United States of America, Africa and Eastern Mediterranean region have the lowest incidence rates [2].

The marked spatio-temporal variations in GC incidence suggest that environmental or lifestyle factors are major contributors to the etiology of this disease.

The stomach is divided into several anatomical sites, including the cardia, fundus, body, pylorus, and the antrum. These areas are distinguished by anatomic demarcations, histological differences, or both. These anatomical sites show a variation between the frequency of cancer incidence and types of tumors seen, influenced by different etiological factors. Also, there are several distinctions between adenocarcinomas arising from the cardia (cardia GC) and other parts of the stomach (non-cardia GC), because they have different epidemiologic patterns and causes.

While the precise etiology is unknown, GC is considered as a multifactorial disease. There are several in common and different risk factors for cancers arising from two anatomical region; cardia and noncardia parts of the stomach. Common risk factors for both cardia and noncardia GC include older age, male sex, cigarette and tobacco smoking, radiation, and family history. While race is a risk factor for each, the direction differs by site. In the United States, Whites are more likely to acquire cardia GC, whereas Hispanics are more likely to be diagnosed with noncardia GC. Factors associated with cardia GC, but not noncardia GC, include obesity and gastroesophageal reflux disease. On the other hand, risk factors that are unique for noncardia GC include *H. pylori* infection (at least in Western countries), low socioeconomic status and dietary factors such as low amount of fruits and vegetable intake and high intake of salty and smoked food [3,4].

There are some other potential risk factors have been investigated in relation to GC but the results are not fullfilling yet. Among these risk factors are poor oral hygiene and tooth loss, opium use and eating pickled vegetables probably because of citric acid and salt [5-7]. On the other hand genetic predisposition is associated with smaller number of cases. The contribution of these various risk factors have been shown to vary by anatomical position of the GC, population and across geographical areas.

3. Pathology of Gastric Cancer

Major pathological descriptors of GC include the anatomic location of the tumor and macroscopic and microscopic features of tumor tissue.

Histologically, gastric carcinoma demonstrates marked heterogeneity at both architectural and cytologic level, often with co-existence of several histologic features. There are several classification systems used for gastric cancers. Although none of them could reach a consensus, the Laurén classification and the World Health Organization (WHO) classification are widely used ones.

Lauren classification devides GC in two major histologic subtypes; intestinal and diffuse type plus indeterminate type of adenocarcinoma of the stomach [8]. These two subtypes present marked differences in pathology, epidemiology, etiology and biological behavior [9]. While the intestinal type is more frequent (54%), the diffuse type is the most aggressive form of gastric cancer and the mortality rate is increasing in spite of the decline of the intestinal type [10,11]. There are indications that the diffuse type gastric carcinoma is more often seen in female and young individuals, while the intestinal type adenocarcinoma is more often associated with intestinal metaplasia and Helicobacter pylori infection [8,12-14].

A more recent classification by WHO divides gastric cancers into four major histologic patterns: tubular, papillary, mucinous and poorly cohesive (including signet ring cell carcinoma),

plus uncommon histologic variants. In addition to the four major histologic subtypes, WHO classification also endorses other uncommon histologic variants, such as adenosquamous carcinoma, squamous carcinoma, hepatoid adenocarcinoma, carcinoma with lymphoid stroma etc. which are not subject to this review. While the tubular and papillary adenocarcinoma is the most common histologic type of early gastric carcinoma. Mucinous adenocarcinoma accounts for 10% of gastric cancers [15].

4. Genetic Predisposition to GC

Environmental factors, including Helicobacter pylori and EBV infections and high-sodium diet are the primary known risk factors for stomach cancer. Only about10 % of GCs are caused by hereditary factors.

In general, the risk for developing gastric neoplasia among relatives of gastric cancer patients is estimated to be 2-3 fold higher than in persons with no familial background of the disease. This, however, should be cautiously analyzed due to the fact that, besides the common genetic background, environmental and cultural factors (e.g. H. pylori, diet, lifestyle behaviors) may be similarly shared among the family members and in some cases are difficult to differentiate.

It is well established that a number of inherited germ-line mutations and genetic syndromes predispose to the development of GCs. Approximately 1 to3 % of all GCs arise in the setting of hereditary gastric cancer syndromes; Hereditary Diffuse Gastric Cancer (HDGC), Familial Intestinal Gastric Cancer (FIGC), Familial Gastric Polyposis and Gastric Adenocarcinoma and Proximal Polyposis of the Stomach (GAPPS) [16].

HDGC, caused by mutations in the CDH1 gene (coding for E-cadherin), greatly increases the risk of developing stomach cancer. HDGC follows an Autosomal Dominant inheritance pattern and the lifetime stomach cancer risk among affected people (people who have a mutation in the CDH1 gene) is about 70% to 80%. Women with this syndrome also have an increased risk of developing lobular type breast cancer. In addition to CDH1, the only gene implicated in the susceptibility to HDGC is CTNNA1 (α1catenin). While mutations in the E-cadherin gene account for 30 - 40 % of HDGCcases, only few HDGC families have been identified carrying mutations in CTNNA1 gene. However, we can estimate that less than 40 % of HDGC is due to known genes and new HDGC susceptibility genes need to be identified [17].

Although genetic basis of HDGC susceptibility is not fully clarified, individuals fulfilling the criteria set by the International Gastric Cancer Consortium (IGCLC) need to be tested for CDH1 gene. Genetic testing criterias for HDGC, defined by the IGCLC are as follows: [1] two or more diffuse gastric cancer cases in first or second degree relatives, with at least one diagnosed before the age of 50, or [2] three or more diffuse gastric cancer cases in first

or second degree relatives independent of age of onset, or [3] an individual diagnosed with diffuse gastric cancer before the age of 40, or [4] individuals and families diagnosed with both diffuse gastric cancer and lobular breast cancer, with one diagnosed before the age of 50 [18].

Gastric cancer is also a known manifestation of some other inherited cancer predisposition syndromes, including Hereditary Nonpolyposis Colon Cancer (HNPCC), Familial Adenomatous Polyposis (FAP), Peutz-Jeghers syndrome (PJS), Mutyh-Associated Polyposis (MAP), Li-Fraumeni Syndrome (LFS1) and Hereditary Breast Ovarian Cancer Syndromes (HBOCS) [19].

5. Genetic and Epigenetic Alterations in GC

Like most solid epithelial cancers, GC is the consequence of a multistep process involving different genetic and epigenetic changes in numerous genes.

Aneuploidy is known to be a common feature of GCs, as 72% of differentiated and 43% of undifferentiated gastric tumors are aneuploid. Frequent Loss of Heterozygosity (LOH) has been identified at 3p, 4p, 4q, 5p, 8q, 13p, 17p, and 18q; in contrast chromosomal arms 8q, 17q, and 20q showed frequent increases in DNA copy number. Many candidate genes located on amplified or deleted chromosomal regions have been analyzed in an attempt to identify the genes that are involved in GC development. The oncogene c-MET, fibroblast growth factor receptor 2 (FGFR2, previously called as K-SAM), fibroblast growth factor 4 (FGF4, previously named as HST-1) and human epidermal growth factor receptor 2 (HER2, also known as c-erbB2 or HER2/neu) were found to be amplified in GCs [20].

The tumor suppressor TP53 gene; located on 17p which is among the most frequently deleted regions in GCs, an important regulator of various cellular functions like cell cycle control, DNA repair, and programmed cell death, is frequently inactivated in GC by deletions or mutations. Up to 60% of GC display genetic alterations in TP53 in different histological subtypes but a link with clinical outcome has not been established [20]. In common with other tumor supressor genes, HDGC gene; CDH1 mutations are frequently seen in sporadic GC cases. Early studies displayed 50% CDH1 mutations in sporadic diffuse GCs, but mixed carcinomas displayed CDH1 mutations less frequently (15%) [21]. APC, a Wnt signaling pathway component that plays a major role in cell adhesion, cell migration, spindle formation and chromosome segregation is also frequently mutated (30-40%) in sporadic GCs [20].

Mutations in bona fide tumor suppressors TP53 and CDH1 often were considered classical driver mutations of gastric cancer but other tumor suppressor and oncogene mutations are also demonstrated. Canonical oncogenes Kras and β-catenin (CTNNB1) mutations also contribute to gastric carcinogenesis [22].

Involvement of mutations of these well defined oncogenes and tumor suppressor genes were confirmed with high-throughput genomic analysisand new mutations, amplifications, deletions and translocations in other genes have been identified. Also, exome sequencing studies of gastric adenocarcinoma have led to identification of new cancer driver genes. Recurrent somatic mutations have been identified in cell adhesion protein coding FAT4 gene and chromosome remodeling genes; ARID1A, MLL and MLL3 [23,24]. Comprehensive genomic studies revealed distinct genomic characteristics of diffuse type gastric carcinoma. Aside from CDH1 mutations, DGC display high frequency of RHOA gene (small GTPase) mutations that are extremely rare in other tumor types, and accumulates low number of somatic mutations and less chromosome instability (CIN) [25,26].

Additional signaling pathways have been recurrently identified as dysregulated in GC including Hedgehog, NF-κB and DNA damage pathways, demonstrating the molecular diversity of GC [20].

Genetic rearrangements are frequent somatic alterations observed mostly in hematologic malignancies. Although rare, translocations due to genetic rearrangements were also reported in solid tumors as well as in GCs. SLC1A2–CD44, SLC34A2–ROS1, CLDN18-ARHGAP26 and BRAF-AGTRAP fusions have been detected in GCs [27-30].

In addition to these genetic changes, epigenetic alterations, including promoter CpG island hypermethylation and histone modifications, associated with the silencing of critical tumor suppressor genes and the activation of oncogenes are the most common molecular alterations in human GCs. H. pylori and Epstein-Barr virus infections are associated with increased methylation in normal gastric mucosae, and it has been shown that increased CpG-island methylation in normal mucosa is a gastric cancer precursor [31].

While genetic inactivation of CDH1 gene is frequent in sporadic and familial GCs, CDH1 promoter hypermethylation has also been observed in 55% of sporadic gastric cancer [32]. On the other hand, promoter hypermethylation is the most common cause of a second hit that inactivates the wild-type CDH1 allele and initiates tumorigenesis in HDGC [33]. CDH1 methylation was associated with H. pylori infection and poor prognosis in diffuse type GCs [34]. CDH4 gene, another cadherin from the cadherin superfamily that encodes for a cell-cell adhesion glycoprotein, is also methylated at a high frequency in gastric cancer cases and may be an early event in tumor progression [35].

The promoter hypermethylation of cell cycle genes p16(INK4a), p15 (INK4b) and CDKN2A have been observed in 30% of GCs and downregulation of p16(INK4a) and CDKN2A was an early event suggesting their role in malignant transformation [36-38].

Genes that are involved in DNA repair such as human mutL homolog 1 (hMLH1) and

methylguanine DNA methyltransferase (MGMT) also frequently undergo epigenetic silencing in GCs [39-41].

Microsatellite instability (MSI) that is defined as a change in the microsatellite sequence size within a tumor in comparison to that of normal tissue, one of the important genomic signatures is observed in 5-10% of diffuse type gastric cancer and in 15-40% of intestinal type of gastric cancer [42]. MSI results from mutational inactivation or epigenetic silencing of DNA mismatch repair genes such as MSH1, MSH2, MLH2, MLH3, MSH6 and PMS2. MSI is one of the major type of genetic instability in GCs that creates a permissive environment for the accumulation of genetic alterations in other genes. Another genomic instability pathway called chromosome instability (CIN) pathway characterized by loss of chromosomal material during carcinogenesis. GCs displaying CIN shows marked aneuploidy, harbor focal amplifications of Receptor Thyrosine Kinase (RTK) genes and recurrent TP53 mutations [20,43].

Noncoding RNAs (ncRNAs) such as microRNA (miRNA) and Long non-coding RNAs (LncRNA) are also involved in carcinogenesis by regulating oncogenes, tumor suppressor genes and important cellular functions. More than 200 miRNAs were found to be associated with GC development. Many miRNAs such as miR-29c, miR-508-3p, miR-448, miR-15a, and miR-485-5p are found to be down regulated in GC tissue. Functional studies of down regulated miRNAs showed their role in proliferation, invasion and/or migration in GC cell lines suggesting their tumor suppressor effects. Contrarily, some miRNAs such as miR-544a, miR-1290 and miR-543 found to be up regulated in GCs and promote gastric tumor cell proliferation or metastasis [44].

Accumulating evidence indicated that LncRNAs are aberrantly expressed in a variety of human cancers and crucial to carcinogenesis. TUSC7 proposed to be a tumor suppressor acting with TP53 and found to be down regulated in GC. Well known LncRNAs HOTAIR, H19 and MALAT1 and some others such as LSINCT5 are found to be overexpressed in GCs and are probable oncogenes driving gastric carcinogenesis [44].

Taken together, genomic and epigenomic studies emphasize comlexity of gastric carcinogenesis and tumour heterogeneity in different GC patients that most probably lead to different responses to therapy.

6. Molecular Classification of GC& Clinical Implications

Over the last decade we have been witnessing very promising improvements in management and survival rates for certain types of cancers. This can be mostly attributed to high-throughput genomic technologies and the data they provide. Advances in the field have clearly translated into better patient care as evidenced by the earlier detection, better prognosis, and new targeted therapies.

Several molecular classifications of GC have been proposed based on the analysis of whole-genome gene expression studies and/or gene copy number studies.

Early studies reported gene expression signatures capable of predicting the prognosis of GC notably prognosis of stage II patients. If stage II patients are identified as "poor prognosis" by the 8 gene signature, they might be prescribed adjuvant chemotherapy while "good prognosis" patient might be treated with surgery alone [45]. Another group published a 6 gene signature along with a risk scoring algorithm to prognosticate recurrence of gastric cancer patients after surgery [46].

The Cancer Genome Atlas (TCGA) study, in addition to identification of new GC genes and pathways, has allowed the researchers to classify GCs into four distinct subtypes; tumors positive for Epstein–Barr virus (EBV), microsatellite unstable tumours (MSI), genomically stable (GS) tumors and tumors with chromosomal instability (CIN). TCGA study showed that each subtype was found throughout the stomach, but the distribution of the molecular subtypes varied between the distinct anatomical regions. In the gastroesophageal junction/cardia, CIN tumours were seen in higher frequency (~65%). At the molecular level, TP53 mutations were observed in 71% of them and RTK-RAS pathway activation was prominent. At the gastric fundus and body, increased frequency of EBV-positive tumours with extreme DNA hypermethylation, PIK3CA mutations, amplification of JAK2, PD-L1 andPD-L2and MSI tumors with mutations in PIK3CA, ERBB3, ERBB2 and EGFR were observed. Microsatellite instability, molecular fingerprint of a deficient DNA mismatch repair system, occurs mostly in the distal part of the stomach. At the antrum and pylorus, MSI and GS tumor frequency with mutations of RHOA and elevated expression of cell adhesion and angiogenesis related pathways were increased. Evaluation of the clinical and histological characteristics of these molecular subtypes in this study revealed enrichment of the diffuse histological subtype in the genomically stable group. One quarter of genomically stable group GCs arise in the antrum, about 20% in the gastroesophageal junction/cardia, and approximately 15% in the gastric body/fundus [43]. Future studies correlating the anatomical location and histological appearance of the tumors with the molecular changes may allow for better classification and may lead to more effective treatments with an integrative approach.

Another study realized by Asian Cancer Research Group (ACRG) allowed researchers to develop a different classification system consisting of four different subgroups based on gene expression signature; the microsatellite stable (MSS)/epithelial-mesenchymal transition (EMT) subtype, microsatellite instable (MSI) subtype, MSS/tumor protein 53 $(TP53)^{+}$ subtype, and MSS/$TP53^{-}$subtype [47].

There are overlapping characteristics between subtypes described by the ACRG and the TCGA, with both groups describing an MSI subtype, and the GS TCGA subtype and epithelial-

to-mesenchymal transition (EMT) ACRG subtype both including predominantly DGCs. The two other ACRG subtypes were defined as being non-MSI, non-EMT and by the status of "TP53 activation", as defined solely by a CDKN1A and MDM2 gene signature [47]. Survival data for the TCGA GC cohort was currently immature but available for ACRG cohort, with the MSI subtype showing the best prognosis, and the lowest frequency of recurrence. The EMT subtype was associated with the poorest prognosis. TP53 active and TP53 inactive types include patients with intermediate prognosis and recurrence rates, with respect to other two subtypes [43,47].

Another recent study classified GCs in two main subtypes based on mutation burden as regular and hyper mutated. The regular mutated subtype is classified further into two subgroups with distinct prognostic outcomes; C1 and C2. C1 is characterized by mutations in TP53, XIRP2, APC, ERBB4 and AKAP6 and associated with a significantly better prognostic outcome whereas C2 is overrepresented by mutations in ARID1A, CDH1, PIK3CA and RHOA [48].

Within the different genes and pathways identified as altered in GC, a number of potentially drug able targets appear.

As mentioned above, overexpression and amplification of HER2 in gastric cancer is reported in 6–23 % of the cases. Overexpression and amplification of HER2 leads to a poor prognosis, related to its role in the invasiveness and metastasic potential of the tumor. But, a significant benefit in advanced gastric cancer could be observed, using the monoclonal antibody Trastuzumab (monoclonal antibody targeting HER2), in combination with chemotherapy [49].

Angiogenesis and major mediators, VEGF and VEGF receptor-2 (VEGFR-2)-mediated signaling contribute to the pathogenesis and progression of GC. The second monoclonal antibody incorporated into the therapeutic arsenal of advanced GC was Ramucirumab, a humanized IgG1 monoclonal antibody that inhibits vascular endothelial growth factor receptor -2 (VEGFR-2) [50].

Immunotherapy, through targeting immune checkpoints holds potential promise for the treatment of EBV+ and MSI + subtype tumors. This is due to the fact that there are a high number of mutations present in MSI+ tumors, which in turn results in the creation of neoantigens that affect the patients' response to immune-checkpoint inhibitors [51]. In the case of EBV+ patients, both the overexpression of PD-L1and activation of the immune pathway provide strong motive to justify the targeting of these molecules and pathways via immunotherapy, as they are likely to be good candidates of an immune response reactivation [52]. Multiple inhibitors of immune checkpoints have been applied to clinical therapy, especially the anti-PD-1 mAb pembrolizumab, which has shown manageable side effects and a 22% (8/36) overall

response rate in a phase I study of PD-L1 expressing AGC patients [53]. Recently, FDA granted accelerated approval for pembrolizumabfor adult and pediatric patients with unrespectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options [54].

Several clinical studies currently evaluating the impact of immune checkpoint inhibitors in GCs. Indeed, a phase 1b trial of Pembrolizumab (KEYNOTE-012) showed durable remissions in a subset of patients with PD-L1 positive advanced GCs and Pembrolizumab monotherapy showed clinical efficacy in patients with advanced gastric cancer, according to data from KEYNOTE-059 that represents a potential treatment option for patients with gastric/GEJ (Gastro-Esophageal Junction) cancer who have progression after at least two prior lines of therapy [50,53].

As mentioned above, in addition to HER2 several RTKs are genetically altered in GCs. Many monoclonal antibodies and small-molecule compounds targeting EGFR, MET and FGFR2 have been studied intensively to assess their therapeutic potential in GC. Although many RTK inhibitors have been approved by the FDA and currently used in clinical practice to treat different cancers, phase II and III clinical trials targeting EGFR, MET and FGFR2 have returned disappointing results [55]. However, all these clinical trials lacked biomarker-assisted patient selection.

RAS/MAP kinase and PI3K/AKT pathways are also important targets for cancer therapy since they are involved in RTK signaling and their mutations affecting Ras, B-Raf, mTOR, PI3K and AKT are common in several types of cancers. Small inhibitor molecules are being developed to target primarily Mek, Raf, PI3K and mTOR in patients with different types of cancers. Unfortunately, most of the RAS/MAP kinase and PI3K/AKT pathway inhibitors have shown low to moderate efficacy in GC preclinical and early clinical studies. Only mTOR inhibitors Rapamycin and Everolimus have been shown to be promising in early-stage studies but phase III studies returned disappointing results [56].

Although progress is being made in GC patient therapy, it was not fast enough compared to other cancers (i.e., breast, colorectal and hematologic malignancies). Several molecular therapies for gastric cancer have entered clinical trials but only Trastuzumab and Ramucirumab have been approved for clinical use in advanced GCs. We strongly believe that, accurately stratified patients bybiomarker-assisted patient selection and properly conducted prospective studies using this genomic information will also improve GC patient outcomes in near feature.

7. References

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