

Review Article

Molecular genetic changes in gastric carcinoma

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ABSTRACT

Gastric cancer remains highly prevalent and accounts for a notable proportion of global cancer mortality and this is associated with poor survival rates. Understanding the molecular genetic changes of gastric carcinoma may offer an insight into its pathogenesis helps in identifying new biomarkers, aid prognostication, and novel treatment targets. Over a past few decades, advances in technology and high throughput analysis have improved understanding of the molecular genetic aspects of gastric cancer. In this article, hierarchy of the changes at genetic and molecular level including several aspects which are heterogenous and represents a wide spectrum such as tumor suppressor genes, oncogenes, cellcycle regulators, apoptosis, cell-adhesion molecules, loss of heterozygosity, microsatellite instability, and epigenetic changes. The classification of gastric carcinoma at molecular and genetic level as well as hereditary gastric carcinoma is elaborated. The molecular genetic aspects regarding pathogenesis, changes and aberrations of all genes and pathways which are involved in gastric cancer are addressed in this review.

Keywords: Gastric carcinoma, Hereditary and acquired, Tumor suppressor genes, Oncogenes, MSI epigenetics

INTRODUCTION

Gastric cancer (GC) remains a worldwide burden as one of the leading causes of cancer-related death in both sexes. The late onset of clinical symptoms is the main reason that the disease is often diagnosed at an advanced stage, which limits the available therapeutic approaches in more than 50% of cases.^[1] At present, GC is a most frequent diagnosed neoplasm worldwide. Although extensive studies have been performed to identify genetic pathways and genes involved in the disease development and progression, the prognosis of GC patients remains poor and little improvement of long-term survival has been achieved. Adenocarcinoma is the major histological type of GC, accounting for 90–95% of all gastric malignancies. The incidence is closely related to environmental factors, reflecting a characteristics geographical distribution.^[2]

GC is a result of complex interaction of environment and multiple genes. The evident risk factors of GC include dietary, the *Helicobacter pylori* infection, the family history, and the genetic factors.^[2,3] Although, there is great progression in the diagnosis and treatment of GC, the survival rate is still stagnant and poor, only about 20% of patients with GC can reach 5-year survival. Thus, a systematic view of the genetic basis of GC is necessary to establish new strategies for prevention and treatment of GC. Genetic factors mainly refer to the susceptible genes of cancer that is involved in multiple genetic and epigenetic alterations of oncogenes, tumor suppressor genes (TSGs), cell cycle regulators, DNA repair genes, and signaling molecules.^[4]

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GC is the second cause of cancer mortality^[5] and the fifth most common malignancy in the world, 50% of the cases are from Eastern Asia^[1] where China has the highest incidence.^[6] The 5-year survival rate is still remain disappointing despite improvements in the diagnosis and treatment of GC as they are usually diagnosed at the advanced stage and usually incurable or rarely curable. The environmental risk factors have been established includes *H. pylori* infection, smoking, consumption of food high in salt, or N-nitroso compounds such as processed or smoked meats and Epstein–Barr virus (EBV) infection. The GC incidence seems to be increasing in younger age groups; however, the cause of phenomenon is still unknown.^[7] Based on the Lauren classification, GC is classified as intestinal and diffuse types, which has different clinicopathological and prognostic features. It not only differs in morphology but also in epidemiology, progression pattern, genetics, and clinical pattern. Recently, it is seen that location of the tumor also matters as there appears to be a difference between proximal and distal non-diffuse GCs because of the distinct or different sets of gene expression level.^[8,9] Surgical treatment is the only therapeutic modality which provides the greatest possibility of cure. Overall, this article aims to cover the molecular and genetic changes, different classification based on several basis genetic and epigenetic changes in GC which are currently understood.

CLASSIFICATION OF GASTRIC CARCINOMA

Early gastric carcinoma is classified based on gross pattern as follows: Type I for the tumor with protruding growth, Type II with superficial growth, Type III with excavating growth, and Type IV for infiltrating growth with lateral spreading. Type II is again divided into IIa (elevated), IIb (flat), and IIc (depressed) proposed by the Japanese endoscopic society.^[10] Another classification, three gross patterns for superficial neoplastic lesions in gastrointestinal tract, the tumor is classified as: Type 0-I for polypoid growth (subcategorized to 0-IP for pedunculated growth and 0-IS for sessile growth), Type 0-II for non-polypoid growth (subcategorized into Type 0-IIa for slightly elevated growth, Type 0-IIb for flat growth, and Type 0-IIc for slight depressed growth), and Type 0-III for excavated growth.^[11] The difference between intramucosal carcinoma and carcinoma *in situ* or high grade dysplasia is very important because the stomach intramucosal unlike the colon intramucosal shows metastasis. The useful important histologic features of intramucosal invasion are single tumor cell in lamina propria and significantly fused neoplastic gland of multiple sizes. The early gastric carcinoma prognosis is excellent and shows 5 years survival rate with about 90%.^[12]

Advanced gastric carcinoma shows invasion upto muscularis propria beyond and has showed worse prognosis with 5-year survival rate at about 60% or less.^[13] The gross appearance can

be exophytic, ulcerated, infiltrative, or combined. Based on Borrmann's classification, the gross appearance is classified into; Type I for polypoid growth, Type II for fungating growth, Type III for ulcerating growth, and Type IV for diffusely infiltrating growth (linitis plastica in signet ring cell carcinoma), this happens when most of the gastric wall is involved by infiltrating tumor cells. In addition, World Health Organization (WHO) classification endorses other uncommon histologic variants, such as adenosquamous carcinoma, squamous carcinoma, hepatoid carcinoma, carcinoma with lymphoid stroma, choriocarcinoma, parietal cell carcinoma, malignant rhabdoid tumor, mucoepidermoid carcinoma, mixed adeno-neuroendocrine carcinoma, endodermal sinus-tumor, embryonal carcinoma, pure gastric yolk sac tumor and oncocytic adenocarcinoma, and micropapillary carcinoma of stomach is a newly recognized histologic variants characterized by small papillary clusters.^[13]

CLASSIFICATION OF GASTRIC CARCINOMA BASED ON GENETIC PROFILE

According to Laurens classification, gastric adenocarcinoma is divided into intestinal, diffuse, mixed, and indeterminate subtypes.^[14] They vary not only in morphology but also in epidemiology, progression pattern, genetics, and clinical pictures. Histopathologically, intestinal type is characterized by malignant epithelial cells that show cohesiveness and glandular differentiation infiltrating the surrounding tissues.^[15] In contrast, the diffuse subtype is characterized by tumor cells that show poor differentiation and lack of cohesion. Intestinal type of GC is felt to be caused mainly by environmental (exogenous) factors whereas the diffuse type is thought to be due to hereditary and genetic (endogenous) factors. These histologic classifications are not sufficient to reflect the molecular and genetic characteristics of GC or to develop personalized treatment strategies in the era of precision medicine. Several molecular classification systems have been proposed, and distinct molecular subtypes have been identified.^[16-18] In 2014, the cancer genome atlas (TCGA) proposed four subtypes: (1) EBV-positive (8.8%), (2) microsatellite unstable/instability (MSI, 21.7%), (3) genomically stable (19.7%), and (4) chromosomally unstable/chromosomal instability (CIN, 49.8%).^[16] Most EBV-positive tumors occurred in male patients and in the gastric fundus or body, displaying extreme DNA hypermethylation and amplification of JAK2 and PD-L1/2, with 80% harboring non-silent PIK3CA mutations. All EBV-positive GCs displayed CDKN2A promoter hypermethylation, while lacking the MLH hypermethylation characteristic of the MSI-associated CpG island methylator phenotype (CIMP).^[16,19]

MSI-subtype tumors tends to occur in female patients, diagnosed at advanced stage, and characterized by elevated mutation rates, including mutations of genes encoding

targetable oncogenic signaling proteins. The genomically stable sub-type lacked numerous molecular alterations, correlated well with the Lauren diffuse histologic variant, but harbored mutations of RHOA or fusions involving RHO-family guanosine triphosphatase (GTPase)-activating proteins. The active GTP-bound form of RHOA activates signal transducer and activator of transcription-3 (*STAT-3*) to promote tumorigenesis. Finally, CIN subtype tumors were frequent at the gastroesophageal junction/cardia, correlated well with the Lauren intestinal histologic variant, showed marked aneuploidy, and harbored focal amplifications of receptor tyrosine kinases (RTKs), in addition to recurrent *TP53* mutations and RTK-RAS activation.^[16]

In 2015, the Asian Cancer Research Group (ACRG) proposed a new classification system associated with distinct genomic alterations, disease progression, and prognosis across multiple GC cohorts.^[17] On the basis of whole-genome sequencing, gene expression profiling, genome-wide copy number microarrays, and targeted gene sequencing, four molecular subtypes were identified: (1) MSI, (2) microsatellite stable with epithelial-to-mesenchymal transition features (MSS/EMT), (3) MSS/*TP53* mutant (MSS/*TP53b*), and (4) MSS/*TP53* wild-type (MSS/*TP53*).^[17] MSI tumors were hypermutated, intestinal, usually antral, and diagnosed at clinical Stage I/II. MSI tumors had the best prognosis; their recurrence rate after surgical resection of primary GC was the lowest among all four subtypes (22%). MSS/*TP53b* tumors were linked to EBV infection and had the next best prognosis, followed by MSS/*TP53*- tumors. MSS/EMT tumors occurred at a younger age, mostly diagnosed at clinical Stage III/IV, and were the Lauren diffuse histologic type. The MSS/EMT subtype had the worst prognosis and the highest recurrence rate (63%), with recurrences located mostly in the peritoneal cavity.^[17] To refine molecular classification, regular mutated and hyper mutated GC types were identified in another recent study. The regular mutated type was further sub classified into two subgroups, C1 and C2. The first subgroup, C1, was enriched in mutations of *TP53*, *XIRP2*, and *APC* and was associated with a significantly better prognostic outcome, whereas C2 was over represented by mutations in *ARID1A*, *CDH1*, *PIK3CA*, *ERBB2*, and *RHOA*.^[17]

MOLECULAR PROFILE IN GASTRIC CARCINOMA

TCGA has characterized 295 cases of gastric adenocarcinoma, the most comprehensive study to date, using multiple high-throughput technologies, which includes somatic copy number analysis, DNA methylation profiling, messenger RNA, and micro-RNA sequencing, reverse phase protein array, microsatellite instability (MSI), and whole genome sequencing.^[16] After this, four GC subtypes were described they are; (1) Tumor positive for EBV, (2) MSI-

high tumors, (3) genomically stable tumors, and (4) tumor with chromosomal instability [Table 1]. Moreover, these GC subtypes showed different epigenetic changes and mutation of several genes like; EBV + tumors show recurrent *PIK3CA* and *ARID1A* mutations, extreme DNA hypermethylation, and high amplification of *JAK2*, *PD-L1*, and *PD-L2*. MSI-H tumors show elevated mutation rates which also include mutation of genes encoding targetable oncogenic signaling proteins.^[17] GS (genomically stable) tumors are enriched by the diffuse histological variants and *CDH1* and *RHOA* mutation or *CLDN18-ARCHGAP* fusion. CIN tumors observed frequently at the gastroesophageal junction/cardia along with recurrent *TP53* mutation and number of amplifications of RTKs genes.

The ACRG has proposed four molecular subtypes which are as follows; (1) MSH-H, (2) MSS/EMT, (3) MSS/*TP53*- mutants, and (4) MSS/*TP53*+ wild type [Table 2].^[17] The recent molecular characterization of GC is evolving. Many molecular classifications being proposed and different molecular subtypes have been identified.^[19] Various RTKs such as human epidermal growth factor (*EGF*) receptor 2, *EGFR1*, mesenchymal epithelial transition factor (*MET*), and fibroblast GF receptor 2 (*FGFR2*) being reported to be amplified in GC

Table 1: Molecular classification of gastric adenocarcinoma based on the cancer genome atlas with salient features of each subtypes.

The cancer genome atlas classification	Defining characteristics
EBV+	<i>PIK3CA</i> , <i>ARID1A</i> , <i>TP53</i> mutation <i>CDKN2A</i> silencing <i>PD-L1/L2</i> overexpression CpG island methylator phenotype Male predominance Increased immune cell signalling More frequently in fundus and body
MSI	Hypermutation <i>TP53</i> , <i>KRAS</i> , <i>PIK3A</i> , <i>ARID1A</i> mutation CpG island methylator phenotype <i>MLH1</i> silencing Mitotic pathways Diagnosed at older age Female predominance
GS	<i>CDH1</i> , <i>RHOA</i> mutations Increased expression of cell adhesion pathways <i>CLDN18-ARHGAP</i> fusion Diagnosed at earlier age
CIN	Diffuse histology RTK-RAS activation Aneuploidy <i>TP53</i> mutation More frequently at gastroesophageal-junction and cardia Intestinal histology

Table 2: Molecular classification of gastric adenocarcinoma based on Asian cancer research group with salient features of each subtype.

Asian cancer research group classification	Defining characteristics
MSI	Primarily intestinal histology Predominantly in antrum Heavy mutational burden Higher rate of liver-limited recurrence Best overall survival, earliest stage at diagnosis
MSS/EMT	Worst overall survival, higher stage at diagnosis Younger age Primarily diffuse histology Highest rate of recurrence, peritoneal spread Lowest mutational burden
MSS/TP53+	Second-best overall survival Highest percentage EBV1 tumors
MSS/TP53-	Higher rate of liver-limited recurrence

and target therapies have been developed.^[20-23] According to GI-screen as the nationwide cancer genome screening project, the frequently detected mutations are *TP53* (47.8%), *PIK3CA* (9.2%), *KRAS* (6.0%), *SMAD 4* (5.1%), *APC* (4.1%), *TET2* (3.9%), and *ERBB2* (3.3%), and copy number variants are *ERBB2* (11.3%), *CCNE1* (11.1%), *KRAS* (3.7%), *FGFR2* (3.3%), *ZNF217* (3.3%), *MYC* (2.7%), *CCND1* (2.3%), and *CDK6* (2.1%).^[24]

The understanding of the molecular aspects of GC is improved by next generation sequencing (NGS) studies which provides high throughput method to know and identify the genetic alteration in GC systematically. Li-Chang *et al.* showed mutations of several driver genes by performing NGS that includes; *TP53*, *PIK3CA*, *CTNNB1*, *CDH1*, *SMAD4*, and *KRAS*.^[25] Few of the TSGs such as *APC*, *CDH1*, *CDH4*, *THBS1*, and *UCHL1* are found to be inactivated by hypermethylation.^[26-29] It has been shown that 59% of GCs shows mutation in chromatin remodeling genes such as *ARID1A*, *PBRM1*, and *SETD2*.^[30] There are new mutated driver genes found, they are (*MUC6*, *CTNN2A*, and *GLI3*) through whole genome sequencing.^[31] It was also found that the genes involved in cell adhesion and chromosome organization showed frequent mutations in gastric adenocarcinoma patients by which it has confirmed that there are 30 candidate driver mutation mutated in primary and lymph node tissues and, on the other hand, seven candidate mutation mutated only in primary tumors. The primary

tumors show more mutations than metastatic tumors because metastatic tumors comes only from the subpopulation of primary tumors but surprisingly researchers do not found any metastatic specific mutations and it is expected that these metastatic tumor cell shows the behavior of continuing the mutations of its genome to gain more growth power. There are some locus at chromosome 17q12 which are frequently amplified in GC and they are as follows; *PPPIRIB-STAR3-T-CAP-PNMT*, *PERLD1-ERBB2-MAC14832-GRB7*.^[32]

In addition to this, there are two genes which are very important located on 9p21 chromosome which encodes *P16* and *P15*, respectively, they are *CDKN2A* and *CDKN2B* showed CN loss (CN=0.8~1.32) as these two genes normally show very crucial function, they inhibit cyclin-dependent kinase (*CDK4*) and *CDK6* and control cellular proliferation by preventing entry into the S phase of the cell cycle and so inactivation of these may leads to uncontrolled growth in cancer.^[33]

GENETIC CHANGES IN GC

Mutation of genes in GC is broadly classified into three categories;

- High frequency drivers which display high rate of recurrence (>5–10%) across multiple GCs
- Low frequency drivers which are recurrently mutated in the 1–10% range but they are still contributing to pathogenesis of disease
- Passenger mutation/bystander which arises as a consequences of underlying mutational processes such as CpG deamination but are not functionally contribute in tumorigenesis.^[34]

Recently, it has been reported the importance of *RTK/RAS/mitogen-activated protein kinase (MAPK)* signaling, frequent mutations in the *ERBB3* *RTK*, and the ligand/*RTK* *NRG1/ERBB4* genes in GCs. The recent NGS studies have been highlighted two new GC genes—*ARID1A* and *RHOA*. *ARID1A* is mutated in 10–15% of GCs which is known to encode a components of *SWI/SNF* chromatin remodellar complex.^[34] Mutation of *ARID1A* are typically inactivating (frameshifts, and non-sense mutation). Consistently acting as a tumor suppressor genes. The normal function of *ARID1A* gene is a role in GC cell proliferation through the control of cell cycle regulators *CCNE1* and *E2F1*. The another genes *RHOA* which has been recently shown that it exhibits recurrent mutations in diffuse type/genome stable GCs.^[34]

The consequences of mutation in both *ARID1A* and *RHOA* are different. *ARID1A* mutation is dispersed throughout the genes, whereas the *RHOA* mutation is localized to an N-terminal hot-spot region (*Ty42*, *Arg5* and *Gly17*). *ARID1A* are predicted to modulate downstream *Rho*-signaling.

Mutation of RHOA suggests after functional studies that they can impart resistance to anoikis (a form of programmed cell death occurring after cellular detachment from a solid substrate). Clinically, RHOA hotspot mutation discovery provides specific inroad for the development of new approaches to target diffuse type GCs (DGCs), traditionally associated with extremely poor prognosis.^[34] Genomic studies also uncovered the long-tail of low frequency driver mutations in GC such as gastric mucin MUC6, BCOR (encoding BCL6 corepressor), FAT 4 (a protocadherin), and RNF43, a *Wnt* pathway regulator. Although they have low mutation frequency, these genes also contributes to the ability of GC to manifest the cancer hallmarks.^[34]

TSG

TSGs normally show protective role in preventing the malignant transformation of cells by repairing DNA inhibiting cell proliferation and initiating programmed cell death (apoptosis). TSGs are involved in the regulation of a range of cell functions including cell adhesion, cell-cell interaction, cytoplasmic signal transduction, and nuclear transcription.^[35] Over recent decades, there is rapid expansion in the member of TSGs which have been identified in relation to a broad range of inherited and non-inherited human cancers. Greater understanding of the pattern of TSG expression in GC may enables the identification of specific biomarkers which could be used for early diagnosis and development of target treatment. The overexpression of P53 and loss of expression of phosphatase and tensin homolog (*PTEN*), E-cadherin, *SMAD4*, *MGMT*, and *CD82* were all significantly associated with poor prognosis in gastric carcinomas.^[36]

1. Tumor protein P53 (*TP53*): It is the most frequently mutated aberration in ~50% of GC cases. The cellular function of *TP53* is a guardian of genomic integrity. The GCs in which *TP53* gene is mutated often exhibit high levels of SCNAs involves both broad chromosomal regions and focal gene regions. The mutations in other canonical oncogenes (*KRAS*, *CTNNB1*, and *PIK3CA*) and tumor suppressor genes (*SMAD4*, *APC*) have also seen in GCs.^[37] The aberrant expression of *TP53* have been found in over half of human cancers including leukemia, breast, colon, and lung carcinoma and alteration in *TP53* is the most common event in human cancers. Normally, *TP53* plays key role in cell cycle progression preventing G1/S transition after DNA damage occurred and allowing DNA repair/cell apoptosis.^[37] Some studies shown that *TP53* abnormalities can occurs in non-neoplastic gastric mucosa with intestinal metaplasia suggesting *TP53* mutation as early phenomena in gastric carcinogenesis^[38]
2. *PTEN*: It is aTSG, discovered in 1997. It functions as a dual-specificity protein and phospholipid phosphatase,

and regulates a variety of cellular processes and signal transduction pathways in a complex network system that can control proliferation, apoptosis, migration, adhesion, and genetic stability. The most detailed study of *PTEN* is focused on its negative regulation on phosphoinositide 3-kinase (PI3K)/Akt signaling pathway, which is important in controlling cell survival, promoting proliferation, and inhibiting apoptosis. It is a lipid phosphatase which phosphorylates phosphotidyl inositol-3,4,5-triphosphate (PIP3) reduces the activation of PI3K and the serin-threonine specific protein kinase Akt.^[39] Hence, loss of expression of *PTEN*, increased level of PIP3, increase AKT activation, and consequently inhibits apoptosis which ultimately results in large number of tumors. There are multiple phenomena due to which *PTEN* inactivation occurs such as gene mutation, loss of heterozygosity (LOH), hypermethylation or miRNA-mediated alterations in gene expression, or post-translational modification.^[40] In gastric adenocarcinoma, *PTEN* protein expression is significantly downregulated compared to normal. On the other hand, PI3K, AKT, MMP-2, MMP-9, and nuclear factor- κ B (*NF- κ Bp65*) protein is overexpressed in GC^[41]

3. *CDH1* (Cadherin 1): Inactivation of E-cadherin found in early stage of gastric carcinogenesis, tumor progression, invasion, and metastasis. *CDH1* gene alteration leads to loss of E-cadherin expression which ultimately results in cancer cell proliferation, invasion, and metastasis which is considered as primary carcinogenic event in hereditary diffuse GC.^[42-44] Somatic alterations in *CDH1* found in approximately 30% of GC patients. About one-third having structural alterations (7.5% LOH, 1.7% mutation) and rest having epigenetic modifications with 18.4% hypermethylation.^[45] Epigenetic modification is more common than structural in diffuse type while intestinal type has approximately similar rates of structural and epigenetic changes.^[45] Wei *et al.*^[46] recently found that mRNA and protein expression level of T-cadherin is lower significantly in gastric tumor tissues compared to normal adjacent tissue.^[46] The decreased T-cadherin expression is correlated with larger tumor size, lymph node metastasis, and higher TNM stage.^[46] Multivariate analysis shows that T-cadherin expression is an independent prognostic factor for overall survival^[46]
4. *SMAD3* is another tumor suppressor gene whose loss of expression is associated to advanced stage and poorer outcome in gastric carcinoma patients which is more common in intestinal type than in diffuse type gastric carcinoma
5. *SMAD4* loss of expression is more common in intestinal type while E-cadherin loss is more common in diffused type.^[36] P53 expression loss is more common in poorly differentiated tumors.^[36] Loss of E-cadherin, *SMAD4*,

CD82, MGMT, and *PTEN* expression is associated with advanced stage of disease while P16 loss expression seen in both early and advanced stages.^[36] Loss of TSG expression accumulation in GC tumor expression which has significant correlation between this accumulation and patient survival.^[36] Apart from mutation, genetic instability, activation of oncogene, and aberrant GF expression/receptor activation all together play role in gastric carcinogenesis

6. Retinoblastoma protein 1 (*RBI*) is involved in negative cell cycle regulation at the G1/S transition. *RBI* shows LOH in advanced stage. *RBI* is known to be a target of miRNA-106b~25 which is involved in promotion of cell proliferation, EMT, cell cycle progression, and exerts an apoptotic effect^[47]
7. Pre-myelocytic leukemia is a TSG controls apoptosis and cell proliferation through P73 and yes associated protein-1 which are found downregulated in GC^[47]
8. *Fbxw7* is a TSG that is responsible for the degradation of several proto-oncogenes and its functional inactivation can dysregulate the cell division process, and potentially lead to tumorigenesis. The inactivation of *Fbxw7* in the progress of human cancer including mutation, deletion, and hypermethylation, of which the *Fbxw7* mutation is most common.^[48] The *Fbxw7* mutation rate in GC tissues was from 3.7% to 6% and did not differ in early or advanced GC, which might play a role in the prognosis of GC.^[49] *Fbxw7* mRNA expression level in cancerous tissues was lower than that in noncancerous tissues. The low *Fbxw7* expression had remarkable poorer prognosis than those with high *Fbxw7* expression.^[50] *Fbxw7* expression was associated with the progressive tumor size, lymph node metastasis, peritoneal dissemination, venous invasion, and clinical stage. *Fbxw7* induced tumor apoptosis and growth arrest and inhibited the EMT in part by downregulating the RhoA signaling pathway in GC.^[51]

Oncogenes

Oncogenes are genes whose normal activity promotes cell proliferation. Oncogenes function through the mechanism of gene mutation, gene insertion, chromosomal translocation, gene amplification, and DNA hypomethylation. Oncogenes can be classified into five broad classes: Secreted GFs; cell surface receptors; components of intracellular signal transduction systems; DNA-binding nuclear proteins; components of the network of cyclins, CDKs, and kinases inhibitors that govern progress through the cell cycle(s).^[52]

Oncogene in humans has the capacity to transform normal cells into malignant ones. These genes are mutated during life make the patients more prone or susceptible to cancer by altering or impairing several phenomenon:^[52] (1) Production

of nuclear transcription factors (TFs) that control cell growth (e.g., *myc*), (2) signal transduction within cells (e.g., *RAS*), (3) interactions of GFs and their receptors (e.g., *her/neu*). Mutations convert proto-oncogenes into oncogene by several processes such as amplification, translocation, and point mutation. Oncogenes are activated by many ways through oncogene amplification, activation by point mutation, and activation by production of chimeric gene products.

RAS

The first human oncogene identified which is involved in 20% of all human malignancies. These are the oncogenes which are not present in normal cells rather they are activated in tumor cells as a consequences of enormous mutations occurs during tumor development. *RAS* gene encodes guanine-nucleotide binding proteins which perform various functions in mitogenic signal transduction and protein activity of *RAS* is controlled by GTP or GDP binding (active – GTP bound and inactive-GDP bound) states.

C-myc

It is an another oncogene which is located on chromosome 8 encodes a nuclear phosphoprotein which acts as TF whose main function is to regulate the transcription of target genes through inducing and repressing the expression.^[53] It also involves in modulation of several genes which are involved in key cellular processes including proliferation, growth, differentiation, and angiogenesis, but also DNA repair and apoptosis,^[54] which if deregulated (*myc* gene) contributes to the formation/genesis of a wide variety of human solid tumors. Overexpression of *C-myc* is found in over 40% of GCs. It is an early event during the genesis of both intestinal and diffuse type of gastric carcinomas, but significantly higher expression in intestinal type than diffuse type. The *C-myc* overexpression is associated with poor survival. It has been found that in benign gastric lesions including chronic atrophic gastritis, gastric ulcer, and *H. pylori* infection, there is high expression of *C-myc* gene.^[55] The another proto-oncogene FOS and JUN gets converted to oncogene is the components of AP-1 complex acts as TF which activates transcription of several targets genes. The FOS and JUN oncogenic proteins are self-sufficient for cell transformation which also have very crucial role in gastric carcinomas.^[56-58]

Prolin rich protein 11 (PRR11)

In 2013, PRR11 identified as novel gene which has been functionally characterized and discovered that, PRR11 plays important role in cell cycle progression and tumorigenesis and its protein has an oncogenic role in GC. The silencing of PRR11 in several gastric cell lines inhibited several proliferation rate, cancer cell migration, and cell colony

formation and tumor growth *in vivo* experiments.^[59,60] The results showed that PRR11 mRNA and protein are upregulated in GC tissues as compared to normal gastric mucosa. PRR11 shows positive expression which was significantly associated with an aggressive cancer phenotypes including tumor with increased amount of invasion, increased tumor differentiation, and advanced disease stage.^[61] However, knockdown of PRR11 in gastric cell lines showed diminished cellular proliferation and decreased colony formation which suggests that PRR11 protein expression plays critical oncogenic role in the development and progression of gastric carcinoma.^[61]

Cell cycle regulators

Cyclins are proteins that control progression through key checkpoints in the cell cycle by binding and activating specific CDKs. From G1-S phase is regulated by the activities of the cyclin D, cyclin E, cyclin A, and their catalytic partners such as CDKs 2,4, and 6. The G2/M transition is regulated by B-type cyclin-associated kinase. The cyclin-CDK complexes stimulate the cell cycle progression and CDKs induce the cell cycle arrest by downregulating CDK activity.^[62] Moreover, the dysregulated expression of these cell cycle related molecules gives rise to uncontrolled proliferation and the malignant transformation of the cell.^[63] Cell cycle control is governed by D-type cyclins which is most commonly mutated pathways in tumor cells. There are accumulating evidences that gastric carcinogenesis frequently involve abnormalities in the expression of cyclin and other cell cycle related genes.^[64]

CyclinD1 (CCND1 gene): Located on chromosome 11q13 encodes members of G1 cyclin family which are involved in regulation of cell cycle and replication. Tumor specific alterations leads to deregulation of cyclin D1 which exhibit oncogenic potential in number of processes such as abnormal growth, angiogenesis, and resistance to apoptosis. Increased cyclin D1 expression has been shown in about 50% of primary gastric tumors. It is associated with signet ring phenotype and poor differentiation in few studies which show no pathologic features, no non-signet ring phenotype.^[65] Cyclin D1 expression found in 50% of GCs, but more commonly in the intestinal type than in the diffuse type.^[65]

Cyclin B1 is seldom studied in gastric tumor. The cyclin B1 expression and its correlation with clinicopathological features and the relationship of its expression to prognosis remain unclear until the present date.^[66] The cyclins expression and negativity for CDK inhibitors are important mechanisms related to gastric carcinogenesis but only cyclins B1 expression is associated with reduced survival. Hence, it can be suggested that the expression of cycle B1 could be considered as a marker of aggressive biological behavior in GC.^[66] The expression of cyclin B1 has been found to play important role in lymph node metastatic potential in GC.^[66]

Cyclin CDKs and CDKIs, tumor suppressor gene P53 and pRb alterations are frequently observed in human neoplasm, including gastric carcinomas.^[67] The decreased expression of CDKI p27^{KIP1} in GC is frequency associated with depth of tumor invasion and the presence of lymph node metastasis.^[68] Cellular proliferation follows an organized and timely regulated progression through the cell cycle, which is controlled by protein complex composed of cyclins and CDKs.

Apoptosis

Apoptosis was initially described by its morphological characteristics, including cell shrinkage, membrane blebbing, chromatin condensation, and nuclear fragmentation.^[69-71] The realization that apoptosis is a gene-directed program has had profound implications in understanding the developmental biology and tissue homeostasis, it implies that cell numbers can be regulated by factors that influence cell survival as well as those that control proliferation and differentiation. Moreover, the genetic basis for apoptosis implies that cell death, like any other metabolic or developmental program, can be disrupted by mutation. In fact, defects in apoptotic pathways are now thought to contribute to a number of human diseases, ranging from neurodegenerative disorders to malignancy.^[72] What triggers apoptosis during tumor development? A variety of signals appear important. Extracellular (EC) triggers include growth/survival factor depletion, hypoxia, radiation, and loss of cell-matrix interactions. Internal imbalances can also trigger apoptosis, including DNA damage (produced by cell cycle checkpoint defects or exogenous toxins), telomere malfunction, and inappropriate proliferative signals produced by oncogenic mutations.

The cloning and characterization of the *Bcl-2* oncogene established the importance of apoptosis in tumor development. *Bcl-2* was first identified at the chromosomal breakpoint of t (14;18) in a human leukemia line.^[73,74] To date, at least 15 *Bcl-2* family member proteins have been identified in mammalian cells, including proteins that promote apoptosis and those that prevent apoptosis.^[75] In addition to *Bcl-2*, *Bcl-x_L* is a potent death suppressor that is upregulated in some tumor types.^[76] *In vitro* studies have shown that both GKN1^[77-81] and GKN2^[81] occur in the gastric mucosa of healthy individuals. On the other hand, in the gastric mucosa of GC patients compared to subjects with superficial gastritis, a decreased GKN1 protein,^[82] and mRNA *GKN1* expression is noted.^[83,84] GKN1 maintains the integrity of the gastric mucosa, protects it against the action of stomach acid, and enzymes as well as mechanical damage, bacteria or foreign antigens.^[78,85] GKN1 has been shown to inhibit tumor cell growth and reduces the number of cell colonies by stopping the G2/M cell cycle instead of inducing

apoptosis.^[80] On the other hand, it was found that in the AGS GC cell line transfected with *GKN1*, inhibits proliferation of these cells and activation of the apoptosis process.^[86]

Cell adhesion molecules

Classical cadherins are transmembrane-spanning adhesion molecules containing five calcium-dependent EC domains that confer homotypic interactions and a cytoplasmic tail that binds to a number of effectors to transduce physical and biochemical signals to the cell.

The names of cadherin were initially based on the cell type in which expression was first described, a consensus nomenclature now defines the classical cadherins as *CDH1* (E-cadherin), *CDH2* (N-cadherin), *CDH3* (P-cadherin), *CDH4* (R-cadherin), and *CDH15* (M-cadherin).^[87] Evidence for a direct function of E-cadherin in cell-cell adhesion was provided when the full-length cDNA of E-cadherin was cloned and expressed in fibroblasts that do normally not express E-cadherin. The pivotal role of E-cadherin during normal epithelial function might form the basis for its function as a tumor suppressor. E-cadherin-inactivating mutations in GC preferentially cause in-frame deletions caused by skipping of exons 7 or 9, or occasional frameshift mutations.

E-cadherin expression is mostly restricted to epithelial cells, whereas cells of neural or mesenchymal origin usually express N-cadherin. Epithelial cells are phenotypically dissimilar from mesenchymal cells; from a cancer point of view the latter are more motile and migratory. Cadherin switching in cancer is defined as loss of E-cadherin and expression of N-cadherin during tumor progression,^[88] which will induce or enhance the metastatic capacity of the invading carcinoma cell. Germline E-cadherin (*CDH1*) mutations are found exclusively in DGC.^[89]

During EMT, Type I cadherin (epithelial-cadherin, E-cadherin, encoded by the *CDH1* gene at human chromosome 16q22.1), which sustains key intracellular binding structures such as desmosomes and claudins, is switched to neural cadherin (N-cadherin, encoded by the *CDH2* gene), which is mostly expressed among mesenchymal cells.^[90] The reduction of E-cadherin with the immunoglobulin-like domain on cellular surface which is able to combine adjacent cells, and the intracellular region which is able to link α - and β -catenin to the actin cytoskeleton sustaining cell shape and polarity, is regarded as an important EMT feature which plays critical roles in EMT by changing the components of intercellular adhesion and regulating diverse signaling pathways.^[91]

E-cadherin downregulation and initiation and execution of EMT are caused by several microRNAs (miRNAs)^[92] and various key EMT-inducing TFs including *Snail1* (Snail), *Snail2* (Slug), *Twist*, *Zeb1*, *Zeb2* (Sip1), forkhead box C2,

E47, Krüppel-like factor (*KLF4*), and *KLF8*, *gooseoid*, *Sox9*, which interact with each other, and which are downstream in important signaling pathways activated by transforming GF- β (*TGF- β*), *Wnt*, *Notch*, *integrin*, interleukin (*IL*)-5, *IL-6*, *FGF*, *STAT-3*, *EGF*, hepatic GF (*HGF*), and *NF- κ B*.^[93,94] EMT is induced in epithelia by diverse EC stimuli including *TGF- β* , *HGF*, TNF- α , hypoxia inducible factor 1- α , and inflammatory signals from surrounding microenvironment, which activate GF and chemokine receptors, the downstream signaling cascades, and several TFs.^[95] Transduction of exogenous WNT5A into GC cells upregulates EMT-related genes, indicating that WNT5A regulates GC EMT.^[96] Downregulation of Wnt5a by EGF, which increases Arf6 and ERK activity, is necessary for EGF-induced EMT in GC cells.^[97] The nuclear expression and transcriptional activity of Snail and β -catenin are increased by inhibitory phosphorylation of glycogen synthase kinase-3 β (GSK-3 β) at Ser9 by FasL-induced EC signal-regulated kinase (ERK)/MAPK signaling. Snail associates with β -catenin in the nucleus and thus increases β -catenin transcriptional activity.^[98] Amplification of *ERBB2*, *MET*, *FGFR2*, *PIK3CA*, *AKT1*, *WNT2*, *WNT2B*, and *WNT8B*, and downregulation of *SFRP1* and *PAR3/PAR6/a* PKC complex could lead to GC EMT also through GSK3 β inhibition.^[99] Aquaporin 3 (AQP3) upregulation represses E-cadherin, thus promoting EMT in human GC. It also upregulates the expression of vimentin and fibronectin *in vitro*. The PI3K/AKT/SNAIL signaling pathway is likely involved in the EMT induction by AQP3 in GC.^[100] Caveolin-1 is modulated by HSP90 and functions as a crucial regulator of GC EMT.^[101] Insulin-like GF I (*IGF-I*) induces EMT by upregulating the levels of *Zeb2*, which is dependent on the PI3K/Akt signaling pathway in GC cells. Positive expression of CD146 is strongly associated with loss of E-cadherin and acquisition of the mesenchymal markers nuclear β -catenin and vimentin, suggesting that CD146 might promote EMT.^[102]

GC is one of the typical oxidative stress-related malignancies.^[103] Hypoxia is also a significant GC EMT inducer. Under hypoxia, E-cadherin decreases and N-cadherin, vimentin, Snail, Sox2, Oct4, and Bmi1 increase, indicating that the hypoxic microenvironment induces EMT, accompanied by cytoskeleton remodeling.^[104] Recent evidence indicates that EMT is a key GC progression driver, and plays a fundamental role during early steps of GC invasion, metastasis, and relapse. EMT-induced cell migration and reach their metastatic niche through both the lymphatic system and blood.^[105] Histone demethylase Jumonji domain containing protein 2B (JMJD2B) promotes EMT and GC invasion and metastasis through β -catenin-induced H3K9 demethylation, which causes vimentin upregulation, implicating JMJD2B as a potential target for reversing EMT and intervening GC progression.^[106]

LOH (Loss of Heterozygosity)

This describes a genetic phenomenon often seen with tumor suppressor genes in cancer. Since the human karyotype is diploid, mutation of one allele of a tumor suppressor gene is not sufficient to cause cancer. In heterozygous individuals, the wildtype allele will provide for a functional phenotype. However, when a “second hit” occurs, for example, through mis-segregation of chromosomes, this individual (or cell) may lose its “heterozygosity,” leading to a full cancerous phenotype. Recently, Karaman *et al.*^[107] found significant correlation between prevalence of 17p (*TP53*) LOH in gastric precancerous lesions, indicating that loss of *TP53* could be an early event in gastric carcinogenesis.^[107]

Research in recent years demonstrated that although *PTEN* mutations in GC are rare, LOH is more frequent. Byun *et al.* (2003) found decreased expression of *PTEN* and upto 47% LOH in 33% (5/15) GC cell lines and 36% (22/55) GC tissue samples.^[108] LOH rate was significantly higher in advanced than in early GC (63–18%); it was also significantly higher in poorly differentiated than highly and moderately differentiated GC (69–29%). This suggests that complete functional inactivation of *PTEN* is not necessary to cause gastric carcinogenesis, loss of one allele is sufficient.^[108] LOH occurs in different stages of GC, genetic mutations could be detected only in advanced GC. Both gene mutations and LOH can promote tumor invasion and metastasis. Some studies have shown that the LOH status of *PTEN* can impact sensitivity to chemotherapy and hence, the prognosis in patients with GC. It may also be used as an independent prognostic tool. There are several putative TSGs, the hypermethylated in cancer 1 (*HIC1*) gene and *TOB1* gene. The *HIC1* gene is an interesting candidate TSG located in the overlapping LOH subregion of LOH in chromosome 17p13.3. It encodes a zinc-finger transcription regulator that contains an NH₂-terminal BTB-POZ domain characteristic of a family of repressors. The gene is ubiquitously expressed in normal tissues, but a decrease or loss of expression was found in several types of tumors excluding GC.^[109] Indeed, there is accumulating evidence indicating that a change in *HIC1* expression through epigenetic mechanisms has an important role in tumor progression. The patterns of monoallelic methylation associated with a partial loss of *HIC1* expression and biallelic methylation, together with a marked loss in *HIC1* expression, were seen in these GCs. The concomitant loss or decreased expression and promoter methylation of *HIC1* indicated that *HIC1* may be silenced by aberrant DNA methylation, and could be a target for inactivation by epigenetic events in gastric tumorigenesis.^[110] Another notable candidate gene in the overlapping LOH subregion in chromosome 17q21.33 (SR₃) is *TOB1* (transducer of ERBB-2, 1). *TOB1* is a member of the *TOB/BTG* antiproliferation protein family. Overexpression of the *TOB* family proteins

results in cell growth retardation,^[109] while *TOB* LOH and decreased *TOB* expression were observed in lung cancer tissues.^[110]

Genetic analyses of LOH helped to identify the chromosomal location of many tumor suppressor genes. LOH analyses have identified several arms and regions of chromosomes that contain or potentially harbor tumor suppressor genes important in gastric tumorigenesis. Chromosome 3p was the most frequently lost locus in LOH analysis of over 100 archived stomach cancers.^[111] Moreover, three distinct regions of chromosome 4q were found to be frequently lost in gastroesophageal junctional adenocarcinomas.^[111] LOH is also a marker of chromosomal instability and might indicate a second inactivation hit of cancer suppressor genes. Several LOH studies demonstrated that the extent of chromosomal loss appeared to be of prognostic significance. LOH has been shown to relate to cancer progression, where a transition from LOH-L to LOH-H is thought to reflect an increase in chromosomal instability during tumor advancement.

The highest LOH frequencies have been identified at 1p, 2q, 3p, 4p, 5q, 6p, 7p, 7q, 8p, 9p, 11q, 12q, 13q, 14q, 17p, 18q, 21q, and 22q chromosome regions in gastric carcinomas.^[111] The main consequence of LOH is loss of genes, such as tumor suppressors, cell cycle regulators, DNA repair genes, and other genes implicated in the maintenance of cell cycle, and/or integrity of DNA. LOH studies have revealed that allelic imbalance of chromosome 17 loci occurs frequently in sporadic GC. Most of the LOH studies performed on chromosome 17 to date have used only a limited number of markers, and a detailed deletion map of overlapping regions on chromosome 17 has not yet been made in sporadic GC.

Microsatellite instability

Another type of genomic instability, commonly recognized in GC, is MSI. MSI is characteristic for hereditary type of GC, developed in the context of a smaller subset of sporadic cancers ranging from 25% to 50%.^[112] Patients with MSI phenotype exhibit a high frequency of replication errors resulting in insertions/deletions of nucleotides within microsatellite repeats in tumor tissues.^[112] In GCs, MSI is mostly caused by the epigenetic alterations in the mismatch repair (MMR) genes.^[113,114] Consequently, the impaired MMR system fails to fulfill its task, resulting in multiple mutations within cell growth regulating genes (*TGF-β RII*, *IGF1R*, *RIZ*, *TCF4*, and *DP2*), apoptosis genes (*BAX*, *BCL10*, *FAS*, *CASPASE5*, and *APAF1*), and DNA repair genes (*hMSH6*, *hMSH3*, *MED1*, *RAD50*, *BLM*, *ATR*, and *MRE11*). The high incidence of MSI in GCs (MSI-H GC) is more likely to occur at an antral location, in the intestinal type, in the expanding type, and with *H. pylori* seropositivity, and correlates with a lower prevalence of lymph-node metastases.^[115-117] These

errors are detected and repaired by a complex of MMR proteins. Inactivation or deficiency of one or more MMR genes, particularly *MLH1* or *MSH2*, induces development of MSI phenotype, which often leads to additional genetic changes, namely, inactivation of tumor suppressor genes and LOH.^[118,119]

Impairment of MMR can occur (1) by mutational inactivation of one or two MMR genes or (2) by epigenetic inactivation of MMR genes (CIMP). Genomes of GCs exhibiting MSI are characterized by the presence of multiple frameshift mutations in many genes at variable frequencies.^[120] Genes that were frequently found to be altered as a consequence of impaired MMR are implicated in cell cycle regulation and apoptosis (*TGF-β RII*, *IGFIIR*, *TCF4*, *RIZ*, *BAX*, *CASPASE5*, *FAS*, *BCL10*, and *APAF1*) or are involved in genomic integrity maintenance (*MSH6*, *MSH3*, *MED1*, *RAD50*, *BLM*, *ATR*, and *MRE11*).^[119,121] The alterations in these genes further promote genetic instability and enhance the development of malignant phenotype. MSI seems to be a promising tool to identify patients with genetic instability and patients with precancerous lesions because it occurs in both gastric adenoma and intestinal metaplasia.^[112]

Epigenetic changes

Broadly, epigenetics refer to alteration in gene expression which is not regulated by the changes in the DNA sequences. DNA methylation and histone modifications are commonly studied epigenetic events. In the present era, the term epigenetics has broadened to include heritable and transient/reversible changes in gene expression that is not accompanied by a change in the DNA sequence. Comprehensive understanding of different biological activity such as DNA methylation, chromatin structure, transcriptional activities, and histone modification has contributed in the development of epigenetics. Two major epigenetic modifications include DNA methylation and chromatin remodeling. While DNA methylation is a chemical change in the DNA sequence that most commonly occurs at cytosine moiety of CpG dinucleotides, chromatin remodeling occurs through histone modifications (primarily on the N-terminal tails) that ultimately affect the interaction of DNA with chromatin modifying protein. Both DNA methylation and histone modifications are associated with silencing critical TSGs and activating oncogenes involved in cancer.^[122-124]

HYPERMETHYLATION

DNA methylation is a reversible chemical modification of the cytosine in the CpG islands of promoter sequences, catalyzed by a family of DNA methyltransferases. DNA

methylation does not change the genetic information but it just alters the readability of the DNA and results in the inactivation of gene by subsequent transcript repression.^[125] In general, CpG island methylation is associated with gene silencing. The methylated CpG Island also recruits histone deacetylases (HDAC) and other factors involved in transcriptional silencing.^[122] Inactivation of TSGs through hypermethylation of CpG islands within promoter regions is a major event in carcinogenesis.^[122] It includes various genes involved in different cellular process such as cell cycle regulation (*p16^{INK4a}*, *CDKN2b/p15^{INK4b}*, and *p14ARF*) which is hypermethylated in human cell lines and primary tumors, DNA repair genes (*hMLH 1* and *MGMT*) were observed aberrantly methylated at their promoter regions, cell-cell/cell matrix adhesion genes (*E-cadherin*, *H-cadherin* and adenomatous polyposis coli [*APC*]), apoptosis (death-associated protein kinase [*DAPK*], *TMS1*, and *Caspase-8*), and angiogenesis (*THBS-1* and *p73*).^[126] Silencing of *p16^{INK4a}* by promoter hypermethylation has also been reported in gastric carcinoma. *CDKN2A* hypermethylation may contribute to the malignant transformation of gastric precursor lesions. Hypermethylation of *DAPK* was observed in intestinal, diffuse, and mixed type of GC and correlated with the presence of LN metastasis, advanced stage, and poor survival.^[127] The epigenetic silencing of *XAF1* gene by aberrant promoter methylation is reported in GC.^[128] *Caspase-1* (*IL-1 beta*-converting enzyme), a member of the cysteine protease family, shows loss of expression in 19.3% cases of gastric carcinoma^[129] and the expression is reversed on 5-aza 2'deoxyctidine and/or trichostatin treatments in GC cell line. The expression of *TSPYL5* mRNA was frequently downregulated due to hypermethylation of its CpG Island.^[129] Recently, Sepulveda *et al.* (2016)^[130] identified 13 genes (*BRINP1*, *CDH11*, *CHFR*, *EPHA5*, *EPHA7*, *FGF2*, *FLI1*, *GALR1*, *HS3ST2*, *PDGFRA*, *SEZ6L*, *SGCE*, and *SNRPN*) which are hypermethylated in gastric carcinoma as compared with normal mucosa, which in turn may be useful in developing diagnostic and prognostic tool for this lethal malignancy.

Hypomethylation of specific genes also contribute to gastric carcinogenesis. Global hypomethylation of the genome was initially thought to be an exclusive event in cancer development.^[131] The loss of methylation in cancer is mainly due to hypomethylation of repetitive DNA sequences. During the development of neoplasm, the degree of hypomethylation of genomic DNA increases as the lesion progress from a benign disease to metastatic.^[122,132] Three mechanisms of DNA hypomethylation have been proposed in the development of cancers. First, it increases the genomic instability, second by reactivation of transposable elements, and third by loss of imprinting. Demethylation of DNA can favour mitotic recombination, leading to deletions, translocations, and chromosomal instability.^[122] Demethylation of *MAGE*,

synuclein- γ (SNCG), and cyclin D2 has been described in gastric carcinoma.^[133] SNCG demethylation is common in cases with LN metastasis.^[130] Hypomethylation of cyclin D2 promoter was found in 71% cases of gastric carcinoma. This event is more common in Stage-III and IV tumor than in Stage-I and II tumor.^[134]

In parallel to global hypomethylation, hypermethylation of CpG Island also has silencing effect on miRNAs. MicroRNAs are short, 18–22 nucleotide, noncoding RNAs that regulate many cellular functions including cell proliferation, apoptosis, and differentiation by silencing specific target genes through translational repression or mRNA degradation.^[127,126]

HISTONE MODIFICATION

In normal cell, a precise balance maintains nucleosomal DNA in either an active/acetylated or an inactive/deacetylated form. This adequate balance is controlled by acetylating enzymes (histone acetyltransferases) and deacetylating enzymes (HDACs). The other modification includes methylation of arginine and lysine residues of histones. This methylation is catalyzed by histone methyltransferase and the process is involved in the regulation of a wide range of gene activities and chromatin structures. In general, lysine methylation at H3K9, H3K27, and H4K20 is associated with gene silencing whereas methylation at H3K4, H3K36, and H3K79 is associated with gene activation.^[124]

Increasing evidence suggests that epigenetic changes play a key role in cancer development including GC. CpG island methylation phenotype as high CIMP (CIMP-H) play an important role in gastric carcinoma progression.

HEREDITARY GC

While the great majority of GCs is sporadic, familial aggregation occurs in about 10% of the cases and out of these only 1–3% clearly constitutes hereditary form. Hereditary GC includes syndromes such as hereditary diffuse GC, gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS), and familial intestinal GC (FIGC). GC has also been identified as part of other hereditary cancer syndromes such as hereditary non-polyposis colorectal cancer, Li-Fraumeni syndrome, familial adenomatous polyposis, and Peutz-jeghers syndrome.^[135]

HDGC is one of the best genetically characterized forms of hereditary GC. Heterozygous germline *CDH1* (E-cadherin) mutations, including frameshifts, splice site, nonsense, and missense mutations as well as large rearrangements, were until recently the only unknown causative alterations of HDGC representing upto 40% of patients belonging to families that fulfill the clinical criteria for HDGC.^[136] Germline variants of genes also cause GCs.^[137] Hereditary

diffuse GC is a well-known familial GC which is linked to *CDH1* (E-cadherin) gene variants.^[137] The physical position of the germline mutation of *CDH1* gene vary according to ethnic and geographical differences.^[137] There are 100 variants of the CTNNA1 (α -catenin), a *CDH1* binding protein also cause HDGC.^[138]

HDGC is an autosomal dominant cancer susceptibility syndrome characterized by DGC which is principally caused by inactivating germline mutations in *CDH1* gene.^[139] *CDH1* gene encodes E-cadherin has major function in cell-cell adhesion, maintenance of epithelial architecture, cell polarity, and regulation of intracellular signaling pathways. The pathogenic mutation in *CDH1* shows 70% risk of developing DGC by age of 80 years by birth.^[140] Histopathology of advanced HDGC is comparable to sporadic DGC, though the presence of typical precursor lesions, *in situ* or pagetoid signet-ring cells, is specific for *CDH1*- mutation related HDGC. Early HDGC shows indolent (showing no real interest/efforts) phenotype, while advanced HDGC display an aggressive phenotype with a mixture of pleomorphic cells, increased proliferation and aberrant P53 expression.^[141] There are 10–40% of families who fulfill current genetic testing criteria have *CDH1* germline mutations.^[144] Although it is not known exactly how many HDGC families with germline *CDH1* mutations have been diagnosed worldwide to date, over 155 different *CDH1* germline mutations have been reported in HDGC families.^[142] These mutations span the length of *CDH1* and no major hotspots have been identified, although some including c.1137G>A and c.792C>T have been observed in several unrelated families.^[140,142]

CDH1 mutation positive patients who postponed a prophylactic gastrectomy, but underwent regular surveillance endoscopy, had a ten-fold increased risk of DGC compared with individuals at risk of HDGC without a mutation.^[143] *CDH1* negative families, fulfilling HDGC criteria, often present monoallelic expression of *CDH1* in the germline, which mimics functionally the presence of a *CDH1* truncating mutation.^[145] The underlying genetic mechanism causing this expression imbalance is still elusive, this finding hints toward *CDH1* involvement in a larger fraction of HDGC families. The complete inactivation of the *CDH1* gene required for tumor initiation occurs mainly through promoter methylation in primary cancer and loss-of-heterozygosity in lymph node metastases.^[145,146] The ongoing search for novel HDGC predisposing germline aberrations identifies new variants in candidate genes using multiplexed panel and whole exome sequencing.^[140,147-149] Germline CTNNA1 mutations have been found in families with HDGC.^[149,150] CTNNA1, encoding α -catenin, is involved in cell adhesion, and forms a complex with β -catenin to bind the cytoplasmic domain of E-cadherin to the cytoskeleton.^[150] New hereditary GC genes are the most promising genes

which seems to be the DNA double-strand break repair genes PALB2, BRCA2, RAD51C, and ATM,^[140,147-151] which earlier reported in breast cancer families. PALB2 and RAD51C are critical in homologous recombination which is a major DNA repair pathway. While designing gene panels to identify HDGC, these new candidate genes can be considered to broaden the understanding of genetic causes of this disease.

Syndromes with fundic gland polyps and increased GC risk include GAPPs, an autosomal-dominant heritable form of GC, which is caused by point mutations in the APC gene promoter 1B.^[152,153] Affected patients shows specific clinicopathologic phenotype of fundic gland with about 10–100s of polyps involving oxyntic mucosa, occasional hyperplastic, and adenomatous polyps, with an increased risk for intestinal-type (WHO tubular type) or mixed-type gastric adenocarcinoma.^[152] The gastric antrum and pylorus are typically spared. In this syndrome other histological features involves hyperproliferative aberrant pits, characterized by disorganized proliferations of oxyntic glands high up in the mucosa with an increased proliferative activity.^[154] The dysplasia in GAPPs is of gastric foveolar phenotype with positive staining for MUC5AC and MUC6.^[154]

FIGC intestinal-type gastric carcinoma patients have also shown familiar clustering. The diagnosis in young patients is strongly associated with a family history of GC in first degree relatives.^[155] Familial aggregation of *H. pylori* infection seems to be related to incidence of intestinal-type GC.^[155] The diagnosis should be considered when there is a history of intestinal-type GC in families without polyposis. A single family with clustering of intestinal-type GC and heterozygous, mutations in the immunity gene IL12RB1 has been reported, but further research is required to determine whether such mutations increase GC risk.^[156]

FIGC follows an autosomal dominant inheritance pattern^[158] and diagnostic criteria^[146] are stratified on the basis of the population GC incidence. In low-incidence countries, diagnosis requires that a patient has at least two first-degree or second-degree relatives with intestinal GC, one by 50 years of age, or has three or more first-degree or second-degree relatives diagnosed with GC at any age.^[158,159] In high-incidence countries, diagnosis requires three criteria: (1) At least three relatives with intestinal GCs with one of them being a first-degree relative of the other two, (2) another criteria are occurrence of GC in at least two generations, and (3) and diagnosis of GC before 50 years of age in at least one patient.^[95,159]

CONCLUSION

GC is a collection of different molecular entities and its landscape is enormously complex, but recent efforts and knowledge

enabled for the development of more modern and solid therapeutic approaches. Several important advances have been achieved in GC genetics. The improvement in our understanding of the molecular genetics of GC has greatly hastened over the last decades, enabling us to redefine the disease at the molecular level. These findings may lead to the identification of high-risk groups that can be targeted for early interventions which may improve our understanding of tumorigenesis and will ultimately lead to improved outcomes for this common malignancy. Molecular classifications, especially TGCA and ACRG, opened the doors wide on the complete comprehension of the complex genetic landscape of GC. Recent genomic and epigenomic profiling studies provides an improved molecular understanding of GC. In the above article, the characterization and classification of GC at molecular and genetic level would further support that this disease is highly heterogeneous. Researchers and clinicians should take advantage of the information provided by these studies to both design and test potential screening markers and new targeted therapeutic approaches.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Conflicts of interest

There are no conflicts of interest.

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