Chapter 4
Prognostic Molecular Markers for Gastrointestinal Cancer

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Abstract Cancer is considered as the most dreadful diseases worldwide. The rate of mortality is increasing every year globally. Among the various cancers, gastric cancer is the fifth most common cancer-causing after the various other cancers like lung, breast, prostate, and even the abdomen. This cancer is the third most cause of cancer death. Various environmental factors like smoking, the role of diet, and some bacterial infections result in gastric cancer. The gastric cancer is unrecognizable at its early stages and is diagnosed only at the advanced stages where the risk of saving a person is unimaginable. There are certain genes in which codes for gastric cancer are mutated which leads to gastric cancer. Identifying correct genes through the biomarkers will help in eradicating the disease at its early stage. Transforming this information from patient care to diagnostic tools remains a challenge for many researchers. Researchers are currently working to translate molecular information into the development of drugs. Before identifying the correct drug, researchers need to focus on identifying the genes which cause gastric cancer and even the pathways associated with detecting cancer at an early stage. Current generation researchers are working on next-generation sequencing which has led to molecular classification systems that are used in designing new targeted therapies and are implemented in clinical trials. This chapter will focus on the latest applications/techniques required in identifying various molecular markers for gastric cancer and even certain metabolic pathways/signalling pathways will be identified/reviewed to identify the correct diagnosis for gastric cancer. This chapter will even focus on the biomarker-targeted therapies that are involved in the treatment of gastric cancer.

Keywords Gastric cancer · Diagnostic markers · Signalling pathways · Enzymes · Pathology · Disease

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4.1 Introduction

Gastric cancer is one of the fatal diseases in the world; it is the second largest cancer in cancer deaths (Jemal et al. 2011). According to WHO, it is reported as 24,590 are affected; nearly 10,720 GC deaths are diagnosed in the USA. Gastric cancer is a nonspecific symptomatic disease; it provides a potential platform to transform and attain oncogenicity (Wagner et al. 2010). The normal symptoms are like stomach ache, anorexia, weight loss, and difficulty in ingestion. The major causes of gastric cancer are diet, *Helicobacter pylori* infection, atrophic gastritis, and intestinal metaplasia. Four somatic modifications in gastric cancer are observed like EBV, microsatellite instability (MSI), genomic stability, and chromosomal instability (CIS). The occurrence of GC was categorized into intestinal and diffuse types by Lauren classification (Wagner et al. 2010). The intestinal GC is glandular with variable differentiation and usually observed in old patients which are formed as a result of causative effects in GC. The diffuse gastric carcinoma, usually motile neoplasm, is infiltrated to different locations of gastric walls. It was observed in young patients.

Diagnosis plays an important role in disease progression and prevention. The conventional methods are used to diagnose GC by laparoscopy and gastroscopy. The reoccurrence of GC was diagnosed by CT scan, echoendoscope (Mihaljevic et al. 2013). The major drawback in diagnosing GC cancer was identifying the stages and reoccurrence of GC. The interventions are measured by various biomarkers and quantified through the pharmacological or normal biological responses. There are other biomarkers like DNA, exosomes, noncoding RNAs, etc. As a result, proteins and genes are exploited to diagnose GC. The biomarkers are characterized into four types such as diagnostic, prognostic, predictive, and therapeutic biomarkers (Matsuoka and Yashiro 2018).

4.2 Conventional Prognostic Markers

The conventional prognostic or biomarkers are identified and characterized based on the surface proteins and genes. There are different cell types that favor the disease progression and pathogenesis (Lin et al. 2012). As a result, they are identified as potential prognostic markers. Likewise, metastatic genes-signalling mediators, immune checkpoint, microsatellite instability.

4.3 Unconventional (Noninvasive Prognostic Markers)

The noninvasive prognostic markers are widely identified by the body fluids like blood, urine, and other body fluids. These are characterized by the site of origin rather than its biological significance.

The major markers are characterized like CTCs, circulating cell-free DNA, miRNA, long-noncoding RNA, and exosomes. These are used as liquid biopsy and help in the identification of stages and quantify the gastric cancer (Siravegna et al. 2017).

4.3.1 Metastatic Genic Prognostic Marker

The metastatic genes, which initiates the transformation of oncogenes with various RTKs and signalling mediators. Thus, the mediators are acting as biomarkers for predictive, prognostic, and diagnostic markers.

4.3.1.1 HER2

HER2 is one of the RTKs, the overexpression potentiates the transformation of oncogene by activating signalling cascade. It is the first biomarker found in GC with poor prognosis and the study highlights the HER amplification is found in patients, who are ranging from 6 to 23%. The HER2 located in the gastroesophageal junction compared to the distal end. The HER2 overexpression is a result of mutations in the erB2 gene that leads to early-stage carcinogenesis.

The role of a biomarker is a bit controversial, in spite of that it has poor prognostic value, it is measured by a chemotherapeutic drug called lapatinib and trastuzumab. The drugs inhibit the HER2 and as a result, progression-free survival was enhanced. The HER2 overexpression was inhabited by various other drugs, and hence, the HER2 acts as a target to the drugs and inhibits the overexpression and inhibits the carcinogenesis (Gomez-Martín et al. 2014). As a result, it can act as a potential prognostic biomarker.

4.3.1.2 MET

MET, is one the receptor tyrosine kinases identified as hepatic growth factor (HGF), it activates various signalling cascade. As a result, it leads to cell proliferation and cell growth. The overexpression of MET leads to over proliferation, angiogenesis, and migration; hence, it is responsible for the poor prognosis of GC (Matsumoto et al. 2017). The MET is characterized as a prognostic and predictive marker for GC by activating signalling cascade likewise, HGF/c-Met signalling cascade. The high

serum HGF can be a possible prognostic marker, where the low levels of HGF are treated with chemotherapeutic drug, i.e., trastuzumab with positive outcomes. These results can highlight the significance of MET as a potential prognostic marker.

4.3.1.3 VEGF

Vascular endothelial growth factor is one of the growth factor responsible for angiogenesis. The neovascularization provides a platform for the formation of new blood vessels for the tissues to attain a normal physiological state. In GC, the VEGF promotes tumor proliferation, survival, and migration by the various signalling cascades. The VEGF has different isoforms, in the recent study highlights the VEGF-2 has potential prognostic value in a ramucirumab treatment. The VEGF-D also can be a promising prognostic marker in the ramucirumab-treated patients (Matsuoka and Yashiro 2018).

4.3.2 MSI

The short repeated nucleotide sequences around 1–6 which are located in the noncoding and protein-coding sequences regulate the expression by addition or deletion of repeating units. As a result, it leads to genomic instability and tumorigenesis. The Gastrointestinal Cancer incidence is estimated by the high and low MSI; the low MSI was characterized by less than 30%; and the high MSI was characterized by more than 30% (Pinto et al. 2000). In Gastrointestinal Cancer, the epigenetic silencing of MLH1 by hypermethylating its promoter. The MSI regulates silencing and activating various expressions of targets which mediates the GC carcinogenesis. The mutations in PIK3CA are observed in MSI-positive GC, which highlights the genomic instability and regulates the targets and their expression. This can be a prognostic biomarker due the differential expressions in the high and low MSI conditions and the extent of chemotherapy treatments and can obtain better clinical outcomes (Smyth et al. 2017).

4.3.3 Genetic Polymorphism

The genetic polymorphism in the carcinogenesis plays an important role in GC; the genetic polymorphism is mainly characterized as SNPs. In GC, the functional similarity between IL-beta and IL-RN in the *Helicobacter pylori* infection induces the progression of chronic gastritis and GC in the Algerian population. CD44, a glycoprotein highly expressed in the GC, has different isoforms involved in GC (Suenaga et al. 2015). In GC, CD44 SNP rs187116 assumed to have high expression

and it can be a prognostic biomarker. Apart from above, other SNPs like TP53, CDH1, and ARID1A are putative targets for prognostic biomarkers as SNPs.

4.3.4 Long-Noncoding RNA

The noncoding RNA containing more than 200 nucleotides are termed as long-noncoding RNA. lncRNA has diverse functions and it regulates transcription, splicing, chromatin remodelling, and post-translational modification. It acts as an oncogene and tumor suppressor. 135 lncRNA are found in dysregulated GC (Fang et al. 2015). As a result, it leads to tumorigenesis, metastasis, and prognosis. The minimal expression of lncRNA like AI364715, GACAT1, and GACAT2 in GC acts as a prognostic biomarker.

4.3.5 Immune Checkpoint

The immune cell inhibition plays a key role in tumor progression and also in GC. The immune activation is attained by PD-1 and PD-2 molecules on the T and B cell surfaces (Sharpe et al. 2007). But in the cancer progression, the T cell and B cell activation are inhibited by PD-L1 and PD-L2. As a result, the activation of cytotoxic T cells are inhibited and immune resistance towards tumor facilitates the tumor survival and progression (Gu et al. 2017). In GC, the inhibition of immune activation in the mucosa of the gastrointestinal tract has poor disease prognosis. PD-L1 is expressed in more than 40% in EB positive condition. According to the study, the PD-L1 expression is high in MSI high condition. The patients treated with the PD-1 inhibitor pembrolizumab have a better survival rate with untreated patients. Therefore, PD-1 can be a potential prognostic biomarker for GC.

4.4 Noninvasive Biomarkers

The differentiation of solid tumors from the patient sample is very tough and determining the stage of the cancer is challenging. To overcome the limitation, researchers found liquid biopsy to characterize and identify the tumor concerning stages and progression. For the liquid biopsy, blood and other body fluids are used.

4.5 CTCs

Circulating tumor cells (CTCs) are the single cells or clusters, identified in the bloodstream which is disseminated from the tumor cells. The CTC can be found in all stages of cancer, majorly in neoplasms. It has metastatic and stems like properties; facilitate the tumor metastasis and tumor renewal respectively. In Gastrointestinal Cancer, the CTCs assumed to have CD44 and other EMT markers which can be evident to have stemness and metastatic conditions.

4.6 Circulating Cell-Free DNA (cfDNA)

The blood is a major carrier of different kinds of cells from normal and cancer cells. The cfDNA is characterized as cell-free extracellular DNA. It was released from neoplasms, primary tumors, and metastatic tumors. The main advantages are specificity, limited sample volume. In GC, the cfDNA originated from the methylated promoter regions of cells and identified by the PCR technique. Likewise, a specific region APC1 in serum and RASSF1A promoter methylations are usual epigenetic modifications of cfDNA. Surprisingly, the study finds the presence of EBV DNA in cfDNA of GC. This infers the potentiality of cfDNA in the GC diagnosis and identification with high specificity.

4.7 miRNA

The short noncoding RNA consists of 18–30 nucleotides in length which bind to 3′UTR of the target sequence and regulates its translation. The miRNA is key molecule that regulates tumor activation and tumor suppression. It affects cell proliferation, cell differentiation, and cell migration. Additionally, the miRNA possesses oncogene activity with the following prognostic markers viz. miR-21, miR-23a, miR-27a, miR-106b-25, miR-199a, miR-215, miR-222-221, and miR-370 respectively. However, the miRNA possessing the tumor-suppressive activity are listed as follows; miR-29a, miR-101, miR-125a, miR-129, miR-148b, miR-181c, miR-212, miR-218, miR-335, miR-375, miR-449, miR-486, miR-512. Therefore, miRNA can be considered as a potential prognostic marker, which has different subsets and localized in blood and plasma.

In a recent study, cfmiRNA was discovered which enhances the functions of the miRNA and can be a potential prognostic marker. The cfmiRNA is derived from the tumor and secreted into the blood and circulated into body fluids. The miRNA expression profiling is examined and several miRNA are found and characterized as important prognostic biomarkers. The miRNA like miR-20b, miR-125a, miR-137, miR-141, miR-146a, miR-196a, miR-206, miR-218, miR-486-5p, and

miR-506. The serum samples are analyzed by RT-PCR and NGS, which can be an ideal diagnostic marker.

4.8 Conclusions and Future Perspectives

The prognostic, diagnostic, predictive biomarkers are very essential for the identification of cancer stages and cancer progression. The prognostic markers are key for diagnosing GC in the early stages. There are different prognostic markers like conventional and nonconventional which are hugely differed based on the detections of tumor markers. The conventional prognostic markers are mainly detected by the tissue sample; unconventional prognostic markers are detected by the blood, plasma, and urine. There are few conventional markers which are potent markers like HER2, MET, VEGF-2, PD-1/2, MSI, and SNP which have a poor prognosis and high prognostic significance. However, employing miRNA, cell-free miRNAs, cell-free DNAs for the early detection of GI cancers can play important role as noninvasive prognostic markers apropos of thier specificity. As a result, the reduction in sample size makes it more prominent and unique. The stages of cancer care in the conventional biomarkers are quantified by the proteins and inhibitors like chemotherapeutic drugs. Conversely among the noninvasive biomarkers, the use cell-free DNA, and cell-free RNA, miRNA are specifically used and quantified for a specific purpose. Paradoxically, the HER2 is the early prognostic marker that does not have any clear evidence yet. The immune checkpoint inhibitors are the important prognostic markers, possessing a diversity of subsets that might play an imperative role in GI cancer prognosis, as depicted in the Table 4.1. The evolution of prognostic markers from the conventional to nonconventional is remarkable, and this highlights the potential use of the nonconventional prognostic markers.

Table 4.1 The significance of miRNA in the detection

	Types	Clinical significance	Detection	References
miRNA	miR-21, miR-23a, miR-106b-25, miR-130b, miR-199a, miR-215, miR-222-221, miR-370, miR-29a, miR-101, miR-125a, miR-129, miR-148b, miR-181c, miR-212, miR218, miR-335,miR-375,miR-449, miR-486, miR-512	Diagnostic/ prognostic	Blood/ plasma	Wu et al. (2014); Zhu et al. (2014)
cfmiRNA	• miR331, miR21	Diagnostic/ prognostic	Blood	Sierzega et al. (2017)
	• miR-20b, 125a,137, 141,146a, 196a, 206,218, 486-5p	Prognostic	Blood/ plasma	Zhang et al. (2017)
	• miR10b-5p, 132-3p,185-5p, 20a-3p,296-5p	Prognostic	Plasma	Huang et al. (2017)

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Conflict of Interest The authors declare that there is no potential conflict of interest.

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