Advances in Biochemistry & Applications in Medicine

Chapter 4

# Recent Updates on Biomarkers of Gastric Cancer

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## Abstract

Gastric cancer (GC) or stomach cancer, is one of the most common malignancies worldwide. It ranks second in terms of global cancer-related mortality and fourth in terms of incidence among various ethnic groups. The etiology of GC is multifactorial and includes dietary as well as non-dietary factors. Despite many efforts, GC remains to be the condition without clear symptoms at the onset, poor prognosis and high recurrence. Thus, there is an urgent need to find efficient assays to identify gastric cancer biomarkers for treatment of the disease. Here, we review the most effective biomarkers for gastric cancer with a potential for the early detection and treatment of GC.

**Keywords:** Cancer; GC biomarkers; Treatment; Biomarker assays

## 1. Introduction

GC is the fourth most common cancer and the second leading cause of cancer related deaths, with nearly one million newly diagnosed cases every year [1]. According to WHO, around 7.6 million people worldwide die of cancer annually, and it is estimated that by 2030, the deaths from cancer will rise to over 11 million [1,2]. The number of deaths due to GC amount to 3.4 per 100,000 per year [3]. GC is an aggressive malignancy that is difficult to be detected at an early stage. It is a complex, multi step process involving several genetic and epigenetic alterations that lead to aberrant expression of specific genes. GC is defined as the

malignancy of the gastric mucosa epithelium with glandular differentiation. About half of the GCs are located in the lower stomach. GC is caused by altered regulation of oncogenes, tumor suppressor genes, DNA repair genes, cell cycle regulators, and signaling molecules [4]. It occurs when cells in the lining of the stomach grow uncontrollably and form tumors that can invade normal tissues and spread to other parts of the body [6]. Anticancer research has led to the development of many novel target molecules and techniques to diagnose and treat cancers [8-11]. As GC is often without the clear symptoms, early diagnosis of GC requires novel biomarkers and techniques with high sensitivity and specificity.

GC can be classified into intestinal and diffuse types based on epidemiological and histo-pathological features [14,15]. Adenocarcinoma is the major histological type of GC, accounting for upto 95% of all gastric malignancies [16]. Tumor node metastasis (TNM) staging system is used worldwide for prognosis and direct therapeutic decisions for patients with GC [17]. Accumulating evidence has implicated the role of *H. pylori* infection in the pathogenesis of GC by causing a chronic gastritis, the precursor to all the pathophysiologic abnormalities characteristic of gastric carcinogenesis [19,20]. Intestinal type GC is associated with *H. pylori* infection, obesity, and certain dietary factors, such as high intake of salt, smoked meats, and food preserved with nitrites or nitrates, smoking and alcohol [18]. During last few decades the studies of GC showed that it results from the complex gene-environment interactions [21,22]. New high-throughput techniques have revealed its association with alterations of many genes, deregulation of signaling pathways, aberrant DNA methylation patterns, and chromosomal imbalances [23]. Despite advances in diagnosis and treatment, the five year survival rate for GC is poor, with only 20% of the patients surviving [18,24].

## 2. GC Biomarkers

GC is a biologically complex disease arises evolves due to various genetic and epigenetic alterations. Therefore, it is essential to understand the molecular variables that affect GC in order to develop clinical strategies for its treatment. A great deal of effort has been made in the search of tumor biomarkers, in order to improve the understanding of GC and to identify biomarkers that would improve cure by early detection and diagnosis. The biomarkers are biological variables that correlate with clinico-biological outcomes. The discovery of cancer biomarkers to devise strategies that target expressed proteins are becoming increasingly popular [25]. Overall, the molecular phenotyping of GC is still in its infancy and the search for novel diagnostic and predictive biomarkers continues [26]. Identifying more biomarkers (e.g. COX-2, c-myc, p27 or p53) will have a major impact for diagnosis and making clinical decisions. Analysis of cancer tissues revealed that miRNAs could be important molecular markers useful for cancer classification, prognosis and therapy. miRNAs also emerged as circulating markers, which may become valuable for early diagnosis and follow-up investigations [27]. miRNAs have become important biomarkers, thanks to their stability and the availability of assays to

quantify them [28,29]. Cancer cells employ multiple and diverse survival pathways and there is an increasing need to define a battery of biomarkers [30]. Such signatures might appropriately represent the breadth of molecular diversity inherent in cancers in general, and pave the way to understand the molecular genetics of gastric carcinogenesis. In the present era of personalized medicine, genomic and proteomic profiling attempts for mining novel biomarkers of GC may provide basis for individualized therapy to cancer patients.

Some of the well-known GC causing factors are the consumption of nitrate- or nitrite-rich food (grilled, salted, or pickled foods) [31], *H. pylori* infection [32], old age (>60 years), and a history of stomach disorders [33]. Proteomics based biomarkers can help in the accurate diagnosis and treatment of GC patients [34,35]. To identify potential target proteins that can serve as novel GC biomarkers, a combination of various proteomics techniques have been used, such as 2-DE, iTRAQ, ICAT, ProteinChip array, hydrophilic interaction liquid chromatography (HILIC), and 2-D LC [36]. The identity of the fractionated proteins has been revealed by MS [37]. Other validating techniques such as western blotting and immunohistochemistry (IHC), can be used to confirm these proteins [38]. Galectins are an important proteomics based GC biomarkers with significant change in expression in GC [39-46].

A number of GC biomarkers have been identified. However, not all have been equally effective. We first describe the two effective assays to treat GC and then list some important GC biomarkers.

### 2.1.1 Bevacizumab assay

Bevacizumab is a humanized monoclonal antibody against human vascular endothelial cell growth factor (VEGF). Bevacizumab is effective against GCs as VEGF is involved in the development of gastric cancers. There are, however, no established methods to predict the clinical efficacy of using bevacizumab. Although patients treated with bevacizumab in addition to chemo-therapy respond well and seem to benefit from it.

The antitumor activity of bevacizumab has been examined in the MKN-45 human gastric xenograft models and was found to show significant effect against MKN-45 tumor growth. The antitumor activity of bevacizumab against colorectal tumors has also been tested and found to be comparable to that of the gastric cancers. The efficacy of bevacizumab in gastric cancers depends on VEGF. VEGF is significantly expressed in bevacizumab-sensitive tumors compared with bevacizumab-insensitive tumors [47]. Bevacizumab also inhibits angiogenesis in gastric cancers

The microvessel density (MVD) measurements suggest that angiogenic factors other than VEGF are involved in angiogenesis in bevacizumab-insensitive tumors. However, expression levels of different angiogenic factors did not significantly correlate with bevacizumab efficacy

[47]. Human cancer xenograft models have been used to study the effects of bevacizumab in gastric cancers for which assays have been developed by inoculating human gastric cancer cells into T-cell-deficient mice [47]. Bevacizumab showed significant antitumor activity in MKN-45. However, the sensitivity of the gastric cancer models to bevacizumab was found to be unrelated to the histological type. Some studies have suggested that bevacizumab directly inhibits the growth of tumor cells [48]. However, in the gastric cancer cell lines the direct antitumor activity of bevacizumab was not observed [47][1]. Thus the levels of VEGF are closely related with the sensitivity of bevacizumab in the tested cell lines.

### 2.1.2 HUVEC pVEGFR2 assay:

Angiogenesis, in which new blood vessels are generated, plays a critical role in tumor development. VEGF triggers angiogenesis by dimerization of the receptor tyrosine kinases. In HUVEC pVEGFR2 assay, tumor samples are homogenized in HuMedia-EB2 basal media. The VEGF concentration in the samples can be measured and HUVEC seeded at a density of approx. $3 \times 10^5$ in HuMedia-EG2. The medium can then be changed to an assay medium which should have been pre-treated with bevacizumab or human IgG and the cells washed with PBS. The cells are lysed and pVEGFR2 detected by immunoblotting.

### 2.2.3 Cancerous inhibitor of protein phosphatase 2A

Cancerous inhibitor of protein phosphatase 2A (CIP2A) is an oncogenic factor that can serve as marker for gastric cancer. It stabilizes c-MYC by inhibiting its degradation and drives the neoplastic transformation [49]. Cancers are characterized by unregulated cell proliferation and differentiation [50]**.** CIP2A expression in malignant gastric tissues can be used to determine the role of CIP2A as a diagnostic marker for gastric cancer. Gastric cancer specimens could be analyzed for CIP2A expression and compared with normal WT expression. In one study, the presence of CIP2A transcripts was significantly higher in 90% of cancer samples analysed [51]. However, there does not seem to be a direct link between CIP2A expression and the stage of tumor.

Unlike other cancers, there is a dearth of novel diagnostic markers for gastric cancer. CIP2A, a potent endogenous inhibitor of PP2A is an oncoprotein required for anchorage-independent growth and transformation of human cells. CIP2A is an important oncogenic factor and may thus serve as a diagnostic marker for gastric cancer. However, a potential link between depletion of CIP2A and induction of senescence remains to be defined. It has recently been showed that the deletion of the c-MYC gene causes premature senescence of human fibroblasts [52]. Intriguingly, CIP2A depletion leads to partial differentiation of leukemic HL60 cells due to the reduced expression of c-MYC in HL60 cells [53] Thus overexpression of CIP2A seen in many tumors may interfere with cellular differentiation leading to malignant

transformation. Since CIP2A gene is highly expressed in gastric cancer tissues compared to normal tissues, it can be an important candidate for the diagnosis and treatment of gastric cancer.

### 2.2.4 *Helicobacter pylori* infection as the indicator of Gastric Cancer

*H. pylori* causes the development of gastric cancer [54]. However, the degree of infection in gastric cancer patients varies in different studies. These variations may be due to differences in the detection methods of *H. pylori* in the gastric cancer patients. Many diagnostic tests for *H. pylori* yield false results, and therefore multiple tests should be carried to provide accurate diagnosis of *H. pylori* infection [55]. In many countries endoscopy is performed for the early detection of gastric cancers. In one study, the relationship between *H. pylori* infection and the incidence of gastric cancer has been studied by endoscopy and biopsy, followed by histological and serologic testing [56]. It showed that gastric cancer developed in patients having *H. pylori* infection whereas uninfected patients did not develop gastric cancer. Other epidemiologic studies have also showed a close relation between *H. pylori* infection and occurrence of gastric cancer. Serum antibodies have also been successfully used to estimate the H. pylori infection in patients with gastric cancers. However, recent studies have demonstrated that serum antibody assay can yield false negatives, thus underestimating the degree of *H. pylori* infection in gastric cancer patients. To circumvent this problem, tissues must be taken from the greater curvature of the uppergastric body that results in fewer false negatives. Hence, H. pylori infection can act as the marker of gastric cancer and eradication of *H. pylori* could prevent the development of new gastric cancers. It has previously been shown that suppression of *H. pylori* infection inhibits the development of gastric cancer or growth of occult cancer.

### 2.2.5 Insulin-like growth factor type 1 receptor, epidermal growth factor receptor and HER2

Insulin-like growth factor type 1 receptor (IGF-IR), epidermal growth factor receptor (EGFR) and HER2 have been linked to several tumors and are considered as important biomarkers of gastric cancer. However, the clinical significance of these gastric cancer biomarkers remains to be investigated beyond doubt. Estimation of IGF-IR levels in surgical GC specimens and diffuse type tumors are significant prognosticators of GC. Thus devising strategies to target IGF-IR may prove valuable in treating GC patients [57]. As GC remains an aggressive malignancy, with an average survival of approx. 10 months in patients with metastatic GC, emphasis on the improved techniques for the diagnosis and treatment may facilitate the therapy which would result in better survival. IGF-IR is a membrane resident receptor activated by IGF-I and IGF-II. IGF-IR signalling functions in cell proliferation and malignant transformation [58]. IGF-IR–directed cancer strategies have been developed for breast cancer and other solid tumors [59]. However, the role of IGF-IR expression in GC is

poorly understood. Many studies suggest that IGF-IR can interact with EGFR to augment the malignancy of tumors [60,61]. EGFR and its homologue HER2 (also called erbB-2) encode for receptor tyrosine kinases. These receptors play a crucial role in cancer cell proliferation, differentiation, survival and angiogenesis [62]. Epidermal growth factors stimulate EGFR for the synthesis of DNA and cell growth affecting various cellular processes. The co-receptor HER2 forms dimers with EGFR and acts synergistically to promote malignant transformation [63]. The expression levels of these receptors is related to many cancers and the ensuing survival rate [64] Immunohistochemical assays to assess the prognostic relevance of these receptors have been designed to determine the expression profile of IGF-IR, EGFR, and HER2 in GC [57]. Therapeutic strategies co-targeting IGF-IR, EGFR or HER2 have resulted in enhanced antitumor activity [65] The roles of anti-EGFR and anti-HER2 monoclonal antibodies (cetuximab and trastuzumab respectively), or a dual inhibitor of EGFR and HER2 (lapatinib) have been reported [66-68]. Therefore it is important to understand the clinical significance of IGF-IR and its molecular interactions with EGFR and HER2 to devise strategies for GC. Although there is significant evidence that IGF-IR, EGFR and HER2 are important predictors of GC, their role as indicators of GC however remains contentious.

### 2.2.6 Circulating micro-RNAs

Plasma microRNAs (miRNAs) can be used to diagnose and monitor GC. Serum tumour markers, such as carcinoembryonic antigen and carbohydrate antigen 19-9 have been used in GC diagnostics [69]. However, these conventional serum markers are non-specific and lack sensitivity to detect early cancer. Recently, several studies have provided evidence that miRNAs involved in tumourigenesis can be detected stably in blood plasma of cancer patients [70-72]. Circulating miRNAs that originate from cancerous tissues and are not lysed by endogenous RNases are stable blood-based biomarkers for the detection of cancer [73]. These serum based biomarkers are interesting molecules for screening of cancers.

A large number of genetic and epigenetic changes are known to be involved in tumour progression and maintenance. Several studies have identified that plasma/serum nucleic acids levels are altered in cancer patients [74-76]. However, recently miRNAs have been identified as novel factors related to oncogenesis [77,78]. The characteristics of miRNAs as tissue-specific molecular signatures and the presence of multiple copies per cell makes miRNAs as potential biomarkers. The diagnostic potential of plasma miRNAs has been validated and the plasma concentrations of *miR-17-5p*, *miR-21*, *miR-106a* and *miR-106b* are significantly higher in GC patients [79].

Plasma miRNA assays can be potentially used to screen high risk patients and monitor disease recurrence in GC. miRNA biomarkers are also powerful tools for evaluating the efficacy of adjuvant therapies. However, further clinical studies using a variety of tumour-

specific plasma miRNAs should be carried out to identify the potential application of these biomarkers in GC diagnosis and treatment.

## 3. Conclusion

Globally, GC continues to be one of the leading causes of cancer related deaths. Despite the development of several novel and effective classes of anticancer drugs, GC remains an aggressive clinical condition, with a median survival of only a few months in patients with unresectable cancers. GC is incurable and chemotherapy can only be palliative once it metastasizes. Therefore, the identification of specific biomarkers to diagnose gastric cancers is important. Modern technologies such as genome sequencing and expression arrays have helped to identify various novel biomarkers with prognostic value. However, advanced clinical trials for the screening of novel biomarkers need to be initiated for better results. Emphasis on the need for improved techniques for identifying GC biomarkers may facilitate the targeted chemotherapy, resulting in better outcomes.

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