Biomekkers of gastric cancer: Current topics and future perspective

Tasuku Matsuoka, Masakazu Yashiro

Article in press: June 2, 2018
Published online: July 14, 2018

Abstract

Gastric cancer (GC) is one of the most prevalent malignant types in the world and an aggressive disease with a poor 5-year survival. This cancer is biologically and genetically heterogeneous with a poorly understood carcinogenesis at the molecular level. Although the incidence is declining, the outcome of patients with GC remains dismal. Thus, the detection at an early stage utilizing useful screening approaches, selection of an appropriate treatment plan, and effective monitoring is pivotal to reduce GC mortalities. Identification of biomarkers in a basis of clinical information and comprehensive genome analysis could improve diagnosis, prognosis, prediction of recurrence and treatment response. This review summarized the current status and approaches in GC biomarker, which could be potentially used for early diagnosis, accurate prediction of therapeutic approaches and discussed the future perspective based on the molecular classification and profiling.

Key words: Biomarkers; Cancer diagnosis; Prognostic marker; Predictive marker; Gastric cancer

Core tip: Gastric cancer (GC) is one of the most common leading causes of cancer death in the world. Hence, any effort in early diagnosis, choice of appropriate therapeutic strategies and efficient monitoring can have a pivotal role in reducing the disease related mortalities. Our review purpose the current trends in GC biomarker which are classified as pathologic signaling, genetic or epigenetic changes within the tumor tissue as well as non-invasive biomarkers such as blood or gastric juice based markers. These biomarkers could facilitate more individualized
treatment approaches.

Matsuoka T, Yashiro M. Biomarkers of gastric cancer: Current topics and future perspective. World J Gastroenterol 2018; 24(26): 2818-2832 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i26/2818.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i26.2818

INTRODUCTION

Gastric cancer (GC) is the fourth most common malignant disease and the second leading cause of cancer-related death worldwide[1]. Despite significant improvements in the survival of patients with GC over the past several decades, GC is often diagnosed at an advanced stage and prognoses is still unsatisfactory due to the high incidence of recurrence[2]. Since GC is mostly asymptomatic until it progresses to advanced stages, the early detection using effective screening approaches is important to impair GC mortalities[2]. Biomarkers are characteristics that are objectively measured and evaluated as an indicator of normal biologic process, pathogenic processes, or pharmacological response to a therapeutic intervention. Various biomarkers related to DNA, RNA, exosome, etc. have been found by recent advances in genome analysis. Development of these biomarkers in the field of cancer treatment is expected to greatly contribute to the progress of cancer; selection of appropriate therapeutic strategies and efficient follow-up programs.

GC is a heterogeneous disease in which each cancer patient exhibits a distinct genetic and molecular profile. Unfortunately, although a numerous studies has been conducted on molecular biomarkers, most of the identified biomarkers failed in the validation studies. Almost patients with advanced GC still cannot be treated with a targeted therapy and currently no diagnostic markers can be seen for secondary prevention. For being able to use GC associated biomarkers in clinical care of patients, comprehensive review to determine the direction for identifying the precise biomarker pinpoint that can be explored for the personalized therapy.

This review aims to classify developing topics for biomarkers in GC, while providing insights on potent candidates based on novel molecular classification that ultimately highlight molecular studies and clinical implementation. These findings should be useful for translating molecular classification and profiling of tumors into therapeutic targets and predictive biomarkers to achieve personalized treatment in the future.

LITERATURE SEARCH

PubMed was searched for English articles using the medical subject heading terms ‘gastric cancer’, and ‘biomarker’. Relevant articles from clinical trials and experimental studies since 1989 were included as well as background articles relevant to the disease processes of interest. Articles which did not include biomarker analysis of GC were excluded from this review.

BIOMARKERS OF GC APPLIED IN CLINICAL PRACTICE

Gastric tumor markers have been used for the diagnosis, the determination of the clinical stage, the evaluation of treatment responses, and the screening for recurrence after successful therapy[3]. Although many biomarkers for GC including carbohydrate antigen (CA) 72-4, alphafetoprotein, carbohydrate antigen (CA)12-5, SLE, BCA-225, hCG and pepsinogen I /II have been reported, carcinoembryonic antigen (CEA) and CA19-9 are still the most frequently used biomarkers in clinical practice for GC.

CEA

CEA is the most widely and frequently used markers in clinical practice in the digestive tract cancer. CEA is known as an independent risk factor for predictive liver metastasis relapse[3]. Increased CEA levels are found in advanced stages of GC in a proportion of all GC patients; therefore, CEA levels are not an effective method of screening. CEA levels in peritoneal lavage fluid are said to accurately predict peritoneal recurrence after a curative resection of GC[4]. The addition of immunohistochemical CEA measurement to conventional cytology resulted in increased sensitivity. Measurement of CEA mRNA using RT-PCR is useful for detecting micrometastasis in the peritoneal cavity[5].

CA19-9

CA19-9 is a glycolipid antigen that has been identified in colorectal cancer, and it is a ligand for E-selectin, which is expressed on the surface of endothelial cells[3]. CA19-9 has previously been a commonly used marker in gastrointestinal cancer; however, it is present in a number of types of cancer, in particular pancreatic and GC. CA19-9-positive GCs demonstrated distinct clinicopathological characteristics such as antral location, differentiated histology, prominent lymphatic and venous invasion, higher proportion of lymph node metastasis, and advanced stage[6]. Previous studies reported that the sensitivity for recurrence of CA19-9 was 56%, with a specificity of 74%[7]. Moreover, the combination of CA19-9 and other tumor markers provided more useful information for prediction of recurrence[8]. The sensitivity was reported to increase to 87% when CA19-9 was combined with CEA.

Other conventional biomarkers

Tumor markers, such as CA72-4, alpha-fetoprotein and CA125 have been widely used for the diagnosis of GC.

Although CA72-4 often represents the superior sensitivity and accuracy compared with CEA, there are few
studies on predictive screening or early detection for CA72-4 under the circumstances. AFP positive GC has the characteristics of high stage and easy occurrence to liver metastasis[3]. AFP producing GC in AFP-positive group also shows the aggressive proliferation and enhanced neovascularization compared with in AFP-negative group[10]. CA12-5 level has been said to be significantly associated with the occurrence of peritoneal dissemination in GC[3]. In patients who have carried out curative surgery, CA125 positivity may serve as the predictor of peritoneal dissemination[11].

**HER2**
HER2 is the first molecular biomarker available for GC patients in clinical practice. HER2, (a proto-oncogene encoded by **ERBB2** on chromosome 17) is a cell membrane surface-bound receptor tyrosine kinase and is one of the four members of the human EGFR family, including EGFR/HER1, HER2/neu, HER3, and HER4[12]. Although the significance of prognostic and predictive value of HER2 is not established in GC, the importance of HER2 as biomarker is known to be emerged. The studied HER2 amplification in patients with GC ranges from 6% to 23%[13-15]. Histological evaluation revealed the HER2 overexpression/amplification rate was predominantly seen in the intestinal-type than in diffuse-type cancers (32% vs 6%)[16-18].

Trastuzumab, a HER2-targeted agent, inhibits HER2-mediated signaling and prevents cleavage of the extracellular domain of HER2[13]. Trastuzumab is the first molecular targeted agent approved as standard treatment in GC. Trastuzumab for Gastric Cancer (ToGA) study, an open-label phase III, randomized controlled trial, showed that an addition of trastuzumab to capetabine or S-FU and cisplatin demonstrated a clinical benefit compared to chemotherapy alone in terms of tumor response and is now considered to be the standard of care for HER2-positive GC[13]. Moreover, assessment of HER2 expression in the primary gastric tumor is a reliable foundation for examining treatment with anti-HER2 agents in patients with secondary focs[17,18]. There are several other HER2-targeted agents such as pertuzumab, lapatinib and trastuzumab emtansine being investigated in randomized clinical trials in patients with HER2-positive GC[10-21]. However, no significant evidence was found yet. Several obstacles, such as determining the suitable dose of trastuzumab, identifying a predictive biomarker, exist for the advancement of HER2-targeted therapy in GC[22]. Some researches proved the usefulness of several factors for monitoring the efficacy of trastuzumab alone or combined chemotherapy, such as p27[91] and HER2-extracellular domain[23,24]. Resistance to trastuzumab is also nowadays topic in HER2 positive GCs. One of the most important mechanisms underlying trastuzumab resistance is dysregulation of phosphatidylinositol-3-kinase (PI3K)/Akt/mTOR pathway. It is well known that PIK3CA mutations and phosphate and tensin homolog (PTEN) inactivation may affect the effectiveness of HER2-targeted therapy[25]. Thus, combination therapy of trastuzumab with PI3K inhibitors may provide substantial benefit in patients with HER2-positive GC. **CCNE1** amplification, one of the most popular co-occurring copy number alteration, are negatively related with the response to HER2-directed therapy, suggesting its potential role as a biomarker of resistance in patients with **ERBB2** amplified GC[26].

**CURRENT TOPICS OF BIOMARKERS IN GC**

The measurement of conventional serum tumor biomarkers has been widely accepted in the diagnosis and prediction of recurrence in GC. However, due to their insufficient specificity and sensitivity, these molecular markers cannot be applied for early GC detection. Therefore, novel and dependable tumor biomarkers are urgently needed.

**Metastasis related genes**

**FGFR2**: With the progression of molecular biological techniques over the last several years, investigators have increased pivotal insights into the oncogenesis mechanisms. Besides the well-known pathogenic factor, a variety of experimental procedures have ascertained numerous oncogenes and tumor suppressor genes, including cell cycle genes in the cell growth and signaling pathways[27-29]. A well-organized clarification of these complexity of molecular and genetic profiles will lead to the precise strategies of personalized treatment. The fibroblast growth factor receptors (FGFR) family consists of four members, FGFR1, FGFR2, FGFR3 and FGFR4. These receptors bind to their high-affinity ligands, the fibroblast growth factors (FGFs)[30]. Gene amplification of FGFR induces receptor overexpression, chromosomal translocation, and point mutation or enhanced kinase activity[31]. Various basic diverse cellular behaviors and cellular processes, such as mitogenesis, differentiation, cell proliferation, angiogenesis and invasion are intermediated though FGFRs signaling pathway[30]. The frequency of overexpression of FGFR2 was 31.1% and was more common than EGFR (23.5%), HER2 (11.8%), MET (24.9%)[32]. Thus, FGFRs should attract substantial attention as a useful therapeutic candidate for targeted anticancer agents. FGFR2 amplification was found to be associated with a higher pT stage, higher pN stage, lymph node metastasis and related to poor overall survival[33]. A recent study described that FGFR expression was positively associated with the recurrent rate more than 5 years in patients with stage II/III GC who undergo curative surgery and adjuvant chemotherapy with S-1[34]. This result indicates that FGFR2 could be the biomarker for predicting long-term failure of adjuvant treatment of S-1 in patients with curative resection for advanced GC.

**E-cadherin**: E-cadherin is a transmembrane molecule...
that is involved in the cellular calcium-mediated adhesion. It is encoded by CDH1 located on the chromosome 16 (q22.1). E-cadherin closely associates to epithelial gastric cells adhesion and differentiation, which is an important prevention against the malignant formation\(^3\). CDH1 is one of the most pivotal tumor suppressor genes in GC, and its disruption of activity has been proven to be closely related with the invasive and metastatic capacity\(^3\). The E-cadherin gene can be inactivated by several mechanisms, including CDH1 mutations, hypermethylation, loss of heterozygosity (LOH), H pylori infection, transcriptional repression binding to the CDH1-E box element, and tyrosine phosphorylation (e.g., EGFR, MET and FGFR)\(^4\). Hereditary diffuse GC (HDGC) is an autosomal dominant cancer syndrome representing approximately 2% of all GCs\(^3\). Germline mutations in the CDH1 gene are identified in HGDC, leading to the histological characteristics similar to diffuse-type GC. The cumulative risk of GC by 80 years of age in male CDH1 mutation carriers is 83% for advanced GC\(^3\). Unfortunately, metastatic HGDC patients show lower survival compared with other sporadic GC. A recent study described that E-cadherin/catenin-EGFR crosstalk is closely associated with HGDC. Enhanced sensitivity to EGFR and PI3K kinase inhibition was induced by loss of E-cadherin/catenin-EGFR interaction in HGDC families with CDH1 germline mutations, suggesting that these inhibitors would be an attractive tool for the targeted therapy in HGC patients in the near future.

Patients with GC showing somatic CDH1 epigenetic and structural alterations have a worse overall survival than patients with tumors negative for CDH1 alterations. This finding indicates that the presence of CDH1 epigenetic and structural alterations in a diagnostic/preoperative biopsy may serve as clinically useful biomarker\(^5\). A recent study examined the diagnostic role of promoter methylation status of CDH1 in blood samples of patients with GC\(^6\). Interestingly, the significant facilitation of promoter methylation of CDH1 was shown in blood samples, suggesting that promoter methylation of CDH1 may be a good candidate of biomarkers in patients with GC.

PI3K/Akt/mTOR: PI3K/Akt/mechanistic target of rapamycin (mTOR) signaling is a crucial mediator of many essential cellular processes; genomic instability, cell cycle, growth, metabolism, survival, metastasis and resistance to chemotherapy\(^7\). The PIK3CA gene encoding the PI3K catalytic isoform p110α is the second most frequently mutated oncogene, and PTEN encoding the major phosphatidylinositol phosphatase is one of the most mutated tumor suppressor genes. Deregulation of the PI3K/Akt/mTOR pathway can occur secondary to oncogenic mutations of PIK3CA\(^8,9\). Genetic deregulations in the PI3K/Akt/mTOR pathway have been identified frequently in GC. PI3K/Akt/mTOR pathway expression has been associated with the lymph node status and poor survival\(^10\). PIK3CA has been reported to be identified in 4%-25% of patients with GC\(^11\). Although PIK3CA mutations have a critical role in resistance to antitumor drugs and acquisition of metastatic potential, its mutations did not likely to have an established efficient on prognosis. It has been reported that no ethnic differences in PIK3CA mutation frequencies exist, whereas the PIK3CA mutations are predominantly found in 80% of Epstein Barr virus (EBV) positive subgroups\(^12\). A recent study pointed that p-AKT negative tumors are more malignant than p-AKT positive but are rescued by the adjuvant chemotherapy for GC patients undergoing gastrectomy regardless of the PIK3CA mutation status\(^13\).

MET: MET is a transmembrane tyrosine kinase receptor identified as the receptor for hepatocyte growth factor/scatter factor (HGF/SF). Activation of MET phosphorylates several signal transduction cascades, leading to cancer cell growth, angiogenesis, migration, and metastases\(^14\). MET amplification and/or overexpression of its secreted protein has been reported to be involved in the carcinogenesis, therapy efficacy, and outcome of GC\(^15,16\). The measurement and assessment of HGF activity have been crucial role in understanding the tumor microenvironment that prompt tumor metastasis and drug resistance\(^17\). The recent immunostaining experiment has presented that MET expression was significantly associated with lymphatic vessel invasion and poor overall survival (OS), implying that the expression of HGF/c-Met pathway might serve as a prospective predictive factor in patients with GC\(^18,19\). Interestingly, patients with a lower pretreatment HGF level showed a positive response to the treatment of trastuzumab. Serum level of HGF was increased in the patients who had no effect on trastuzumab compared with the pre-treated level\(^20\). In the meanwhile, MET may be a useful predictive marker for chemotherapy, because MET signaling positively related with chemoresistance of GC therapy via increasing UGT1A1 level\(^21\).

Vascular endothelial growth factor: Several signal transduction pathways are proved to be associated with tumor-associated angiogenesis, including vascular endothelial growth factor (VEGF)\(^22\). VEGF is a pivotal growth factor and signaling molecule to promote formation of new blood vessels. Binding to its receptor, VEGFR, activates a complex cascade of downstream signaling pathways, which leads to neovascularization, vasodilation\(^23\). Inhibition of VEGF and/or VEGFR activity impaired these pathways, which results in reduction of tumor proliferation, survival, and invasion. VEGF and its receptors are upregulated in 40% to 36% of cases, respectively in GC\(^24\). Antibodies against VEGF and VEGFR have been shown to yield anti-tumor effect, and to date, combined therapy with cytotoxic chemotherapy are adapted as standard first- or second-line treatment of GC. Ramucirumab is a recombinant humanized monoclonal antibody (mAb) specific for VEGFR-2 and impairs its activity by VEGF. Ramucirumab has provided anti-tumor effect in clinical practice as a single agent (REGARD trial) and
in combination with paclitaxel (RAINBOW trial)\textsuperscript{[56,57]}. In a recent, VEGFR-2 as predictive/prognostic biomarkers has been shown in two independent phase-III studies evaluating the role of ramucirumab in GC. In the RAISE study, second-line treatment with remucirumab combined with FOLFIRI presented that the group of high expression of VEGF-D had a longer survival compared with that of low expression of VEGF-D in colorectal cancer\textsuperscript{[58]}. Therefore, it could be plausible that VEGF-D would be a promising predictive biomarker for ramucirumab efficacy in GC.

TP53: TP53 gene is an extremely crucial tumor suppressor which plays a role as an important regulator of different cellular processes including growth arrest and apoptosis, DNA damage, and aberrant proliferative signals\textsuperscript{[59]}. The mutational site of p53 in GC is wide and the reported incidence of p53 mutations ranges from 3.2% to 65%\textsuperscript{[60]}. The incidence of p53 mutation was significantly lower in EBV-GC (n = 1) when compared with non-EBV-GCs (n = 10)\textsuperscript{[61]}. TP53 mutation is identified most often in the intestinal type of GC\textsuperscript{[62]}. TP53 codon 72 single nucleotide polymorphism (SNP) Arg72Pro was correlated with a shorter outcome in patients with GC. TP53 codon 72 SNP was shown to predict the response to chemotherapy, and related with the time to progression in advanced GC patients treated with paclitaxel and cisplatin chemotherapy\textsuperscript{[63]}.  

**Immune checkpoint**

The programmed cell death 1 (PD-1) and 2 (PD-2) are key immune checkpoint receptors expressed on activated T and B lymphocytes, natural killer T cells, and monocytes\textsuperscript{[64]}. Binding of its two ligand, programmed death-1 ligands (PD-Ls) 1 and 2 to PD-1 on activated T cells leads to downregulation of cytotoxic T-cell activity and also induce immune tolerance to tumor. The expression of PD-L1 in patients with GC is ranged in 15% to 70% of cases, and they are correlated with poor outcome\textsuperscript{[65]}. Targeting the PD-1 pathway and immune checkpoint blockade has proved to be a novel tool for GC treatment. Pembrolizumab and nivolumab are an anti-PD1 monoclonal antibody, and they facilitated the capacity of the immune system. A phase II study (KEYNOTE-059) demonstrated that application of pembrolizumab alone showed clinical efficacy in previously treated advanced GC\textsuperscript{[66]}. Treatment of pembrolizumab showed a higher overall response rate (ORR) for patients with PD-L1 positive tumors, than in patients with PD-L1 negative tumors. Interestingly, patients with microsatellite-high (MSI-High) revealed higher response compared with those with MSI-High tumors, suggesting the level of PD-L1 and MSI-High may serve as predictive biomarkers for efficacy of pembrolizumab. Besides, up-regulated expression of PD-L1/2 has been shown in the EBV-positive sub-type of tumors\textsuperscript{[67]}. The results of these studies have facilitated the adaptation of immune checkpoint inhibitors generally in patients with GC.

**Comprehensive gene analysis**

Whole genome sequencing to targeted sequencing has played a crucial role in the identification of the genetic variations and anomalies, which leads to the development of GC. Initiation of GC is closely associated with epigenetic modifications and genome alterations. Recently, human genome project was completed and examination of gene expression profiling has been developed. Several critical genes as biomarker have been identified through genome-wide expression profile for GC\textsuperscript{[68-70]}. For genome analysis, cDNA microarrays and serial analysis of gene expression (SAGE) have been mainly utilized\textsuperscript{[71]}. Similar the microarray technique, SAGE is a powerful technique for worldwide analysis of gene expression in a quantitative manner without previous understanding of the gene sequences\textsuperscript{[72]}. A recent cDNA microarray analysis assumed that seven genes exclusively expressed in patients with positive lymph node metastasis and five genes entirely expressed in lymph node negative patients. Genes (including Egr-1) which involved in cell growth, transcription and vascularization were up-regulated, whereas those in apoptosis and cell differentiation was down-regulated\textsuperscript{[73]}. Up-regulation of CEACAM6, APOC1, and YF13H12 have been shown to be frequently up-regulated in GC\textsuperscript{[74]}. In the meanwhile, significant correlation of FUS, CDH17, COLIA1, COLIA2, and APOE with invasion and metastasis was proved. A recent comprehensive analysis using SAGE and Escherichia coli ampicillin secretion trap (CAST) detected several gene alterations in GC. Among them, CDH17, REG4, OLFM4, HOXA10, DSC2, TSPAN8 and TM9SF3 were upregulated and CLDN18 was downregulated. These molecules may not serve as just biomarkers but therapeutic target.

**MSI**

Microsatellites are repeating 1-6 nucleotide long units of DNA sequences that can be detected in both non-coding and protein coding sequences of DNA\textsuperscript{[75]}. MSI is stated as somatic alterations in microsatellite sequences due to the insertion or deletion of those repeat units, which lead to genomic instability and increasing the susceptibility for the tumor development. Tumors showing 10%-29% of unstable microsatellite are considered MSI-low while tumors with ≥ 30% of unstable microsatellite are classified as MSI-high. In GC, 15%-30% of tumor display MSI, mainly due to epigenetic silencing thorough promoter hypermethylation of the MLH1\textsuperscript{[76]}. A recent comprehensive analysis from Korea have found that more than 63% of the MSI-high GC identified the mutations within mononucleotide tracts in TGFB2R, CEP164, MIS18BP1, RNPC3, KIAA2018, CNOT1 and CCDC150 genes\textsuperscript{[77]}. The high status of PIK3CA mutations in MSI positive GCs has shown the efficiency of PIK3CA inhibitors in the personalized treatment of MSI positive patients\textsuperscript{[79]}. Studies have shown a strong association of MSI loci
in GC with intestinal type, which undergoes more genomic instability in comparison to the diffuse type\[80\]. Interestingly, MSI-high tumors had a better prognosis than MSI-low tumors because MSI-high tumors showed an inferior capacity of invasion and lymph node metastases\[81\]. A recent randomized clinical trial (MAGIC trial) reported that the prognosis of patients with MSI-high gastroesophageal cancer showed significantly higher survival compared with those with MSS/MSI-low when treated with surgery alone. In contrast, when patients had a treatment with surgery and perioperative chemotherapy, the prognosis was shorter in patients with MSI-high, suggesting that perioperative chemotherapy may not provide a benefit in patients with MSI-high\[82\]. These showing results suggest that MSI frequency may be a beneficial predictive and prognostic biomarker in patients with GC.

**Epigenetic alterations**

Abnormality in the epigenetic system has been caused to pathogenic mechanism, which lead to the carcinogenesis of several cancers. Numerous of research has been performed linking aberrant DNA methylation profiles and histone modifications to progressive diseases, including cancers. The most widely studied epigenetic alteration in cancer is aberrant DNA methylation\[83\]. In humans, DNA methylation occurs at cytosine residues that precede guanines, called CpG dinucleotides (C-phosphodiester-G). Abnormal DNA methylation in the promoter region of genes, resulted in the inactivation of tumor suppressor and other cancer-relevant genes is the most well-defined epigenetic band in GC. Various risk factors such age, chronic inflammation, and infection with *H. Pylori* and EBV can cause the aberrant gene methylation in GC\[84\]. Defective DNA methylation in *CDH1*, *CHFR*, *DAPK*, *GSTP1*, *p15*, *p16*, *RARβ*, *RASSF1A*, *RUNX3* and *TP73* has been considered as a serum biomarker for the diagnosis of GC\[84,85\]. Among them, the mitotic checkpoint gene, CHFR methylation has been found significantly elevated in mucosa from patients with GC in comparison to mucosa from normal gastric tissue. CHFR promoter methylation is related with tumor differentiation and lymph node involving\[86\]. Aberrant DNA methylation in noncancerous gastric mucosa has been implicated in gastric carcinogenesis and could be a useful biomarker for the assessing risk of GC. A recent study revealed that defect of expression of *FAT4* gene was found in highly methylated GC cell lines and impairment of methylation reduced its expression. *H. Pylori* infection has also related to methylation frequency of *FAT4* gene\[87\]. The understandings gained from genetic studies on molecular pathogenesis of GC may serve as the inciting cause of various experiments to identify different genetic biomarkers for early diagnosis and prognosis of this type of malignancy.

**Genetic polymorphism**

Genetic polymorphisms have a pivotal role in human malignancies, and the close association between cancer and genetic polymorphism for tumor initiation has been demonstrated in a variety of experimental studies\[88\]. One of the important genetic polymorphisms in GC is Interleukin-1β (IL1-β). IL1-β and IL1RN polymorphisms with *H. pylori* infection has been shown to provide the progression of chronic atrophic gastritis and GC in an Algerian population\[89\]. To date, advancements of research have proved the importance of SNP in showing individual specific variations of gene aberrations. A recent study presented that the *CD44* SNP genotype, rs187116 was a meaningful prognostic factor for early recurrent GC and CD44 isoform switching from CD44v to CD44s was closely related with this effect of *CD44* rs187116 on tumor recurrence\[90\]. Furthermore, this *CD44* SNP was an independent risk factor for disease free survival, suggesting that *CD44* rs187116 may serve as a useful biomarker in GC patient in a Japanese population. A study to detect copy number variations and mutations found that the top mutated genes revealing high frequency were *TP53*, *SYNE1*, *CSMD3*, *LRP1B*, *CDH1*, *PIK3CA*, *RASSF1A*, *ARID1A* and *PKHD*. Copy number variation has been identified for KRAS, *JAK2*, *CD274* and *PDCD1LG2* genes using single cell resequencing amplified by different three whole genome amplification\[92\].

**NON-INVASIVE BIOMARKERS; LIQUID BIOPSIES**

The main problem to the diagnosis, treatment and surveillance of solid cancers is the necessity for getting appropriate tumor volume frequently and derived tumors does not fully represent the character of total tumor. A ‘liquid biopsy’ is in principle a sample of any body fluid that may contain genetic material from a tumor, for instance blood, urine, saliva or cerebrospinal fluid\[93\]. Progress in the field of liquid biopsies may solve the challenges with tissue biopsies by using body fluids to investigate disease biomarkers. Among the liquid biopsy options, blood samples are the most widely studied\[93\]. Peripheral blood samples from patients with cancer contain circulating tumor cells (CTCs), cell-free DNA, micro RNA, cell -free RNA and cell - derived vesicles, such as exosomes.

**CTCs**

CTCs are disseminated tumor cells as single cells or, less commonly, as cell clusters, derived from either primary tumors or metastases which are circulating in the bloodstream\[94\]. The existence of CTCs has been said to be clinically related with progressive or metastatic disease. Hence, CTCs can be used to monitor advanced stage disease without other surveillance markers. In particular, CTCs can be detected at an early stage before the metastasis occurs\[94,95\]. CTCs can thus identify patients who would have more advantage from adjuvant...
treatment after surgery of primary cancer\textsuperscript{[94]}. In GC, a recent meta-analysis of CTCs in patients with GC suggested associations of CTCs with prognosis, tumor staging, histologic type, and lymphovascular invasion\textsuperscript{[96]}. A subset of detected CTCs with stem cell-like characteristics or epithelial-mesenchymal transition (EMT) properties, which should have the capacity for surviving and migrating to secondary foci, may play a pivotal role in tumor stage evaluation and prediction of recurrence. CD44 has been identified as a marker of GC stem cells and increased resistance for chemotherapy- or irradiation-induced cell death was found in the CD44-positive GC cells\textsuperscript{[97]}. The expression of epithelial markers pan-CK, E-cadherin were decreased, and mesenchymal markers N-cadherin, vimentin were overexpressed in gastric CTCs, which may provide more useful information for prediction of recurrence\textsuperscript{[98]}. To date, unfortunately, utilizing CTCs in GC is not still established in clinical practice. The novel innovative approaches for detecting EMT CTCs or circulating stem cells are needed to be developed and evaluation in clinical trials should be necessary. Interestingly, a recent phase II study presented that preselected patients whose primary tumors were HER2- but who had HER2+ CTCs had response rates equivalent to those reported in the trastuzumab-plus-chemotherapy arm of the ToGA study\textsuperscript{[99]}.

**Circulating cell-free DNA**

Circulating cell-free DNA (cfDNA) is cell-free extracellular DNA originating from normal and cancerous cells identifiable in the blood (the plasma or the serum)\textsuperscript{[100]}. The fraction of cell-free DNA that derived from primary tumors, metastases or from CTCs is called ctDNA. Currently, the utility of ctDNA in cancer treatment is the most extensively studied issue in cfDNA research. Compared to the restrictions of conventional biopsy which leads to significant trauma and produces small sample size, ctDNA detection displays several benefits including convenient sampling, minimal invasiveness and high repeatability. Moreover, ctDNA has been shown to be more sensitive than CTC\textsuperscript{[100]}. The potential diagnostic and/or prognostic values of quantifying cfDNA in GC patients compared to the healthy controls, have been evaluated in a variety of researches.

In GC, methylated promoter regions have been used extensively to identify ctDNA in both serum and plasma by methylation-specific PCR. A recent meta-analysis study showed that detection of ctDNA had an obvious advantage in GC diagnosis specificity, although no superiority of ctDNA over conventional protein biomarkers was detected in sensitivity, such as CEA, CA125 and CA72-4\textsuperscript{[101]} With regard to prognostic value, significantly poorer DFS and OS in patients were identified. A recent study described that serum APC promoter 1A and RASSF1A promoter hypermethylation in cfDNA was a frequent epigenetic event in patients with early operable GC\textsuperscript{[102]}. Interestingly, cfDNA showing Epstein-Barr virus (EBV) DNA has been proved to be useful for identifying the EBV-associated GC subtype, monitoring tumor development, and managing response in patients with this subtype\textsuperscript{[103]}. Tumor responses to lapatinib plus Capecitabine were closely related with changes in plasma-detected ERBB2 copy number through serial cfDNA sequencing\textsuperscript{[104]}.

**MicroRNA**

Dysregulations in non-coding regulatory RNAs can contribute to cancer initiation and development\textsuperscript{[105]}. A class of small cellular RNAs, termed microRNAs (miRNAs) are 18 to 24 nucleotides noncoding RNA fragments whose function is to bind the 3’UTR region of their target gene and regulate its expression by impairing the translation\textsuperscript{[105-107]}. MicroRNAs are key players in regulating several biological processes of the cell proliferation, differentiation, migration, and invasion\textsuperscript{[105]}.

Expression profiling of microRNAs have shown the distinctive signatures of these small regulatory RNAs in different cancers including GC\textsuperscript{[108]}. Numerous microRNAs have been identified and recognized to be implicated in GC\textsuperscript{[108,109]}. MiRNAs can have a critical role in cancer cell progression through EMT into metastases. The miR-200 family promotes EMT, resulting in cancer cell migration by suppressing E-cadherin and ZEB2 expressions\textsuperscript{[110]}. It is known that miRNAs can increase the expression of oncogenes or reduce the expression of tumor suppressor genes\textsuperscript{[111]}. Abundant differentially expressed miRNAs have been associated with different stages of GC. miRNAs such as miR-21, miR-23a, miR-27a, miR-106b-25, miR-130b, miR-199a, miR-215, miR-222-221 and miR-370 were associated with oncogenic activity of GC. Whereas, miR-29a, miR-101, miR-125a, miR-129, miR-148b, miR-181c, miR-212, miR-218, miR-335, miR-375, miR-449, miR-486 and miR-512 reveal tumor suppressive activity\textsuperscript{[108]}.

Recently, the research for miRNA as biomarker in human malignancies has facilitated because of the unique feature of miRNAs. Cell-free miRNAs (cfmRNAs) can be derived from cancer cells to body fluids via secreting exosomes particles, which lead to protected from RNase-mediated degradation in circulation, and thus are easily extractable from a variety of body fluids including blood, saliva, urine, feces etc. Thus, cf-miRNA could be a useful noninvasive biomarker for diagnosis and relapse of GC. Recent experimental analyses have validated expression levels of cfmRNAs in serum are consistent with gastric tumor tissue\textsuperscript{[112]}. A study based on analysis of comprehensive expression profiling of miRNAs presented that high expression of two potential biomarkers (miR-331 and miR-21) was observed in peripheral blood than in the vein draining the primary tumor and suggested as a potential diagnostic biomarker\textsuperscript{[113]}. A significantly poorer OS was shown in highly miR-21 expressed group compared with low miR-21 expressed group in meta-analysis study. Several other miRNAs showed significant prognostic value in this study. Among them, miR-20b, 125a, 137, 141, 146a, 196a, 206, 218, 486-5p and 506
showed convincing as prognostic biomarkers in patients with GC\textsuperscript{[114]}. Overexpression of six serum-based miRNAs (miR10b-5p, miR132-3p, miR185-5p, miR215-5p, miR196a, and miR296-5p) was shown in GC compared with normal controls by using qRT-PCR-based Exiqon panel\textsuperscript{[115]}. In the arm not receiving chemotherapy, high expression of miR10b-5p or miR296-5p in tissues correlated with shorter OS. Consequently, cfmiRNAs would play an increasingly important role in the diagnosis, prognosis and/or prediction of recurrence of GC. In contrast, it has been said to be difficult to utilize a miRNA as a cancer biomarker in clinical practice\textsuperscript{[116]}. However, to date, clinical study are ongoing to analyze the expression level of miRNA using next generation sequencing (NGS) in GC tissue and blood by chemotherapy response (NCT03253107). Similarly, a phase II study to elucidate whether response to pralatrexate can be predicted by miR-215-5p is currently underway (NCT02050178). When these trials will complete with convincing evidence, miRNAs would be promising markers or new therapeutic targets for drug response prediction and control as well as modification of conventional adjuvant therapy.

Long noncoding RNAs

Long noncoding RNAs (IncRNAs) are sequences of nucleotides longer than 200, that can function as oncogenic or tumor-suppressor\textsuperscript{[117]}. The IncRNAs act as transcriptional mediator, splicing regulator, posttranscriptional processor, enhancer, molecular sponge for miRNAs, chromatin remodeler. The IncRNAs are frequently expressed in a disease - or developmental - specific manner and thus submit potential as a biomarker\textsuperscript{[111]}. Nowadays over 56000 human IncRNAs populating the human genome have been identified and about 135 IncRNAs have been recognized as dysregulated in GC, so they are closely related to tumorigenesis, metastases, and prognosis\textsuperscript{[117,118]}. Impaired expression of nCruPAR significantly associated with lymph node metastasis, distant metastasis, tumor size and TNM stage in patients with GC\textsuperscript{[119]}. A downregulation in the expression of A1364715, GACAT1, and GACAT2 in GC tissues could also serve as a prognostic marker\textsuperscript{[120]}. LncRNA PVT1 was markedly overexpressed in GC tissues compared with that in the normal control and could be an independent prognostic marker\textsuperscript{[121,122]}. However, further studies about IncRNAs are needed in order to identify their possible clinical utilization.

Exosomes

Exosomes, small cell-derived vesicles, can protect RNAs and miRNAs, from being degraded\textsuperscript{[123-127]}. When exosomes were exposed to RNase the contained RNAs were protected from degradation while cellular RNA was degraded by the same RNase\textsuperscript{[126]}. Exosomes hold great potential for both diagnosis and prognosis of diseases and are exceptionally useful as cancer biomarkers\textsuperscript{[128]}. mir-19b and miR-106a, identified in serum-circulating exosomes, remarkably overexpressed in individuals with GC compared to healthy controls. Furthermore, the validated miRNAs were correlated to lymphatic metastasis and expressed at higher levels in stages III and IV compared to I and II stages in GC\textsuperscript{[129]}. Similarly, increased expressions of exosomal miR-21 and miR-1225-5p, isolated peritoneal lavage fluid, were exhibited in patients with T4-stage cancer compared with that in T1- to T3-stage patients\textsuperscript{[130]}. These findings suggest that exosomes may serve as novel diagnostic and therapeutic biomarkers for GC.

STOMACH SPECIFIC BIOMARKER

Gastric Washes/Gastric Juice

Because many mucosal cells can be found in stomach juice, the detection of molecular markers in stomach juice is a possible noninvasive approach to screening for GC. Gastric juice could serve as an excellent source of GC biomarkers, because these are directly released by the tumor without being excluded by the liver. Thus, gastric washes represent an alternative source for detecting aberrant DNA methylation. The analysis for the methylation levels of six genes (ADAM23, GDNF, MINT25, MLF1, PRDM5, RORA) demonstrated that a combination of the markers MINT25, PRDM5 and GDNF achieved a high sensitivity (95%) and specificity (92%)\textsuperscript{[131]}. As well, the levels of PVT1 in gastric juice from gastric patients were significantly higher than those from normal subjects. PVT1 might serve as a promising biomarker for early detection and prognosis prediction of GC\textsuperscript{[121]}. Gastric Juice miR-421, miR-21, miR-106a and miR-129 represent a potential biomarker for screening GC\textsuperscript{[129]}

Other Specific Biomarker

Micro-aerophilic, spiral-shaped Gram-negative bacterium Helicobacter pylori (H.pylori) infection has been said to be associated with the initiation of GC in clinico-epidemiological studies\textsuperscript{[134]}. H. pylori Cytotoxic-associated gene A (CagA) is the first identified bacterial protein playing a positive role in the progression of GC\textsuperscript{[135]}. The molecular mechanism underlying CagA-positive H. pylori-induced GC has been widely studied. CagA induces dysregulation of a variety of signaling pathways, including Wnt/β-catenin, PI3K/Akt, NF-κB, Hedgehog, JAK/ATAT has been identified, which results in the carcinogenesis of GC\textsuperscript{[136]}. Interestingly, the development of EBV-positive GC has been shown to be prompted by H. pylori CagA activity, via SHP1 inhibition through exhibition of FTRN6 hypermethylation\textsuperscript{[137]}. In similar, H. pylori producing another bacterial toxin vacuolating toxin A (vacA) infection were meaningfully associated with increased risk of GC\textsuperscript{[138]}. Gastrokine 1 (GKN1) is a tissue-specific 18 kDa protein that significantly expressed in gastric tissue and...
| Marker | Alteration | Clinical purpose | Detection method | Ref. |
|--------|------------|------------------|------------------|------|
| Metastasis related genes | Overexpression | Diagnostic/prognostic/therapeutic | Tissue | [16-18,25,32,33,44-46,55,58] |
| HER2, FGFR, PI3K/Akt/mTOR (PIK3CA), MET, VEGF (VEGFR-2, VEGF-D) | Overexpression | Diagnostic/prognostic/therapeutic | Tissue | [16-18,25,32,33,44-46,55,58] |
| Cell cycle regulation | Mutation | Diagnostic | Tissue | [60,61,63] |
| TP53 | Mutation | Diagnostic | Tissue | [60,61,63] |
| Adhesion molecule | Mutation/epigenetic alteration | Diagnostic/prognostic | Tissue/blood | [39,40] |
| E-cadherin (CDH1) | Mutation | Diagnostic | Tissue | [60,61,63] |
| Immune checkpoint | Mutation | Diagnostic | Tissue | [60,61,63] |
| PD-L1 | Up-regulated | Diagnostic/prognostic/therapeutic | Tissue | [89,90] |
| Comprehensive gene analysis | Up-regulated | Diagnostic/prognostic/therapeutic | Tissue | [89,90] |
| CDH2, CDH3, TP53, SYNE1, CDX1, LRP1B, CDH1, PIK3CA, ARID1A, PKHD, KRASJ1, AK2, CD274, PDCH12, RASSF1A, RINX3, TPPI2 | Hypermethylation | Diagnostic | Tissue | [84-86] |
| Genetic polymorphism | SNP | Prognostic | Tissue | [89,90] |
| IL1-β, IL-1RN, CDN4 | SNP | Prognostic | Tissue | [89,90] |
| Circulating tumor cells | Overexpression | Diagnostic/therapeutic | Blood | [96] |
| CD44, N-cadherin, vimentin | Overexpression | Diagnostic/therapeutic | Blood | [97] |
| HER2 | Decreased expression | Therapeutic | Blood | [99] |
| Circulating cell-free DNA | Hypermethylation | Diagnostic | Blood/plasma | [102] |
| APC promoter 1, RASSF1A | SNP | Prognostic | Tissue | [91,92] |
| ERBB2 | SNP | Prognostic | Tissue | [91,92] |
| MicroRNA | Up-regulated | Diagnostic/prognostic/therapeutic | Blood/plasma | [108,111] |
| miR-21, miR-23a, miR-27a, miR-106b-25, miR-130b, miR-199a, miR-215, miR-222-221, miR-370, miR-25a, miR-125a, miR-129, miR-529a, miR-486, miR-512 | Up-regulated | Diagnostic/prognostic/therapeutic | Blood/plasma | [108,111] |
| Cell-free miRNAs | Up-regulated | Prognostic | Blood | [111] |
| miR-33-1 and miR-21 | Up-regulated | Prognostic | Blood | [111] |
| miR-20b, 20c-5p, 15c, 19b-3p, 96, 188b | Up-regulated | Prognostic | Blood | [111] |
| Long noncoding RNAs | Down-regulated | Diagnostic/prognostic | Blood | [119] |
| ncRIPAR | Down-regulated | Prognostic | Tissue | [120] |
| AL64715, GACAT1, GACAT2 | Down-regulated | Prognostic | Tissue | [120] |
| PVT1 | Down-regulated | Prognostic | Tissue | [121] |
| Exosomes | Up-regulated | Diagnostic/prognostic | Plasma | [129] |
| MiR-19b, miR-10a | Up-regulated | Diagnostic/prognostic | Plasma | [130] |
| Stomach specific biomarker | Up-regulated | Diagnostic/prognostic | Plasma | [130] |
| ADAM23, GDNF, MNT25, MLF1, PRDM5, RORA | Hypermethylation | Diagnostic | Gastric wash | [131] |
| BARHL2 | Hypermethylation | Diagnostic | Gastric wash/juice | [132] |
| PVT1 | Up-regulated | Diagnostic | Gastric wash | [132] |
| miR-421, miR-21, miR-106a, miR-129 | Up-regulated | Diagnostic | Gastric juice | [132] |
| CagA | Up-regulated | Diagnostic | Tissue | [133] |
| VacA | Up-regulated | Diagnostic | Tissue | [133] |
| Gastrokine 1 | Inactivation | Prognostic | Tissue | [139] |
| HER2: Human epidermal growth factor receptor 2; PLF: Peritoneal lavage fluid; FGFR: Fibroblast growth hormone receptor; PI3K: Phosphatidylinositol-3-kinase; mTOR: Mechanistic target of rapamycin; VEGF: Vascular endothelial growth factor; PD-1L: Programmed death-1 ligands; MSI: Microsatellite instability; CagA: Cytotoxin-associated gene A; VacA: Vacuolating toxin A. |
is secreted into the stomach but is absent in GC. Its biological function is still unclear, but it is considered to serve as the replenishment of the surface lumen epithelial cell layer, in maintaining mucosal integrity. GKN1 acts as a tumor suppressor and a modulator of apoptotic signals in GC. Due to a facilitated risk of gastric carcinogenesis in patients who have a lower expression of the protein, GKN1 could also be considered a biomarker for cancer specific to stomach. Epigenetic mechanisms leading to the inactivation of GKN1 play a key role in the multi-step process of gastric carcinogenesis.

CONCLUSION

Through recent rapid advanced understanding of cancer biology, particularly in the field of molecular cell signaling and genetic and/or epigenetic dysregulation, the pattern of gastric carcinogenesis, and the pathways involved have become clearer. These findings may provide precious objectives for the early diagnosis of GC. Reliable prognostic and predictive markers as mentioned above may contribute to improved outcome of advanced GC. Current topics of GC biomarker based on a variety of molecular and genetic feature in this review article were summarized in Table 1. We also classified these biomarkers for early diagnosis, recurrence forecast and chemotherapy benefits assessment (Supplementary Table 1). The use of these new biomarkers such as evaluation of expression levels of various proteins and genes (i.e., FGFR, CDH1, PI3K, MET, VEGFR, TP53, and PD-1) and various body fluid samples (CTC, cfDNA, miRNAs and exosomes) have opened new opportunity for diagnosis and monitoring patients with GC. And these markers will continue to be tested, developed from knowledge of novel approach, such as NGS.

This would facilitate more individualized treatment approaches.

FUTURE PERSPECTIVES

Although biological researchers have shown a lot of new findings in regard to biomarkers of GC to numerous publications, only conventional biomarkers (CEA, CA19-9, etc.) and HER2 are still in clinical use. It is urgently expected to develop biomarkers that are conventional, noninvasive, highly specific, capable of early detection and leading to treatment choice. Ideal biomarkers for early detection of cancer should be up-regulated in majority of patients with high level in cancerous tissues.

GC is a highly heterogeneous disease where even similar clinical and pathologic features lead to different outcomes, suggesting that previous staging systems may have extended to their limit of benefit for predicting patients’ outcome and therapy. Thus, the novel classification of patients with GC to provide preventive and therapeutic approaches based on the genome analysis and clinical evidences are needed. In a recent, the genomic characterization of GC has led to the development of new classification by The Cancer Genome Atlas (TCGA) Research Network. The division of GC into four molecular types: (1) Tumors positive for EBV, (2) MSI-high tumors, (3) genomically stable tumors, and (4) tumors with chromosomal instability, allows identifying patients on the basis of the molecular features. Future strategies aiming to translate molecular classification and profiling of tumors into therapeutic targets and predictive biomarkers in GC will be useful. The subtype of EBV-positive cancer is characterized by recurrent PIK3CA and ARID1A mutations, and high expression of PD-L1 and PD-L2, extreme DNA hypermethylation, which should be the good candidate as the diagnostic and therapeutic biomarkers. Inhibition of DNA methylation, and the suppression of immune checkpoints are promising target of this subtype. The MSI-high subtype reveals often mutation of multiple genes such as HER2 and HER3. Thus, besides the MSI, ErB family may be considerable as biomarker of this subtype. As mentioned previously, gastric MSI-high tumors represent a high frequency of PD-L1 expression. Hence, this subtype may be a pivotal candidate to anti-PD-1 therapy. The genomically stable subtype has a few somatic copy-number alterations but involves ARID1A and RHOA mutations or CLDN18-ARHGAP gene fusions. RhoA and its related genes could act as the therapeutic biomarker of this subtype. The subtype with chromosomal instability is rich in TP53 mutations, and has relatively abundant amplifications of RTK genes. Therefore, this subtype can be the target therapy for RTKs, including EGFR and VEGF. The molecular classification of GC will further highlight the need for the identification and use of molecular biomarkers.

Genome wide investigation of cancer transcriptomes identified many new candidate genes. On the contrast, the candidate gene lists generated from comprehensive gene analysis vary considerably among individual studies. Therefore, it is essential to pinpoint the key players that can be explored for the development of biomarkers and leads for better cancer management. On the other hand, with regard to molecular targeting agents, their target molecules and related genes would be suitable for predicting treatment response more accurately.

The discovery of precise biomarker closely related with GC development can also be applied to treatment. We hope that this article will help design to identify the robust biomarkers in clinical care of patients and they can be relevant for the ultimate prevention and treatment of GC.

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90 [PMID: 21296855 DOI: 10.3322/cace.20107]
2. Wagner AD, Unverzagt S, Grothe W, Kleber G, Grothey A, Haerting J, Fleig WE. Chemotherapy for advanced gastric cancer. Cochrane Database Syst Rev 2010; 3: CD004064 [PMID: 20238327 DOI: 10.1002/14651858.CD004064.pub3]
3. Shimada H, Noie T, Ohashi M, Oba K, Takahashi Y. Clinical significance of serum tumor markers for gastric cancer: a systematic review of literature by the Task Force of the Japanese Gastric Cancer Association. Gastric Cancer 2014; 17: 26-33 [PMID: 23572188 DOI: 10.1007/s10120-013-0259-5]
Matsuoka T et al. Biomarker of gastric cancer

4 Asao T, Fukuda T, Yazawa S, Nagamachi Y. Carcinomembrane antigen levels in peritoneal washings can predict peritoneal recurrence after curative resection of gastric cancer. Cancer 1991; 68: 44–47 [PMID: 2049751 DOI: 10.1002/1071-4122(19910701)68:1<44::AID-AN20>3.0.CO;2-K]

5 Zhang YS, Xu J, Luo GH, Wang RC, Zhu J, Zhang XY, Nilsson-Ehle P, Xu N. Detection of carcinoembryonic antigen mRNA in peritoneal washes from gastric cancer patients and its clinical significance. World J Gastroenterol 2006; 12: 1408–1411 [PMID: 16552810 DOI: 10.3748/wjg.v12.i9.1408]

6 Kannagi R, Yin J, Miyazaki K, Izawa M. Current relevance of incomplete synthesis and neo-synthesis for cancer-associated alteration of carbohydrate determinants—Hakomori’s concepts revisited. Biochim Biophys Acta 2008; 1780: 525–531 [PMID: 17980710 DOI: 10.1016/j.bbadeng.2007.10.007]

7 Marrelli D, Pinto E, de Stefano A, Farnetani M, Garosi L, Roviello F. Clinical utility of CEA, CA 19-9, and CA 72-4 in the follow-up of patients with resectable gastric cancer. Ann Surg 2001; 181: 16–19 [PMID: 11248169 DOI: 10.1096/0003-4932(2001)86<00549-3>

8 Song YK, Huang XZ, Gao P, Sun JX, Chen XW, Yang YC, Zhang C, Liu HP, Wang HC, Wang ZN. Clinicopathologic and Prognostic Value of Serum Carbohydrate Antigen 19-9 in Gastric Cancer: A Meta-Analysis. Dis Markers 2015; 2015: 549843 [PMID: 26576068 DOI: 10.1155/2015/549843]

9 Kono K, Arimori H, Sekikawa T, Izuka H, Takahashi A, Fujii H, Matsumoto Y. Clinicopathologic features of gastric cancers producing alpha-fetoprotein. Dig Surg 2002; 19: 359–365; discussion 365 [PMID: 12435906 DOI: 10.1080/074358402101956838]

10 Koide N, Nishio A, Igarashi J, Kajikawa S, Adachi W, Amano J. Alpha-fetoprotein-producing gastric cancer: histochemical analysis of cell proliferation, apoptosis, and angiogenesis. Am J Gastroenterol 1999; 94: 1658–1663 [PMID: 10346040 DOI: 10.1111/j.1572-0241.1999.00158.x]

11 Namikawa T, Kawanishi F, Yusa K, Mikage K, Iwabu I, Munekage M, Maeda H, Kitagawa H, Kobayashi M, Hanakazi K. Serum carbohydrate antigen 125 is a significant prognostic marker in patients with unresectable advanced or recurrent gastric cancer. Surg Today 2018; 48: 388–394 [PMID: 29043453 DOI: 10.1007/s10535-017-1598-3]

12 Akiyama T, Sado C, Ogawa H, Toyoshima K, Yamamoto T. The product of the human c-erbB-2 gene: a 185-kilodalton glycoprotein with tyrosine kinase activity. Science 1986; 232: 1644-1646 [PMID: 3012781 DOI: 10.1126/science.3012781]

13 Bang YJ, van Cutsem E, Feyereislova A, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Shu S, Harada N, Fujimoto-Ouchi K. Pertuzumab plus trastuzumab and docetaxel in HER2-positive advanced gastric cancer. N Engl J Med 2012; 366: 660–666 [PMID: 24960402 DOI: 10.1056/j.bjc.2014.356]

14 Matsuoka T, Yashiro M. Recent advances in the HER2 targeted therapy of gastric cancer. World J Clin Cases 2015; 3: 42–51 [PMID: 25660149 DOI: 10.12998/wjcc.v3.i1.42]

15 Nahta R, Takahashi T, Ueno NT, Hung MC, Esteve FJ. P27(kip1) down-regulation is associated with trastuzumab resistance in breast cancer cells. Cancer Research 2004; 64: 3981–3986 [PMID: 15173011 DOI: 10.1158/0008-5472.CAN-03-3900]

16 Oyama K, Fushida S, Tsukada T, Kinoshita J, Watanabe T, Shoji M, Nakamura S, Okamoto K, Sakai S, Makino I, Nakamura K, Hayashi H, Inokuchi M, Nagaokawa H, Miyashita T, Tajima H, Takahara H, Ninomiya I, Kitagawa H, Fujimura T, Tajiri R, Oos A, Ohta T. Evaluation of serum HER2-ECD levels in patients with gastric cancer. J Gastroenterol 2015; 41: 45–49 [PMID: 10007655-014-0941-3]

17 Matsuoka T, Yashiro M. The Role of PI3K/Akt/mTOR Signaling in Gastric Carcinoma. Cancers (Basel) 2014; 6: 1441-1463 [PMID: 25003395 DOI: 10.3390/cancers6031441]

18 Kim ST, Banks KC, Pectasides D, Kim SY, Kim K, Lamann RB, Talasaz A, An J, Choi MG, Lee JH, Sohn TS, Bae JM, Kim S, Park SH, Park JO, Lim HY, Kim NKD, Park W, Lee H, Bass AJ, Kang K, Kim W, Lee J. Impact of genomic alterations on lapatinib therapy in HER2+ gastric cancer patients. Mol Clin Oncol 2018; 22: 175–181 [PMID: 28282805 DOI: 10.1007/s00535-017-1598-3]

19 Yashiro M, Kondo T, Hase T, Ito H, Yamashita-Kashima Y, Ochiai A. Expression profiles of HER2, EGFR, MET and FGFR2 receptors in cancer. Biochim Biophys Acta 2008; 1780: 549843 [PMID: 26576068 DOI: 10.1155/2015/549843]

20 Koide N, Nishio A, Igarashi J, Kajikawa S, Adachi W, Amano J. Alpha-fetoprotein-producing gastric cancer: histochemical analysis of cell proliferation, apoptosis, and angiogenesis. Am J Gastroenterol 1999; 94: 1658–1663 [PMID: 10346040 DOI: 10.1111/j.1572-0241.1999.00158.x]

21 Namikawa T, Kawanishi F, Yusa K, Mikage K, Iwabu I, Munekage M, Maeda H, Kitagawa H, Kobayashi M, Hanakazi K. Serum carbohydrate antigen 125 is a significant prognostic marker in patients with unresectable advanced or recurrent gastric cancer. Surg Today 2018; 48: 388–394 [PMID: 29043453 DOI: 10.1007/s10535-017-1598-3]

22 Bang YJ, van Cutsem E, Feyereislova A, Chung HC, Shen L, Shu S, Harada N, Fujimoto-Ouchi K. Pertuzumab plus trastuzumab and docetaxel in HER2-positive advanced gastric cancer. J Clin Oncol 2015; 33: 2415–2423 [PMID: 26937130 DOI: 10.3748/wjg.v17.i3.290]
assessmnet of downstream pathway activation. *Virology* Arch 2014; 464: 145-156 [PMID: 24304695 DOI: 10.1007/s00428-013-1517-y]

34 Hosoda K, Yamashita K, Ushiki H, Ena A, Moriya H, Mienen H, Washio M, Watanabe M. Prognostic relevance of FGFR2 expression in stage II-III gastric cancer with curative resection and S-1 chemotherapy. *Oncol Lett* 2018; 15: 1853-1860 [PMID: 29434882 DOI: 10.3892/ol.2017.7515]

35 Carneiro P, Fernandes MS, Figueredo J, Caldeira J, Carvalho J, Pinheiro H, Leite M, Melo S, Oliveira P, Simões-Correia J, Oliveira MJ, Carneiro F, Figueredo C, Paredes J, Oliveira C, Seruca R. E-cadherin dysfunction in gastric cancer—cellular consequences, clinical applications and open questions. *FEBS Lett* 2012; 586: 2981-2989 [PMID: 22481718 DOI: 10.1016/j.febslet.2012.07.045]

36 Chan AO. E-cadherin in gastric cancer. *World J Gastroenterol* 2006; 12: 199-203 [PMID: 16482618 DOI: 10.3748/wjg.v12.i2.199]

37 Lynch HT, Kaurah P, Wirtzfeld D, Rubinstein WS, Weissman S, Lynch JF, Grady W, Wyrick S, Senz J, Huntsman DG. Hereditary diffuse gastric cancer: diagnosis, genetic counseling, and prophylactic total gastrectomy. *Cancer* 2008; 112: 2655-2663 [PMID: 18442100 DOI: 10.1002/cncr.23501]

38 Pharoah PD, Guilford P, Caldas C, International Gastric Cancer Linkage Consortium. Incidence of gastric cancer and breast cancer in CDH1 (E-cadherin) mutation carriers from hereditary diffuse gastric cancer families. *Gastroenterology* 2001; 121: 1348-1353 [PMID: 11729114 DOI: 10.1053/gast.2001.29611]

39 Corso G, Carvalho J, Marrelli D, Vindigni C, Carvalho B, Seruca R, Roviello F, Oliveira C. Somatic mutations and deletions of the E-cadherin gene predict poor survival of patients with gastric cancer. *J Clin Oncol* 2013; 31: 868-875 [PMID: 23341533 DOI: 10.1200/JCO.2012.44.4612]

40 Wen J, Zheng T, Hu K, Zhu C, Gao L, Ye G. Promoter methylation of tumor-related genes as a potential biomarker using blood samples for gastric cancer detection. *Oncotarget* 2017; 8: 77783-77793 [PMID: 29100425 DOI: 10.18632/oncotarget.20782]

41 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; 144: 646-674 [PMID: 21376320 DOI: 10.1016/j.cell.2011.02.013]

42 Samuels Y, Ericson K. Oncogenic PI3K and its role in cancer. *Curr Opin Oncol* 2006; 18: 77-82 [PMID: 16357568 DOI: 10.1097/01.coon.0000198021.93474.b9]

43 Song MS, Salmena L, PandolﬁPP. The functions and regulation of the PTEN tumour suppressor. *Nat Rev Mol Cell Biol* 2012; 13: 283-296 [PMID: 22473468 DOI: 10.1038/nrm3330]

44 Xu DZ, Geng QR, Tian Y, Cai MY, Fang XJ, Zhan YQ, Zhou ZW, Li W, Chen BY, Sun XW, Guan YX, Li YF, Lin TY. Activated proteins in advanced gastric cancer. *World J Gastroenterol* 2010; 16: 536 [PMID: 20929255 DOI: 10.3748/wjg.v16.i2.536]

45 lizasa H, Nanko A, Nishikawa J, Jinushi M, Yoshihama H. Epstein-Barr Virus (EBV)-associated gastric carcinoma. *Viruses* 2012; 4: 3420-3439 [PMID: 23342366 DOI: 10.3390/v4123420]

46 Ito C, Nishizaka SS, Ishida K, Usugi N, Sugai T, Tamura G, Koeda K, Sasaki A. Analysis of PIK3CA mutations and PI3K pathway proteins in advanced gastric cancer. *J Surg Res* 2017; 212: 195-204 [PMID: 28550907 DOI: 10.1016/j.jss.2017.01.018]

47 Matsumoto K, Uimitsu M, De Silva DM, Roy A, Bottaro DP. Hepatocyte growth factor/MET in cancer progression and biomarker discovery. *Cancer Sci* 2017; 108: 296-307 [PMID: 28064454 DOI: 10.1111/cas.13156]

48 Graziano F, Arduini F, Ruzzo A, Beearzi I, Humar B, More H, Silva R, Muretto P, Guilford P, Testa E, Mari D, Magnani M, Cescdin S. Prognostic analysis of E-cadherin gene promoter hypermethylation in patients with surgically resected, curative-resection and S-1 chemotherapy. *Carcinogenesis* 2006; 27: 2784-2789 [PMID: 15102685 DOI: 10.1118/1078-0432.CCR-03-0320]

49 Lee HE, Kim MA, Lee HS, Jung EJ, Yang HK, Lee BL, Bang YJ, Kim WH. MET in gastric carcinomas: comparison between protein expression and gene copy number and impact on clinical outcome. *Br J Cancer* 2012; 107: 325-333 [PMID: 22644302 DOI: 10.1038/bjc.2012.237]
 dependent on the genetic pathways. *J Pathol* 2000; 191: 257-263 [PMID: 10878546 DOI: 10.1002/1096-9896(20000999-9999::AID-PATHE)31.3.0.CO;2-2]

63 Li QF, Yao RV, Liu KW, Lv HY, Jiang T, Liang J. Genetic polymorphism of GSTP1: prediction of clinical outcome to oxaliplatin/S-FU-based chemotherapy in advanced gastric cancer. *J Korean Med Sci* 2010; 25: 846-852 [PMID: 20514304 DOI: 10.3346/jkms.2010.25.6.846]

64 Sharpe AH, Wherry EJ, Ahmed R, Freeman GJ. The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. *Nat Immunol* 2007; 8: 239-245 [PMID: 17304324 DOI: 10.1038/ni0103-1443]

65 Gu L, Chen M, Guo D, Zhu H, Zhang W, Pan J, Zhong X, Li X, Qian H, Wang X, PD-L1 and gastric cancer prognosis: A systematic review and meta-analysis. *PLoS One* 2012; 12: e0182692 [PMID: 28796808 DOI: 10.1371/journal.pone.0182692]

Curea FG, Hebbbar M, Ilie SM, Bacinesci HE, Trifanesci O, Bacinschi XE, Trifanesci OG, 1996; Yamashita S, Niwa T, Mori A, Ito S, Ichinose M, 2004; Matsuoka T, 2011; Noyo K, 80]

70 Hippo Y, Taniguchi H, Tsutsui S, Machida N, Chong JM, Fukayama M, Kodama T, Aburatani H. Global gene expression patterns in human cancer. *Nucleic Acids Res* 2011; 39: 1197-1207 [PMID: 20965966 DOI: 10.1093/nar/gkp690]

71 DeRisi J, Penland L, Brown PO, Bittner ML, Meltzer PS, Ray M, 14]

72 Velculescu VE, Zhang L, Vogelstein B, Kinzler KW. Serial analysis of gene expression. *Science* 1995; 270: 484-487 [PMID: 7570003 DOI: 10.1126/science.270.5235.484]

73 Ojetti V, Persiani R, Cananzi FC, Sensi C, Piscaglia AC, Saulnier F, Botnariuc I, Anghel RM. Current Targeted Therapies in HER2-

74 DeRisi J, Penland L, Brown PO, Bittner ML, Meltzer PS, Ray M, 14]

75 DeRisi J, Penland L, Brown PO, Bittner ML, Meltzer PS, Ray M, 14]

76 Velculescu VE, Zhang L, Vogelstein B, Kinzler KW. Serial analysis of gene expression. *Science* 1995; 270: 484-487 [PMID: 7570003 DOI: 10.1126/science.270.5235.484]

77 Ojetti V, Persiani R, Cananzi FC, Sensi C, Piscaglia AC, Saulnier F, Botnariuc I, Anghel RM. Current Targeted Therapies in HER2-

78 DeRisi J, Penland L, Brown PO, Bittner ML, Meltzer PS, Ray M, 14]

79 Ding Y, Lian HF, Du Y. Clinicopathological significance of CHFR promoter methylation in gastric cancer: a meta-analysis. *Oncotarget* 2017; 9: 10083-10090 [PMID: 29515792 DOI: 10.18632/oncotarget.23394]

80 Yoshida S, Yamashita S, Niwa T, Mori A, Ito S, Ichinose M, Ushijima T. Epigenetic inactivation of FAT4 contributes to gastric carcinogenesis. *Gastroenterology* 2013; 145: 239-245 [PMID: 23922292 DOI: 10.1016/j.gastro.2013.05.002]

81 Dixon K, Kopras E. Genetic alterations and DNA repair in human carcinogenesis. *Semin Cancer Biol* 2004; 14: 441-448 [PMID: 15489137 DOI: 10.1016/j.semcancer.2004.06.007]

82 Drici Ael-M, Mouslehoul S, Tifiti A, Daf M, Turki DK, Bachir M, Tou A. Effect of IL-1β and IL-1R polymorphisms in carcinogenesis of the gastric mucosa in patients infected with Helicobacter pylori in Algeria. *Lymph J Med* 2016; 11: 31576 [PMID: 27340011 DOI: 10.3402/ljm.v11.31576]

83 Suenaga M, Yamada S, Fuchu BC, Fuji T, Kanda M, Tanaka C, Kobayashi D, Fujisawa M, Tanabe K, Koder Y. CD44 single nucleotide polymorphism and isofom switching may predict gastric cancer recurrence. *J Surg Oncol* 2015; 112: 622-628 [PMID: 26416034 DOI: 10.1002/jso.24056]

84 Kuboki Y, Yamashita S, Niwa T, Usui J, Nagatsuna A, Kawata T, Yoshino T, Doi T, Ochiai A, Ohtsu A. Comprehensive analyses using next-generation sequencing and immunohistochemistry enable precise treatment in advanced gastric cancer. *Ann Oncol* 2016; 27: 127-133 [PMID: 26489445 DOI: 10.1016/j.annonc.2015.10.008]

85 Hou Y, Wu K, Shi X, Li F, Song L, Wu H, Dean M, Li G, Tsang S, Jiang R, Zhang X, Li B, Liu G, Bedekar N, Lu N, Xie G, Liang H, Chang L, Wang T, Chen J, Li Y, Zhang X, Yang H, Xu X, Wang L, Wang J. Comparison of variations detection between whole-genome amplification methods used in single-cell resequencing. *Gigascience* 2015; 4: 3 [PMID: 26251608 DOI: 10.1101/giga.315765.100]
Matsuoka T et al. Biomarker of gastric cancer

125 Skog J, Würdingen T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, Curry WT Jr, Carter BS, Krichovsky AM, Breakefield XO. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat Cell Biol 2008; 10: 1470-1476 [PMID: 19011622 DOI: 10.1038/ncli8000]

126 Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007; 9: 654-659 [PMID: 17486113 DOI: 10.1038/ncli596]

127 Cheng L, Sharples RA, Scicluna BJ, Hill AF. Exosomes provide a protective and enriched source of mRNA for biomarker profiling compared to intracellular and cell-free blood. J Extracell Vesicles 2014; 3: [PMID: 24683445 DOI: 10.3402/ev.j.3.23743]

128 Khan S, Bennit HF, Wall NR. The emerging role of exosomes in survivin secretion. Histol Histopathol 2015; 30: 43-50 [PMID: 25020159 DOI: 10.14670/HH-30.43]

129 Wang N, Wang L, Yang Y, Gong L, Xiao B, Liu X. A serum exosomal microRNA panel as a potential biomarker test for gastric cancer. Biochem Biophys Res Commun 2017; 493: 1322-1328 [PMID: 28986250 DOI: 10.1016/j.bbrc.2017.10.003]

130 Tsuchiya M, Ichikawa Y, Kosaka N, Oikawa R, Morita R, Yoshida Y, Yamamoto H, Jelinek J, Issa JP. Sensitive and specific detection of early gastric cancer with DNA methylation analysis of gastric washes. Gastroenterology 2009; 136: 2149-2158 [PMID: 19375421 DOI: 10.1053/j.gastro.2009.02.085]

131 Yamamoto H, Watanabe Y, Oikawa R, Morita R, Yoshida Y, Maehata T, Yasuda H, Itoh F. BARHL2 Methylation Using Gastric Wash DNA or Gastric Juice Exosomal DNA is a Useful Marker For Early Detection of Gastric Cancer in an H. pylori-Independent Manner. Clin Transl Gastroenterol 2016; 7: e184 [PMID: 27441821 DOI: 10.1038/ctg.2016.40]

132 Virgilio E, Giacobbe E, Giovagnoli MR, Montagnini M, Proietti A, D’Urso R, Mercantini P, Balducci G, Cavallini M. Gastric Juice MicroRNAs as Potential Biomarkers for Screening Gastric Cancer: A Systematic Review. Anticancer Res 2018; 38: 613-616 [PMID: 29374683 DOI: 10.21873/antcancers.12265]

133 Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. Helicobacter pylori infection and the risk of gastric carcinoma. N Engl J Med 1991; 325: 1127-1131 [PMID: 1891020 DOI: 10.1056/NEJM1991101732515603]

134 Hatakeyama M. Structure and function of Helicobacter pylori CagA, the first-identified bacterial protein involved in human cancer. Proc Jpn Acad Ser B Phys Biol Sci 2017; 93: 196-219 [PMID: 28413197 DOI: 10.2183/pjab.93.013]

135 Yong X, Tang B, Li BS, Xie R, Hu CJ, Luo G, Qin Y, Dong H, Yang SM. Helicobacter pylori virulence factor CagA promotes tumorigenesis of gastric cancer via multiple signaling pathways. Cell Commun Signal 2015; 13: 30 [PMID: 26160167 DOI: 10.1186/s12964-015-0111-0]

136 Saju P, Murata-Kamiya N, Hayashi T, Senda Y, Nagase L, Noda S, Matussaka K, Funata S, Kunita A, Urabe M, Seto Y, Fukayama M, Kaneda A, Hatakeyama M. Host SHP1 phosphatase antagonizes Helicobacter pylori CagA and can be downregulated by Epstein-Barr virus. Nat Microbiol 2016; 1: 16026 [PMID: 27572445 DOI: 10.1038/nmicrobiol.2016.26]

137 Pormmohammad A, Ghotsaslou R, Leylabadlo HE, Nasiri MJ, Dabiri H, Hashemi A. Risk of gastric cancer in association with Helicobacter pylori different virulence factors: A systematic review and meta-analysis. Microb Pathog 2018; 118: 214-219 [PMID: 29510208 DOI: 10.1016/j.micpath.2018.03.004]

138 Attieri F, Di Stadio CS, Federico A, Miselli G, De Palma M, Rippa E, Arcari P. Epigenetic alterations of gastrokine 1 gene expression in gastric cancer. Oncotarget 2017; 8: 16899-16911 [PMID: 28129645 DOI: 10.18632/oncotarget.14817]

139 Verma R, Sharma PC. Next generation sequencing-based emerging trends in molecular biology of gastric cancer. Am J Cancer Res 2018; 8: 207-225 [PMID: 29511593]

P- Reviewer: Chen JQ, Hwang CM, Lin JY, Luyer MD
S- Editor: Gong ZM
E- Editor: Filipodia
