Biomarkers of gastric cancer: Current topics and future perspective

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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; Ccancer 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.

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 APLLIED 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, alpha-fetoprotein, 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 clinico-pathological 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[9]. 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%)[15-18].

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 capecitabine or 5-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 foci[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[19-21]. 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[Kip1] 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 applicated 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[35]. *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[36]. The E-cadherin gene can be inactivated by several mechanisms, including *CDH1* mutations, hyper-methylation, loss of heterozygosity (LOH), *H pylori* infection, transcriptional repression binding to the CDH1-E box element, and tyrosine phospholyration (*e.g.*, EGFR, MET and FGFR)[36]. Hereditary diffuse GC (HDGC) is an autosomal dominate cancer syndrome representing approximately 2% of all GCs[37]. 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[38]. 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 HDGC. Enhanced sensitivity to EGFR and PI3K kinase inhibition was induced by loss of E-cadherin/catenin-EGFR interaction in HDGC 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 *CDHI* alterations. This finding indicates that the presence of *CDH1* epigenetic and structural alterations in a diagnostic/preoperative biopsy may serve as clinically useful bio-marker[39]. A recent study examined the diagnostic role of promoter methylation status of CDH1 in blood samples of patients with GC[40]. 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[41]. The *PIK3CA* gene encoding the PI3K catalytic isoform p110α is the second most frequently mutated oncogene, and *PTEN* encoding the major phosphatidylisositol 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*[42,43]. Genetic deregulations in the PI3K/Akt/mTOR pathway have been identified frequently in GC. PI3K/Akt/mTOR expression has been associated with the lymph node status and poor survival[44]. The *PI3KCA* has been reported to be identified in 4%-25% of patients with GC[25]. 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[45]. 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[46].

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[47]. MET amplification and/or overexpression of its secreted protein has been reported to be involved in the carcinogenesis, therapy efficacy, and outcome of GC[48,49]. The measurement and assessment of HGF activity have been crucial role in understanding the tumor microenvironment that prompt tumor metastasis and drug resistance[47]. 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[50,51]. 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 tastuzumab compared with the pre-treated level[52]. 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[53].

Vascular endothelial growth factor: Several signal transduction pathways are proved to be associated with tumor-associated angiogenesis, including vascular endothelial growth factor (VEGF)[54]. 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[54]. 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[55].

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 VEGF-R2 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)[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 FOLFORI 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[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[59]. The mutational site of p53 in GC is wide and the reported incidence of p53 mutations ranges from 3.2% to 65%[60]. The incidence of p53 mutation was significantly lower in EBV-GC (n = 1) when compared with non-EBV-GCs (n = 10)[61]. TP53 mutation is identified most often in the intestinal type of GC[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[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[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[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[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 in those with non-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[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[68-70]. For genome analysis, cDNA microarrays and serial analysis of gene expression (SAGE) have been mainly utilized[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[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 downregulated[73]. Up-regulation of *CEACEM6, APOC1*, and *YF13H12* have been shown to be frequently up-regulated in GC[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 in GC[75]. 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[76]. 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[77]. A recent comprehensive analysis from Korea have found that more than 63% of the MSI-high GC identified the mutations within mononucleotide tracts in *TGFBR2, CEP164, MIS18BP1, RNPC3, KIAA2018, CNOT1* and *CCDC150* genes[78]. 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[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 longer 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 *TFPI2* 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 IL-1RN have a lot of functionally related polymorphism which is associated with the secretion of IL1-β. Existence of IL-1β and IL-1RN 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*, *ARID1A* and *PKHD*[91]. 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[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[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 radiation-induced cell death was found in the CD44-positive GC cells[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[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[99].

Circulating cell-free DNA

Circulating cell-free DNA (cfDNA) is cell-free extracellular DNA originating from normal and cancerous cells identicalable in the blood (the plasma or the serum)[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[100]. The potential diagnostic and /or prognostic values of quantifying cf-DNA in GC patients compared to the healthy controls, have been evaluates 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[101.] With regard to prognostic value, significantly poorer DFS and OS in patients were identified. A recent study described that serum APC promotor 1A and RASSF1A promoter hypermethylation in *cf*DNA was a frequent epigenetic event in patients with early operable GC[102]. Interestingly, *cf*DNA 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[103]. Tumor responses to lapatinib plus Capecitabine were closely related with changes in plasma-detected *ERBB2* copy number through serial cfDNA sequencing[26].

MicroRNA

Dysregulations in non-coding regulatory RNAs can contribute to cancer initiation and development[104]. 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[105-107]. MicroRNAs are key players in regulating several biological processes of the cell proliferation, differentiation, migration, and invasion[105].

Expression profiling of microRNAs have shown the distinctive signatures of these small regulatory RNAs in different cancers including GC[108]. Numerous microRNAs have been identified and recognized to be implicated in GC[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[110]. It is known that miRNAs can increase the expression of oncogenes or reduce the expression of tumor suppressor genes[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[108].

Recently, the research for miRNA as biomarker in human malignancies has facilitated because of the unique feature of miRNAs. Cell-free miRNAs (cfmiRNAs) 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 cfmiRNAs in serum are consistent with gastric tumor tissue[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[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[114]. Overexpression of six serum-based miRNAs (miR10b-5p, miR132-3p, miR185-5p, miR195-5p, miR-20a3p, and miR296-5p) was shown in GC compared with normal controls by using qRT-PCR-based Exiqon panel[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[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 (lncRNAs) are sequences of nucleotides longer than 200, that can function as oncogenic or tumor-suppressor[117]. The lncRNAs act as transcriptional mediator, splicing regulator, posttranscriptional processor, enhancer, molecular sponge for miRNAs, chromatin remodeler. The lncRNAs are frequently expressed in a disease‑ or developmental‑specific manner and thus submit potential as a biomarker[111]. Nowadays over 56000 human lncRNAs populating the human genome have been identified and about 135 lncRNAs have been recognized as dysregulated in GC, so they are closely related to tumorigenesis, metastases, and prognosis[117,118]. Impaired expression of ncRuPAR significantly associated with lymph node metastasis, distant metastasis, tumor size and TNM stage in patients with GC[119]. A downregulation in the expression of AI364715, GACAT1, and GACAT2 in GC tissues could also serve as a prognostic marker[120]. LncRNA PVT1 was markedly overexpressed in GC tissues compared with that in the normal control and could be an independent prognostic marker[121,122]. However, further studies about lncRNAs are needed in order to identify their possible clinical utilization.

Exosomes

Exosomes, small cell-derived vesicles, can protect RNAs and miRNAs, from being degraded[123-127]. When exosomes were exposed to RNase the contained RNAs were protected from degradation while cellular RNA was degraded by the same RNase[126]. Exosomes hold great potential for both diagnosis and prognosis of diseases and are exceptionally useful as cancer biomarkers[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[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[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%)[131]. As well, BARHL2 methylation in gastric wash DNA or gastric juice exosomal DNA significantly attenuated after endoscopic resection, suggesting that BARHL2 methylation could be useful for predicting tumor relapse[132]. 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[121]. *Gastric juice miR-421, miR-21, miR-106a and miR-129 represent a potential biomarker for screening GC*[133].

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[134]. *H. pylori* Cytotoxin-associated gene A (CagA) is the first identified bacterial protein playing a positive role in the progression of GC[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, JNK, NF-κB, Hedgehog, JAK/ATAT has been identified, which results in the carcinogenesis of GC[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 *PTPN6* hypermethylation[137]. In similar, *H. pylori* producing another bacterial toxin vacuolating toxin A (vacA) infection were meaningfully associated with increased risk of GC[138].

Gastrokine 1 (GKN1) is a tissue-specific 18 kDa protein that significantly expressed in gastric tissue and

Table 1 Current topics of molecular markers associated with diagnosis, prognosis, prediction of therapeutic response of gastric cancer

Marker	Alteration	Clinical purpose	Detection method	Ref.
Metastasis related genes				
Growth factors				
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				
TP53	Mutation	Diagnostic	Tissue	[60,61,63]
Adhesion molecule				
E-cadherin (*CDH1*)	Mutation/epigenetic alteration	Diagnostic/prognostic	Tissue/blood	[39,40]
Immune checkpoint				
PD-L1	Mutation	Prognostic/therapeutic	Tissue	[66,67]
Comprehensive gene analysis				
CEACEM6, APOC1, YF13H12, CDH17, REG4, OLFM4, HOXA10, DSC2, TSPAN8, TM9SF3, FUS, COLIA1, COLIA2, APOE	Up-regulated	Diagnostic/prognostic/ therapeutic	Tissue	[74,75]
ATP4B, S100A9, CYP20A1, ARPC3, DDX5 CLDN18	Down-regulated	Diagnostic/prognostic/ therapeutic	Tissue	[74,75]
Microsatellite instability	High level	Prognostic/therapeutic	Tissue	[79,81,82]
Epigenetic alterations				
CDH1, CHFR, DAPK, GSTP1, p15, p16, RARβ, RASSF1A, RUNX3, TFPI2	Hypermethylation	Diagnostic	Tissue	[84-86]
Genetic polymorphism				
IL1-β, IL-1RN, CD44	SNP	Prognostic	Tissue	[89,90]
TP53, SYNE1, CSMD3, LRP1B, CDH1, PIK3CA, ARID1A, PKHD, KRAS, JAK2, CD274, PDCD1LG2	Copy number variations/ mutations	Diagnostic/prognostic/ therapeutic	Tissue	[91,92]
Circulating tumor cells				
CD44, N-cadherin, vimentin	Overexpression	Diagnostic/therapeutic	Blood	[96]
pan-CK, E-cadherin	Decreased expression	EMT process	Blood	[97]
HER2	Overexpression	Therapeutic	Blood	[99]
Circulating cell-free DNA				
APC promotor 1, RASSF1A	Hypermethylation	Diagnostic	Blood/plasma	[102]
ERBB2	Copy number variations	Therapeutic	Plasma	[26]
MicroRNA				
miR-21, miR-23a, miR-27a, miR-106b-25, miR-130b, miR-199a, miR-215, miR-222-221, miR-370	Up-regulated	Diagnostic/prognostic/ therapeutic	Blood/plasma	[108,111]
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	Up-regulated	Diagnostic/prognostic/ therapeutic	Blood/plasma	[108,111]
Cell-free miRNAs				
miR-331 and miR-21	Up-regulated	Diagnostic/Prognostic	Blood	[113]
miR-20b, 125a, 137, 141, 146a, 196a, 206, 218, 486-5p	Up-regulated	Prognostic	Blood/plasma	[114]
miR10b-5p, miR132-3p, miR185-5p, miR195-5p, miR-20a3p, miR296-5p	Up-regulated	Prognostic	Plasma	[115]
Long noncoding RNAs				
ncRuPAR	Down-regulated	Diagnostic/prognostic	Tissue	[119]
AI364715, GACAT1, GACAT2	Down-regulated	Prognostic	Tissue	[120]
PVT1	Up-regulated	Prognostic	Tissue	[121]
Exosomes				
MiR-19b, miR-106a	Up-regulated	Diagnostic/prognostic	Plasma	[129]
miR-21, miR-1225-5p	Up-regulated	Diagnostic/therapeutic	PLF	[130]
Stomach specific biomarker				
ADAM23, GDNF, MINT25, MLF1, PRDM5, RORA	Hypermethylation	Diagnostic	Gastric wash	[131]
BARHL2	Hypermethylation	Diagnostic/therapeutic	Gastric wash/juice	[132]
PVT1	Up-regulated	Diagnostic/prognostic	Gastric juice	[121]
miR-421, miR-21, miR-106a, miR-129	Up-regulated	Diagnostic	Gastric juice	[133]
CagA	Up-regulated	Diagnostic	Tissue	[137]
VacA	Up-regulated	Diagnostic	Tissue	[138]
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[139]. 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[140]. 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[67]. 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 acts 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/caac.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]

4   **Asao T**, Fukuda T, Yazawa S, Nagamachi Y. Carcinoembryonic antigen levels in peritoneal washings can predict peritoneal recurrence after curative resection of gastric cancer. *Cancer* 1991; **68**: 44-47 [PMID: 2049751 DOI: 10.1002/1097-0142(19910701)68: 1<44::AID-CNCR2820680109>3.0.CO;2-J]

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.bbagen.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. *Am J Surg* 2001; **181**: 16-19 [PMID: 11248169 DOI: 10.1016/S0002-9610(00)00549-3]

8   **Song YX**, 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**, Amemiya H, Sekikawa T, Iizuka 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: 10.1159/000065838]

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: 10364040 DOI: 10.1111/j.1572-0241.1999.01158.x]

11   **Namikawa T**, Kawanishi Y, Fujisawa K, Munekage E, Iwabu J, Munekage M, Maeda H, Kitagawa H, Kobayashi M, Hanazaki 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/s00595-017-1598-3]

12   **Akiyama T**, Sudo C, Ogawara 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, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Rüschoff J, Kang YK; ToGA Trial Investigators. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet* 2010; **376**: 687-697 [PMID: 20728210 DOI: 10.1016/S0140-6736(10)61121-X]

14   **Sheng WQ**, Huang D, Ying JM, Lu N, Wu HM, Liu YH, Liu JP, Bu H, Zhou XY, Du X. HER2 status in gastric cancers: a retrospective analysis from four Chinese representative clinical centers and assessment of its prognostic significance. *Ann Oncol* 2013; **24**: 2360-2364 [PMID: 23788757 DOI: 10.1093/annonc/mdt232]

15   **Gravalos C**, Jimeno A. HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target. *Ann Oncol* 2008; **19**: 1523-1529 [PMID: 18441328 DOI: 10.1093/annonc/mdn169]

16   **Moelans CB**, van Diest PJ, Milne AN, Offerhaus GJ. Her-2/neu testing and therapy in gastroesophageal adenocarcinoma. *Pathol Res Int* 2010; **2011**: 674182 [PMID: 21188213 DOI: 10.4061/2011/674182]

17   **Janjigian YY**, Werner D, Pauligk C, Steinmetz K, Kelsen DP, Jäger E, Altmannsberger HM, Robinson E, Tafe LJ, Tang LH, Shah MA, Al-Batran SE. Prognosis of metastatic gastric and gastroesophageal junction cancer by HER2 status: a European and USA International collaborative analysis. *Ann Oncol* 2012; **23**: 2656-2662 [PMID: 22689179 DOI: 10.1093/annonc/mds104]

18   **Bozzetti C**, Negri FV, Lagrasta CA, Crafa P, Bassano C, Tamagnini I,

Gardini G, Nizzoli R, Leonardi F, Gasparro D, Camisa R, Cavalli S, Silini EM, Ardizzoni A. Comparison of HER2 status in primary and paired metastatic sites of gastric carcinoma. *Br J Cancer* 2011; **104**: 1372-1376 [PMID: 21487407 DOI: 10.1038/bjc.2011.121]

19   **Kang YK**, Rha SY, Tassone P, Barriuso J, Yu R, Szado T, Garg A, Bang YJ. A phase IIa dose-finding and safety study of first-line pertuzumab in combination with trastuzumab, capecitabine and cisplatin in patients with HER2-positive advanced gastric cancer. *Br J Cancer* 2014; **111**: 660-666 [PMID: 24960402 DOI: 10.1038/bjc.2014.356]

20   **Shimoyama S**. Unraveling trastuzumab and lapatinib inefficiency in gastric cancer: Future steps (Review). *Mol Clin Oncol* 2014; **2**: 175-181 [PMID: 24649329 DOI: 10.3892/mco.2013.218]

21   **Yamashita-Kashima Y**, Shu S, Harada N, Fujimoto-Ouchi K. Enhanced antitumor activity of trastuzumab emtansine (T-DM1) in combination with pertuzumab in a HER2-positive gastric cancer model. *Oncol Rep* 2013; **30**: 1087-1093 [PMID: 23783223 DOI: 10.3892/or.2013.2547]

22   **Matsuoka T**, Yashiro M. Recent advances in the HER2 targeted therapy of gastric cancer. *World J Clin Cases* 2015; **3**: 42-51 [PMID: 25610849 DOI: 10.12998/wjcc.v3.i1.42]

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

24   **Oyama K**, Fushida S, Tsukada T, Kinoshita J, Watanabe T, Shoji M, Nakanuma S, Okamoto K, Sakai S, Makino I, Nakamura K, Hayashi H, Inokuchi M, Nakagawara H, Miyashita T, Tajima H, Takamura H, Ninomiya I, Kitagawa H, Fujimura T, Tajiri R, Ooi A, Ohta T. Evaluation of serum HER2-ECD levels in patients with gastric cancer. *J Gastroenterol* 2015; **50**: 41-45 [PMID: 24557054 DOI: 10.1007/s00535-014-0941-3]

25   **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]

26   **Kim ST**, Banks KC, Pectasides E, Kim SY, Kim K, Lanman RB, Talasaz A, An J, Choi MG, Lee JH, Sohn TS, Bae JM, Kim S, Park SH, Park JO, Park YS, Lim HY, Kim NKD, Park W, Lee H, Bass AJ, Kim K, Kang WK, Lee J. Impact of genomic alterations on lapatinib treatment outcome and cell-free genomic landscape during HER2 therapy in HER2+ gastric cancer patients. *Ann Oncol* 2018; **29**: 1037-1048 [PMID: 29409051 DOI: 10.1093/annonc/mdy034]

27   **Hamashima C**, Shibuya D, Yamazaki H, Inoue K, Fukao A, Saito H, Sobue T. The Japanese guidelines for gastric cancer screening. *Jpn J Clin Oncol* 2008; **38**: 259-267 [PMID: 18344316 DOI: 10.1093/jjco/hyn017]

28   **Nobili S**, Bruno L, Landini I, Napoli C, Bechi P, Tonelli F, Rubio CA, Mini E, Nesi G. Genomic and genetic alterations influence the progression of gastric cancer. *World J Gastroenterol* 2011; **17**: 290-299 [PMID: 21253387 DOI: 10.3748/wjg.v17.i3.290]

29   **Leung WK**, Wu MS, Kakugawa Y, Kim JJ, Yeoh KG, Goh KL, Wu KC, Wu DC, Sollano J, Kachintorn U, Gotoda T, Lin JT, You WC, Ng EK, Sung JJ; Asia Pacific Working Group on Gastric Cancer. Screening for gastric cancer in Asia: current evidence and practice. *Lancet Oncol* 2008; **9**: 279-287 [PMID: 18308253 DOI: 10.1016/S1470-2045(08)70072-X]

30   **Yashiro M**, Matsuoka T. Fibroblast growth factor receptor signaling as therapeutic targets in gastric cancer. *World J Gastroenterol* 2016; **22**: 2415-2423 [PMID: 26937130 DOI: 10.3748/wjg.v22.i8.2415]

31   **Greulich H**, Pollock PM. Targeting mutant fibroblast growth factor receptors in cancer. *Trends Mol Med* 2011; **17**: 283-292 [PMID: 21367659 DOI: 10.1016/j.molmed.2011.01.012]

32   **Nagatsuma AK**, Aizawa M, Kuwata T, Doi T, Ohtsu A, Fujii H, Ochiai A. Expression profiles of HER2, EGFR, MET and FGFR2 in a large cohort of patients with gastric adenocarcinoma. *Gastric Cancer* 2015; **18**: 227-238 [PMID: 24626858 DOI: 10.1007/s10120-014-0360-4]

33   **Betts G**, Valentine H, Pritchard S, Swindell R, Williams V, Morgan S, Griffiths EA, Welch I, West C, Womack C. FGFR2, HER2 and cMet in gastric adenocarcinoma: detection, prognostic significance and

assessment of downstream pathway activation. *Virchows Arch* 2014; **464**: 145-156 [PMID: 24306956 DOI: 10.1007/s00428-013-1517-y]

34 **Hosoda K**, Yamashita K, Ushiku H, Ema A, Moriya H, Mieno 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, Figueiredo J, Caldeira J, Carvalho J, Pinheiro H, Leite M, Melo S, Oliveira P, Simões-Correia J, Oliveira MJ, Carneiro F, Figueiredo 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: 22841718 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, Wiyrick 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, Guo 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: 21376230 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.cco.0000198021.99347.b9]

43 **Song MS**, Salmena L, Pandolfi PP. 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 YB, Sun XW, Guan YX, Li YF, Lin TY. Activated mammalian target of rapamycin is a potential therapeutic target in gastric cancer. *BMC Cancer* 2010; **10**: 536 [PMID: 20929525 DOI: 10.1186/1471-2407-10-536]

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

46 **Ito C**, Nishizuka SS, Ishida K, Uesugi 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**, Umitsu 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, Bearzi I, Humar B, More H, Silva R, Muretto P, Guilford P, Testa E, Mari D, Magnani M, Cascinu S. Prognostic analysis of E-cadherin gene promoter hypermethylation in patients with surgically resected, node-positive, diffuse gastric cancer. *Clin Cancer Res* 2004; **10**: 2784-2789 [PMID: 15102685 DOI: 10.1158/1078-0432.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]

50 **Noguchi E**, Saito N, Kobayashi M, Kameoka S. Clinical significance of hepatocyte growth factor/c-Met expression in the assessment of gastric cancer progression. *Mol Med Rep* 2015; **11**: 3423-3431 [PMID: 25592281 DOI: 10.3892/mmr.2015.3205]

51 **Huang X**, Wang C, Sun J, Luo J, You J, Liao L, Li M. Clinical value of CagA, c-Met, PI3K and Beclin-1 expressed in gastric cancer and their association with prognosis. *Oncol Lett* 2018; **15**: 947-955 [PMID: 29422968 DOI: 10.3892/ol.2017.7394]

52 **Takahashi N**, Furuta K, Taniguchi H, Sasaki Y, Shoji H, Honma Y, Iwasa S, Okita N, Takashima A, Kato K, Hamaguchi T, Shimada Y, Yamada Y. Serum level of hepatocyte growth factor is a novel marker of predicting the outcome and resistance to the treatment with trastuzumab in HER2-positive patients with metastatic gastric cancer. *Oncotarget* 2016; **7**: 4925-4938 [PMID: 26716644 DOI: 10.18632/oncotarget.6753]

53 **Yashiro M**, Nishii T, Hasegawa T, Matsuzaki T, Morisaki T, Fukuoka T, Hirakawa K. A c-Met inhibitor increases the chemosensitivity of cancer stem cells to the irinotecan in gastric carcinoma. *Br J Cancer* 2013; **109**: 2619-2628 [PMID: 24129235 DOI: 10.1038/bjc.2013.638]

54 **Ylä-Herttuala S**, Rissanen TT, Vajanto I, Hartikainen J. Vascular endothelial growth factors: biology and current status of clinical applications in cardiovascular medicine. *J Am Coll Cardiol* 2007; **49**: 1015-1026 [PMID: 17349880 DOI: 10.1016/j.jacc.2006.09.053]

55 **Lieto E**, Ferraraccio F, Orditura M, Castellano P, Mura AL, Pinto M, Zamboli A, De Vita F, Galizia G. Expression of vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) is an independent prognostic indicator of worse outcome in gastric cancer patients. *Ann Surg Oncol* 2008; **15**: 69-79 [PMID: 17896140 DOI: 10.1245/s10434-007-9596-0]

56 **Fuchs CS**, Tomasek J, Yong CJ, Dumitru F, Passalacqua R, Goswami C, Safran H, Dos Santos LV, Aprile G, Ferry DR, Melichar B, Tehfe M, Topuzov E, Zalcberg JR, Chau I, Campbell W, Sivanandan C, Pikiel J, Koshiji M, Hsu Y, Liepa AM, Gao L, Schwartz JD, Tabernero J; REGARD Trial Investigators. Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): an international, randomised, multicentre, placebo-controlled, phase 3 trial. *Lancet* 2014; **383**: 31-39 [PMID: 24094768 DOI: 10.1016/S0140-6736(13)61719-5]

57 **Wilke H**, Muro K, Van Cutsem E, Oh SC, Bodoky G, Shimada Y, Hironaka S, Sugimoto N, Lipatov O, Kim TY, Cunningham D, Rougier P, Komatsu Y, Ajani J, Emig M, Carlesi R, Ferry D, Chandrawansa K, Schwartz JD, Ohtsu A; RAINBOW Study Group. Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (RAINBOW): a double-blind, randomised phase 3 trial. *Lancet Oncol* 2014; **15**: 1224-1235 [PMID: 25240821 DOI: 10.1016/S1470-2045(14)70420-6]

58 **Tabernero J**, Hozak RR, Yoshino T, Cohn AL, Obermannova R, Bodoky G, Garcia-Carbonero R, Ciuleanu TE, Portnoy DC, Prausová J, Muro K, Siegel RW, Konrad RJ, Ouyang H, Melemed SA, Ferry D, Nasroulah F, Van Cutsem E. Analysis of angiogenesis biomarkers for ramucirumab efficacy in patients with metastatic colorectal cancer from RAISE, a global, randomized, double-blind, phase Ⅲ study. *Ann Oncol* 2018; **29**: 602-609 [PMID: 29228087 DOI: 10.1093/annonc/mdx767]

59 **Levine AJ**. p53, the cellular gatekeeper for growth and division. *Cell* 1997; **88**: 323-331 [PMID: 9039259 DOI: 10.1016/S0092-8674(00)81871-1]

60 **Oki E**, Zhao Y, Yoshida R, Egashira A, Ohgaki K, Morita M, Kakeji Y, Maehara Y. The difference in p53 mutations between cancers of the upper and lower gastrointestinal tract. *Digestion* 2009; **79** Suppl 1: 33-39 [PMID: 19153488 DOI: 10.1159/000167864]

61 **Lee J**, van Hummelen P, Go C, Palescandolo E, Jang J, Park HY, Kang SY, Park JO, Kang WK, MacConaill L, Kim KM. High-throughput mutation profiling identifies frequent somatic mutations in advanced gastric adenocarcinoma. *PLoS One* 2012; **7**: e38892 [PMID: 22723903 DOI: 10.1371/journal.pone.0038892]

62 **Endoh Y**, Sakata K, Tamura G, Ohmura K, Ajioka Y, Watanabe H, Motoyama T. Cellular phenotypes of differentiated-type adenocarcinomas and precancerous lesions of the stomach are

dependent on the genetic pathways. *J Pathol* 2000; **191**: 257-263 [PMID: 10878546 DOI: 10.1002/1096-9896(2000)9999:9999<:: AID-PATH631>3.0.CO;2-2]

63   **Li QF**, Yao RY, Liu KW, Lv HY, Jiang T, Liang J. Genetic polymorphism of GSTP1: prediction of clinical outcome to oxaliplatin/5-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: 17304234 DOI: 10.1038/ni1443]

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* 2017; **12**: e0182692 [PMID: 28796808 DOI: 10.1371/journal.pone.0182692]

66   **Curea FG**, Hebbar M, Ilie SM, Bacinschi XE, Trifanescu OG, Botnariuc I, Anghel RM. Current Targeted Therapies in HER2- Positive Gastric Adenocarcinoma. *Cancer Biother Radiopharm* 2017; **32**: 351-363 [PMID: 29265917 DOI: 10.1089/cbr.2017.2249]

67   **Cancer Genome Atlas Research Network**. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014; **513**: 202-209 [PMID: 25079317 DOI: 10.1038/nature13480]

68   **Cho JY**, Lim JY, Cheong JH, Park YY, Yoon SL, Kim SM, Kim SB, Kim H, Hong SW, Park YN, Noh SH, Park ES, Chu IS, Hong WK, Ajani JA, Lee JS. Gene expression signature-based prognostic risk score in gastric cancer. *Clin Cancer Res* 2011; **17**: 1850-1857 [PMID: 21447720 DOI: 10.1158/1078-0432.CCR-10-2180]

69   **Cui J**, Chen Y, Chou WC, Sun L, Chen L, Suo J, Ni Z, Zhang M, Kong X, Hoffman LL, Kang J, Su Y, Olman V, Johnson D, Tench DW, Amster IJ, Orlando R, Puett D, Li F, Xu Y. An integrated transcriptomic and computational analysis for biomarker identification in gastric cancer. *Nucleic Acids Res* 2011; **39**: 1197-1207 [PMID: 20965966 DOI: 10.1093/nar/gkq960]

70   **Hippo Y**, Taniguchi H, Tsutsumi S, Machida N, Chong JM, Fukayama M, Kodama T, Aburatani H. Global gene expression analysis of gastric cancer by oligonucleotide microarrays. *Cancer Res* 2002; **62**: 233-240 [PMID: 11782383]

71   **DeRisi J**, Penland L, Brown PO, Bittner ML, Meltzer PS, Ray M, Chen Y, Su YA, Trent JM. Use of a cDNA microarray to analyse gene expression patterns in human cancer. *Nat Genet* 1996; **14**: 457-460 [PMID: 8944026 DOI: 10.1038/ng1296-457]

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 N, Biondi A, Gasbarrini A, D'Ugo D. cDNA-microarray analysis as a new tool to predict lymph node metastasis in gastric cancer. *World J Surg* 2014; **38**: 2058-2064 [PMID: 24696059 DOI: 10.1007/ s00268-014-2529-8]

74   **Yasui W**, Oue N, Ito R, Kuraoka K, Nakayama H. Search for new biomarkers of gastric cancer through serial analysis of gene expression and its clinical implications. *Cancer Sci* 2004; **95**: 385-392 [PMID: 15132764 DOI: 10.1111/j.1349-7006.2004. tb03220.x]

75   **Oue N**, Sentani K, Sakamoto N, Yasui W. Clinicopathologic and molecular characteristics of gastric cancer showing gastric and intestinal mucin phenotype. *Cancer Sci* 2015; **106**: 951-958 [PMID: 26033320 DOI: 10.1111/cas.12706]

76   **Aaltonen LA**, Peltomäki P, Leach FS, Sistonen P, Pylkkänen L, Mecklin JP, Järvinen H, Powell SM, Jen J, Hamilton SR. Clues to the pathogenesis of familial colorectal cancer. *Science* 1993; **260**: 812-816 [PMID: 8484121 DOI: 10.1126/science.8484121]

77   **Pinto M**, Oliveira C, Machado JC, Cirnes L, Tavares J, Carneiro F, Hamelin R, Hofstra R, Seruca R, Sobrinho-Simões M. MSI-L gastric carcinomas share the hMLH1 methylation status of MSI-H carcinomas but not their clinicopathological profile. *Lab Invest* 2000; **80**: 1915-1923 [PMID: 11140703 DOI: 10.1038/labinvest.3780201]

78   **Yoon K**, Lee S, Han TS, Moon SY, Yun SM, Kong SH, Jho S, Choe J, Yu J, Lee HJ, Park JH, Kim HM, Lee SY, Park J, Kim WH, Bhak J, Yang HK, Kim SJ. Comprehensive genome- and transcriptome- wide analyses of mutations associated with microsatellite instability in Korean gastric cancers. *Genome Res* 2013; **23**: 1109-1117 [PMID: 23737375 DOI: 10.1101/gr.145706.112]

79   **Zang ZJ**, Cutcutache I, Poon SL, Zhang SL, McPherson JR, Tao J, Rajasegaran V, Heng HL, Deng N, Gan A, Lim KH, Ong CK, Huang D, Chin SY, Tan IB, Ng CC, Yu W, Wu Y, Lee M, Wu J, Poh D, Wan WK, Rha SY, So J, Salto-Tellez M, Yeoh KG, Wong WK, Zhu YJ, Futreal PA, Pang B, Ruan Y, Hillmer AM, Bertrand D, Nagarajan N, Rozen S, Teh BT, Tan P. Exome sequencing of gastric adenocarcinoma identifies recurrent somatic mutations in cell adhesion and chromatin remodeling genes. *Nat Genet* 2012; **44**: 570-574 [PMID: 22484628 DOI: 10.1038/ng.2246]

80   **Shokal U**, Sharma PC. Implication of microsatellite instability in human gastric cancers. *Indian J Med Res* 2012; **135**: 599-613 [PMID: 22771588]

81   **dos Santos NR**, Seruca R, Constância M, Seixas M, Sobrinho- Simões M. Microsatellite instability at multiple loci in gastric carcinoma: clinicopathologic implications and prognosis. *Gastroenterology* 1996; **110**: 38-44 [PMID: 8536886 DOI: 10.1053/ gast.1996.v110.pm8536886]

82   **Smyth EC**, Wotherspoon A, Peckitt C, Gonzalez D, Hulkki-Wilson S, Eltahir Z, Fassan M, Rugge M, Valeri N, Okines A, Hewish M, Allum W, Stenning S, Nankivell M, Langley R, Cunningham D. Mismatch Repair Deficiency, Microsatellite Instability, and Survival: An Exploratory Analysis of the Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) Trial. *JAMA Oncol* 2017; **3**: 1197-1203 [PMID: 28241187 DOI: 10.1001/ jamaoncol.2016.6762]

83   **Kim H**, Wang X, Jin P. Developing DNA methylation-based diagnostic biomarkers. *J Genet Genomics* 2018; **45**: 87-97 [PMID: 29496486 DOI: 10.1016/j.jgg.2018.02.003]

84   **Qu Y**, Dang S, Hou P. Gene methylation in gastric cancer. *Clin Chim Acta* 2013; **424**: 53-65 [PMID: 23669186 DOI: 10.1016/ j.cca.2013.05.002]

85   **Sapari NS**, Loh M, Vaithilingam A, Soong R. Clinical potential of DNA methylation in gastric cancer: a meta-analysis. *PLoS One* 2012; **7**: e36275 [PMID: 22558417 DOI: 10.1371/journal.pone.0036275]

86   **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]

87   **Yoshida S**, Yamashita S, Niwa T, Mori A, Ito S, Ichinose M, Ushijima T. Epigenetic inactivation of FAT4 contributes to gastric field cancerization. *Gastric Cancer* 2017; **20**: 136-145 [PMID: 26792292 DOI: 10.1007/s10120-016-0593-5]

88   **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]

89   **Drici Ael-M**, Moulessehoul S, Tifrit A, Diaf M, Turki DK, Bachir M, Tou A. Effect of IL-1β and IL-1RN polymorphisms in carcinogenesis of the gastric mucosa in patients infected with Helicobacter pylori in Algeria. *Libyan J Med* 2016; **11**: 31576 [PMID: 27340011 DOI: 10.3402/ljm.v11.31576]

90   **Suenaga M**, Yamada S, Fuchs BC, Fujii T, Kanda M, Tanaka C, Kobayashi D, Fujiwara M, Tanabe KK, Kodera Y. CD44 single nucleotide polymorphism and isoform switching may predict gastric cancer recurrence. *J Surg Oncol* 2015; **112**: 622-628 [PMID: 26416034 DOI: 10.1002/jso.24056]

91   **Kuboki Y**, Yamashita S, Niwa T, Ushijima T, Nagatsuma A, Kuwata 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: 10.1093/annonc/mdv508]

92   **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**: 37 [PMID: 26251698 DOI: 10.1186/s13742-015-0068-3]

93 **Siravegna G**, Marsoni S, Siena S, Bardelli A. Integrating liquid biopsies into the management of cancer. *Nat Rev Clin Oncol* 2017; **14**: 531-548 [PMID: 28252003 DOI: 10.1038/nrclinonc.2017.14]

94 **Allard WJ**, Matera J, Miller MC, Repollet M, Connelly MC, Rao C, Tibbe AG, Uhr JW, Terstappen LW. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. *Clin Cancer Res* 2004; **10**: 6897-6904 [PMID: 15501967 DOI: 10.1158/1078-0432. CCR-04-0378]

95 **Glaves D**. Correlation between circulating cancer cells and incidence of metastases. *Br J Cancer* 1983; **48**: 665-673 [PMID: 6639858 DOI: 10.1038/bjc.1983.248]

96 **Huang X**, Gao P, Sun J, Chen X, Song Y, Zhao J, Xu H, Wang Z. Clinicopathological and prognostic significance of circulating tumor cells in patients with gastric cancer: a meta-analysis. *Int J Cancer* 2015; **136**: 21-33 [PMID: 24803400 DOI: 10.1002/ijc.28954]

97 **Takaishi S**, Okumura T, Tu S, Wang SS, Shibata W, Vigneshwaran R, Gordon SA, Shimada Y, Wang TC. Identification of gastric cancer stem cells using the cell surface marker CD44. *Stem Cells* 2009; **27**: 1006-1020 [PMID: 19415765 DOI: 10.1002/stem.30]

98 **Yuan D**, Xia H, Zhang Y, Chen L, Leng W, Chen T, Chen Q, Tang Q, Mo X, Liu M, Bi F. P-Akt/miR-200 signaling regulates epithelial-mesenchymal transition, migration and invasion in circulating gastric tumor cells. *Int J Oncol* 2014; **45**: 2430-2438 [PMID: 25200917 DOI: 10.3892/ijo.2014.2644]

99 **Mishima Y**, Matsusaka S, Chin K, Mikuniya M, Minowa S, Takayama T, Shibata H, Kuniyoshi R, Ogura M, Terui Y, Mizunuma N, Hatake K. Detection of HER2 Amplification in Circulating Tumor Cells of HER2-Negative Gastric Cancer Patients. *Target Oncol* 2017; **12**: 341-351 [PMID: 28508152 DOI: 10.1007/s11523-017-0493-6]

100 **Qi Q**, Pan YF, Shen JJ, Gu XQ, Han SW, Liao HH, Jiang YZ, Zhong LP. Circulating DNA for detection of gastric cancer. *Eur Rev Med Pharmacol Sci* 2016; **20**: 2558-2564 [PMID: 27383305]

101 **Gao Y**, Zhang K, Xi H, Cai A, Wu X, Cui J, Li J, Qiao Z, Wei B, Chen L. Diagnostic and prognostic value of circulating tumor DNA in gastric cancer: a meta-analysis. *Oncotarget* 2017; **8**: 6330-6340 [PMID: 28009985 DOI: 10.18632/oncotarget.14064]

102 **Balgkouranidou I**, Matthaios D, Karayiannakis A, Bolanaki H, Michailidis P, Xenidis N, Amarantidis K, Chelis L, Trypsianis G, Chatzaki E, Lianidou ES, Kakolyris S. Prognostic role of APC and RASSF1A promoter methylation status in cell free circulating DNA of operable gastric cancer patients. *Mutat Res* 2015; **778**: 46-51 [PMID: 26073472 DOI: 10.1016/j.mrfmmm.2015.05.002]

103 **Shoda K**, Ichikawa D, Fujita Y, Masuda K, Hiramoto H, Hamada J, Arita T, Konishi H, Kosuga T, Komatsu S, Shiozaki A, Okamoto K, Imoto I, Otsuji E. Clinical utility of circulating cell-free Epstein-Barr virus DNA in patients with gastric cancer. *Oncotarget* 2017; **8**: 28796-28804 [PMID: 28430637 DOI: 10.18632/oncotarget.15675]

104 **Calin GA**, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer* 2006; **6**: 857-866 [PMID: 17060945 DOI: 10.1038/nrc1997]

105 **Han TS**, Hur K, Xu G, Choi B, Okugawa Y, Toiyama Y, Oshima H, Oshima M, Lee HJ, Kim VN, Chang AN, Goel A, Yang HK. MicroRNA-29c mediates initiation of gastric carcinogenesis by directly targeting ITGB1. *Gut* 2015; **64**: 203-214 [PMID: 24870620 DOI: 10.1136/gutjnl-2013-306640]

106 **Su ZX**, Zhao J, Rong ZH, Wu YG, Geng WM, Qin CK. Diagnostic and prognostic value of circulating miR-18a in the plasma of patients with gastric cancer. *Tumour Biol* 2014; **35**: 12119-12125 [PMID: 25416437 DOI: 10.1007/s13277-014-2516-6]

107 **Xu L**, Hou Y, Tu G, Chen Y, Du YE, Zhang H, Wen S, Tang X, Yin J, Lang L, Sun K, Yang G, Tang X, Liu M. Nuclear Drosha enhances cell invasion via an EGFR-ERK1/2-MMP7 signaling pathway induced by dysregulated miRNA-622/197 and their targets LAMC2 and CD82 in gastric cancer. *Cell Death Dis* 2017; **8**: e2642 [PMID: 28252644 DOI: 10.1038/cddis.2017.5]

108 **Wu HH**, Lin WC, Tsai KW. Advances in molecular biomarkers for gastric cancer: miRNAs as emerging novel cancer markers. *Expert Rev Mol Med* 2014; **16**: e1 [PMID: 24456939 DOI: 10.1017/erm.2013.16]

109 **Liu HS**, Xiao HS. MicroRNAs as potential biomarkers for gastric cancer. *World J Gastroenterol* 2014; **20**: 12007-12017 [PMID: 25232237 DOI: 10.3748/wjg.v20.i34.12007]

110 **Korpal M**, Lee ES, Hu G, Kang Y. The miR-200 family inhibits epithelial-mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2. *J Biol Chem* 2008; **283**: 14910-14914 [PMID: 18411277 DOI: 10.1074/jbc.C800074200]

111 **Zhu X**, Lv M, Wang H, Guan W. Identification of circulating microRNAs as novel potential biomarkers for gastric cancer detection: a systematic review and meta-analysis. *Dig Dis Sci* 2014; **59**: 911-919 [PMID: 24337687 DOI: 10.1007/s10620-013-2970-9]

112 **Mitchell PS**, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci U S A* 2008; **105**: 10513-10518 [PMID: 18663219 DOI: 10.1073/pnas.0804549105]

113 **Sierzega M**, Kaczor M, Kolodziejczyk P, Kulig J, Sanak M, Richter P. Evaluation of serum microRNA biomarkers for gastric cancer based on blood and tissue pools profiling: the importance of miR-21 and miR-331. *Br J Cancer* 2017; **117**: 266-273 [PMID: 28641313 DOI: 10.1038/bjc.2017.190]

114 **Zhang Y**, Guan DH, Bi RX, Xie J, Yang CH, Jiang YH. Prognostic value of microRNAs in gastric cancer: a meta-analysis. *Oncotarget* 2017; **8**: 55489-55510 [PMID: 28903436 DOI: 10.18632/oncotarget.18590]

115 **Huang Z**, Zhu D, Wu L, He M, Zhou X, Zhang L, Zhang H, Wang W, Zhu J, Cheng W, Chen Y, Fan Y, Qi L, Yin Y, Zhu W, Shu Y, Liu P. Six Serum-Based miRNAs as Potential Diagnostic Biomarkers for Gastric Cancer. *Cancer Epidemiol Biomarkers Prev* 2017; **26**: 188-196 [PMID: 27756776 DOI: 10.1158/1055-9965.EPI-16-0607]

116 **Baniak N**, Senger JL, Ahmed S, Kanthan SC, Kanthan R. Gastric biomarkers: a global review. *World J Surg Oncol* 2016; **14**: 212 [PMID: 27514667 DOI: 10.1186/s12957-016-0969-3]

117 **Fang XY**, Pan HF, Leng RX, Ye DQ. Long noncoding RNAs: novel insights into gastric cancer. *Cancer Lett* 2015; **356**: 357-366 [PMID: 25444905 DOI: 10.1016/j.canlet.2014.11.005]

118 **Song H**, Sun W, Ye G, Ding X, Liu Z, Zhang S, Xia T, Xiao B, Xi Y, Guo J. Long non-coding RNA expression profile in human gastric cancer and its clinical significances. *J Transl Med* 2013; **11**: 225 [PMID: 24063685 DOI: 10.1186/1479-5876-11-225]

119 **Liu L**, Yan B, Yang Z, Zhang X, Gu Q, Yue X. ncRuPAR inhibits gastric cancer progression by down-regulating protease-activated receptor-1. *Tumour Biol* 2014; **35**: 7821-7829 [PMID: 24817013 DOI: 10.1007/s13277-014-2042-6]

120 **Chandra Gupta S**, Nandan Tripathi Y. Potential of long non-coding RNAs in cancer patients: From biomarkers to therapeutic targets. *Int J Cancer* 2017; **140**: 1955-1967 [PMID: 27925173 DOI: 10.1002/ijc.30546]

121 **Yuan CL**, Li H, Zhu L, Liu Z, Zhou J, Shu Y. Aberrant expression of long noncoding RNA PVT1 and its diagnostic and prognostic significance in patients with gastric cancer. *Neoplasma* 2016; **63**: 442-449 [PMID: 26925791 DOI: 10.4149/314_150825N45]

122 **Fan QH**, Yu R, Huang WX, Cui XX, Luo BH, Zhang LY. The has-miR-526b binding-site rs8506G>a polymorphism in the lincRNA-NR_024015 exon identified by GWASs predispose to non-cardia gastric cancer risk. *PLoS One* 2014; **9**: e90008 [PMID: 24595048 DOI: 10.1371/journal.pone.0090008]

123 **Koga A**, Aoyagi K, Imaizumi T, Miyagi M, Shirouzu K. Comparison between the gastric cancer cell line MKN-45 and the high-potential peritoneal dissemination gastric cancer cell line MKN-45P. *Kurume Med J* 2011; **58**: 73-79 [PMID: 22531121 DOI: 10.2739/kurumemedj.58.73]

124 **Pegtel DM**, Cosmopoulos K, Thorley-Lawson DA, van Eijndhoven MA, Hopmans ES, Lindenberg JL, de Gruijl TD, Würdinger T, Middeldorp JM. Functional delivery of viral miRNAs via exosomes. *Proc Natl Acad Sci USA* 2010; **107**: 6328-6333 [PMID: 20304794 DOI: 10.1073/pnas.0914843107]

125 **Skog J**, Würdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, Curry WT Jr, Carter BS, Krichevsky 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/ncb1800]

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/ncb1596]

127 **Cheng L**, Sharples RA, Scicluna BJ, Hill AF. Exosomes provide a protective and enriched source of miRNA for biomarker profiling compared to intracellular and cell-free blood. *J Extracell Vesicles* 2014; **3**: [PMID: 24683445 DOI: 10.3402/jev.v3.23743]

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 **Tokuhisa M**, Ichikawa Y, Kosaka N, Ochiya T, Yashiro M, Hirakawa K, Kosaka T, Makino H, Akiyama H, Kunisaki C, Endo I. Exosomal miRNAs from Peritoneum Lavage Fluid as Potential Prognostic Biomarkers of Peritoneal Metastasis in Gastric Cancer. *PLoS One* 2015; **10**: e0130472 [PMID: 26208314 DOI: 10.1371/journal.pone.0130472]

131 **Watanabe Y**, Kim HS, Castoro RJ, Chung W, Estecio MR, Kondo K, Guo Y, Ahmed SS, Toyota M, Itoh F, Suk KT, Cho MY, Shen L, 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]

132 **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]

133 **Virgilio E**, Giarnieri 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/anticanres.12265]

134 **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/NEJM199110173251603]

135 **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]

136 **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]

137 **Saju P**, Murata-Kamiya N, Hayashi T, Senda Y, Nagase L, Noda S, Matsusaka 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]

138 **Pormohammad A**, Ghotaslou 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]

139 **Altieri 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]

140 **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]

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