World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i41.11636

World J Gastroenterol 2015 November 7; 21(41): 11636-11653
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2015 Advances in Gastric Cancer

Emerging blood-based biomarkers for detection of gastric cancer

Zane Kalniņa, Irēna Meistere, Ilze Kikuste, Ivars Tolmanis, Pawel Zayakin, Aija Linē

Zane Kalniņa, Irēna Meistere, Pawel Zayakin, Aija Linē, Latvian Biomedical Research and Study Centre, Riga, 1067 LV, Latvia

Ilze Kikuste, Ivars Tolmanis, Digestive Diseases Centre GASTRO, Riga, 1006 LV, Latvia

Ilze Kikuste, Faculty of Medicine, University of Latvia, Riga, 1586 LV, Latvia

Author contributions: Kalniņa Z and Meistere I equally contributed to the collection of data and writing the manuscript; Kikuste I and Tolmanis I contributed to collection of data and writing individual sections of the manuscript; Zayakin P assisted in collection of data; Linē A supervised the work and revised the manuscript.

Supported by ERDF project, No. 2013/0052/2DP/2.1.1.1.0/13APIA/VIAA/019.

Conflict-of-interest statement: The authors have no conflicts of interest to report.

Correspondence to: Aija Linē, PhD, Latvian Biomedical Research and Study Centre, Cancer Biomarker Group, Ratsupites Str 1, k-1, LV-1067, Riga, 1067 LV, Latvia. aija@biomed.lu.lv
Telephone: +371-7808208
Fax: +371-7442407

Received: May 9, 2015
Peer-review started: May 11, 2015
First decision: June 23, 2015
Revised: July 8, 2015
Accepted: September 30, 2015
Article in press: September 30, 2015
Published online: November 7, 2015

Abstract

Early detection and efficient monitoring of tumor dynamics are prerequisites for reducing disease burden and mortality, and for improving the management of patients with gastric cancer (GC). Blood-based biomarker assays for the detection of early-stage GC could be of great relevance both for population-wide or risk group-based screening programs, while circulating biomarkers that reflect the genetic make-up and dynamics of the tumor would allow monitoring of treatment efficacy, predict recurrences and assess the genetic heterogeneity of the tumor. Recent research to identify blood-based biomarkers of GC has resulted in the identification of a wide variety of cancer-associated molecules, including various proteins, autoantibodies against tumor associated antigens, cell-free DNA fragments, mRNAs and various non-coding RNAs, circulating tumor cells and cancer-derived extracellular vesicles. Each type of these biomarkers provides different information on the disease status, has different advantages and disadvantages, and distinct clinical usefulness. In the current review, we summarize the recent developments in blood-based GC biomarker discovery, discuss the origin of various types of biomarkers and their clinical usefulness and the technological challenges in the development of biomarker assays for clinical use.

Key words: Gastric cancer; Biomarker; Liquid biopsy; Cell-free DNA; Cell-free RNA; Extracellular vesicles; Autoantibodies; Proteomics

Core tip: The identification of blood-based biomarkers that could reliably detect the presence of early-stage gastric cancer or provide means to monitor the tumor dynamics is an unmet clinical need. Recently, considerable effort has been devoted to discovering various types of cancer-associated molecules in the blood of gastric cancer patients, and this has resulted in establishing biomarker models with remarkably high sensitivity and specificity. However, a validation in large-scale studies and a head-to-head comparison of the biomarker models and technologies are required before these biomarkers can be used in routine clinical practice.

Kalniņa Z, Meistere I, Kikuste I, Tolmanis I, Zayakin P, Linē A. Emerging blood-based biomarkers for detection of gastric cancer. *World J Gastroenterol* 2015; 21(41): 11636-11653 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i41/11636.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i41.11636

INTRODUCTION

Although the incidence of gastric cancer (GC) has decreased in most parts of the world, with estimated 952000 new cases and 723000 deaths from GC in 2012, it still accounts for approximately 6.8% of all cases and 8.8% of cancer-related deaths worldwide[1]. The incidence rates vary significantly across the globe, being the highest in Eastern Asia, followed by Central and Eastern Europe and rates are the lowest in North America and Western Africa[1,2]. The main type of GC is adenocarcinoma (approximately 95%), which can be further categorized into an intestinal and a diffuse type according to Lauren's classification[3]. Intestinal-type gastric adenocarcinoma, the most common subtype of GC, develops through a well-described sequence of histopathological stages from normal mucosa to chronic gastritis, chronic atrophic gastritis followed by intestinal metaplasia, dysplasia and finally to adenocarcinoma, with *H. pylori* infection, which is recognized as the main underlying cause of pan-gastric mucosal inflammation[4,5]. Thus, the main risk factors for GC are chronic infection with *H. pylori* and the presence of the above-listed precancerous lesions, whereas a relatively smaller proportion of GC cases are linked to a genetic predisposition and dietary factors[6,7].

The high mortality rate in GC mostly results from its detection at late stages. Most GC cases are detected at stage ⅢA-Ⅳ, when the estimated 5-year survival ranges from 7%-27% and the median survival is less than 12 mo[8,9]. On the contrary, early GC that is limited to the submucosal layer is curable by endoscopic mucosal dissection or minimally-invasive surgery[10]. Early GC detection, however, is hampered by the lack of specific symptoms before it has spread beyond the original site. Thus, organized screening programs that aim to detect pre-cancerous lesions and early-stage GC seem to be a main tool for reducing GC-related mortality, but such programs have been implemented only in some Asian countries[11]. Upper endoscopy is the primary screening technique in most of the programs and the gold standard for confirmation of the diagnosis[6,11]. However, endoscopy is an invasive technique with uncommon but serious side effects and a relatively high cost, and the results are highly dependent on the skill of the endoscopist[6]. Therefore, GC screening in low GC incidence areas and low-income countries is not practical, and is likely to be associated with low participation rates in the screening programs.

Currently, the only non-invasive test that has been used for GC detection is the pepsinogen (PG) test. PGs are pro-enzymes that are converted into the proteolytic enzyme pepsin. PGs are mainly synthesized and secreted by the gastric chief cells and their serum levels indirectly reflect secretion in the stomach[12]. PGI is exclusively produced by the corpus mucosa, while PGⅡ is also secreted by the cardiac and pyloric glands and the proximal duodenal mucosa[13]. Low PGI levels and a low PGⅠ/PGⅡ ratio are indicators of atrophic changes in the gastric corpus. PG tests can detect gastric mucosal atrophy with a sensitivity of 66.7%-84.6% and a specificity of 73.5%-87.1%[14-16], whereas the sensitivity for GC detection ranges from 36.8%-62.3%[17-19], which is not acceptable in population-based screening settings. Thus, the PG test can be administered in a two-stage screening approach as a primary screening test to identify individuals who are at an elevated GC risk, and these high-risk individuals are then referred for endoscopic examination followed by the histological analysis of gastric biopsy[11].

In recent years, considerable effort has been devoted to the discovery of novel blood-based biomarkers that are suitable for the development of non-invasive tests to detect GC at an early stage or to monitor tumor dynamics. Such biomarkers may include quantitatively- or structurally-altered proteins, cancer-associated autoantibodies, cell-free nucleic acids (cfNAs), circulating tumor cells (CTCs), cancer-derived extracellular vesicles (EVs) and metabolites. In the current review, we provide an overview of recently-discovered blood-based GC biomarkers, and discuss their origin and mechanisms of release into the bloodstream, and also their potential clinical usefulness.

CRITERIA FOR BIOMARKERS APPLICABLE TO CANCER CONTROL PROGRAMS

In 2013, a Working Group of international experts established by the International Agency for Research on Cancer made recommendations for GC control

and concluded that a decisive public health action to include GC in cancer control programs is required; however, interventions should be tailored to the local conditions, taking into account the prevalence, cost-benefit ratio and adverse consequences[20]. Prevention strategies should aim to reduce both GC incidence and mortality. Primary prevention strategies are focused on preventing exposure to GC risk factors, for example, by eradicating *Helicobacter pylori* (*H. pylori*) infection or modifying patients' diet and lifestyle, while secondary prevention strategies aim to identify patients with early-stage GC or precancerous lesions, who would then undergo endoscopic surveillance[6,11]. Tertiary prevention aims to control the symptoms and morbidity of established cancer. Blood-based biomarkers for the detection of early-stage, residual or recurrent cancers could be highly relevant for both secondary and tertiary prevention strategies.

Ideally, a biomarker that is used in population-wide screening programs should be stable and robustly measurable in plasma or serum using routine laboratory equipment, appear in the bloodstream before the clinical signs and symptoms arise, should discriminate between cancer and inflammatory diseases and should have high positive and negative predictive values. However, the relatively low prevalence of GC in most parts of the world, except for Eastern Asia, suggests that even biomarker assays with high sensitivity and specificity would have a low positive predictive value (PPV). For example, if a hypothetical biomarker assay with a sensitivity of 95% and specificity of 98% would be applied to screen 100000 asymptomatic individuals in a medium-incidence area such as Eastern Europe (with GC prevalence of 0.04%), 38 true positives, 2 false negatives and approximately 2000 false positives would be detected, thus yielding PPV of only 1.87%.

A biomarker for detecting residual or recurrent cancer, however, must reflect the tumor dynamics. For example, it should be rapidly cleared from the circulation after complete tumor removal, and it should be able to detect incompletely-resected tumor and to increase in the circulation before the clinical signs of recurrence.

PROTEOMIC BIOMARKERS

Proteomic analyses can provide information on a complex composition of proteins that are differentially expressed in blood specimens from cancer patients and healthy donors that could be used for cancer biomarker discovery. The flowchart of serum proteomic analysis usually consists of protein extraction and separation performed by 2-dimensional gel electrophoresis (2-DE), difference gel electrophoresis (2D-DIGE), surface-enhanced laser desorption/ionization (SELDI), Liquid Chip and other approaches. These are followed by diagnostic model determination or protein identification through MS and bioinformatics, after which identified proteins are verified using conventional techniques such as Western blot and ELISA (technology approaches reviewed by Liu *et al*[21]). The current challenges in blood-borne biomarker discovery include variability of sample preparation and pre-treatment as well as inter-laboratory analytical variability of different instruments used in discovery and validation studies. Another issue is the choice of sample type used for proteome analyses - serum seems to be the most common choice because of its availability in biobanks and thus, it is frequently used in studies. However, the Human Proteome Organisation recommends the use of plasma for proteomic studies to reduce the variability caused by the coagulation process[22].

Many proteomic studies of serum biomarkers for GC detection have been published in the last 10 years (reviewed in detail by Liu *et al*[21] and Lin *et al*[23]), and examples of biomarker models are listed in Table 1. In one of the pioneering studies, Ebert *et al*[24] analyzed serum from GC patients with SELDI-TOF-MS and Protein-Chip technology in combination with a pattern-matching algorithm and built a classifier ensemble that consists of 50 decision trees that achieved 100% sensitivity and 96.7% specificity (including both, intestinal and diffuse type GC). Moreover, this classifier could detect early stage GC with sensitivity of 89.9%. Liu *et al*[25] showed that there were three differentially-expressed peaks identified by screening serum samples from 65 GC and 53 cancer-free individuals, including patients with chronic superficial gastritis and chronic atrophic gastritis. The combined use of the three biomarkers, which were identified as fibrinogen α chain, apolipoprotein A-Ⅱ and apolipoprotein C-I, distinguished the cancer group from the control group with a sensitivity of 93.85% and a specificity of 94.34% in an independent validation set. In another study, Li *et al*[26] found a six-feature proteomic model by applying SELDI-TOF-MS analysis that effectively distinguished GC samples from control samples with a sensitivity of 93.5% and specificity of 91.6%. In addition, they observed that three of the peaks were differentially expressed between patients with stage Ⅰ GC and advanced GC (accuracy 88.9%). Other groups have reported using the SELDI-MS application to analyze the serum profile from GC patients, and they showed an overall high sensitivity and specificity (over 90% and 80%, respectively)[27-32]. However, these promising results have to be validated in larger multicenter studies because the SELDI-MS approach has several disadvantages, as follows: the results lack consistency among research groups, the reproducibility is low and it cannot directly identify proteins[33].

Other approaches besides SELDI-MS have been used. Yang *et al*[34], using magnetic beads, separated peptidome from GC patients' serum using matrix-assisted laser desorption/ionization - time of flight (MALDI-TOF) MS, and they found 11 differentially-expressed proteins and the two most promising of them could detect GC patients with 95.2% sensitivity

Table 1 Proteomics-based biomarker models for detection of gastric cancer

Biomarker model	Approach used	Sample size and type (cancer/controls)	Diagnostic value[1]	Ref.
Five peaks - 3316, 6629, 3217, 3952, 6431 Da	MB-WCX, MALDI-TOF-MS	T: GC = 32/HC = 32 V: GC = 30 (GC Ⅰ-Ⅱ = 8)/HC = 30	AUC = 0.86-0.99 for individual features (P < 0.001), Sn = 79.3%, Sp = 86.5% Sn = 71.7% for early stage GC	Yang *et al*[148], 2012
1546 Da (SERPINA1) 5335 Da (ENOSF1)	MB-WCX, MALDI-TOF-MS; ELISA for validation	T: GC = 70/HC = 72 V: GC = 36/HC = 36, BGD = 30, other cancers = 108	AUC (1546 Da) = 0.83 (P < 0.001), AUC (5335 Da) = 0.87 (P < 0.001) - calculated for training set; in validation set SERPINA1 concentration was significantly higher for GC patients than for all other controls (P < 0.001) and ENOSF1 concentration was significantly higher for GC patients than HC (P < 0.001)	Yang *et al*[34], 2015
Fibrinogen α-chain, apolipoprotein A-Ⅱ and apolipoprotein C-Ⅰ	HPLC, LC-MS/MS	T: GC = 65/HC = 30, BGD = 23 V: GC = 44/ HC = 30, BGD = 23	Sn = 90.9%, Sp = 90.6% (P = NA)	Liu W *et al*[37], 2012
Six peaks at 2873, 3163, 4526, 5762, 6121 and 7778 m/z; For stage Ⅰ three peaks at 2873, 6121 and 7778 m/z	Protein Chip SELDI-TOF-MS	GC = 169 (GC Ⅰ = 27)/ HC = 83	Sn = 93.5%, Sp = 91.6% Accuracy for stage Ⅰ - 88.9%, (P = NA)	Li *et al*[26], 2012
EGFR, proApoA1, ApoA1, TTR, RANTES, VN, DD, IL-6, A2M, CRP, PAI1	xMAP (Luminex), ELISA	T: GC = 120/BGD = 101, HC = 19 V: GC = 95 (GC Ⅰ-Ⅱ = 75)/BGD = 43, HC = 8	AUC = 0.95, (P < 0.05) Sn = 88.8%, Sp = 89.7% Sn (Ⅰ-Ⅱ) = 92.3% Sn (tumor size ⩽ 2 cm) = 81.8%	Ahn *et al*[35], 2012
Four peaks at 1867 (tubulin beta chain), 2701 (thymosin beta4 like protein3), 2094 (cytochrom b-c1 subunit), 1467 Da	MB-WCX, MALDI-TOF-MS	T: GC = 40/HC = 39 V: GC = 40/GA = 30, HC = 39	AUC (1867 Da) = 1, AUC (1467 Da) = 0.83 AUC (2701 Da) = 0.71 AUC (2094 Da) = 0.70 (P < 0.05) Sn = 95.0%, Sp = 97.1%	Fan *et al*[30], 2013
50 decision trees, 28 masses Three peaks at 3946, 3503 and 15958 Da	Protein Chip SELDI-TOF-MS,	T: GC = 41/HC = 49 V: GC = 28; GC Ⅰ = 9/HC = 30	Sn = 100%, Sp = 96.7% For stage Ⅰ Sn = 89.9% (P = NA) Sn = 92.8%, Sp = 86.7% For stage Ⅰ Sn = 89.9% (P = NA)	Ebert *et al*[24], 2004

[1]Diagnostic values listed for validation set, if not otherwise stated. A2M: Alfa 2 macroglobulin; Apo: Apolipoprotein; AUC: Area under the curve; BGD: Benign gastric diseases; CRP: C reactive protein; DD: D-dimer; ENOSF1: Isoform 2 of mitochondrial enolase superfamily member 1; EGFR: Epidermal growth factor receptor; GA: Gastric adenoma; GC (Ⅰ-Ⅳ): Gastric cancer (TNM stages); HC: Healthy control; HPLC: High performance liquid chromatography; IL-6: Interleukin 6; LC: Liquid chromatography; MALDI-TOF-MS: Matrix-assisted laser desorption/ionization-time of flight-mass spectrometry; MB-WCX: Magnetic bead based weak cation-exchange chromatography; NA: Not available; PAI1: Plasminogen activator inhibitor 1; proApo: Proapolopoprotein; RANTES: Regulated upon activation, normally T-expressed and presumably secreted; SELDI-TOF-MS: Surface-enhanced laser desorption/ionization-time of flight-mass spectrometry; SERPINA1: Serpin peptidase inhibitor clade A (alpha-1 antiproteinase, antitrypsin), member 1; Sn: Sensitivity; Sp: Specificity; T: Training set; TTR: Transthyretrin; V: Validation set; VN: Vitronectin.

and 93.6% specificity. In another study, Ahn *et al*[35] constructed a 29-plex array platform based on antibodies against 11 proteins discovered using proteomic approaches and 18 known cancer-associated proteins, and used it to examine serum from 120 GC patients and 120 non-cancerous individuals including 98 gastritis or ulcer patients. They used multivariate classification analysis including 11 analytes (listed in Table 1) that differed between the above-mentioned groups (P value < 0.001). They obtained an accuracy > 85% in an independent validation sample set (95 GC and 51 controls).

By evaluating the known individual serum proteins identified using proteomic approaches from the cancer biomarker perspective, the complexity of the plasma proteome has to be taken into account; it has a wide dynamic range covering 10 orders of magnitude starting from albumins as the most abundant proteins and ending with cytokines and interleukins[36]. Some groups have tried to reduce the plasma proteome complexity by depleting highly abundant protein fractions using different means; however, the results obtained are rather ambiguous. For example, to focus on lower-abundance proteins that might be relevant to cancer, Liu *et al*[37] depleted serum of predominant protein fractions and compared GC and healthy donor specimens using 2D-DIGE followed by MS. They detected 12 differentially expressed proteins including plasminogen, apolipoprotein A-Ⅳ, kininogen-1, clusterin and complement component C4A. Chong *et al*[38] used a combination of proteomic techniques that included highly abundant protein removal and found that plasma protein C9 was significantly increased in GC patients compared with

the healthy donor group. Increased C9 levels have also been reported in serum samples from patients with acute leukemia and sarcoma as well as autoimmune diseases[38]. Ebert *et al*[39] used MALDI-TOF-MS for screening whole serum samples from 14 GC patients and 14 healthy individuals and found that a peptide fragment increased in cancer patients' serum; the peptide was later identified as fibrinopeptide A (FpA). The authors confirmed its level in serum using ELISA in a larger cohort of GC patients (n = 99), high-risk individuals (n = 13) and controls (n = 111), and they observed increased levels in cancer patients and high-risk individuals compared to normal controls. FpA is a blood coagulation protein that is also reported to be a putative biomarker for GC staging[32,40]. The above-mentioned apolipoprotein C-Ⅰ together with C-Ⅲ have been previously reported as diagnostic biomarkers for GC, and the analysis of serum from 103 GC patients and 54 cancer free controls showed decreased expression in the cancer group versus the control group; these results were confirmed using ELISA. The level of apolipoproteins in blood has been reported to be a potential biomarker for various cancers[41]. Yang *et al*[34] identified two peptides that were later characterized as fragments of SERPINA1, an inflammation acute phase protein, and ENOSF1 as the most significantly increased peptides in GC patients. Generally, most proteins mentioned above represent highly abundant plasma proteins and their roles as GC-specific diagnostic markers have to be interpreted with caution, because they are known to be part of a blood coagulation system or represent acute phase inflammatory proteins and they have been reported to be associated with other types of cancer. Evidence from a study using a mouse model of breast cancer showed that the host cell and tumor microenvironment-derived protein signature in plasma differs from the signature associated with inflammatory conditions that are not related to cancer, and therefore could be used for early stage cancer detection[42].

Some studies are focused on posttranslational modification of the serum proteome, such as protein glycosylation[43-47], because it is known that alterations in protein glycosylation are a common feature of tumor cells. Bones *et al*[43], using a combination of glycomic techniques and 2D-DIGE, demonstrated an increased level of sialyl Lewis X epitopes that are presented on triantennary glycans in serum from 80 GC patients compared with 10 patients who had benign gastric diseases and 20 healthy donors, and core fucosylated biantennary agalactosyl glycans were present on extracted immunoglobulin G molecules that were associated with increased TNM stage. Ozcan *et al*[45], by analyzing serum N-glycan profiles using MALDI-TOF-MS, identified 19 glycans that were differentially expressed among patients with GC, non-atrophic gastritis and duodenal ulcers. The glycan profile of the duodenal ulcer group was similar to that in the GC group. In another study, the serum immunoglobulin G glycosylation profile was analyzed using Nano-LC-MS, and eight glycans that can distinguish GC from non-atrophic gastritis, eight glycans that differed between GC and duodenal ulcer and three glycans that differentiated between the non-atrophic gastritis and duodenal ulcer groups were identified[47]. Roy *et al*[46] used an on-chip lectin microarray-based glycomic approach to analyze tissue and serum samples from patients with GC, chronic gastritis and healthy individuals. They showed that the glycoprofile obtained from the tissue samples deviated more than that from the serum samples. It is likely that the altered glycan profile in serum from cancer patients is related to the inflammatory processes and the host defense response mechanisms during carcinogenesis in general[43,45,47].

Although highly promising proteomic diagnostic biomarkers have been identified, especially for early GC diagnosis, there are currently no proteomic-based serum biomarker tests available for clinical application. It has become apparent that large-scale validation studies are critical to evaluate the accumulated proteomic data. Currently, the field of proteomic techniques is rapidly evolving, and continuously-improving technical performance provides constant and reliable high throughput analysis and increasing technical sensitivity for low concentration plasma protein measurements[36,48].

CANCER-ASSOCIATED AUTOANTIBODIES

The human immune system senses the presence of cancer before manifestation of the disease[49]. High-titer IgG class autoantibodies against specific tumor associated antigens (TAAs) have been found in patients' blood even up to five years before clinical diagnosis, thus demonstrating their potential for the detection of early stage cancer[50-52]. In addition, autoantibodies have other promising biomarker qualities: they are found in all tumor types that have been analyzed so far[53,54] and they are highly stable, antigen specific. Unlike the known GC biomarkers such as pepsinogens, CEA and CA19-9, autoantibodies are qualitative, not quantitative, biomarkers. Testing autoantibody reactivity against panels of TAAs using multiplex immunoassays has been shown to be feasible[55] and this aspect might substantially foster their transition from experimental to clinical medicine.

Accumulating evidence has shown that any individual cancer-associated autoantibody biomarker has a limited diagnostic value. Autoantibody repertoires in cancer patients are diverse, and the frequency of antibodies against any particular antigen typically ranges from 1%-15%[56-58]. Among the most studied individual markers in GC, there are autoantibodies against well-known TAAs such as p53 (*e.g.*, 13 studies summarized by Werner *et al*[58] report a biomarker sensitivity range of 8.1-32.1% and specificity range of

Table 2 Autoantibody signatures with diagnostic value for gastric cancer

Biomarker signature description	Technology	Study design	Sample size (GC/controls)	Diagnostic value	Ref.
2 TAAs – p62, Koc	ELISA	GC *vs* HC	135/82	Sn = 19.3%, Sp = 97.6%, $P < 0.01$	Zhang *et al*[62], 2001
3 TSAs - IQGAP3, KRT23 and REG3A	PARSE assay	GC *vs* HC (age and sex matched)	48/46	Sn = 22.9%, Sp = 100%, $P < 0.001$	Xu *et al*[149], 2012
3 TAAs – p16, p53, c-myc	ELISA	GC *vs* HC	74/82	Sn = 21.6%, Sp = 97.6%; $P < 0.001$	Looi *et al*[150], 2006
7 TAAs - p53, C-myc, p16, IMP1, Koc, p62 and Survivin	ELISA	Cardia GC *vs* HC	88/140	AUC = 0.73, Sn = 64%, Sp = 87%, $P < 0.001$	Zhou *et al*[68], 2015
7 TAAs - C-myc, Cyclin B1, IMP1, Koc, P53, p62 and Survivin	ELISA, fixed cut-off	GC *vs* HC	91/346	Sn = 52.7%, Sp = 89.9%, $P < 0.01$	Zhang *et al*[63], 2003
	ELISA, individual cut-off (recursive partitioning)	GC *vs* HC	91/346	Sn = 98.9%, Sp = 93.1%, $P < 0.001$	Koziol *et al*[151], 2003
45 T7 phage-displayed TAA clones (including NY-ESO-1, DDX53, MAGE antigens *etc*.)	T7 phage displayed TAA microarray	GC *vs* HC (age and sex matched)	T:100/100 V:235/213	AUC = 0.79, Sn = 59%, Sp = 90%, $P < 0.001$	Zayakin *et al*[56], 2013
		GC *vs* gastritis	235/100	AUC = 0.64, Sn = 58.7%, Sp = 55%, $P < 0.001$	
		GC *vs* gastric ulcer	235/54	AUC = 0.76, Sn = 58.7%, Sp = 81.5%, $P < 0.001$	

AUC: Area under the curve; GC: Gastric cancer; HC: Healthy controls; ND: Not determined; Sn: Sensitivity; Sp: Specificity; TAA: Tumor associated antigen; TSA: Tumor specific antigen; T: Training; V: Validation.

95.25%-100%), NY-ESO-1[59,60], MUC1[61], Koc, p62[62], C-myc and Survivin[63,64] and others[58].

The development of high-throughput proteomic techniques, such as various native and recombinant protein microarrays and bead-based technologies (reviewed by Meistere *et al*[65]), has enabled the simultaneous detection of autoantibodies against many different TAAs. This has allowed systematic analysis and comparison of the heterogeneous repertoires of circulating autoantibodies within large patient cohorts, which has resulted in selection for cancer-associated biomarker signatures and discarding of those that are induced by other immune processes such as tissue damage, viral infections or possible autoimmune conditions[66,67]. To the best of our knowledge, seven studies have been published on the diagnostic values of different GC-associated autoantibody biomarker combinations (overviewed in Table 2). Within these studies, the identified biomarker signatures could discriminate GC from healthy controls with relatively high specificity (ranging from 87-100%) but with variable sensitivity (19.3%-98.9%). AUC was reported in only two studies: Zhou *et al*[68] showed that autoantibody reactivity against seven known TAAs was able to distinguish between patients with cardia GC from healthy controls with an AUC of 0.73, while Zayakin *et al*[56] reported that 45 GC-associated autoantibody classifiers distinguished GC (all stages with similar sensitivity) from healthy controls with an AUC of 0.79. However, these studies vary greatly in regard to various important aspects, such as the multiplexing level (2-45 autoantibodies), the method used for autoantibody detection, definition of appropriate control group(s), and approaches used for data normalization and cut-off definition. Altogether, these issues may greatly hamper the introduction of the identified biomarkers into clinical practice.

The most relevant biomarkers for early GC diagnosis would be those capable of detecting cancer in high-risk individuals. Only some studies have addressed the GC-associated autoantibody repertoire overlap with that found in patients with benign gastric lesions. For example, a study by Zayakin *et al*[56] found that, within the diagnostic 45-autoantibody signature, the identified biomarker pattern was partially shared between GC and gastritis patients, and was not found in patients with peptic ulcer and healthy controls. Two smaller studies addressed the p53 autoantibody specificity regarding benign gastric diseases and in both cases it was shown that this biomarker specifically detect GC in approximately 32% of the cases and that it is not found in the control patients[69,70]. Another issue is that GC-associated serologically active antigens have been shown to elicit B cell responses in variety of other malignancies[71]. The overlap of the identified GC-associated autoantibody signatures with those found in patients with other (gastrointestinal) cancer types has been addressed only partially and remains to be systematically analyzed within further studies to ascertain their clinical value.

In summary, cancer-associated autoantibody biomarkers have been shown to have high specificity, but moderate sensitivity, which would hinder their use in clinical practice for population-based screening. The limitations of the autoantibody biomarker sensitivity from the biological point of view are currently unknown. In a previous study, we analyzed autoantibody responses against 45 TAAs in 235 GC patients and found no serum-reactivity in 41% of the patients[56]. We then performed extensive screening of cDNA expression libraries with serum samples that did not react against the 45 TAA panel. The screening results showed that up to 10% of the GC patients either generally do not mount an antibody response against tumor antigens or did not have detectable autoantibody levels at the given time

point (unpublished results), thus demonstrating the biological limits for the sensitivity of autoantibody-based diagnostic assays. In addition, heterogeneity of TAA repertoires between cancer patients is high, and each individual autoantibody biomarker generally has a low frequency of detection. Thus, currently-published studies are most likely statistically underpowered. Rare cancer-specific autoantibodies that individually do not reach statistical significance, but are incorporated into the diagnostic biomarker panels, lead to the low reproducibility of initially-obtained results and this lowers the diagnostic value of a diagnostic autoantibody signature, which may be improved by analyzing the proposed biomarker combinations within cohorts with sufficient statistical power.

However, autoantibodies may be important players in the stratification of risk group patients. One of their strengths over other biomarker classes is that the adaptive immune system senses the tumor development early on[49] and can mount high titer antibody responses even to minute amounts of antigen while the presence of other biomarkers (*e.g.*, circulating tumor cells, protein biomarkers, cancer exosomes, cell-free nucleic acids) is gradually increasing in circulation during the progression of cancer. Moreover, the autoantibody repertoires elicited by GC have not been previously analyzed in the context of IgG subclasses. This may be an important aspect because each of the IgG1-4 subclasses have different affinities for activating and inhibiting Fcγ receptors, which eventually has an impact on the activating/inhibitory balance of the infiltrating immune effector cells. This may result in either host-protective or tumor-promoting immune responses, and thus the diagnostic value could be assigned to the specific IgG subclass itself and not only to the antigen specificity of total IgG, as was shown for melanoma[72]. In addition, the analyses of a TAA-specific secreted IgA repertoire might reveal possible novel biomarker candidates because mucosal linings are known to produce more IgA than all other types of antibodies combined.

CELL-FREE NUCLEIC ACIDS

Although the presence of cell-free nucleic acid (cfNA) in human blood was first described by Mandel and Métais[73] in 1948, researchers only began to realize the clinical significance of this finding half a century later[74]. During the past decade, the idea that cfNAs could serve as blood-based biomarkers of cancer has attracted increasing attention. cfNAs may serve as a "liquid biopsy" of cancer reflecting the genetic make-up of tumors, thus enabling detection of drug targets and tracking evolving genetic alterations throughout the course of the disease. Numerous studies have investigated the diagnostic and prognostic potential of total cfDNA levels, gene copy number, DNA integrity, cancer-associated DNA methylation markers or somatic mutations and expression levels of mRNAs, miRNAs and other non-coding RNAs in the blood of cancer patients.

cfNAs can be released into the circulation *via* various forms of cell death such as apoptosis, necrosis, autophagy and necroptosis[74-76] or actively secreted by packaging into extracellular vesicles (EVs)[77-80]. Most of the cfDNA is fragmented and the size distribution of the fragments varies from 150-350 bp to > 10000 bp[81]. The shorter fragments correspond to the mono- and dinucleosomal DNA fragments released from apoptotic cells, while the larger fragments are likely to be released from necrotic cells[81]. Increased cfDNA integrity (*i.e.*, higher ratio of longer to shorter DNA fragments), presumably reflecting an increased rate of necrotic cell death in cancer, has been found in several types of cancer and has been shown to have a diagnostic relevance[82-84]. However, the fraction of tumor-derived DNA has been shown to vary from only 3% to as much as 93% of total cfDNA in different patients[81] and the cellular source of cfDNA is still controversial.

Circulating cfRNA, in particular miRNA, has been found to be remarkably resistant to endogenous and exogenous RNase activity, extreme pH conditions and freeze-thaw cycles[85]. This suggests that cfRNA may be protected from degradation by packaging into various EVs, including exosomes, microvesicles and apoptotic bodies. Studies evaluating the proportion of vesicle-enclosed and vesicle-free miRNA in human plasma, however, have come to controversial conclusions: several studies have showed that the majority of circulating miRNAs are concentrated in exosomes and exosome isolation improves the sensitivity and consistency of miRNA analysis in biofluids[86,87], while other studies showed that only a few miRNAs are enclosed into exosomes[88] and, on average, there is less than one molecule of a given miRNA per exosome[89]. Currently, the reason for such a discrepancy is unclear and more detailed studies on the content, localization and stoichiometry of various RNA species in distinct EV subtypes are required.

Total cfDNA level

Several studies have reported increased levels of total cfDNA in plasma of GC patients compared with healthy controls[90-93] (Table 3). The cfDNA levels could distinguish between GC and control plasma with an AUC varying from 0.75[90] to 0.991[92]. Because the measurement of cfDNA levels does not require any *a priori* knowledge of genetic alterations in the tumor tissue, such an approach could be highly relevant to the development of non-invasive assays for the early detection of GC. However, the size of patient cohorts was relatively small in all of these studies, and therefore validation of the findings in large, well characterized cohorts is required to draw conclusions about clinical utility of cfDNA levels. In addition,

Table 3 Cell-free DNA as biomarkers for detection of gastric cancer

Candidate biomarkers	Sample size and type	Method/technology	Diagnostic value/outcome	Ref.
Total cell-free DNA level				
β-actin (total cf DNA level)	GC = 53, HC = 21, plasma	qPCR	AUC = 0.75, $P < 0.0001$	Sai *et al*[90], 2007
DNA integrity		qPCR (ratio of long *vs* short b-actin amplicons)	No significant difference between GC and HC	
Alu DNA sequences	GC = 54, HC = 59; plasma	Alu81-qPCR	AUC = 0.784, Sn = 75%, Sp = 63%	Park *et al*[91], 2012
Total cfDNA level	Early GC = 16; advanced GC = 14; HC = 34; plasma	Measurement of cfDNA concentration	AUC = 0.991, Sn = 96.67%, Sp = 94.11% for GC *vs* HC	Kim *et al*[92], 2014
Gene amplification				
MYC gene copy number (*MYC/GAPDH* ratio)	GC = 57, HC = 39; tissues and plasma	qPCR	AUC = 0.816; strong positive correlation between MYC levels in GC tissues and plasma ($r = 0.342$; $P = 0.009$)	Park *et al*[99], 2009
HER2 gene copy number (*HER2/RPPH1* ratio)	Discovery: GC = 52 (pre and post-operative treatment), HC = 40; plasma and tissues	qPCR	AUC = 0.746, Sn = 53.9%, Sp = 96.7%; Positive correlation between GC tissues and plasma ($r = 0.424$; $P = 0.00721$); decrease in post-treatment plasma in HER2 + GC cases	Shoda *et al*[100], 2014
	Validation: GC = 25 plasma		Sn = 66.7%, Sp = 100%	
DNA methylation markers				
RPRM (Reprimo)	GC = 43, HC = 31; GC tissues and plasma	MSP	95.3% GC, 9.7% HC, $P < 0.00001$; Strong correlation between methyl status in tissues and plasma	Bernal *et al*[107], 2008
RUNX3	GC (preoperative) = 65, GC (postoperative) = 43, HC = 50, tissues and serum	qMSP	AUC = 0.8651, Sn = 95.5%, Sp = 62.5%; decrease after surgical resection	Sakakura *et al*[152], 2009
KCNA4 + CYP26B1	GC = 46, GPL = 46, HC = 30; serum	Discovery: Methylation microarray in tissues; Testing: MSP	AUC = 0.917, Sn = 91.3%, Sp = 92.1%	Zheng *et al*[109], 2011
SLC19A3	Discovery: GC = 45, HC = 60; plasma	MSRED-qPCR	Increased in GC, $P < 0.0001$	Ng *et al*[153], 2011
	Validation: GC = 20, HC = 20		AUC = 0.82, Sn = 85%, Sp = 85%	
FAM5C + MYLK	GC = 58, GPL = 46, HC = 30; serum	Discovery: MeDIP in cell lines; Testing: MSP	AUC = 0.838, Sn = 77.6%, Sp = 90% for GC *vs* HC; Sn = 30.4% for GPL *vs* HC; decrease after surgical resection	Chen *et al*[154], 2012
XAF1	GC = 202, HC = 88, tumor tissues and serum	qMSP	AUC = 0.909, $P < 0.0001$; 83.9% concordance between GC tissues and serum	Ling *et al*[108], 2013

AUC: Area under the curve; GC: Gastric cancer; GPL: Gastric precancerous lesions; HC: Healthy controls; MeDIP: Methylated DNA immunoprecipitation; MSP: Methylation-specific PCR; MSRED-qPCR: Methylation-sensitive restriction enzyme digestion and real-time quantitative PCR; Sn: Sensitivity; Sp: Specificity.

elevated cfDNA levels have also been detected in patients with inflammatory diseases[94], infections[95] and cardiovascular disorders[96] and in healthy individuals after exercise[97], thus indicating that this phenomenon is not strictly cancer-specific. Similarly, a recent study by Hamakawa *et al*[98] demonstrated that the quantity of DNA fragments harboring cancer-specific somatic mutations in *TP53* gene (circulating tumor DNA, ctDNA) did not correlate with the level of total plasma cfDNA, and only ctDNA showed a good correlation with the GC disease status.

Cancer-specific gene amplification

More specific approaches for measuring total cfDNA levels could be the assessment of cancer-specific genetic alterations in the circulating cfDNA. Several studies have used qPCR to quantify the copy number of genes known to be amplified in GC tissues, such as *MYC*[99] and *HER2*[100,101], in cell-free plasma from GC patients (Table 3). An increased *MYC/GAPDH* ratio in plasma significantly correlated with that in the GC tissues and could distinguish between GC patients and healthy controls with an AUC of 0.816[99]. Similarly, the *HER2* level showed a high correlation in plasma and GC tissues, when quantified using qPCR, and had an AUC of 0.746 for detecting GC[100]. Meanwhile, Lee *et al*[101] reported that the *HER2* copy number in tumor tissues determined by FISH was not significantly associated with the plasma *HER2* level, thus calling into question how well ctDNA levels reflect gene copy numbers in the tumor tissue. The diagnostic usefulness of such tests is limited to detecting GC in patients who harbor the respective genetic amplifications, and therefore they are unlikely to be widely implemented in routine diagnostic examinations. However, they might prove to be highly relevant for detecting the presence or loss of therapeutic targets, and for monitoring treatment efficacy and the course of the disease. Further studies are needed to assess to what extent the ctDNA levels reflect the intratumoral heterogeneity and what factors affect the stability and half-life of the DNA fragments in the plasma.

DNA methylation markers

Several other studies have explored the possibility of detecting cancer-associated hypermethylated DNA fragments in the cfDNA of cancer patients. Methylation markers in the bloodstream were first discovered in breast and lung cancer patients in 1999[102,103]. Lee *et al*[104] demonstrated, for the first time, the feasibility of detecting aberrant methylation in serum from GC patients. This study reported that promoter region hypermethylation of genes encoding DAP-kinase, E-cadherin, GSTP1, p15 and p16 was detected in serum of 48.1%, 57.4%, 14.8%, 55.6% and 51.9% of GC patients, respectively. Subsequently, multiple studies showed hypermethylated genes in the plasma or serum of GC patients. These studies have been systematically summarized in recent reviews by Tsujiura *et al*[105] and Toiyama *et al*[106] and examples of key studies are given in Table 3. Hypermethylated genes showing the highest diagnostic value for detecting GC include *RPRM*[107], *XAF1*[108] and a combination of *KCNA4* and *CYP26B1*[109]. *RPRM* encodes Reprimo, a TP53-dependent cell cycle regulator, and is frequently silenced in GC *via* methylation of its promoter[110]. Bernal *et al*[107] reported that methylated *RPRM* was detected in plasma from 95.3% of GC patients but in only 9.7% of healthy controls, thus yielding a sensitivity of 95.3% and specificity of 90.3%. *XAF1*, a negative regulator of apoptosis inhibitor, has been shown to be downregulated by hypermethylation in cancer tissues of over 83% of GC patients and the agreement between the methylation status in tumor tissues and corresponding serum was 83.9%. Methylated *XAF1* promoter fragments were detected in the serum from 141 out of 202 GC patients, while all 88 cancer-free controls were negative (AUC, 0.909; 95%CI: 0.875-0.942, $P < 0.0001$)[108]. Zheng *et al*[109] used methylation CpG island microarray technology to search for hypermethylated genes in GC tissues and then selected five candidate genes in the serum of 46 GC patients, 46 patients with precancerous lesions and 30 healthy controls. A combination of two methylation markers, *CYP26B1* and *KCNA4*, could distinguish GC from the control serum with a sensitivity of 91.3%, specificity of 92.1% and AUC of 0.917 (95%CI: 0.858-0.976, $P < 0.001$).

These studies have shown several promising methylation markers that warrant further validation in independent cohorts of patients to establish which of the individual markers or combination of markers has the highest diagnostic value. There are also several technical issues that have to be resolved before these assays could be used in a clinical setting. Most of the studies are based on the treatment of DNA with sodium bisulfite, which converts unmethylated cytosine residues to uracil but leaves methylated cytosines unaffected. The modified DNA is analyzed by methylation-specific PCR (MSP) or DNA sequencing. However, these techniques are prone to false-positive results arising mostly from incomplete conversion of unmethylated cytosine residues to uracil[111,112]. Recently, several quantitative techniques for methylation analysis, such as MS-HRM, SMART-MSP, methyl-BEAMing and bisulfite pyrosequencing, have been established[112-114], but their performance in a clinical setting still needs to be validated.

Cell-free RNAs

In 2008, Mitchell *et al*[85] used a mouse model to demonstrate that miRNAs originating from human prostate cancer xenografts enter the blood circulation, thus providing proof of principle that cancer cells release miRNAs that can be detected in the blood. Chen *et al*[115] reported results obtained by deep sequencing of serum miRNAs in patients with diabetes, lung and colorectal cancer and healthy individuals. This study revealed that serum from patients had distinct patterns of disease-specific miRNAs that were absent in the healthy controls and suggested that several diseases may leave specific miRNA-fingerprints in the blood of patients. Recently, more than 20 studies[116] have explored the usefulness of circulating miRNAs for detecting GC. Examples of key studies are given in Table 4. Most of these studies were focused on candidate miRNAs that were selected from previous analysis of GC tissues, while others used a hypothesis-free approach, where miRNA profiling is performed in a discovery sample set using high throughput techniques such as TaqMan arrays, microarrays or deep sequencing, and the diagnostic value of the selected candidate miRNAs is then determined using qRT-PCR in an independent validation set.

Tsujiura *et al*[117] for the first time demonstrated the usefulness of circulating miRNAs for diagnosing and monitoring GC. The levels of five GC-associated miRNAs (miR-17-5p, miR-21, miR-106a, miR-106b and let-7a) were studied in plasma from GC patients and the results showed that the former four miRNAs were present at significantly higher levels while let-7a was decreased in the plasma from GC patients compared to the controls, and the miR-106a/let-7a ratio could distinguish between patients and controls with an AUC of 0.879. Although the authors found relatively good correlation between the miRNA expression levels in the blood and tumor tissue, several subsequent studies showed that only a subset of miRNAs that are highly expressed in tumors show elevated levels in serum or plasma, while other miRNA species are selectively released or retained by the cell[118]. The same group then compared miRNA profiles in pre- and post-operative plasma samples from GC patients using microarray analysis and identified a list of miRNAs that were markedly decreased in post-operative plasma and therefore are likely to be associated with the presence of cancer[119]. Two candidate miRNAs, miR-451 and miR-486, were tested in a cohort of 56 GC patients and 30 healthy controls, and the ROC curve analyses

Table 4 Cell-free RNAs as biomarkers for detection of gastric cancer

Candidate biomarkers	Sample size and type	Method/technology	Diagnostic value/outcome	Ref.
Circulating cell-free miRNAs				
miR-106a/let-7a ratio	GC = 69, HC = 30; plasma	qRT-PCR	AUC = 0.879, Sn = 85.5%, Sp = 80%	Tsujiura *et al*[117], 2010
5-miRNA signature: miR-1, miR-20a, miR-27a, miR-34, miR-423-5p	Discovery: GC = 20, HC = 20; Validation: GC = 142, HC = 105; Serum	Discovery: Solexa sequencing; Testing: qRT-PCR	AUC = 0.831 (validation set)	Liu *et al*[155], 2011
miR-451	Discovery: pre- and post-operative plasma, GC = 3;	Discovery: microarray	AUC = 0.96, Sn = 96%, Sp = 100%; decreased in 90% of post-operation plasma samples	Konishi *et al*[119], 2012
miR-486	Validation: GC = 56, HC = 30	Testing: qRT-PCR	AUC = 0.92, Sn = 86%, Sp = 97%; decreased in 93% of post-operation plasma samples	
miR-378	Discovery: GC = 7, CRC = 7, HC = 10;	Discovery: microarray	AUC = 0.861, Sn = 87.5%, Sp = 70.73%;	Liu *et al*[122], 2012
	Validation: GC = 40, HC = 41; serum	Testing: qRT-PCR	No significant differences across stages Ⅰ-Ⅳ	
miR-223	Test set: GC = 10, HC = 10;	qRT-PCR	AUC = 0.9089	Li *et al*[121], 2012
miR-21	Validation: GC = 60, HC = 60;		AUC = 0.7944	
miR-218	plasma		AUC = 0.7432	
3 miRNA combined			AUC = 0.9531, Sn = 84.29%, Sp = 92.86% No significant differences across stages Ⅰ-Ⅳ	
3-miRNA signature: miR-221, miR-744, and miR-376c	Discovery: GC = 14, HC = 14; Validation Ⅰ: GC = 68, HC = 68 Validation Ⅱ: DYS = 46, HC = 46 Pre-diagnosis serum samples, GC = 58	Discovery: TaqMan array, validation: qRT-PCR	Sn = 82.4%, Sp = 58.8% (for GC *vs* HC) Sn = 73.3% (for early GC) miR-221 elevated in DYS, no difference from HC for miR-376c and miR-744; Increase during GC development; Sn = 79.3% (for GC 2-5 years before diagnosis)	Song *et al*[124], 2012
miR-106b	Discovery: GC = 30, HC = 30	qRT-PCR	AUC = 0.773 (all in validation set)	Cai *et al*[156], 2013
miR-20a	Validation: GC = 60, HC = 60;		AUC = 0.859	
miR-221	plasma		AUC = 0.796	
miR-223	GC = 50, HC = 47; serum	qRT-PCR	AUC = 0.85, Sn = 81%, Sp = 78%; Increased in advanced stages	Wang *et al*[157], 2014
miR-16			AUC = 0.90, Sn = 79%, Sp = 78%	
miR-100			AUC = 0.71, Sn = 71%, Sp = 58% Increased in advanced stages	
miR-16	Discovery: stage Ⅰ non-cardia GC = 40, HC = HC;	Discovery: TaqMan array, validation: qRT-PCR	AUC = 0.768 (all in validation set)	Zhu *et al*[120], 2014
miR-25			AUC = 0.694	
miR-92a	Validation: stage Ⅰ non-cardia GC = 48, HC = 102		AUC = 0.732	
miR-451			AUC = 0.790	
miR-486-5p			AUC = 0.779	
5 miRNA combined			AUC = 0.812, Sn = 72.9%, Sp = 89.2%; In vitro evidence that miR-16, miR-25 and miR92a but not miR-451 and miR486-5p are secreted from cancer cells	
miR-222	GC = 114, HC = 56; plasma	qRT-PCR	AUC = 0.850, Sn = 66.1%, Sp = 88.3%	Fu *et al*[158], 2014
miR-18a	GC = 82, HC = 65, plasma	qRT-PCR	AUC = 0.907, Sn = 80.5%, Sp = 84.6%; no association with stage	Su *et al*[123], 2014
miR-18a	GC = 104, HC = 65, plasma and GC tissues	qRT-PCR	AUC = 0.8059, Sn = 84.6%, Sp = 69.2% Overexpressed in GC; in vitro evidence that miR-18a is released by cancer cells; decreased in postoperative plasma	Tsujiura *et al*[159], 2015
Circulating cell-free mRNAs and long non-coding RNAs				
hTERT mRNA	GC = 118, CAG = 40, HC = 58; plasma	qRT-PCR	AUC = 0.891, Sn = 66%, Sp = 87%; strong positive correlation with advanced stage of GC	Kang *et al*[125], 2013
MACC1 mRNA	GC = 76, HC = 54, plasma	qRT-PCR	Sn = 68%, Sp = 89%	Burock *et al*[160], 2015
LINC00152	Pre- and post-operative plasma GC = 79, GED = 31, HC = 81	qRT-PCR	AUC = 0.657, Sn = 48.1%, Sp = 85.2%	Li *et al*[131], 2015

AUC: Area under the curve; DYS: Intestinal dysplasia; GC: Gastric cancer; HC: Healthy controls; Sn: Sensitivity; Sp: Specificity.

showed an AUC of 0.96 and 0.92, respectively, thus demonstrating their relevance for diagnosing GC and monitoring the course of the disease. However, Zhu *et al*[120] found that these two miRNAs had a lower diagnostic performance (AUC of 0.790 and 0.779, respectively) for detecting early stage non-cardia GC. Surprisingly, both miRNAs were downregulated in GC tissues compared with adjacent normal tissues[119], and their cellular source and the mechanism of release into the circulation remains unknown[120].

Subsequent studies have resulted in the identification of several individual miRNAs or miRNA signatures

that show significant diagnostic values, with an AUC as high as 0.953[121]. Some of the studies report no significant differences in the miRNA levels across GC stages, thus suggesting that these miRNA biomarkers appear in patients' blood at an early stage of cancer development and could be suitable for the detection of early GC[121-123]. A retrospective study by Song *et al*[124] demonstrated an increasing trend in expression of three serum miRNAs (miR-221, miR-744 and miR-376c) over a 15-year timeframe before GC diagnosis and showed that the 3-miRNA panel could classify serum samples collected 2-5 years before the clinical diagnosis of GC with 79.3% accuracy.

Several other studies have explored the possibility of using circulating mRNAs, long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs) for the detection of GC. Despite the presence of RNases in human blood, all these RNA species turned out to be stable and robustly detectable in plasma or serum samples and some of them have shown a relatively high diagnostic value. For example, Kang *et al*[125] reported that elevated *hTERT* mRNA levels could distinguish between GC and healthy controls, with an AUC of 0.891, sensitivity of 66% and specificity of 87%. LncRNAs and circRNAs are recently-discovered categories of non-coding RNAs that regulate gene expression at the transcriptional and posttranscriptional levels and accumulating evidence suggests that they may play key roles in the development of cancer[126,127]. Several recent studies reported that their expression is deregulated in GC tissues and some can be detected in patients' blood[128-132], and thus, they may represent a novel source for circulating biomarker discovery. However, a deeper understanding in their biology, mode of action and mechanism of release into the circulation is required to evaluate their clinical significance.

However, there is a little overlap among the identified miRNAs in various studies and, with a few exceptions such as miR-223 or miR-18a, most of the results have not been reproduced by other studies to date. One of the main reasons for variability and inconsistency among the findings is the approach used to normalize qRT-PCR data. Currently, there is no consensus on housekeeping genes in serum or plasma that could be used as internal controls for this normalization. Several studies have used U6 snRNA or miR-16 as a normalization control, but other studies have shown large fluctuations in their levels in serum and plasma, and they concluded that these RNAs are not suitable as endogenous controls[133,134]. An alternative approach for controlling the technical variability is based on synthetic spike-ins. In this approach, miRNAs without a sequence homology to human miRNAs, such as cel-miR-39, are spiked into the serum/plasma samples before RNA extraction and amplified together with the target miRNAs. The target miRNA levels are then normalized to the sample volume and spike-ins, but this approach does not control for the preanalytical variability. Hemolysis has been shown to alter miRNA content in plasma. For example, miR-16 and miR-451 have been shown to be released by red blood cells and their levels were proportional to the degree of hemolysis[135]. This suggests that assessing the degree of hemolysis is a crucial step in assays that quantify circulating RNA levels.

OTHER POTENTIAL BIOMARKERS

Circulating tumor cells

Detection of the presence of CTCs in the peripheral blood of cancer patients has a promising clinical value in the predictive and prognostic setting, but currently, it has a rather limited potential for detection of early stage cancer. Accumulating evidence shows variable overall GC detection rates based on CTC isolation and characterization of their mRNA expression (ranging from 9.6%-71.2%). Current results are summarized in recent review by Tsujiura *et al*[105]. Studies have shown that the number of CTCs analyzed in peripheral blood from patients with metastatic gastrointestinal cancer is generally lower (1-2 CTCs/7.5 mL of blood) than that found in other malignancies, such as in patients with metastatic prostate cancer (3-5 CTCs/7.5 mL of blood) or breast cancer (6-7 CTCs/7.5 mL of blood)[136-138]. Although novel approaches for rare CTC detection in a small amount of peripheral blood are emerging, their sensitivity for early stage GC is still limited. For example Kolostova *et al*[139] demonstrated that there are biologically inherent limitations to the CTC-based test application for GC detection.

To date, the CellSearch system (Veridex) is the first and only FDA approved test that has been shown to be useful for detecting CTCs in patients with metastatic breast, prostate or colorectal cancer. It enables the enumeration of CTCs of epithelial origin (CD45-, EpCAM+, and cytokeratins 8, 18+, and/or 19+) in whole blood. The usefulness of the CellSearch system in GC detection has recently been evaluated by Uenosono *et al*[140]. The authors showed that the test could detect stage Ⅰ and Ⅱ GC patients in only in 1.6% (1/64) and 3.9% (1/26) of the cases, respectively (P = 0.0002); however, the data indicated that CTC detection in peripheral blood may be a useful tool for predicting tumor progression, prognosis, and the effect of chemotherapy in patients with GC. Besides the CellSearch system, novel and more sensitive experimental approaches for rare CTC detection are being developed; however, the data on their sensitivity for early stage GC is still limited. For example, Kolostova *et al*[139] used the MetaCell® approach, which is based on physical sorting and cultivation of isolated CTCs, to detect one out of three stage Ⅰ GC and two out of four stage Ⅱ GC cases.

Taken together, although numerous studies have been performed, the research on this type of "liquid biopsy" for GC detection remains in its infancy. Further

studies involving larger patient/control cohorts, a deeper understanding of CTC biology and significance and progress in techniques linked to CTC isolation and characterization could enhance their usefulness as biomarkers in future.

Cancer-derived extracellular vesicles

Cancer-derived EVs are gaining increasing attention in the cancer biomarker field[141]. Currently, they are under intense investigation for their composition, biological functions and distribution, along with their diagnostic and therapeutic potential. Either secreted or shed from cancer cells, they are considered to be a liquid tumor biopsy because they are found in elevated levels in the circulation and they have been shown to carry cancer cell-derived lipids, proteins, mRNAs, non-coding and structural RNAs and even genomic DNA, which at least partially reflect parental cells and represents attractive shuttles for cancer biomarkers[142,143]. Studies from several groups have demonstrated the diagnostic potential of cancer-derived EV for the detection of various cancer types, including but not limited to melanoma, prostate, ovarian and colorectal cancer (reviewed by Zocco *et al*[144]). However, there is little data on circulating GC EVs; to the best of our knowledge, only one study has been published regarding the analyses of circulating EVs in patients with stomach cancer. Baran *et al*[145] attempted to characterize the EVs isolated from platelet-depleted plasma samples from 37 GC patients, compared to those from 10 healthy controls. They demonstrated that GC patients, compared with controls, have: (1) a significantly higher number of total circulating EVs (except for patients with stage I GC) ($P < 0.001$); (2) EVs with significantly higher expression of GC-associated proteins MAGE-1 and Her-2/*neu*+ (only late stage patients analyzed, $n = 13$; $P < 0.05$); and (3) EVs with upregulated CRC6 and downregulated CXCR4 surface expression ($P < 0.05$). However, they made no attempt to set a diagnostic value based on these findings. Considering the current advances in this field, further studies on EVs released in patients with GC are warranted.

CONCLUSION

Over the last decade, considerable effort has been dedicated to discovering various types of cancer-associated molecules in the blood of GC patients. Several of the identified biomarkers have remarkably high sensitivity and specificity that greatly outperform the previously-known GC serum biomarkers such PGs, CA 72-4, CA19-9 and CEA[146,147], and therefore have the potential to complement or replace the existing endoscopy, X-ray or biopsy-based examinations. Each type of biomarker has a different origin, provides various types of information and has their own strengths and weaknesses, thus suggesting different clinical applications. For example, autoantibodies against TAAs are qualitative and highly specific markers for the presence of cancer, and they have been identified in the circulation several years before the clinical manifestation of the cancer. Autoantibodies against TAAs, therefore, seem to be an excellent biomarker for the detection of early-stage cancer. However, there is a subset of GC patients with no humoral immunity against tumor antigens that limits the use of autoantibody-based assays for population-based screening programs. Moreover, antibodies are relatively stable and they may remain in the circulation for several months, even years; therefore, they likely have limited potential for monitoring the disease. However, detection of cancer-specific genetic or epigenetic alterations in the cfNA would provide an excellent tool for monitoring cancer dynamics, while their diagnostic use is limited to those patients who have the respective alterations. In addition, these assays may fail to detect evolving cancer cell clones that have lost the respective marker. Several of the proteomics-based biomarker models have demonstrated high sensitivity and specificity for detecting GC; however, it is not clear if most of these proteins are directly and causally involved in the development of cancer and therefore further mechanistic studies are required to validate them as cancer-associated biomarkers.

We suggest that new bio-fluid testing systems, which will combine various types of biomarkers, will be developed in the future and will allow collection of all the information on the disease status, genetic make-up of the tumor and the status of patients' immune system using a single blood test. However, there are several technical issues that have to be resolved before such a device could meet the regulatory requirements. Thus, the next goal would be to perform a head-to-head comparison of various biomarker models and technological platforms in large, well-characterized cohorts of patients and controls to select the biomarkers with highest clinical relevance. This would require a collaborative effort among the research groups to establish standardized pre-analytical and analytical procedures and guidelines for reporting the results.

REFERENCES

1 **Ferlay J**, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11. Lyon, France: International Agency for Research on Cancer, 2013

2 **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]

3 **Lauren P**. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. an attempt at a histo-clinical classification. *Acta Pathol Microbiol Scand* 1965; **64**: 31-49 [PMID: 14320675]

4 **Correa P**, Haenszel W, Cuello C, Tannenbaum S, Archer M. A model for gastric cancer epidemiology. *Lancet* 1975; **2**: 58-60 [PMID:

49653]
5 **Hishida A**, Matsuo K, Goto Y, Hamajima N. Genetic predisposition to Helicobacter pylori-induced gastric precancerous conditions. *World J Gastrointest Oncol* 2010; **2**: 369-379 [PMID: 21160888]
6 **Park JY**, von Karsa L, Herrero R. Prevention strategies for gastric cancer: a global perspective. *Clin Endosc* 2014; **47**: 478-489 [PMID: 25505712 DOI: 10.5946/ce.2014.47.6.478]
7 **Oliveira C**, Pinheiro H, Figueiredo J, Seruca R, Carneiro F. Familial gastric cancer: genetic susceptibility, pathology, and implications for management. *Lancet Oncol* 2015; **16**: e60-e70 [PMID: 25638682 DOI: 10.1016/S1470-2045(14)71016-2]
8 **Orditura M**, Galizia G, Sforza V, Gambardella V, Fabozzi A, Laterza MM, Andreozzi F, Ventriglia J, Savastano B, Mabilia A, Lieto E, Ciardiello F, De Vita F. Treatment of gastric cancer. *World J Gastroenterol* 2014; **20**: 1635-1649 [PMID: 24587643 DOI: 10.3748/wjg.v20.i7.1635]
9 **Rosati G**, Ferrara D, Manzione L. New perspectives in the treatment of advanced or metastatic gastric cancer. *World J Gastroenterol* 2009; **15**: 2689-2692 [PMID: 19522017]
10 **Yoshida K**, Yamaguchi K, Okumura N, Osada S, Takahashi T, Tanaka Y, Tanabe K, Suzuki T. The roles of surgical oncologists in the new era: minimally invasive surgery for early gastric cancer and adjuvant surgery for metastatic gastric cancer. *Pathobiology* 2011; **78**: 343-352 [PMID: 22104206 DOI: 10.1159/000328197]
11 **Leja M**, You W, Camargo MC, Saito H. Implementation of gastric cancer screening - the global experience. *Best Pract Res Clin Gastroenterol* 2014; **28**: 1093-1106 [PMID: 25439074 DOI: 10.1016/j.bpg.2014.09.005]
12 **di Mario F**, Cavallaro LG. Non-invasive tests in gastric diseases. *Dig Liver Dis* 2008; **40**: 523-530 [PMID: 18439884 DOI: 10.1016/j.dld.2008.02.028]
13 **Dinis-Ribeiro M**, Yamaki G, Miki K, Costa-Pereira A, Matsukawa M, Kurihara M. Meta-analysis on the validity of pepsinogen test for gastric carcinoma, dysplasia or chronic atrophic gastritis screening. *J Med Screen* 2004; **11**: 141-147 [PMID: 15333273]
14 **Leja M**, Kupcinskas L, Funka K, Sudraba A, Jonaitis L, Ivanauskas A, Janciauskas D, Kiudelis G, Chiu HM, Lin JT. The validity of a biomarker method for indirect detection of gastric mucosal atrophy versus standard histopathology. *Dig Dis Sci* 2009; **54**: 2377-2384 [PMID: 19731026 DOI: 10.1007/s10620-009-0947-5]
15 **Kikuchi S**, Kato M, Katsuyama T, Tominaga S, Asaka M. Design and planned analyses of an ongoing randomized trial assessing the preventive effect of Helicobacter pylori eradication on occurrence of new gastric carcinomas after endoscopic resection. *Helicobacter* 2006; **11**: 147-151 [PMID: 16684261 DOI: 10.1111/j.1523-5378.2006.00392.x]
16 **Kitahara F**, Kobayashi K, Sato T, Kojima Y, Araki T, Fujino MA. Accuracy of screening for gastric cancer using serum pepsinogen concentrations. *Gut* 1999; **44**: 693-697 [PMID: 10205207]
17 **Kang JM**, Kim N, Yoo JY, Park YS, Lee DH, Kim HY, Lee HS, Choe G, Kim JS, Jung HC, Song IS. The role of serum pepsinogen and gastrin test for the detection of gastric cancer in Korea. *Helicobacter* 2008; **13**: 146-156 [PMID: 18321304 DOI: 10.1111/j.1523-5378.2008.00592.x]
18 **Yanaoka K**, Oka M, Mukoubayashi C, Yoshimura N, Enomoto S, Iguchi M, Magari H, Utsunomiya H, Tamai H, Arii K, Ohata H, Fujishiro M, Takeshita T, Mohara O, Ichinose M. Cancer high-risk subjects identified by serum pepsinogen tests: outcomes after 10-year follow-up in asymptomatic middle-aged males. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 838-845 [PMID: 18398025 DOI: 10.1158/1055-9965.EPI-07-2762]
19 **Mizuno S**, Kobayashi M, Tomita S, Miki I, Masuda A, Onoyama M, Habu Y, Inokuchi H, Watanabe Y. Validation of the pepsinogen test method for gastric cancer screening using a follow-up study. *Gastric Cancer* 2009; **12**: 158-163 [PMID: 19890696 DOI: 10.1007/s10120-009-0522-y]
20 **Herrero R**, Park JY, Forman D. The fight against gastric cancer - the IARC Working Group report. *Best Pract Res Clin Gastroenterol* 2014; **28**: 1107-1114 [PMID: 25439075 DOI: 10.1016/j.bpg.2014.10.003]
21 **Liu W**, Yang Q, Liu B, Zhu Z. Serum proteomics for gastric cancer. *Clin Chim Acta* 2014; **431**: 179-184 [PMID: 24525212 DOI: 10.1016/j.cca.2014.02.001]
22 **Omenn GS**. THE HUPO Human Plasma Proteome Project. *Proteomics Clin Appl* 2007; **1**: 769-779 [PMID: 21136733 DOI: 10.1002/prca.200700369]
23 **Lin LL**, Huang HC, Juan HF. Discovery of biomarkers for gastric cancer: a proteomics approach. *J Proteomics* 2012; **75**: 3081-3097 [PMID: 22498886 DOI: 10.1016/j.jprot.2012.03.046]
24 **Ebert MP**, Meuer J, Wiemer JC, Schulz HU, Reymond MA, Traugott U, Malfertheiner P, Röcken C. Identification of gastric cancer patients by serum protein profiling. *J Proteome Res* 2004; **3**: 1261-1266 [PMID: 15595736 DOI: 10.1021/pr049865s]
25 **Liu C**, Pan C, Liang Y. Screening and identification of serum proteomic biomarkers for gastric adenocarcinoma. *Exp Ther Med* 2012; **3**: 1005-1009 [PMID: 22970007 DOI: 10.3892/etm.2012.515]
26 **Li P**, Zhang D, Guo C. Serum biomarker screening for the diagnosis of early gastric cancer using SELDI-TOF-MS. *Mol Med Rep* 2012; **5**: 1531-1535 [PMID: 22427025 DOI: 10.3892/mmr.2012.834]
27 **Poon TC**, Sung JJ, Chow SM, Ng EK, Yu AC, Chu ES, Hui AM, Leung WK. Diagnosis of gastric cancer by serum proteomic fingerprinting. *Gastroenterology* 2006; **130**: 1858-1864 [PMID: 16697748 DOI: 10.1053/j.gastro.2006.02.011]
28 **Qian HG**, Shen J, Ma H, Ma HC, Su YH, Hao CY, Xing BC, Huang XF, Shou CC. Preliminary study on proteomics of gastric carcinoma and its clinical significance. *World J Gastroenterol* 2005; **11**: 6249-6253 [PMID: 16419150]
29 **Liang Y**, Fang M, Li J, Liu CB, Rudd JA, Kung HF, Yew DT. Serum proteomic patterns for gastric lesions as revealed by SELDI mass spectrometry. *Exp Mol Pathol* 2006; **81**: 176-180 [PMID: 16828742 DOI: 10.1016/j.yexmp.2006.04.008]
30 **Fan NJ**, Li K, Liu QY, Wang XL, Hu L, Li JT, Gao CF. Identification of tubulin beta chain, thymosin beta-4-like protein 3, and cytochrome b-c$_1$ complex subunit 1 as serological diagnostic biomarkers of gastric cancer. *Clin Biochem* 2013; **46**: 1578-1584 [PMID: 23747515 DOI: 10.1016/j.clinbiochem.2013.05.068]
31 **Lu HB**, Zhou JH, Ma YY, Lu HL, Tang YL, Zhang QY, Zhao CH. Five serum proteins identified using SELDI-TOF-MS as potential biomarkers of gastric cancer. *Jpn J Clin Oncol* 2010; **40**: 336-342 [PMID: 20089528 DOI: 10.1093/jjco/hyp175]
32 **Su Y**, Shen J, Qian H, Ma H, Ji J, Ma H, Ma L, Zhang W, Meng L, Li Z, Wu J, Jin G, Zhang J, Shou C. Diagnosis of gastric cancer using decision tree classification of mass spectral data. *Cancer Sci* 2007; **98**: 37-43 [PMID: 17052262 DOI: 10.1111/j.1349-7006.2006.00339.x]
33 **Wang P**, Whiteaker JR, Paulovich AG. The evolving role of mass spectrometry in cancer biomarker discovery. *Cancer Biol Ther* 2009; **8**: 1083-1094 [PMID: 19502776]
34 **Yang J**, Xiong X, Wang X, Guo B, He K, Huang C. Identification of peptide regions of SERPINA1 and ENOSF1 and their protein expression as potential serum biomarkers for gastric cancer. *Tumour Biol* 2015; **36**: 5109-5118 [PMID: 25677901 DOI: 10.1007/s13277-015-3163-2]
35 **Ahn HS**, Shin YS, Park PJ, Kang KN, Kim Y, Lee HJ, Yang HK, Kim CW. Serum biomarker panels for the diagnosis of gastric adenocarcinoma. *Br J Cancer* 2012; **106**: 733-739 [PMID: 22240791 DOI: 10.1038/bjc.2011.592]
36 **Surinova S**, Schiess R, Hüttenhain R, Cerciello F, Wollscheid B, Aebersold R. On the development of plasma protein biomarkers. *J Proteome Res* 2011; **10**: 5-16 [PMID: 21142170 DOI: 10.1021/pr1008515]
37 **Liu W**, Liu B, Cai Q, Li J, Chen X, Zhu Z. Proteomic identification of serum biomarkers for gastric cancer using multi-dimensional liquid chromatography and 2D differential gel electrophoresis. *Clin Chim Acta* 2012; **413**: 1098-1106 [PMID: 22446497 DOI: 10.1016/j.cca.2012.03.003]
38 **Chong PK**, Lee H, Loh MC, Choong LY, Lin Q, So JB, Lim KH, Soo RA, Yong WP, Chan SP, Smoot DT, Ashktorab H, Yeoh KG, Lim YP. Upregulation of plasma C9 protein in gastric cancer patients. *Proteomics* 2010; **10**: 3210-3221 [PMID: 20707004 DOI:

10.1002/pmic.201000127]
39 **Ebert MP**, Niemeyer D, Deininger SO, Wex T, Knippig C, Hoffmann J, Sauer J, Albrecht W, Malfertheiner P, Röcken C. Identification and confirmation of increased fibrinopeptide a serum protein levels in gastric cancer sera by magnet bead assisted MALDI-TOF mass spectrometry. *J Proteome Res* 2006; **5**: 2152-2158 [PMID: 16944926 DOI: 10.1021/pr060011c]
40 **Zhang MH**, Xu XH, Wang Y, Linq QX, Bi YT, Miao XJ, Ye CF, Gao SX, Gong CY, Xiang H, Dong MS. A prognostic biomarker for gastric cancer with lymph node metastases. *Anat Rec* (Hoboken) 2013; **296**: 590-594 [PMID: 23382154 DOI: 10.1002/ar.22642]
41 **Cohen M**, Yossef R, Erez T, Kugel A, Welt M, Karpasas MM, Bones J, Rudd PM, Taieb J, Boissin H, Harats D, Noy K, Tekoah Y, Lichtenstein RG, Rubin E, Porgador A. Serum apolipoproteins C-I and C-III are reduced in stomach cancer patients: results from MALDI-based peptidome and immuno-based clinical assays. *PLoS One* 2011; **6**: e14540 [PMID: 21267442 DOI: 10.1371/journal.pone.0014540]
42 **Pitteri SJ**, Kelly-Spratt KS, Gurley KE, Kennedy J, Buson TB, Chin A, Wang H, Zhang Q, Wong CH, Chodosh LA, Nelson PS, Hanash SM, Kemp CJ. Tumor microenvironment-derived proteins dominate the plasma proteome response during breast cancer induction and progression. *Cancer Res* 2011; **71**: 5090-5100 [PMID: 21653680 DOI: 10.1158/0008-5472.CAN-11-0568]
43 **Bones J**, Byrne JC, O'Donoghue N, McManus C, Scaife C, Boissin H, Nastase A, Rudd PM. Glycomic and glycoproteomic analysis of serum from patients with stomach cancer reveals potential markers arising from host defense response mechanisms. *J Proteome Res* 2011; **10**: 1246-1265 [PMID: 21142185 DOI: 10.1021/pr101036b]
44 **Gomes C**, Almeida A, Ferreira JA, Silva L, Santos-Sousa H, Pinto-de-Sousa J, Santos LL, Amado F, Schwientek T, Levery SB, Mandel U, Clausen H, David L, Reis CA, Osório H. Glycoproteomic analysis of serum from patients with gastric precancerous lesions. *J Proteome Res* 2013; **12**: 1454-1466 [PMID: 23312025 DOI: 10.1021/pr301112x]
45 **Ozcan S**, Barkauskas DA, Renee Ruhaak L, Torres J, Cooke CL, An HJ, Hua S, Williams CC, Dimapasoc LM, Han Kim J, Camorlinga-Ponce M, Rocke D, Lebrilla CB, Solnick JV. Serum glycan signatures of gastric cancer. *Cancer Prev Res* (Phila) 2014; **7**: 226-235 [PMID: 24327722 DOI: 10.1158/1940-6207.CAPR-13-0235]
46 **Roy B**, Chattopadhyay G, Mishra D, Das T, Chakraborty S, Maiti TK. On-chip lectin microarray for glycoprofiling of different gastritis types and gastric cancer. *Biomicrofluidics* 2014; **8**: 034107 [PMID: 24959308 DOI: 10.1063/1.4882778]
47 **Ruhaak LR**, Barkauskas DA, Torres J, Cooke CL, Wu LD, Stroble C, Ozcan S, Williams CC, Camorlinga M, Rocke DM, Lebrilla CB, Solnick JV. The Serum Immunoglobulin G Glycosylation Signature of Gastric Cancer. *EuPA Open Proteom* 2015; **6**: 1-9 [PMID: 25685702 DOI: 10.1016/j.euprot.2014.11.002]
48 **Parker CE**, Borchers CH. Mass spectrometry based biomarker discovery, verification, and validation--quality assurance and control of protein biomarker assays. *Mol Oncol* 2014; **8**: 840-858 [PMID: 24713096 DOI: 10.1016/j.molonc.2014.03.006]
49 **Schreiber RD**, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science* 2011; **331**: 1565-1570 [PMID: 21436444 DOI: 10.1126/science.1203486]
50 **Chapman C**, Murray A, Chakrabarti J, Thorpe A, Woolston C, Sahin U, Barnes A, Robertson J. Autoantibodies in breast cancer: their use as an aid to early diagnosis. *Ann Oncol* 2007; **18**: 868-873 [PMID: 17347129 DOI: 10.1093/annonc/mdm007]
51 **Zhong L**, Coe SP, Stromberg AJ, Khattar NH, Jett JR, Hirschowitz EA. Profiling tumor-associated antibodies for early detection of non-small cell lung cancer. *J Thorac Oncol* 2006; **1**: 513-519 [PMID: 17409910]
52 **Li Y**, Karjalainen A, Koskinen H, Hemminki K, Vainio H, Shnaidman M, Ying Z, Pukkala E, Brandt-Rauf PW. p53 autoantibodies predict subsequent development of cancer. *Int J Cancer* 2005; **114**: 157-160 [PMID: 15523685 DOI: 10.1002/ijc.20715]
53 **Preuss KD**, Zwick C, Bormann C, Neumann F, Pfreundschuh M. Analysis of the B-cell repertoire against antigens expressed by human neoplasms. *Immunol Rev* 2002; **188**: 43-50 [PMID: 12445280]
54 **Chen YT**. Identification of human tumor antigens by serological expression cloning: an online review on SEREX. *Cancer Immun* 2004
55 **Chapman CJ**, Healey GF, Murray A, Boyle P, Robertson C, Peek LJ, Allen J, Thorpe AJ, Hamilton-Fairley G, Parsy-Kowalska CB, MacDonald IK, Jewell W, Maddison P, Robertson JF. EarlyCDT®-Lung test: improved clinical utility through additional autoantibody assays. *Tumour Biol* 2012; **33**: 1319-1326 [PMID: 22492236 DOI: 10.1007/s13277-012-0379-2]
56 **Zayakin P**, Ancāns G, Siliņa K, Meistere I, Kalniņa Z, Andrejeva D, Endzeliņš E, Ivanova L, Pismennaja A, Ruskule A, Doniņa S, Wex T, Malfertheiner P, Leja M, Linē A. Tumor-associated autoantibody signature for the early detection of gastric cancer. *Int J Cancer* 2013; **132**: 137-147 [PMID: 22684876 DOI: 10.1002/ijc.27667]
57 **Gnjatic S**, Ritter E, Büchler MW, Giese NA, Brors B, Frei C, Murray A, Halama N, Zörnig I, Chen YT, Andrews C, Ritter G, Old LJ, Odunsi K, Jäger D. Seromic profiling of ovarian and pancreatic cancer. *Proc Natl Acad Sci USA* 2010; **107**: 5088-5093 [PMID: 20194765 DOI: 10.1073/pnas.0914213107]
58 **Werner S**, Chen H, Tao S, Brenner H. Systematic review: serum autoantibodies in the early detection of gastric cancer. *Int J Cancer* 2015; **136**: 2243-2252 [PMID: 24615018 DOI: 10.1002/ijc.28807]
59 **Fujiwara S**, Wada H, Kawada J, Kawabata R, Takahashi T, Fujita J, Hirao T, Shibata K, Makari Y, Iijima S, Nishikawa H, Jungbluth AA, Nakamura Y, Kurokawa Y, Yamasaki M, Miyata H, Nakajima K, Takiguchi S, Nakayama E, Mori M, Doki Y. NY-ESO-1 antibody as a novel tumour marker of gastric cancer. *Br J Cancer* 2013; **108**: 1119-1125 [PMID: 23403818 DOI: 10.1038/bjc.2013.51]
60 **Zeng G**, Aldridge ME, Wang Y, Pantuck AJ, Wang AY, Liu YX, Han Y, Yuan YH, Robbins PF, Dubinett SM, deKernion JB, Belldegrun AS. Dominant B cell epitope from NY-ESO-1 recognized by sera from a wide spectrum of cancer patients: implications as a potential biomarker. *Int J Cancer* 2005; **114**: 268-273 [PMID: 15540228 DOI: 10.1002/ijc.20716]
61 **Kurtenkov O**, Klaamas K, Mensdorff-Pouilly S, Miljukhina L, Shljapnikova L, Chuzmarov V. Humoral immune response to MUC1 and to the Thomsen-Friedenreich (TF) glycotope in patients with gastric cancer: relation to survival. *Acta Oncol* 2007; **46**: 316-323 [PMID: 17450466 DOI: 10.1080/02841860601055441]
62 **Zhang JY**, Chan EK, Peng XX, Lu M, Wang X, Mueller F, Tan EM. Autoimmune responses to mRNA binding proteins p62 and Koc in diverse malignancies. *Clin Immunol* 2001; **100**: 149-156 [PMID: 11465943 DOI: 10.1006/clim.2001.5048]
63 **Zhang JY**, Casiano CA, Peng XX, Koziol JA, Chan EK, Tan EM. Enhancement of antibody detection in cancer using panel of recombinant tumor-associated antigens. *Cancer Epidemiol Biomarkers Prev* 2003; **12**: 136-143 [PMID: 12582023]
64 **Megliorino R**, Shi FD, Peng XX, Wang X, Chan EK, Tan EM, Zhang JY. Autoimmune response to anti-apoptotic protein survivin and its association with antibodies to p53 and c-myc in cancer detection. *Cancer Detect Prev* 2005; **29**: 241-248 [PMID: 15896923 DOI: 10.1016/j.cdp.2005.03.002]
65 **Meistere I**, Kalnina, Z, Silina, K, Line, A. Cancer-associated Autoantibodies as Biomarkers for Early Detection and Prognosis is Cancer: An Update. *Curr Canc Ther Rev* 2013; **9**: 10 [DOI: 10.2174/1573394709041404180936 12]
66 **Preiss S**, Kammertoens T, Lampert C, Willimsky G, Blankenstein T. Tumor-induced antibodies resemble the response to tissue damage. *Int J Cancer* 2005; **115**: 456-462 [PMID: 15700321 DOI: 10.1002/ijc.20914]
67 **Ludewig B**, Krebs P, Metters H, Tatzel J, Türeci O, Sahin U. Molecular characterization of virus-induced autoantibody responses. *J Exp Med* 2004; **200**: 637-646 [PMID: 15353556 DOI: 10.1084/jem.20040358]

68 **Zhou SL**, Ku JW, Fan ZM, Yue WB, Du F, Zhou YF, Liu YL, Li Y, Tang S, Hu YL, Hu XP, Hou ZC, Liu J, Liu Y, Feng XS, Wang LD. Detection of autoantibodies to a panel of tumor-associated antigens for the diagnosis values of gastric cardia adenocarcinoma. *Dis Esophagus* 2015; **28**: 371-379 [PMID: 24612004 DOI: 10.1111/dote.12206]

69 **Shiota G**, Ishida M, Noguchi N, Takano Y, Oyama K, Okubo M, Katayama S, Harada K, Hori K, Ashida K, Kishimoto Y, Hosoda A, Suou T, Ito H, Kawasaki H. Clinical significance of serum P53 antibody in patients with gastric cancer. *Res Commun Mol Pathol Pharmacol* 1998; **99**: 41-51 [PMID: 9523354]

70 **Qiu LL**, Hua PY, Ye LL, Wang YC, Qiu T, Bao HZ, Wang L. The detection of serum anti-p53 antibodies from patients with gastric carcinoma in China. *Cancer Detect Prev* 2007; **31**: 45-49 [PMID: 17292563 DOI: 10.1016/j.cdp.2006.12.005]

71 **Reuschenbach M**, von Knebel Doeberitz M, Wentzensen N. A systematic review of humoral immune responses against tumor antigens. *Cancer Immunol Immunother* 2009; **58**: 1535-1544 [PMID: 19562338 DOI: 10.1007/s00262-009-0733-4]

72 **Karagiannis P**, Gilbert AE, Josephs DH, Ali N, Dodev T, Saul L, Correa I, Roberts L, Beddowes E, Koers A, Hobbs C, Ferreira S, Geh JL, Healy C, Harries M, Acland KM, Blower PJ, Mitchell T, Fear DJ, Spicer JF, Lacy KE, Nestle FO, Karagiannis SN. IgG4 subclass antibodies impair antitumor immunity in melanoma. *J Clin Invest* 2013; **123**: 1457-1474 [PMID: 23454746 DOI: 10.1172/JCI65579]

73 **Mandel P**, Metais P. Les acides nucléiques du plasma sanguin chez l'homme. *C R Seances Soc Biol Fil* 1948; **142**: 241-243 [PMID: 18875018]

74 **Schwarzenbach H**, Hoon DS, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. *Nat Rev Cancer* 2011; **11**: 426-437 [PMID: 21562580 DOI: 10.1038/nrc3066]

75 **Su Z**, Yang Z, Xu Y, Chen Y, Yu Q. MicroRNAs in apoptosis, autophagy and necroptosis. *Oncotarget* 2015; **6**: 8474-8490 [PMID: 25893379]

76 **Ivanov A**, Pawlikowski J, Manoharan I, van Tuyn J, Nelson DM, Rai TS, Shah PP, Hewitt G, Korolchuk VI, Passos JF, Wu H, Berger SL, Adams PD. Lysosome-mediated processing of chromatin in senescence. *J Cell Biol* 2013; **202**: 129-143 [PMID: 23816621 DOI: 10.1083/jcb.201212110]

77 **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]

78 **György B**, Szabó TG, Pásztói M, Pál Z, Misják P, Aradi B, László V, Pállinger E, Pap E, Kittel A, Nagy G, Falus A, Buzás EI. Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. *Cell Mol Life Sci* 2011; **68**: 2667-2688 [PMID: 21560073 DOI: 10.1007/s00018-011-0689-3]

79 **Sadovska L**, Bajo CS, Kalniņa Z, Linē A. Biodistribution, Uptake and Effects Caused by Cancer-derived Extracellular Vesicles. *JCB* 2015; **4**: 1-15 [DOI: 10.5772/60522]

80 **Zandberga E**, Kozirovskis V, Ābols A, Andrējeva D, Purkalne G, Linē A. Cell-free microRNAs as diagnostic, prognostic, and predictive biomarkers for lung cancer. *Genes Chromosomes Cancer* 2013; **52**: 356-369 [PMID: 23404859 DOI: 10.1002/gcc.22032]

81 **Jahr S**, Hentze H, Englisch S, Hardt D, Fackelmayer FO, Hesch RD, Knippers R. DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells. *Cancer Res* 2001; **61**: 1659-1665 [PMID: 11245480]

82 **Wang BG**, Huang HY, Chen YC, Bristow RE, Kassauei K, Cheng CC, Roden R, Sokoll LJ, Chan DW, Shih IeM. Increased plasma DNA integrity in cancer patients. *Cancer Res* 2003; **63**: 3966-3968 [PMID: 12873992]

83 **Umetani N**, Giuliano AE, Hiramatsu SH, Amersi F, Nakagawa T, Martino S, Hoon DS. Prediction of breast tumor progression by integrity of free circulating DNA in serum. *J Clin Oncol* 2006; **24**: 4270-4276 [PMID: 16963729 DOI: 10.1200/JCO.2006.05.9493]

84 **Umetani N**, Kim J, Hiramatsu S, Reber HA, Hines OJ, Bilchik AJ, Hoon DS. Increased integrity of free circulating DNA in sera of patients with colorectal or periampullary cancer: direct quantitative PCR for ALU repeats. *Clin Chem* 2006; **52**: 1062-1069 [PMID: 16723681 DOI: 10.1373/clinchem.2006.068577]

85 **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 USA* 2008; **105**: 10513-10518 [PMID: 18663219 DOI: 10.1073/pnas.0804549105]

86 **Gallo A**, Tandon M, Alevizos I, Illei GG. The majority of microRNAs detectable in serum and saliva is concentrated in exosomes. *PLoS One* 2012; **7**: e30679 [PMID: 22427800 DOI: 10.1371/journal.pone.0030679]

87 **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]

88 **Arroyo JD**, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, Mitchell PS, Bennett CF, Pogosova-Agadjanyan EL, Stirewalt DL, Tait JF, Tewari M. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proc Natl Acad Sci USA* 2011; **108**: 5003-5008 [PMID: 21383194 DOI: 10.1073/pnas.1019055108]

89 **Chevillet JR**, Kang Q, Ruf IK, Briggs HA, Vojtech LN, Hughes SM, Cheng HH, Arroyo JD, Meredith EK, Gallichotte EN, Pogosova-Agadjanyan EL, Morrissey C, Stirewalt DL, Hladik F, Yu EY, Higano CS, Tewari M. Quantitative and stoichiometric analysis of the microRNA content of exosomes. *Proc Natl Acad Sci USA* 2014; **111**: 14888-14893 [PMID: 25267620 DOI: 10.1073/pnas.1408301111]

90 **Sai S**, Ichikawa D, Tomita H, Ikoma D, Tani N, Ikoma H, Kikuchi S, Fujiwara H, Ueda Y, Otsuji E. Quantification of plasma cell-free DNA in patients with gastric cancer. *Anticancer Res* 2007; **27**: 2747-2751 [PMID: 17695442]

91 **Park JL**, Kim HJ, Choi BY, Lee HC, Jang HR, Song KS, Noh SM, Kim SY, Han DS, Kim YS. Quantitative analysis of cell-free DNA in the plasma of gastric cancer patients. *Oncol Lett* 2012; **3**: 921-926 [PMID: 22741019 DOI: 10.3892/ol.2012.592]

92 **Kim K**, Shin DG, Park MK, Baik SH, Kim TH, Kim S, Lee S. Circulating cell-free DNA as a promising biomarker in patients with gastric cancer: diagnostic validity and significant reduction of cfDNA after surgical resection. *Ann Surg Treat Res* 2014; **86**: 136-142 [PMID: 24761422 DOI: 10.4174/astr.2014.86.3.136]

93 **Kolesnikova EV**, Tamkovich SN, Bryzgunova OE, Shelestyuk PI, Permyakova VI, Vlassov VV, Tuzikov AS, Laktionov PP, Rykova EY. Circulating DNA in the blood of gastric cancer patients. *Ann N Y Acad Sci* 2008; **1137**: 226-231 [PMID: 18837952 DOI: 10.1196/annals.1448.009]

94 **Coimbra S**, Catarino C, Costa E, Oliveira H, Figueiredo A, Rocha-Pereira P, Santos-Silva A. Circulating cell-free DNA levels in Portuguese patients with psoriasis vulgaris according to severity and therapy. *Br J Dermatol* 2014; **170**: 939-942 [PMID: 24245854 DOI: 10.1111/bjd.12738]

95 **Outinen TK**, Kuparinen T, Jylhävä J, Leppänen S, Mustonen J, Mäkelä S, Pörsti I, Syrjänen J, Vaheri A, Hurme M. Plasma cell-free DNA levels are elevated in acute Puumala hantavirus infection. *PLoS One* 2012; **7**: e31455 [PMID: 22347483 DOI: 10.1371/journal.pone.0031455]

96 **Jylhävä J**, Lehtimäki T, Jula A, Moilanen L, Kesäniemi YA, Nieminen MS, Kähönen M, Hurme M. Circulating cell-free DNA is associated with cardiometabolic risk factors: the Health 2000 Survey. *Atherosclerosis* 2014; **233**: 268-271 [PMID: 24529155 DOI: 10.1016/j.atherosclerosis.2013.12.022]

97 **Breitbach S**, Tug S, Simon P. Circulating cell-free DNA: an up-coming molecular marker in exercise physiology. *Sports Med* 2012; **42**: 565-586 [PMID: 22694348 DOI: 10.2165/11631380-000000000-00000]

98 **Hamakawa T**, Kukita Y, Kurokawa Y, Miyazaki Y, Takahashi T, Yamasaki M, Miyata H, Nakajima K, Taniguchi K, Takiguchi S, Mori M, Doki Y, Kato K. Monitoring gastric cancer progression with

circulating tumour DNA. *Br J Cancer* 2015; **112**: 352-356 [PMID: 25490524 DOI: 10.1038/bjc.2014.609]

99 **Park KU**, Lee HE, Park do J, Jung EJ, Song J, Kim HH, Choe G, Kim WH, Lee HS. MYC quantitation in cell-free plasma DNA by real-time PCR for gastric cancer diagnosis. *Clin Chem Lab Med* 2009; **47**: 530-536 [PMID: 19302034 DOI: 10.1515/CCLM.2009.126]

100 **Shoda K**, Masuda K, Ichikawa D, Arita T, Miyakami Y, Watanabe M, Konishi H, Imoto I, Otsuji E. HER2 amplification detected in the circulating DNA of patients with gastric cancer: a retrospective pilot study. *Gastric Cancer* 2015; **18**: 698-710 [PMID: 25322965 DOI: 10.1007/s10120-014-0432-5]

101 **Lee HE**, Park KU, Yoo SB, Nam SK, Park do J, Kim HH, Lee HS. Clinical significance of intratumoral HER2 heterogeneity in gastric cancer. *Eur J Cancer* 2013; **49**: 1448-1457 [PMID: 23146959 DOI: 10.1016/j.ejca.2012.10.018]

102 **Silva JM**, Dominguez G, Garcia JM, Gonzalez R, Villanueva MJ, Navarro F, Provencio M, San Martin S, España P, Bonilla F. Presence of tumor DNA in plasma of breast cancer patients: clinicopathological correlations. *Cancer Res* 1999; **59**: 3251-3256 [PMID: 10397273]

103 **Esteller M**, Sanchez-Cespedes M, Rosell R, Sidransky D, Baylin SB, Herman JG. Detection of aberrant promoter hypermethylation of tumor suppressor genes in serum DNA from non-small cell lung cancer patients. *Cancer Res* 1999; **59**: 67-70 [PMID: 9892187]

104 **Lee TL**, Leung WK, Chan MW, Ng EK, Tong JH, Lo KW, Chung SC, Sung JJ, To KF. Detection of gene promoter hypermethylation in the tumor and serum of patients with gastric carcinoma. *Clin Cancer Res* 2002; **8**: 1761-1766 [PMID: 12060614]

105 **Tsujiura M**, Ichikawa D, Konishi H, Komatsu S, Shiozaki A, Otsuji E. Liquid biopsy of gastric cancer patients: circulating tumor cells and cell-free nucleic acids. *World J Gastroenterol* 2014; **20**: 3265-3286 [PMID: 24696609 DOI: 10.3748/wjg.v20.i12.3265]

106 **Toiyama Y**, Okugawa Y, Goel A. DNA methylation and microRNA biomarkers for noninvasive detection of gastric and colorectal cancer. *Biochem Biophys Res Commun* 2014; **455**: 43-57 [PMID: 25128828 DOI: 10.1016/j.bbrc.2014.08.001]

107 **Bernal C**, Aguayo F, Villarroel C, Vargas M, Díaz I, Ossandon FJ, Santibáñez E, Palma M, Aravena E, Barrientos C, Corvalan AH. Reprimo as a potential biomarker for early detection in gastric cancer. *Clin Cancer Res* 2008; **14**: 6264-6269 [PMID: 18829507 DOI: 10.1158/1078-0432.CCR-07-4522]

108 **Ling ZQ**, Lv P, Lu XX, Yu JL, Han J, Ying LS, Zhu X, Zhu WY, Fang XH, Wang S, Wu YC. Circulating Methylated XAF1 DNA Indicates Poor Prognosis for Gastric Cancer. *PLoS One* 2013; **8**: e67195 [PMID: 23826230 DOI: 10.1371/journal.pone.0067195]

109 **Zheng Y**, Chen L, Li J, Yu B, Su L, Chen X, Yu Y, Yan M, Liu B, Zhu Z. Hypermethylated DNA as potential biomarkers for gastric cancer diagnosis. *Clin Biochem* 2011; **44**: 1405-1411 [PMID: 21945024 DOI: 10.1016/j.clinbiochem.2011.09.006]

110 **Ooki A**, Yamashita K, Yamaguchi K, Mondal A, Nishimiya H, Watanabe M. DNA damage-inducible gene, reprimo functions as a tumor suppressor and is suppressed by promoter methylation in gastric cancer. *Mol Cancer Res* 2013; **11**: 1362-1374 [PMID: 23982217 DOI: 10.1158/1541-7786.MCR-13-0091]

111 **Rand K**, Qu W, Ho T, Clark SJ, Molloy P. Conversion-specific detection of DNA methylation using real-time polymerase chain reaction (ConLight-MSP) to avoid false positives. *Methods* 2002; **27**: 114-120 [PMID: 12095268]

112 **Kristensen LS**, Mikeska T, Krypuy M, Dobrovic A. Sensitive Melting Analysis after Real Time- Methylation Specific PCR (SMART-MSP): high-throughput and probe-free quantitative DNA methylation detection. *Nucleic Acids Res* 2008; **36**: e42 [PMID: 18344521 DOI: 10.1093/nar/gkn113]

113 **Lim AM**, Candiloro IL, Wong N, Collins M, Do H, Takano EA, Angel C, Young RJ, Corry J, Wiesenfeld D, Kleid S, Sigston E, Lyons B, Rischin D, Solomon B, Dobrovic A. Quantitative methodology is critical for assessing DNA methylation and impacts on correlation with patient outcome. *Clin Epigenetics* 2014; **6**: 22 [PMID: 25859283 DOI: 10.1186/1868-7083-6-22]

114 **Li M**, Chen WD, Papadopoulos N, Goodman SN, Bjerregaard NC, Laurberg S, Levin B, Juhl H, Arber N, Moinova H, Durkee K, Schmidt K, He Y, Diehl F, Velculescu VE, Zhou S, Diaz LA, Kinzler KW, Markowitz SD, Vogelstein B. Sensitive digital quantification of DNA methylation in clinical samples. *Nat Biotechnol* 2009; **27**: 858-863 [PMID: 19684580 DOI: 10.1038/nbt.1559]

115 **Chen X**, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo X, Li Q, Li X, Wang W, Zhang Y, Wang J, Jiang X, Xiang Y, Xu C, Zheng P, Zhang J, Li R, Zhang H, Shang X, Gong T, Ning G, Wang J, Zen K, Zhang J, Zhang CY. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res* 2008; **18**: 997-1006 [PMID: 18766170 DOI: 10.1038/cr.2008.282]

116 **Wang R**, Wen H, Xu Y, Chen Q, Luo Y, Lin Y, Luo Y, Xu A. Circulating microRNAs as a novel class of diagnostic biomarkers in gastrointestinal tumors detection: a meta-analysis based on 42 articles. *PLoS One* 2014; **9**: e113401 [PMID: 25406082 DOI: 10.1371/journal.pone.0113401]

117 **Tsujiura M**, Ichikawa D, Komatsu S, Shiozaki A, Takeshita H, Kosuga T, Konishi H, Morimura R, Deguchi K, Fujiwara H, Okamoto K, Otsuji E. Circulating microRNAs in plasma of patients with gastric cancers. *Br J Cancer* 2010; **102**: 1174-1179 [PMID: 20234369 DOI: 10.1038/sj.bjc.6605608]

118 **Palma J**, Yaddanapudi SC, Pigati L, Havens MA, Jeong S, Weiner GA, Weimer KM, Stern B, Hastings ML, Duelli DM. MicroRNAs are exported from malignant cells in customized particles. *Nucleic Acids Res* 2012; **40**: 9125-9138 [PMID: 22772984 DOI: 10.1093/nar/gks656]

119 **Konishi H**, Ichikawa D, Komatsu S, Shiozaki A, Tsujiura M, Takeshita H, Morimura R, Nagata H, Arita T, Kawaguchi T, Hirashima S, Fujiwara H, Okamoto K, Otsuji E. Detection of gastric cancer-associated microRNAs on microRNA microarray comparing pre- and post-operative plasma. *Br J Cancer* 2012; **106**: 740-747 [PMID: 22262318 DOI: 10.1038/bjc.2011.588]

120 **Zhu C**, Ren C, Han J, Ding Y, Du J, Dai N, Dai J, Ma H, Hu Z, Shen H, Xu Y, Jin G. A five-microRNA panel in plasma was identified as potential biomarker for early detection of gastric cancer. *Br J Cancer* 2014; **110**: 2291-2299 [PMID: 24595006 DOI: 10.1038/bjc.2014.119]

121 **Li BS**, Zhao YL, Guo G, Li W, Zhu ED, Luo X, Mao XH, Zou QM, Yu PW, Zuo QF, Li N, Tang B, Liu KY, Xiao B. Plasma microRNAs, miR-223, miR-21 and miR-218, as novel potential biomarkers for gastric cancer detection. *PLoS One* 2012; **7**: e41629 [PMID: 22860003 DOI: 10.1371/journal.pone.0041629]

122 **Liu H**, Zhu L, Liu B, Yang L, Meng X, Zhang W, Ma Y, Xiao H. Genome-wide microRNA profiles identify miR-378 as a serum biomarker for early detection of gastric cancer. *Cancer Lett* 2012; **316**: 196-203 [PMID: 22169097 DOI: 10.1016/j.canlet.2011.10.034]

123 **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]

124 **Song MY**, Pan KF, Su HJ, Zhang L, Ma JL, Li JY, Yuasa Y, Kang D, Kim YS, You WC. Identification of serum microRNAs as novel non-invasive biomarkers for early detection of gastric cancer. *PLoS One* 2012; **7**: e33608 [PMID: 22432036 DOI: 10.1371/journal.pone.0033608]

125 **Kang Y**, Zhang J, Sun P, Shang J. Circulating cell-free human telomerase reverse transcriptase mRNA in plasma and its potential diagnostic and prognostic value for gastric cancer. *Int J Clin Oncol* 2013; **18**: 478-486 [PMID: 22527847 DOI: 10.1007/s10147-012-0405-9]

126 **Qi P**, Du X. The long non-coding RNAs, a new cancer diagnostic and therapeutic gold mine. *Mod Pathol* 2013; **26**: 155-165 [PMID: 22996375 DOI: 10.1038/modpathol.2012.160]

127 **Peng L**, Yuan XQ, Li GC. The emerging landscape of circular RNA ciRS-7 in cancer (Review). *Oncol Rep* 2015; **33**: 2669-2674 [PMID: 25873049 DOI: 10.3892/or.2015.3904]

128 **Arita T**, Ichikawa D, Konishi H, Komatsu S, Shiozaki A, Shoda K, Kawaguchi T, Hirajima S, Nagata H, Kubota T, Fujiwara H,

Okamoto K, Otsuji E. Circulating long non-coding RNAs in plasma of patients with gastric cancer. *Anticancer Res* 2013; **33**: 3185-3193 [PMID: 23898077]

129 **Liu Z**, Shao Y, Tan L, Shi H, Chen S, Guo J. Clinical significance of the low expression of FER1L4 in gastric cancer patients. *Tumour Biol* 2014; **35**: 9613-9617 [PMID: 24961353 DOI: 10.1007/s13277-014-2259-4]

130 **Zeng X**, Shi H, Wang J, Cui S, Tang H, Zhang X. Long noncoding RNA aberrant expression profiles after cytoreductive surgery and hyperthermic intraperitoneal chemotherapy of AGC ascertained by microarray analysis. *Tumour Biol* 2015; **36**: 5021-5029 [PMID: 25652469 DOI: 10.1007/s13277-015-3153-4]

131 **Li Q**, Shao Y, Zhang X, Zheng T, Miao M, Qin L, Wang B, Ye G, Xiao B, Guo J. Plasma long noncoding RNA protected by exosomes as a potential stable biomarker for gastric cancer. *Tumour Biol* 2015; **36**: 2007-2012 [PMID: 25391424 DOI: 10.1007/s13277-014-2807-y]

132 **Li P**, Chen S, Chen H, Mo X, Li T, Shao Y, Xiao B, Guo J. Using circular RNA as a novel type of biomarker in the screening of gastric cancer. *Clin Chim Acta* 2015; **444**: 132-136 [PMID: 25689795 DOI: 10.1016/j.cca.2015.02.018]

133 **Xiang M**, Zeng Y, Yang R, Xu H, Chen Z, Zhong J, Xie H, Xu Y, Zeng X. U6 is not a suitable endogenous control for the quantification of circulating microRNAs. *Biochem Biophys Res Commun* 2014; **454**: 210-214 [PMID: 25450382 DOI: 10.1016/j.bbrc.2014.10.064]

134 **Keller A**, Leidinger P, Gislefoss R, Haugen A, Langseth H, Staehler P, Lenhof HP, Meese E. Stable serum miRNA profiles as potential tool for non-invasive lung cancer diagnosis. *RNA Biol* 2011; **8**: 506-516 [PMID: 21558792]

135 **Kirschner MB**, Kao SC, Edelman JJ, Armstrong NJ, Vallely MP, van Zandwijk N, Reid G. Haemolysis during sample preparation alters microRNA content of plasma. *PLoS One* 2011; **6**: e24145 [PMID: 21909417 DOI: 10.1371/journal.pone.0024145]

136 **Hiraiwa K**, Takeuchi H, Hasegawa H, Saikawa Y, Suda K, Ando T, Kumagai K, Irino T, Yoshikawa T, Matsuda S, Kitajima M, Kitagawa Y. Clinical significance of circulating tumor cells in blood from patients with gastrointestinal cancers. *Ann Surg Oncol* 2008; **15**: 3092-3100 [PMID: 18766405 DOI: 10.1245/s10434-008-0122-9]

137 **Toss A**, Mu Z, Fernandez S, Cristofanilli M. CTC enumeration and characterization: moving toward personalized medicine. *Ann Transl Med* 2014; **2**: 108 [PMID: 25489582 DOI: 10.3978/j.issn.2305-5839.2014.09.06]

138 **Moreno JG**, O'Hara SM, Gross S, Doyle G, Fritsche H, Gomella LG, Terstappen LW. Changes in circulating carcinoma cells in patients with metastatic prostate cancer correlate with disease status. *Urology* 2001; **58**: 386-392 [PMID: 11549486]

139 **Kolostova K**, Matkowski R, Gurlich R, Grabowski K, Soter K, Lischke R, Schutzner J, Bobek V. Detection and cultivation of circulating tumor cells in gastric cancer. *Cytotechnology* 2015; Epub ahead of print [PMID: 25862542 DOI: 10.1007/s10616-015-9866-9]

140 **Uenosono Y**, Arigami T, Kozono T, Yanagita S, Hagihara T, Haraguchi N, Matsushita D, Hirata M, Arima H, Funasako Y, Kijima Y, Nakajo A, Okumura H, Ishigami S, Hokita S, Ueno S, Natsugoe S. Clinical significance of circulating tumor cells in peripheral blood from patients with gastric cancer. *Cancer* 2013; **119**: 3984-3991 [PMID: 23963829 DOI: 10.1002/cncr.28309]

141 **Katsuda T**, Kosaka N, Ochiya T. The roles of extracellular vesicles in cancer biology: toward the development of novel cancer biomarkers. *Proteomics* 2014; **14**: 412-425 [PMID: 24339442 DOI: 10.1002/pmic.201300389]

142 **Thakur BK**, Zhang H, Becker A, Matei I, Huang Y, Costa-Silva B, Zheng Y, Hoshino A, Brazier H, Xiang J, Williams C, Rodriguez-Barrueco R, Silva JM, Zhang W, Hearn S, Elemento O, Paknejad N, Manova-Todorova K, Welte K, Bromberg J, Peinado H, Lyden D. Double-stranded DNA in exosomes: a novel biomarker in cancer detection. *Cell Res* 2014; **24**: 766-769 [PMID: 24710597 DOI: 10.1038/cr.2014.44]

143 **Fais S**, Logozzi M, Lugini L, Federici C, Azzarito T, Zarovni N, Chiesi A. Exosomes: the ideal nanovectors for biodelivery. *Biol Chem* 2013; **394**: 1-15 [PMID: 23241589 DOI: 10.1515/hsz-2012-0236]

144 **Zocco D**, Ferruzzi P, Cappello F, Kuo WP, Fais S. Extracellular vesicles as shuttles of tumor biomarkers and anti-tumor drugs. *Front Oncol* 2014; **4**: 267 [PMID: 25340037 DOI: 10.3389/fonc.2014.00267]

145 **Baran J**, Baj-Krzyworzeka M, Weglarczyk K, Szatanek R, Zembala M, Barbasz J, Czupryna A, Szczepanik A, Zembala M. Circulating tumour-derived microvesicles in plasma of gastric cancer patients. *Cancer Immunol Immunother* 2010; **59**: 841-850 [PMID: 20043223 DOI: 10.1007/s00262-009-0808-2]

146 **Schneider J**, Schulze G. Comparison of tumor M2-pyruvate kinase (tumor M2-PK), carcinoembryonic antigen (CEA), carbohydrate antigens CA 19-9 and CA 72-4 in the diagnosis of gastrointestinal cancer. *Anticancer Res* 2003; **23**: 5089-5093 [PMID: 14981971]

147 **Carpelan-Holmström M**, Louhimo J, Stenman UH, Alfthan H, Haglund C. CEA, CA 19-9 and CA 72-4 improve the diagnostic accuracy in gastrointestinal cancers. *Anticancer Res* 2002; **22**: 2311-2316 [PMID: 12174919]

148 **Yang J**, Song YC, Dang CX, Song TS, Liu ZG, Guo YM, Li ZF, Huang C. Serum peptidome profiling in patients with gastric cancer. *Clin Exp Med* 2012; **12**: 79-87 [PMID: 21739109 DOI: 10.1007/s10238-011-0149-2]

149 **Xu QW**, Zhao W, Wang Y, Sartor MA, Han DM, Deng J, Ponnala R, Yang JY, Zhang QY, Liao GQ, Qu YM, Li L, Liu FF, Zhao HM, Yin YH, Chen WF, Zhang Y, Wang XS. An integrated genome-wide approach to discover tumor-specific antigens as potential immunologic and clinical targets in cancer. *Cancer Res* 2012; **72**: 6351-6361 [PMID: 23135912 DOI: 10.1158/0008-5472.CAN-12-1656]

150 **Looi K**, Megliorino R, Shi FD, Peng XX, Chen Y, Zhang JY. Humoral immune response to p16, a cyclin-dependent kinase inhibitor in human malignancies. *Oncol Rep* 2006; **16**: 1105-1110 [PMID: 17016600]

151 **Koziol JA**, Zhang JY, Casiano CA, Peng XX, Shi FD, Feng AC, Chan EK, Tan EM. Recursive partitioning as an approach to selection of immune markers for tumor diagnosis. *Clin Cancer Res* 2003; **9**: 5120-5126 [PMID: 14613989]

152 **Sakakura C**, Hamada T, Miyagawa K, Nishio M, Miyashita A, Nagata H, Ida H, Yazumi S, Otsuji E, Chiba T, Ito K, Ito Y. Quantitative analysis of tumor-derived methylated RUNX3 sequences in the serum of gastric cancer patients. *Anticancer Res* 2009; **29**: 2619-2625 [PMID: 19596937]

153 **Ng EK**, Leung CP, Shin VY, Wong CL, Ma ES, Jin HC, Chu KM, Kwong A. Quantitative analysis and diagnostic significance of methylated SLC19A3 DNA in the plasma of breast and gastric cancer patients. *PLoS One* 2011; **6**: e22233 [PMID: 21789241 DOI: 10.1371/journal.pone.0022233]

154 **Chen L**, Su L, Li J, Zheng Y, Yu B, Yu Y, Yan M, Gu Q, Zhu Z, Liu B. Hypermethylated FAM5C and MYLK in serum as diagnosis and pre-warning markers for gastric cancer. *Dis Markers* 2012; **32**: 195-202 [PMID: 22377736 DOI: 10.3233/DMA-2011-0877]

155 **Liu R**, Zhang C, Hu Z, Li G, Wang C, Yang C, Huang D, Chen X, Zhang H, Zhuang R, Deng T, Liu H, Yin J, Wang S, Zen K, Ba Y, Zhang CY. A five-microRNA signature identified from genome-wide serum microRNA expression profiling serves as a fingerprint for gastric cancer diagnosis. *Eur J Cancer* 2011; **47**: 784-791 [PMID: 21112772 DOI: 10.1016/j.ejca.2010.10.025]

156 **Cai H**, Yuan Y, Hao YF, Guo TK, Wei X, Zhang YM. Plasma microRNAs serve as novel potential biomarkers for early detection of gastric cancer. *Med Oncol* 2013; **30**: 452 [PMID: 23307259 DOI: 10.1007/s12032-012-0452-0]

157 **Wang H**, Wang L, Wu Z, Sun R, Jin H, Ma J, Liu L, Ling R, Yi J, Wang L, Bian J, Chen J, Li N, Yuan S, Yun J. Three dysregulated microRNAs in serum as novel biomarkers for gastric cancer screening. *Med Oncol* 2014; **31**: 298 [PMID: 25367852 DOI: 10.1007/s12032-014-0298-8]

158 **Fu Z**, Qian F, Yang X, Jiang H, Chen Y, Liu S. Circulating miR-222 in plasma and its potential diagnostic and prognostic value in gastric cancer. *Med Oncol* 2014; **31**: 164 [PMID: 25129310 DOI: 10.1007/

s12032-014-0164-8]

159 **Tsujiura M**, Komatsu S, Ichikawa D, Shiozaki A, Konishi H, Takeshita H, Moriumura R, Nagata H, Kawaguchi T, Hirajima S, Arita T, Fujiwara H, Okamoto K, Otsuji E. Circulating miR-18a in plasma contributes to cancer detection and monitoring in patients with gastric cancer. *Gastric Cancer* 2015; **18**: 271-279 [PMID: 24626859 DOI: 10.1007/s10120-014-0363-1]

160 **Burock S**, Herrmann P, Wendler I, Niederstrasser M, Wernecke KD, Stein U. Circulating Metastasis Associated in Colon Cancer 1 transcripts in gastric cancer patient plasma as diagnostic and prognostic biomarker. *World J Gastroenterol* 2015; **21**: 333-341 [PMID: 25574109 DOI: 10.3748/wjg.v21.i1.333]

P- Reviewer: Huang KH, Smith SM **S- Editor**: Yu J
L- Editor: A **E- Editor**: Wang CH

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 1007-9327
41>