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Review Article

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Urinary Biomarkers for the Noninvasive Detection of Gastric Cancer

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ABSTRACT

Gastric cancer (GC) is associated with high morbidity and mortality rates. Thus, early diagnosis is important to improve disease prognosis. Endoscopic assessment represents the most reliable imaging method for GC diagnosis; however, it is semi-invasive and costly and heavily depends on the skills of the endoscopist, which limit its clinical applicability. Therefore, the search for new sensitive biomarkers for the early detection of GC using noninvasive sampling collection methods has attracted much attention among scientists. Urine is considered an ideal biofluid, as it is readily accessible, less complex, and relatively stable than plasma and serum. Over the years, substantial progress has been made in screening for potential urinary biomarkers for GC. This review explores the possible applications and limitations of urinary biomarkers in GC detection and diagnosis.

Keywords: Gastric cancer; Diagnostic; Noninvasive detection; Urinary biomarkers

INTRODUCTION

Gastric cancer (GC) is a malignant tumor originating from the gastric mucosa and associated with high morbidity and mortality [1,2]. Surgical resection is still considered the best treatment approach for GC. However, patients with early-stage cancer are often asymptomatic and thus lose their chance to undergo surgery. Therefore, early diagnosis is crucial for improving clinical outcomes and prognosis [3,4]. Endoscopic assessment is the most reliable imaging method for GC diagnosis, which allows clinicians to collect tissue biopsy and perform endoscopic ultrasound to determine the depth of invasion (tumor or T stage). However, it is semi-invasive and costly and heavily depends on the skills of the endoscopist, which limits its clinical applicability [5]. Other common diagnostic approaches include magnetic resonance imaging, X-ray pepsinogen I, and X-ray pepsinogen II. These approaches offer lower sensitivity and specificity and are costly. Thus, the search for novel noninvasive biomarkers, especially for early-stage GC, has become a hot topic among scientists.

Urine, an ideal biofluid, has gained increasing attention in biomarker discovery. Urine is a highly desirable biospecimen for biomarker analysis; it can be easily obtained when compared with plasma and serum [6,7]. The application of urinary biomarkers in tumors

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of the excretory or genitourinary cancer system, such as bladder cancer, prostate cancer, and upper urinary tract urothelial carcinoma, has gradually matured, and some urinary biomarkers have already completed the confirmatory stages of clinical use [8,9]. Over the years, substantial progress has been made in screening for potential urinary biomarkers for GC, especially early-stage tumors. However, urine may be affected by age, sex, diet, hormonal status, and physical activity [10]. Therefore, the universal applicability of potential biomarkers requires further verification, and experimental protocols must be standardized. Given that there are different components in the urine, this paper summarizes the research field, possible applications, and limitations of urinary biomarkers for GC detection.

DETECTION TECHNIQUES OF BIOMARKERS IN THE URINE

Continuous improvements in urine testing technologies have enabled the identification of many substances in urine, especially low-abundance substances, thus further promoting the discovery of new biomarkers. Over the last two decades, urine RNomics, proteomics, and metabolomics have developed rapidly in parallel with advanced omics and medical tests [11]. Microarray technologies, quantitative real-time polymerase chain reaction (PCR), and next-generation RNA sequencing have prompted the discovery of many urinary microRNAs (miRNAs) in cancer [12]. Additionally, breakthroughs in analytical technologies have supported metabolic profiling, making it one of the most rapidly expanding disciplines in cancer research. Significant progress has been made in acquiring metabolomic data, sampling techniques, experimental techniques, and data characterization [13,14]. Furthermore, urinary metabolomics has been advanced by recent technological developments in mass spectrometry (MS), nuclear magnetic resonance (NMR), gas and liquid chromatography (LC), and capillary electrophoresis (CE), thus improving reproducibility and metabolome coverage [15]. Meanwhile, there are several different techniques for proteomic studies, including tandem MS (MS/MS), LC-MS, CE-MS, surface-enhanced laser desorption ionization MS, and array technology have been implemented for proteomics analysis of urine and biomarker discovery [16]. **Fig. 1** summarizes the applications of urine detection technologies for GC urinary biomarker research.

MICRORNAs IN URINE

miRNAs are a class of 21–28 nucleotide noncoding RNAs that mediate gene expression post-transcriptionally and are involved in carcinogenesis [17,18]. To date, a number of miRNAs have been discovered, some of which are candidate biomarkers for early diagnosis [19] and are highly stable in tissues and body fluids, including urine [20]. Moreover, studies have shown that urinary miRNAs remained unchanged even after seven cycles of freezing and thawing or incubation at room temperature for 72 hours [21]. Various technologies such as microarray, quantitative real-time PCR, and next-generation RNA sequencing have been widely used to analyze miRNA expression profiles in both biofluids and tissues [22-24]. Iwasaki *et al.* demonstrated higher levels of miR-6807-5p and miR-6856-5p in the urine of patients with GC than in control subjects. A combination of miR-6807-5p and miR-6856-5p achieved an area under the curve (AUC) of 0.748, suggesting that these miRNAs could be used to diagnose early-stage GC [25]. Another study showed that urinary miR-376c was also significantly increased in 20 patients with GC when compared with that of 11 healthy individuals, and it displayed 64% specificity and 60% sensitivity, with an AUC of 0.70

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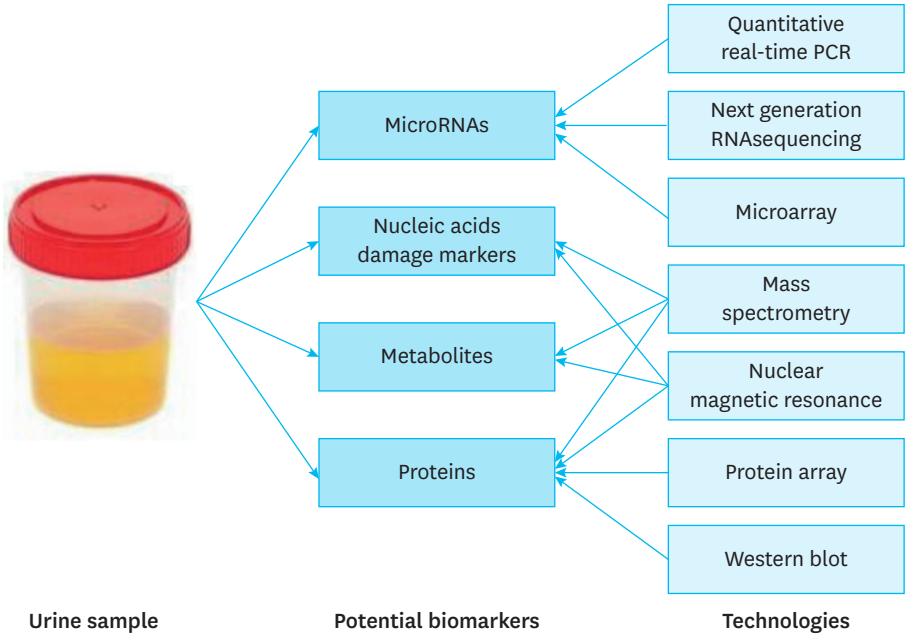

Fig. 1. Several techniques for gastric cancer biomarker discovery.
PCR = polymerase chain reaction.

for GC diagnosis [26]. Moreover, Kao *et al.* [27] performed a quantitative stem-loop PCR assay of miR-21-5pin urinary samples from healthy individuals, preoperative patients, and postoperative patients with GC. Compared with healthy controls, patients with GC had significantly upregulated miR-21-5p, and urinary miR-21-5p levels showed a clear downward trend after tumor tissue resection. Interestingly, another study reported no urinary miR-21-5p in patients with GC and healthy controls [25]. The different results may be explained as follows: 1) The sample sizes were different and could significantly affect the results. Therefore, large-scale multicenter studies are warranted to validate these biomarkers. 2) Cancer biomarkers vary across stages of disease progression, and studies involving patients at different stages may report different results. 3) GC is a multifactorial disease, and environmental and genetic factors may affect its etiology. There are differences in the incidence of GC among different regions and races. Whether or not biomarkers reflect disease status across diverse ethnic groups remains unknown. 4) Biomarkers may exhibit different expression levels in different subtypes.

In summary, all these data suggest that miRNA in urine may be a promising noninvasive diagnostic biomarker of the disease; however, their significance needs to be validated in further independent large-scale cohorts.

Table 1 summarizes the literature on urinary miRNAs in GC, focusing on the main aspects of the studies presented (i.e., study design, biological function, and results).

DNA AND RNA OXIDATIVE DAMAGE MARKERS IN URINE

Nucleic acids are continuously oxidized in the cell [28], and oxidative modifications of nucleic acids are associated with various diseases including cancer [29]. Oxidized nucleosides

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Table 1. Summary of potential urinary miRNAs for the early diagnosis of gastric cancer

Type	Biomarker	Study design	Biological function	AUC	Sensitivity/Specificity	Study
miRNA	miR-6807-5p/miR-6856-5p/*H. pylori*+	Case control design: training cohort: 95 GC cases, 95 healthy controls; validation cohort: 54 GC cases, 54 healthy controls	Upregulated in GC; correlated with *H. pylori* status	GC: 0.885 Stage I GC: 0.748	GC: 76.9%/88.9% Stage I GC: –/–	Iwasaki et al. [25]
miRNA	miR-376c	Case control design: 20 GC cases and 11 healthy controls	Upregulated in GC; correlated with proliferation, migration, and anchorage-independent growth	0.70	60%/64%	Hung et al. [26]
miRNA	miR-21-5p	Case control design: 50 GC cases and healthy controls	Upregulated in GC; correlated with disease status	-	-	Kao et al. [27]

miRNA = microRNA; AUC = area under the curve; *H. pylori* = *Helicobacter pylori*; GC = gastric cancer; - = no data available.

are fairly water-soluble, are generally excreted into the urine, and do not undergo further metabolism [30]. 8-Oxo-7,8-dyhydroguanine (8-oxoGua) and 8-hydroxyguanosine(8-OHG) are typical markers of oxidative modification of RNA, while 8-oxo-7′8-dihydro-2′-deoxyguanosine (8-oxodG) and 8-hydroxy-2′-deoxyguanosine (8-OHdG) are markers of oxidative modification of DNA. Their urinary concentrations reflect the mean rate of oxidatively generated modifications of RNA and DNA in organism [31].

Roszkowski *et al.* [29] investigated the daily urinary excretion of 8-oxoGua and 8-oxodG in a large cohort of 222 patients with malignant cancer, including gastrointestinal cancer, and found that the urinary levels of 8-oxoGua and 8-oxodG were significantly higher in the GC group than in healthy control group. Furthermore, Borrego *et al.* [32] confirmed that urinary 8-oxo-2′-deoxyguanosine(8-oxo-dG) levels were significantly elevated in patients with GC and progressively declined after gastrectomy. The latest research successfully quantified 8-OHdG and 8-OHG in urine using robust solid-phase extraction (SPE) combined with ultra-performance LC-MS/MS in 70 healthy individuals and 60 patients with GC and found that the concentrations of urinary 8-OHdG and 8-OHG were increased dramatically in patients with GC, with AUC of 0.777 and 0.841, respectively [33].

Table 2 summarizes urinary DNA and RNA oxidative damage markers for GC.

ENDOGENOUS METABOLITES IN URINE

Metabolites are small substrates and products of metabolism with mass units below 2000 that drive essential cellular functions [34]. Metabolites represent the integrated outputs of the genome, transcriptome, and proteome. Moreover, they reflect the upstream input from various external factors, including the environment, diet, lifestyle, and drug exposure

Table 2. Summary of potential DNA and RNA oxidative damage markers for the early diagnosis of gastric cancer

Type	Biomarker	Study design	Biological function	AUC	Sensitivity/Specificity	Study
RNA	8-oxoGua	Case control design: 11 gastrointestinal cancer cases and 85 healthy controls	Upregulated in GC; correlated with oxidative stress situation	-	-	Roszkowski et al. [29]
RNA	8-OHG	Case control design: 60 GC cases and 70 healthy controls	Upregulated in GC; correlated with occurrence and development	0.841	-	Chen et al. [33]
DNA	8-oxodG	Case control design: 11 cases of gastrointestinal cancer and 85 healthy controls	Upregulated in GC; correlated with oxidative stress situation	-	-	Roszkowski et al. [29]
		Case control design: 48 preoperative cases of GC, 48 postoperative cases, and 48 healthy controls	Upregulated in GC; correlated with disease status	-	-	Borrego et al. [32]
DNA	8-OHdG	Case control design: 60 GC cases and 70 healthy controls	Upregulated in GC; correlated with occurrence and development	0.777	-	Chen et al. [33]

AUC = area under the curve; GC = gastric cancer; - = no data available.

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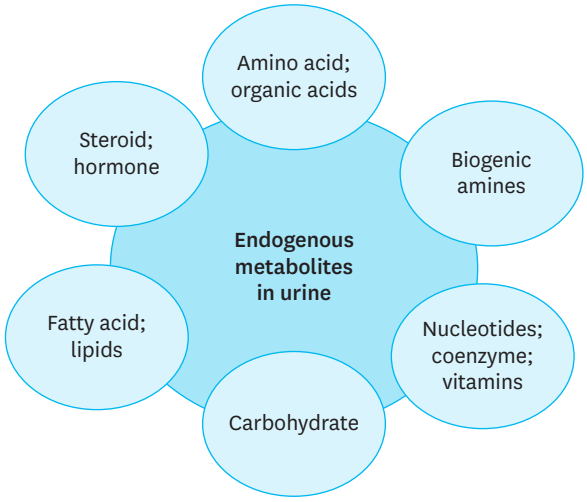

Fig. 2. Common endogenous metabolites in urine.

[35]. Metabolic alterations can be used to detect variations in the biology and morphology of cancers to guide clinical management decisions [15]. Urine is commonly used for profiling metabolic and screening clinical biomarkers [36,37]. To date, various endogenous metabolites involved in multiple metabolic pathways have been detected in urine (**Fig. 2**). For example, metabolomics has been used to analyze urine for GC biomarkers [38]. GC's distinct urinary metabolomic profile identification of GC could provide an efficient, non-invasive diagnostic modality.

Several studies have examined urinary metabolites for GC detection. Amino acids, bile acids, and oxidative nucleic acid metabolites may be used as diagnostic biomarkers for GC. A previous study analyzed metabolites in 293 urine samples by gas chromatography coupled to mass spectrometry (GC-MS) and found that the urine levels of 10 amino acids (namely, valine, alanine, proline, tryptophan, isoleucine, serine, threonine, tyrosine, methionine, and glycine) were significantly higher in patients with GC and showed diagnostic ability with an AUC from 0.693 to 0.823 [39]. Moreover, Chan *et al.* detected increased urinary alanine concentrations in patients with GC when compared with those in healthy individuals. They also established a diagnostic model using alanine, 2-hydroxyisobutyrate (2-HIB), and 3-indoxylsulfate (3-IS) for GC, with an AUC of corresponding receiver operating characteristic curve of 0.95, specificity of 80%, and sensitivity of 95% for predicting GC [40]. A study comparing the concentrations of 44 metabolites in the urine of 50 patients with GC and 50 healthy individuals revealed that alanine, tyrosine, glycolate, glycine, methionine, phenylalanine, and arginine levels were significantly increased in patients with GC; moreover, the combination of alanine, acetate, 4-hydroxyphenylacetate, and phenylacetyl glycine showed high sensitivity and specificity (sensitivity: 86%, specificity: 92%) for GC prediction [41]. A further CE-MS metabolomics study found increased lactic acid, valine, leucine, arginine, and isoleucine levels in patients with GC when compared with control subjects. However, histidine, aspartate, citric acid, succinate, malic acid, methionine, and serine were markedly decreased in patients with GC [42]. Kwon *et al.* [43] employed NMR metabolomics to urine samples to compare urinary metabolites in 103 patients with GC and 100 age- and sex-matched healthy control subjects. In this study, the population included more patients with stage I disease (66.99%). They found that phenylalanine, alanine, creatinine, hippurate, citrate, glycerol, creatine, 3-hydroxybutyrate, and taurine were

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significantly different between healthy individuals and patients with GC, with AUCs ranging from 0.632 to 0.936. Furthermore, the early-stage GC diagnostic model exhibited a specificity of 97% and a sensitivity of 94.7%. They also found that urinary metabolomics had a higher diagnostic value than CEA, CA19-9, and CA72-4 levels. A more recent study demonstrated that the levels of D-serine (D-Ser) and D-isoleucine (D-Ile) were significantly higher in the GC group than in the healthy group, while the levels of β-(pyrazol-1-yl)-L-alanine (L-PA) in the GC group were lower than those in the HC group. Univariable analysis of age, L-PA, D-Ser, and D-Ile showed that their AUC values ranged from 0.760 to 0.895, while multivariate model analysis showed that the AUC of the combined indicators was 0.977, showing great potential in diagnosing GC [38].

Lyu *et al.* [44] used an SPE column that contains a covalent organic framework material coupled to LC-MS/MS to quantitatively analyze samples from patients with GC and healthy control subjects. They found that the levels of hyodeoxycholic acid, cholic acid, and chenodeoxycholic acid were significantly higher in patients with GC, while the glycochenodeoxycholic acid level in patients with GC was significantly lower than that in control subjects. These bile acids achieved favorable diagnostic performance with AUCs of 0.854, 0.851, 0.753, and 0.769, respectively.

Table 3 summarizes the urinary metabolites used for GC detection.

EXTRACELLULAR VESICLES (EVs) AND EXOSOMES IN URINE

EVs are nano-sized membrane vesicles containing nucleic acids, lipids, and proteins, which play important roles in intercellular communication by transferring their components to

Table 3. Summary of potential urinary metabolites for the early diagnosis of gastric cancer

Type	Biomarker	Study design	Biological function	AUC	Sensitivity/ Specificity	Study
Metabolites	10 amino acids (alanine, glycine, valine, isoleucine, serine, threonine, proline, methionine, tyrosine, and tryptophan)	Case control design: 112 GC cases and 87 healthy controls	Upregulated in GC; correlated with occurrence and prognosis	0.693–0.823	62.3%–91.5%/ 41.4%–78.2%	Chen et al. [39]
Metabolites	2-hydroxyisobutyrate (2-HIB), 3-indoxylsulfate(3-IS), and alanine	Case control design: 43 GC cases, 40 BN controls, and 40 healthy controls	Correlated with establishing diagnostic regression model	0.95	95%/80%	Chan et al. [40]
Metabolites	Alanine, acetate, 4-hydroxyphenylacetate, phenylacetyl glycine	Case control design: 50 GC cases and 50 healthy controls	Upregulated in GC; correlated with T stage	-	86%/92%	Jung et al. [41]
Metabolites	Methionine, arginine, leucine, serine, aspartate, valine, isoleucine, histidine, succinate, citric acid, malic acid, lactic acid	Case control design: 26 GC cases and 14 healthy controls	5 metabolites were upregulated in GC and 7 metabolites were downregulated in GC; correlated with disease stage	1.000	-	Chen et al. [42]
Metabolites	Alanine, citrate, creatine, creatinine, glycerol, hippurate, phenylalanine, taurine, and 3-hydroxybutyrate	Case control design: 103 GC cases and 100 healthy controls	6 metabolites were upregulated in GC and 3 metabolites were downregulated in GC; correlated with specific genes	0.632–0.936	50%–90%/ 70%–90%	Kwon et al. [43]
Metabolites	β-(pyrazol-1-yl)-L-alanine, D-serine, D-isoleucine	Case control design: 84 GC cases and 80 healthy controls	2 metabolites were upregulated in GC and 1 metabolite was downregulated in GC; correlated with *H. pylori* status	0.670–0.889	-	Huang et al. [38]
Metabolites	Hyodeoxycholic acid, cholic acid, glycochenodeoxycholic acid, and chenodeoxycholic acid	Case control design: 76 GC cases and 32 healthy controls	3 metabolites were upregulated in GC and 1 metabolite was downregulated in GC	0.753–0.854	-	Lyu et al. [44]

AUC = area under the curve; GC = gastric cancer; BN = benign gastric disease; - = no data available.

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recipient cells [45]. EVs secreted from cancer cells participate in fibrosis, angiogenesis, metastasis, and evasion of immune surveillance [46,47]. EVs can be found in various body fluids such as plasma, urine, breast milk, saliva, semen, lymphatic fluid, cerebrospinal fluid, sputum, amniotic fluid, and synovial fluid [48]. Urinary EVs appear to be particularly promising for the early diagnosis of GC. A prospective study performed metagenome analysis using body fluid samples (gastric juice, urine, and blood) to examine the distinct microbial composition of bacteria-derived EVs from patients with GC. Among the four sample types of prediction models, the model using urine samples showed the highest AUC of 0.823, with 67.7% sensitivity, 84.9% specificity, and 76.1% accuracy [49].

Exosomes are EVs of 30–150 nm in diameter that are present in almost all body fluids and contain miRNAs, mRNA, lncRNAs, and proteins [50,51]. Exosomes can regulate the expression of target genes, signal pathways, and cell transformation of receptor cells by mediating information transmission between tumor cells and the tumor microenvironment, which have become important mediators of tumorigenesis, tumor growth, angiogenesis, and metastasis [52] and have been identified as prognostic and diagnostic biomarkers for cancer (**Fig. 3**). Qian *et al.* [53] applied next-generation sequencing technology to identify exosomal miRNAs in the serum and urine of patients with GC and healthy individuals and found urinary exosomal hsa-miR-1246 upregulation and hsa-miR-139-5p and hsa-miR-345-5p downregulation in GC.

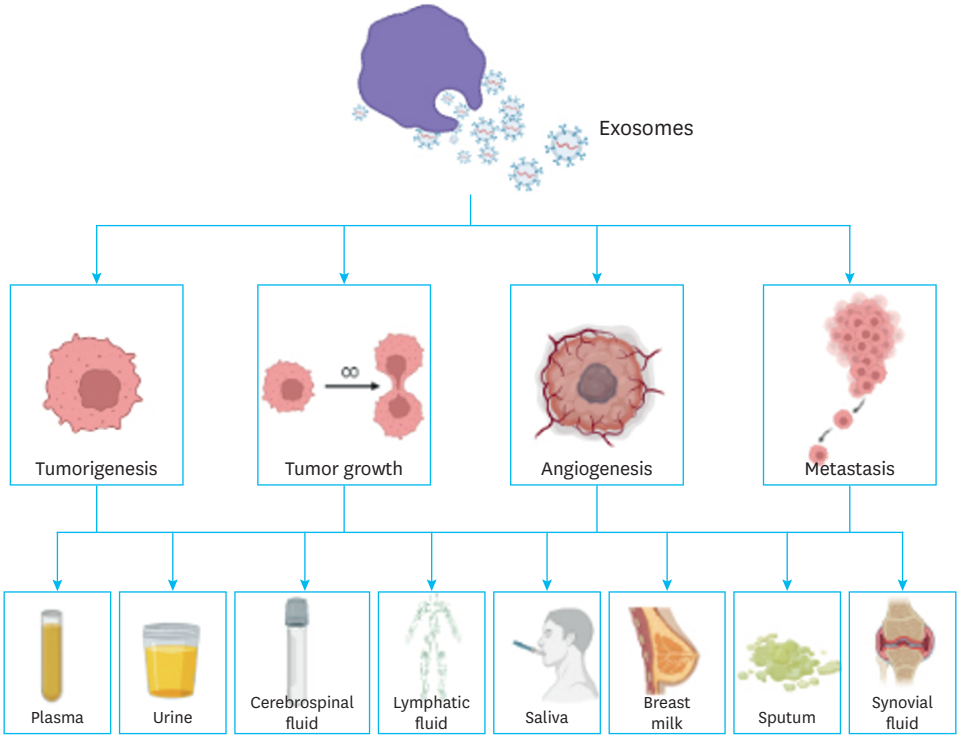

Fig. 3. Exosomes take part in *multiple* biochemical processes involved in cancer and are present in almost all body fluids. This image was created using BioRender (http://biorender.com/; accessed on June 29, 2020).

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PROTEINS IN URINE

Urinary proteins may be used for the early diagnosis of GC. Dong *et al.* [54] found that the protein expression levels of endothelial lipase (EL) in the GC group were significantly lower than those in the normal groups, and EL was proposed to act as a promising diagnostic marker of GC, because it achieved an AUC of 0.967 and a 95% confidence interval (CI) of (0.942–0.993). A study based on a computational method for the prediction of excretory proteins confirmed that urinary EL was substantially reduced in patients with GC, obtaining an AUC greater than 0.9, with true positive and false positive rates of 85% and 9.5%, respectively [55].

Metalloproteinases, a group of zinc-dependent proteinases, activate a water molecule that performs a nucleophilic attack on the scissile peptide bond [56]. Matrix metalloproteinases (MMPs) belong to the family M10 of metalloproteinases [57], which degrade various proteins in the extracellular matrix and regulate growth factors, cytokines, chemokines, and cytoskeletal proteins [58]. MMPs are involved in a wide range of biological processes such as cellular differentiation, tissue repair morphogenesis, embryogenesis, cell mobility, angiogenesis, cell proliferation, migration, wound healing, apoptosis, and main reproductive events, such as ovulation and endometrial proliferation [59]. MMPs are recognized as boosters in tumorigenesis [60]. ADAMs (a disintegrin and metalloproteases), a family of MMP related to metalloproteinases, are involved in cell adhesion, cell signaling, and proteolytic processing of numerous transmembrane proteins and play important roles in tumor progression and metastasis [61]. A previous study found increased MMP-9/NGAL (neutrophil gelatinase-associated lipocalin) complex and ADAM12 in the urine of patients with GC compared to healthy control subjects, and a combination of MMP-9/NGAL complex and ADAM12 showed 77.1% sensitivity and 82.9% specificity, with an AUC of 0.825 for the diagnosis of GC [62].

Many proteomics-based biomarkers that rely on single proteins are currently being used for clinical diagnosis. However, because of the lack of specificity of single biomarkers, a step has been made toward identifying and validating panels of biomarkers rather than attempting to identify a unique ideal diagnostic candidate that might not exist [63]. Urinary proteomics used to search for early markers has gained increasing attention because the complexity of the urinary proteome is lower than that of the plasma proteome, making it easier to detect low-abundance protein changes [64]. A proteomics study was used to screen urine diagnostic markers of GC; the study revealed that urinary levels of TFF1 (trefoil factor 1), ADAM12 (a disintegrin and metalloproteinase domain–containing protein 12), PGA3 (pepsinogen 3), and BARD1 (BRCA1-associated RING domain 1) were significantly higher in the GC group than in the healthy control group. Moreover, uTFF1and uADAM12 appeared to be significant independent proteins for GC diagnosis. In addition, these combination biomarkers displayed an important diagnostic value for GC (AUC of uTFF1+uADAM12 0.815, 95% CI, 0.754-0.877; AUC of uTFF1 + uADAM12+ *Helicobacter pylori* 0.832, 95% CI, 0.773–0.892). These proteins display sex-specific effects; for male GC, the panel of uTFF1/uADAM12/*H. pylori* demonstrated good performance with an AUC of 0.858, whereas for female GC, another combination of uTFF1/uBARD1/*H. pylori* also achieved an AUC of 0.893 [65].

Despite some progress, urinary proteomics research and clinical translation remain in their infancy, as some major problems have not yet been resolved. Specimen collection, processing, and fractionation schemas, as well as analytical platform differences and data

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Table 4. Summary of potential urinary proteins for the early diagnosis of gastric cancer

Type	Biomarker	Study design	Biological function	AUC	Sensitivity/Specificity	Study
Protein	Endothelial lipase	Case control design: 90 GC cases and 57 healthy controls	Downregulated in GC	0.967	79%/100%	Dong et al. [54]
		Case control design: 21 GC cases and 21 healthy controls	Downregulated in GC	0.9	85%/91.5%-	Hong et al. [55]
Protein	MMP-9/NGAL/ADAM12	Case control design: 35 GC cases and 35 healthy controls	Upregulated in GC; correlated with tumor presence and status	0.825	77.1%/82.9%	Shimura et al. [62]
Protein panel	uTFF1/uADAM12/*H. pylori* status for males; uTFF1/uBARD1/*H. pylori* status for females	Case control design: 144 GC cases and 138 healthy controls	Upregulated in GC; correlated with disease stage	0.858 for males; 0.893 for females; 0.805 for males of Stage I; 0.845 for females of Stage I	91.5%/52.5% for males of Stage I; 100%/51.6% for females of Stage I	Shimura et al. [65]

AUC = area under the curve; GC = gastric cancer; *H. pylori* = *Helicobacter pylori*; MMP = matrix metalloprotein; - = no data available.

reduction method variables, create barriers to interlaboratory comparisons [66]. Hence, standardization processes and applicable data normalization methods are required. Defining urinary protein levels in healthy individuals remains an important and challenging problem. Age, sex, diet, exercise, diurnal variation, and hormone status contribute to differences in the proteomics of normal urine [67]. Large-scale longitudinal studies of individuals are needed to establish a reference interval for urinary proteomics.

Table 4 summarizes the urinary proteins used for GC detection.

FUTURE PERSPECTIVE

Urine is an ideal biofluid for biomarker discovery in GC. Urinary miRNAs, proteins, and metabolites have all been reported as possible biomarkers of GC. The current large research output and financial investment in this area undoubtedly confirm the great expectations for the potential urinary analysis might have. Nevertheless, owing to a lack of robust validation, evidence is insufficient to support their clinical use. Most studies on urinary biomarkers for GC diagnosis have been small-scale. Therefore, further research with a larger sample size is required. Choosing a greater number of patients, including low-prevalence populations and premalignant conditions such as intestinal metaplasia and atrophic gastritis, helps represent the areal screening population. With rapid developments in computer technology and medicine, using artificial intelligence to combine "signals" from multiple patterns will facilitate the process of discovery and verification. In addition, combining different biomarker values, clinical evidence, and biochemical parameters will be a great strategy to increase the diagnostic accuracy.

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