Clinica Chimica Acta 498 (2019) 76–83

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Review

Current status of urinary diagnostic biomarkers for colorectal cancer

Hiroyasu Iwasaki, Takaya Shimura*, Hiromi Kataoka

Department of Gastroenterology and Metabolism, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

ARTICLE INFO

Keywords:
Biomarker
Diagnosis
Colorectal cancer
Urine

ABSTRACT

Fecal occult blood test (FOBT) and flexible sigmoidoscopy are the currently using screening methods for colorectal cancer (CRC). However, these methods still have problems of high false positive rates in FOBT and increased invasiveness and cost associated with endoscopy. The development of non-invasive biomarkers is thus important for the diagnosis of CRC. Urine is one of the most commonly used samples for mass screening owing to its non-invasive and simple process of collection; however, the discovery of urinary diagnostic biomarkers for malignancies is still challenging and developing. Since urine contains abundant substances reflecting systemic body condition, urinary biomarker might contribute to detect CRC in a completely non-invasive manner. In this review, we describe the current utility of urinary diagnostic biomarkers for CRC.

1. Introduction

Colorectal cancer (CRC) is one of the most frequent causes of cancer-related deaths worldwide with an incidence rate of 31.6 (male) and 21.2 (female)/100,000 person-years and a death rate of 15.5 (male) and 10.5 (female)/100,000 person-years [1]. Since patients with early stage CRC can be cured with minimally invasive therapy including endoscopic resection, it is very important to identify patients with early stage CRC by mass screening. Fecal occult blood test (FOBT) and flexible sigmoidoscopy have been established as screening methods and there is strong evidence that these methods can help reduce disease-specific morbidity and mortality in CRC [2,3]. The American Cancer Society recommends either stool-based tests (FOBT or DNA) or structural tests [colonoscopy or computed tomography colonography (CTC)] for adults aged 50 years and older [4]. However, FOBT is well known to have many false positives and is easily affected by non-specific bleeding, thus initiating unnecessary invasive examinations [5]. In addition to its invasiveness, colonoscopy is also costly and time consuming. These reasons make it difficult to disseminate colonoscopy globally as a method of medical checkup. Although CTC might be proposed for patients who refuse colonoscopy, benefits of CTC in terms of accuracy and comfortability are unclear [6,7].

Research for non-invasive detection of CRC has been conducted using various materials in serum/plasma, feces, and urine. Serum carcinoembryonic antigen (CEA) and carbohydrate antigen 19–9 (CA19–9) are commonly used as less-invasive biomarkers in clinical practice; however, their sensitivities are very low, and they cannot accomplish early stage detection [8]. Despite the less-invasiveness, serum sample collection still needs special instruments and healthcare professionals to draw blood. Compared to reports regarding serum and fecal samples, there are much fewer reports using urine samples as a diagnostic biomarker of CRC. However, urine containing various components is considered to be an ideal sample for medical checkup because it can be collected completely non-invasively without requiring the patients to visit the clinic (Fig. 1) [9,10]. In addition, the urinary tract is so clean biologically that the materials in urine might be less affected by microorganisms than in feces. The discovery of urinary biomarker is thus desirable for the diagnoses of several malignancies including CRC. We have had longstanding interest in the discovery of urinary diagnostic biomarkers for gastrointestinal cancers including gastric cancer and CRC [11–14]. In this review, we summarize and discuss urinary biomarkers that can be used for the detection of CRC.

Abbreviations: CRC, colorectal cancer; FOBT, fecal occult blood test; CTC, computed tomography colonography; CEA, carcinoembryonic antigen; CA19–9, carbohydrate antigen 19–9; TF, tissue factor; HC, healthy control; AUC, area under the curve; ctDNA, circulating tumor DNA; MT-sDNA, multitarget stool DNA; NMR, nuclear magnetic resonance; MS, mass spectrometry; VOMs, volatile metabolites; 5-ALA, 5-aminolevulinic acid; HPLC, high-performance liquid chromatography; PGE2, prostaglandin E2; COX-2, cyclooxygenase-2; ROS, reactive oxygen species; PGE-M, metabolites of PGE2; LC, liquid chromatography; *C. elegans*, *Caenorhabditis elegans*; ARS, arylsulfatase; DiAcSpm *N1*, *N12*-diacetylspermine

* Corresponding author at: Department of Gastroenterology and Metabolism, Nagoya City University Graduate School of Medical Sciences, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan.

E-mail address: tshimura@med.nagoya-cu.ac.jp (T. Shimura).

https://doi.org/10.1016/j.cca.2019.08.011
Received 11 July 2019; Received in revised form 13 August 2019; Accepted 13 August 2019
Available online 14 August 2019
0009-8981/ © 2019 Elsevier B.V. All rights reserved.

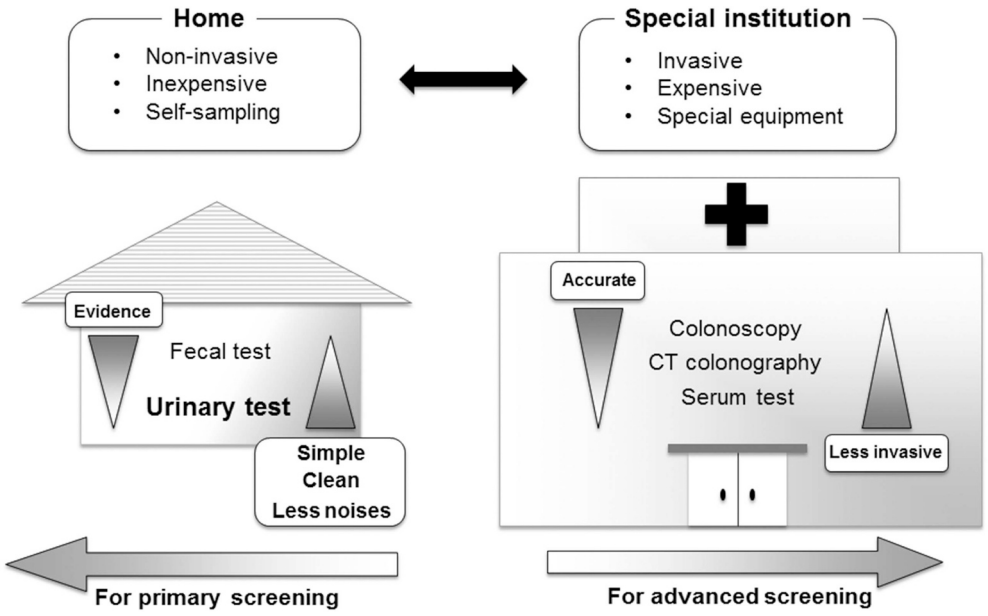

Fig. 1. Current screening tools for colorectal cancer.

2. Protein markers

Since excreted proteins are not abundant in urine, a small amount of urinary protein may be a disadvantage in the detection of CRC because it is sometimes difficult to identify these slight differences in the concentration of target proteins. On the other hand, this small amount of target protein is an advantage in the identification of reliable biomarkers because the urinary target protein that emerges after glomerular filtering and tubular resorption in kidney is not contaminated with large amounts of other non-relevant proteins including albumin.

Table 1 shows urinary protein biomarkers that can be used in the detection of CRC. Carty et al. have described that the urinary procoagulant activity caused by tissue factor (TF) was higher in the urine samples of patients with CRC than in those from healthy controls (HCs) [15].

There is another report in which the level of CRC specific neoantigen detected using BAC 18.1, a murine monoclonal antibody was significantly higher not only in colonic effluents, but also in urine from patients with CRC compared to the urine samples from HCs [16]. However, the number of evidences in favor and credibility of this biomarker are quite low owing to the small sample size and lack of accurate data.

El-Masry and colleagues investigated urinary CEA using ELISA and revealed that it was more detectable in patients with CRC than in HCs [17]. Since the molecular size of CEA might be much larger (180 kDa) than the glomerulus pore size, the amount of CEA exuded would be limited. This study did not refer to the diagnosis of early stage CRC. Additionally, measurement of serum CEA and its use in clinical practice is not recommended as a method of screening for CRC owing to its low sensitivity [8,18,19].

We recently reported that urinary levels of Cyr61 and TFF3 were significantly higher in patients with CRC than in HCs, and the diagnostic panel consisted of Cyr61 and TFF3 in both HC and CRC samples [area under the curve (AUC) = 0.753 in the training cohort and 0.720 in the validation cohort] [13]. This study included around 30% of patients with stage 0/I CRC in the CRC cohort. Cyr61 is a secreted protein that is associated with angiogenesis, cell proliferation, differentiation, apoptosis, and extracellular matrix formation. Serum Cyr61 [20] and serum TFF3 [21,22] have also been reported as candidates for diagnostic biomarkers for CRC. The molecular sizes of both proteins are < 40 kDa. Therefore, it is possible that the proteins secreted from CRC tissues to the serum are ultimately discharged into the urine.

There is one report using proteomics for exploring urinary diagnostic biomarker for CRC. In that, the logistic regression model using the significant peaks of m/z ratio showed good efficacy with AUC = 0.88, 78% sensitivity, and 87% specificity calculated by leave-one-out cross-validation method [23].

Some fecal proteins have also been introduced as candidates for non-invasive biomarkers [24]; however, fecal proteins would not be suitable as biomarkers because feces contain protein-rich residues and intestinal microorganisms that can interfere with the detection of specific proteins.

3. Genetic markers

DNA methylation regulates transcription and its abnormality could cause carcinogenesis. Furthermore, DNA methylation is also expected to be useful as a diagnostic marker and therapeutic target for CRC [25–27]. It is possible that mutated DNAs that originate from cancer tissues are excreted into the urine either within enclosed microvesicles such as exosomes or in the naked form [28,29]. As shown in Table 2, there are several reports of urinary genetic markers using methylated or mutated genes identified in serum, fecal, or tissue samples of CRC patients.

Song et al. investigated urinary methylated vimentin and found that it was detectable in 15 out of 20 patients with CRC (75%), whereas it was detectable in only 2 out of the 20 HCs [30]. However, the significance of early diagnosis remained unclear because of the limited number of samples from patients with early stage CRC. Another study analyzed methylated Wif-1, ALX-4, and vimentin in the urine samples from patients with CRC and HCs [31]. Although the specificity of methylated Wif-1 was over 90%, its sensitivity was quite low. The third study comprising of relatively high percentages of patients with stage I and II CRC identified that urinary methylated NDRG4 is a diagnostic biomarker for CRC with 72.6% sensitivity and 85.0% specificity [32]. In the gene mutation biomarker study, KRAS mutation in both urinary and plasma circulating tumor DNA (ctDNA) showed excellent diagnostic performance for Stage II-IV CRC with AUC ≥ 0.95. However, for stage I CRC, AUC was around 0.7 [33].

Kits for the detection of methylated SEPT9 in serum (ColoVantage®) and methylated vimentin in stool (ColoSure®) are commercially available. Two studies indicated that serum vimentin methylation has been

Table 1
Protein markers.

Urinary biomarkers	Molecular size	Examination modality	N		AUC		Sensitivity (%)		Specificity (%)		Ref.No.
			CRC (Stage 0/I)	HC	Training	Validation	Training	Validation	Training	Validation	
Tissue factor	33.1 kDa	Chromogenic assays	70 (NA)	79	NA	NA	NA	NA	NA	NA	15
Organ specific neoantigen	NA	ELISA	27 (NA)	29	NA	NA	NA	NA	NA	NA	16
CEA	180 kDa	Slot-Blot-ELISA	90 (NA)	50	NA	NA	76.7	NA	78.0	NA	17
Cyr61	42 kDa	ELISA	Total 88 (27)	Total 88[a]	0.745 (0.653-0.838)	0.696 (0.571–0.822)	NA	NA	NA	NA	13
TFF3	10.2 kDa	ELISA	Training 53	Training 53	0.576 (0.466–0.686)	0.639 (0.508–0.770)	NA	NA	NA	NA	
Cyr61 + TFF3			Validation 35	Validation 35	0.753 (0.659–0.847)	0.720 (0.599–0.841)	75.5	71.4	69.8	74.3	
Proteomics	NA	MALDI SELDI	67	72	0.880	NA	78[b]	NA	87[b]	NA	23

AUC, area under the curve; CRC, colorectal cancer; HC, healthy control; Ref No, reference number; NA, not available; MALDI, matrix assisted laser desorption/ionization time-of-flight mass spectrometry; SELDI, surface enhanced laser desorption/ionization time-of-flight mass spectrometry.

[a] Age, sex-matched.

[b] Calculated by leave-one-out cross-validation method.

detected in 9.1–32.6% of patients with CRC, which was lower than the detection rate of urinary vimentin methylation in their previous report [34,35]. Multitarget stool DNA (MT-sDNA) screening test capable of detecting CRC-related gene changes, including aberrant methylated BMP3 and NDRG4, KRAS mutation, beta-actin, and fecal hemoglobin, has been approved by the U.S. Food and Drug Administration (Cologuard®). Moreover, this test may aid in the detection of colorectal neoplasms [36].

Since gene alterations are generally much more common in advanced stages, CRC screening using only genetic biomarkers may be inefficient in the early detection of CRC. A combination of genetic biomarkers and other tools may generate synergistic effects.

4. Metabolomics

Since urinary metabolites are derived from glomerular filtration of serum and secretion in the renal tubule, it is possible that the metabolites secreted into the blood from cancer tissues are detected in urine of the patients with CRC. The development of metabolomics using nuclear magnetic resonance (NMR) and mass spectrometry (MS) has made it possible to analyze a large number of metabolites [37,38].

Table 3 lists the urinary metabolite biomarkers used in the detection of CRC. Notably, despite its small sample size, Silva et al. analyzed urinary volatile metabolites (VOMs) using MS and found 16 VOMs that differed significantly in quantities in patients with CRC and HC [39]. Cheng et al. focused on MS-based metabolomics and made a model that consisted of 7 metabolites showing great performance with an AUC of almost 1 [40]. Another recent MS-based metabolomics study reported the results from a relatively large sized multicenter study that established a panel of 17 urinary metabolites that may be detected in patients with CRC. These metabolites also have an excellent AUC of 0.967 and 0.868 in the training and validation sets, respectively [41].

Another small study revealed higher levels of some porphyrin metabolites, including 5-aminolevulinic acid (5-ALA), uroporphyrinogen I, and coproporphyrinogen, upon 5-ALA administration, in patients with CRC compared with that in HCs [42].

Two studies using NMR-based metabolomics have established urinary biomarker panels that consisted of many metabolites with significant differences between patients with CRC and HCs. These biomarkers exhibited an excellent diagnostic performance for CRC with an AUC $>$ 0.8 [43,44].

There are several reports focusing on modified DNA bases (nucleosides) that are generated by DNA damage and are related to the development of cancer. All reports used high-performance liquid chromatography (HPLC) to detect urinary nucleosides. One study identified 12 nucleosides that were present in higher quantities in patients with CRC, using the column switching HPLC method [45], and the other study identified 1-methylguanosine and pseudouridine as a good predictor with AUC = 0.896 and 0.816, using reverse-phase HPLC [46]. In another comprehensive analysis of urinary nucleosides using HPLC-tandem MS, it has been found that the levels of 3 nucleotides were higher in CRC patients than in HCs [47]. Rozalski et al. reported that the model consisted of 8-oxo-7,8-dihydroguanine, 8-oxo-7,8-dihydro-2′-deoxyguanosine, and 5-hydroxymethyluracil distinguished between CRC patients and HCs with AUC = 0.778 [48].

Compared with other type of biomarkers, urinary metabolites seem to show higher diagnostic performance. Indeed, since metabolites have very low molecular weight, urine contains plenty of metabolites. This is a huge advantage of using urinary metabolites as cancer biomarkers because it can reflect the systemic metabolic condition including cancer homeostasis. In contrast, complex instruments and processors for metabolomics analysis result in problems of applying mass screening for large population. Additionally, the reported metabolomics biomarkers are usually composed of so many parameters that it is difficult to extrapolate the result to clinical use. Biomarkers with fewer factors are ideal for use in a clinical setting.

Table 2
Genetic markers.

Urinary biomarkers	Molecular size	Examination modality	N		AUC		Sensitivity (%)		Specificity (%)		Ref.No.
			CRC (Stage 0/I)	HC	Training	Validation	Training	Validation	Training	Validation	
mVim	1.401kbp	qPCR (MethyLight assay)	20 (4)	20	NA	NA	75.0	NA	90.0	NA	30
mWif-1	1.140kbp	qPCR (MethyLight assay)	Total 90[b] (NA)	Total 157[d]	NA	NA	52.0	0.27	93.0	99.0	31
mALX-4	1.236kbp		Training 48	Training 14	NA	NA	15.0	NA	100	NA	
mVim	1.401kbp		Validation 90[e]	Validation 157[e]	NA	NA	8.0	NA	100	NA	
mNDRG4	1.059kbp	n-MSP	84 (48[a])	16[c]	NA	NA	72.6	NA	85.0	NA	32
mtKRAS	0.570kbp	ddPCR	150 (30)	40	Stage I 0.691 StageII 0.960 StageIII 0.989 StageIV 0.994	NA	NA	NA	NA	NA	33

CRC, colorectal cancer; HC, healthy control; AUC, area under the curve; Ref No, reference number; mVim, methylated Vimentin; mWif-1, methylated Wif-1; mALX-4, methylated ALX-4; mNDRG4, methylated NDRG4; mtKRAS, mutated KRAS; n-MSP, nested methylation-specific polymerase chain reaction; ddPCR, droplet digital polymerase chain reaction.

[a] Indicating the number of the patients with Stage I and II.

[b] Indicating the number of the patients with advanced neoplasms (CRC or advanced adenoma [measuring > 10 mm and/or with high-grade dysplasia]).

[c] Age-matched.

[d] Indicating the number of the participants with normal colon or small adenomas measuring ≤ 10 mm.

[e] Including training cohort.

Although several studies have shown different profiles of fecal metabolites in HCs and patients with CRC [49,50], the reproducibility of the results was reportedly low for many of the metabolites (within-subject intraclass correlation coefficient ≤ 0.5) [51]. Indeed, cancer-specific metabolites may be directly excreted from cancer tissue to the feces; however, since digestive enzymes and enterobacterium are abundantly contained in feces, these may modulate the fecal metabolites component. Urine sample may have advantage over feces sample as a metabolomics biomarker. Although many urinary metabolites derived from the gut microbiome were affected by tumor resection in the previous CRC study, it is unclear whether these alterations reflected the metabolites originated from cancer itself or the microenvironmental changes caused by cancer occurrence [52].

5. Prostaglandin/reactive oxygen species

It has been reported that prostaglandin E2 (PGE2) plays crucial roles in promoting angiogenesis and carcinogenesis under the regulation of cyclooxygenase-2 (COX-2), consequently resulting in proliferation and metastasis [53,54]. Meanwhile, reactive oxygen species (ROS) generated by oxidative stress such as inflammation can induce nucleic acid damage and ROS are associated with carcinogenesis [55–57]. Based on these reports, some studies identified the diagnostic biomarkers related to PGE2 and ROS (Table 4). Two studies quantified the urinary metabolites of PGE2 (PGE-M) using liquid chromatography (LC)-MS [58,59]. Urinary PGE-M could distinguish patients with CRC from the patients with benign polyps and HCs effectively in women, but not in men [58]. Another study showed that the urinary level of PGE-M was significantly higher in CRC compared with HC and the relative risk of CRC incidence increased with increase in the level of urinary PGE-M [59]. Chandramathi et al. analyzed 4 urinary factors involved in oxidative reaction, namely, advanced oxidative protein product, hydrogen peroxide, malondialdehyde, and ferric-reducing antioxidant power. The levels of these showed aberrant differences between patients with CRC and HCs [60].

From these results, PGE-M and ROS-related metabolites might help in distinguishing between patients with CRC and HCs; however, the real significance of these as diagnostic biomarkers remains obscure because of the limited number of reports and limited data of diagnostic power.

6. Other markers

Other specific biomarkers are summarized in Table 5. Hirotsu et al. reported a very unique method that used *Caenorhabditis elegans* (*C. elegans*) having chemotaxis to the urine from patients with CRC. Albeit a small sample size of CRC, *C. elegans* showed an excellent diagnostic performance with 100% sensitivity and 95% specificity [61]. There is another unique method that uses canine scent in the detection of CRC from breath and fecal samples with over 90% sensitivity and specificity [62]. Although the causal substances are unclear, excrements from patients with CRC might contain some odorants that are attractive for specific animals such as *C. elegans* and canines.

In terms of enzyme activity, despite the small sample size, one study described that the urinary activities of *N*-acetyl-β-D-hexosaminidase and its isoenzyme A and B are higher in patients with CRC than in HCs [63]. Another study measured arylsulfatase (ARS) activity using urine samples from CRC patients and HCs. Urinary ARS activity distinguished between patients with CRC and HCs efficiently with over 0.8 AUC [64]. As for other samples, fecal tumor M2 pyruvate kinase showed good performance in distinguishing patients with CRC from HCs with AUC = 0.762–0.925, 73.0–85.0% sensitivity, and 71.1–75.0% specificity [65–68].

Polyamines including spermine play an important role in cell proliferation and differentiation by binding to DNA [69,70]. There are 2 reports that introduced urinary spermine as a diagnostic biomarker for CRC. In these studies, the urinary level of *N1, N12*-diacetylspermine (DiAcSpm) was higher in patients with CRC than that in HCs. These biomarkers had 69.6–75.8% sensitivity for CRC diagnosis [71,72], which was higher than the sensitivities of both serum CEA (39.5%) and CA19–9 (14.1%) [71].

In terms of other samples, DiAcSpm level was higher in CRC tissues than in adjacent normal tissues [73]. Additionally, spermidine/spermine N^1-acetyltransferase, which catalyzes the acetylation of spermidine and spermine, is also expressed in CRC tissues [74] and inhibits CRC cell growth [75]. Taken together, it is possible that spermine generated aberrantly in CRC tissue flows into body fluids and thus may be used as a diagnostic biomarker. However, its diagnostic power seems to be limited.

7. Conclusions

The present review provided the candidates for urinary diagnostic

Table 3
Markers using metabolomics.

Urinary biomarkers	Molecular size	Examination modality	N		AUC		Sensitivity (%)		Specificity (%)		Ref.No
			CRC (Stage 0/I)	HC	Training	Validation	Training	Validation	Training	Validation	
Volatile organic metabolites	NA	SPME GC–MS	12 (NA)	21	NA	NA	NA	NA	NA	NA	39
MS-metabolomics	NA	GC-TOFMS UPLC-QTOFMS	Total 101 (24) Training 61 Validation 40	Total 103[d] Training 62 Validation 41	0.993[i]	0.998[i]	97.5	97.6	97.5	100	40
17 metabolites[a]	NA	LC-MS	171	171[f]	0.967	0.868	99.2	74.0	80	80	41
4 metabolites[c]					0.903	0.873	82.6	72.0	80	80	
2 metabolites[b]					0.864	0.851	80.0	74.0	80	80	
5-ALA	0.131 kDa	HPLC	33 (12)	24[e]	0.67	NA	NA	NA	NA	NA	42
UP I	0.837 kDa				0.84						
CP III	0.661 kDa				0.65						
NMR-metabolomics	NA	NMR	55 (23[g])	40[d]	0.933[j]	NA	87.5	NA	91.3	NA	43
NMR-metabolomics	NA	NMR	92[h] (24/8)	156	0.842[k]	NA	100	NA	96.2	NA	44
Nucleosides	NA	HPLC	52 (NA)	60	NA	NA	NA	NA	NA	NA	45
m1G	NA	RP-HPLC	56 (NA)	62	0.896	NA	71.2[l]	NA	93.3[l]	NA	46
Pseu					0.816						
Nucleosides	NA	HPLC/MS	26 (NA)	45	NA	NA	69.0[m]	NA	98.0[m]	NA	47
8-oxoGua	0.151 kDa	HPLC	56 (NA)	72	0.694	NA	78.6[n]	NA	75.0[n]	NA	48
8-oxodGuo	0.283 kDa				0.635						
5-hmUra	0.112 kDa				0.669						
8-oxoGua + 8-oxodGuo + 5-hmUra					0.778[n]						

SPME, solid-phase microextraction; GC, gas chromatography; MS, mass spectrometry; TOFMS, time-of-flight mass spectrometry; UPLC, ultra performance liquid chromatography; QTOFMS, quadrupole TOFMS; HPLC, high-performance liquid chromatography; NMR, nuclear magnetic resonance; RP-HPLC, reverse-phase HPLC; 5-ALA, 5-aminolevulinic acid; UP I, uroporphyrinogen I; CP III, coproporphyrinogen III; m1G, 1-methylganosine; Pseu, pseudouridine; 8-oxoGua, 8-oxo-7,8-dihydroguanine; 8-oxodGuo, 8-oxo-7,8-dihydro-2′-deoxyguanosine; 5-hmUra, 5-hydroxymethyluracil;

[a] Including Proline, Diacetylspermine, C14.1, Kynurenine, Glutamate, Beta-Hydroxybutyric acid, HPHPA, DOPA, c4-OH.Proline, Putrescine, Indole acetic acid, Citric acid, Hippuric acid, Sarcosine, and Butyric acid.

[b] Including Proline, Diacetylspermine, Kynurenine, and Glucose.

[c] Including Diacetylspermine and Kynurenine.

[d] Age, sex-matched.

[e] Including 8 healthy adults and 16 preoperative benign disease surgery patients.

[f] Sex-matched.

[g] Indicating the number of the patients with CRC Stage I/II.

[h] Including 56 patients with CRC and 36 patients with advanced adenomas (measuring ≥ 1 cm).

[i] Indicating the value gained from the model consisted of citrate, hippurate, p-cresol, 2-aminobutyrate, myristate, putrescine, and kynurenate.

[j] Indicating the value gained from the model consisted of analine, glutamine, asparagic acid, and acetoacetate.

[k] Indicating the value gained from ROC curve with 3-Aminoisobutyrate for detecting the patients with CRC stage 0 or advanced adenomas (measuring ≥ 1 cm).

[l] Calculated by Jackknife method using the model consisted of m1G and Pseu.

[m] Calculated using the model consisted of cytidine, 3-methylcytidine, 1-methyladenosine, 2-deoxyguanosine, adenosine, and inosine.

[n] Indicating the value gained from the model consisted of 8-oxoGua, 8-oxodGuo, and 5-hmUra.

Table 4
Markers using prostaglandin and reactive oxygen species.

Urinary biomarkers	Molecular size	Examination modality	N		AUC		Sensitivity (%)		Specificity (%)		Ref.No
			CRC (Stage 0/I)	HC	Training	Validation	Training	Validation	Training	Validation	
PGE-M	0.352 kDa	LC-qMS	58 (NA)	72	Total 0.64[a] Male 0.54[a] Female 0.84[a]	NA	Total 90[a] Male 88[a] Female 92[a]	NA	Total 45[a] Male 35[a] Female 78[a]	NA	58
PGE-M	0.352 kDa	LC/tandem MS	150 (NA)	150[b]	NA	NA	NA	NA	NA	NA	59
AOPP	NA	Spectro-photometry	49 (0)	95	NA	NA	NA	NA	NA	NA	60
H_2O_2	0.034 kDa										
MDA	0.072 kDa										
FRAP	NA										

PGE-M, Prostaglandin E2; AOPP, Advanced oxidative protein product; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; FRAP, ferric-reducing antioxidant power; LC-qMS, liquid chromatography-quadrupole mass spectrometry.

[a] CRC vs. patients with benign polyps and healthy controls.

[b] Incidence density method.

Table 5
Other markers.

Category	Urinary biomarkers	Molecular size	Examination modality	N		AUC		Sensitivity (%)		Specificity (%)		Ref.No
				CRC (Stage 0/I)	HC	Training	Validation	Training	Validation	Training	Validation	
Odorous substance	*C. elegans*	NA	Chemotactic behavior	10 (1)	218	NA	NA	100	NA	95.0	NA	61
Enzyme	HEX	62 kDa	Spectro-photometry	32 (NA)	20	0.864 (0.739–0.989)	NA	85.0	NA	85.7	NA	63
	HEX A							85.0		85.7		
	HEX B							75.0		57.1		
						0.939 (0.864–1.000)						
						0.686 (0.505–0.866)						
Enzyme	arylsulfatase	72 kDa	Spectro-photometry	119 (NA)	300	Male 0.89 (0.83–0.95)	NA	Male 63.4	NA	Male 96.4	NA	64
						Female 0.87 (0.79–0.94)		Female 81.3		Female 95.1		
Spermines	DiAcSpm	0.2 kDa	ELISA	248 (60)	52	NA	NA	75.8	NA	NA	NA	71
Spermines	DiAcSpm	0.2 kDa	ELISA	33 (5)	33	NA	NA	69.6	NA	NA	NA	72
	DiAcSpd	0.2 kDa			29	NA	NA	36.3	NA	NA	NA	

C. elegans, *Caenorhabditis elegans*; HEX, *N*-acetyl-β-D-hexosaminidase; HEX A, hexosaminidase isoenzyme A; HEX B, hexosaminidase isoenzyme B; DiAcSpm, *N1*, *N12*-diacetylspermine; DiAcSpd, *N1,N8*-diacetylspermidine.

biomarkers for the diagnosis of CRC. Although none of them have been clinically applied because of the lack of validation, accumulating evidence indicates the potency of urinary test as a screening tool for CRC. Although the fecal assays including FOBT and MT-sDNA are more advanced for CRC diagnosis, urine samples still have some advantages compared to faces and blood samples. First, as urine contains very few protein and microorganisms, the degradation of specific materials by various enzymes can be avoided. Second, it can be collected in high volumes and non-invasively, despite urine samples reflecting the systemic metabolic status and a part of gene expression. Recently, technological and analytic advances have boosted scientific biomarker research. In the near future, we expect the advent of novel urinary assays with high efficacy that would reduce CRC mortality.

Declaration of Competing Interest

None of the authors declare any conflicts of interest. No financial support was received for this study.

Acknowledgements

The authors would like to thank Honyaku Center Inc. (https://www.honyakucenter.jp/) for the English language review. Financial support was not received for this study.

References

[1] C. Global Burden of Disease Cancer, C. Fitzmaurice, T.F. Akinyemiju, F.H. Al Lami, T. Alam, R. Alizadeh-Navaei, C. Allen, U. Alsharif, N. Alvis-Guzman, E. Amini, B.O. Anderson, O. Aremu, A. Artaman, S.W. Asgedom, R. Assadi, T.M. Atey, L. Avila-Burgos, A. Awasthi, H.O. Ba Saleem, A. Barac, J.R. Bennett, I.M. Bensenor, N. Bhakta, H. Brenner, L. Cahuana-Hurtado, C.A. Castaneda-Orjuela, F. Catala-Lopez, J.J. Choi, D.J. Christopher, S.C. Chung, M.P. Curado, L. Dandona, R. Dandona, J. das Neves, S. Dey, S.D. Dharmaratne, D.T. Doku, T.R. Driscoll, M. Dubey, H. Ebrahimi, D. Edessa, Z. El-Khatib, A.Y. Endries, F. Fischer, L.M. Force, K.J. Foreman, S.W. Gebrehiwot, S.V. Gopalani, G. Grosso, R. Gupta, B. Gyawali, R.R. Hamadeh, S. Hamidi, J. Harvey, H.Y. Hassen, R.J. Hay, S.I. Hay, B. Heibati, M.K. Hiluf, N. Horita, H.D. Hosgood, O.S. Ilesanmi, K. Innos, F. Islami, M.B. Jakovljevic, S.C. Johnson, J.B. Jonas, A. Kasaeian, T.D. Kassa, Y.S. Khader, E.A. Khan, G. Khan, Y.H. Khang, M.H. Khosravi, J. Khubchandani, J.A. Kopec, G.A. Kumar, M. Kutz, D.P. Lad, A. Lafranconi, Q. Lan, Y. Legesse, J. Leigh, S. Linn, R. Lunevicius, A. Majeed, R. Malekzadeh, D.C. Malta, L.G. Mantovani, B.J. McMahon, T. Meier, Y.A. Melaku, M. Melku, P. Memiah, W. Mendoza, T.J. Meretoja, H.B. Mezgebe, T.R. Miller, S. Mohammed, A.H. Mokdad, M. Moosazadeh, P. Moraga, S.M. Mousavi, V. Nangia, C.T. Nguyen, V.M. Nong, F.A. Ogbo, A.T. Olagunju, M. Pa, E.K. Park, T. Patel, D.M. Pereira, F. Pishgar, M.J. Postma, F. Pourmalek, M. Qorbani, A. Rafay, S. Rawaf, D.L. Rawaf, G. Roshandel, S. Safiri, H. Salimzadeh, J.R. Sanabria, M.M. Santric Milicevic, B. Sartorius, M. Satpathy, S.G. Sepanlou, K.A. Shackelford, M.A. Shaikh, M. Sharif-Alhoseini, J. She, M.J. Shin, I. Shiue, M.G. Shrime, A.H. Sinke, M. Sisay, A. Sligar, M.B. Sufiyan, B.L. Sykes, R. Tabares-Seisdedos, G.A. Tessema, R. Topor-Madry, T.T. Tran, B.X. Tran, K.N. Ukwaja, V.V. Vlassov, S.E. Vollset, E. Weiderpass, H.C. Williams, N.B. Yimer, N. Yonemoto, M.Z. Younis, C.J.L. Murray, M. Naghavi, Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted Life-Years for 29 cancer groups, 1990 to 2016: a systematic analysis for the global burden of disease study, JAMA Oncol. 4 (11) (2018) 1553–1568.

[2] P. Hewitson, P. Glasziou, E. Watson, B. Towler, L. Irwig, Cochrane systematic review of colorectal cancer screening using the fecal occult blood test (hemoccult): an update, Am. J. Gastroenterol. 103 (6) (2008) 1541–1549.

[3] R.E. Schoen, P.F. Pinsky, J.L. Weissfeld, L.A. Yokochi, T. Church, A.O. Laiyemo, R. Bresalier, G.L. Andriole, S.S. Buys, E.D. Crawford, M.N. Fouad, C. Isaacs, C.C. Johnson, D.J. Reding, B. O'Brien, D.M. Carrick, P. Wright, T.L. Riley, M.P. Purdue, G. Izmirlian, B.S. Kramer, A.B. Miller, J.K. Gohagan, P.C. Prorok, C.D. Berg, P.P. Team, Colorectal-cancer incidence and mortality with screening flexible sigmoidoscopy, N. Engl. J. Med. 366 (25) (2012) 2345–2357.

[4] A.M.D. Wolf, E.T.H. Fontham, T.R. Church, C.R. Flowers, C.E. Guerra, S.J. LaMonte, R. Etzioni, M.T. McKenna, K.C. Oeffinger, Y.T. Shih, L.C. Walter, K.S. Andrews, O.W. Brawley, D. Brooks, S.A. Fedewa, D. Manassaram-Baptiste, R.L. Siegel, R.C. Wender, R.A. Smith, Colorectal cancer screening for average-risk adults: 2018 guideline update from the American Cancer Society, CA Cancer J. Clin. 68 (4) (2018) 250–281.

[5] A. Kapidzic, A.H. van Roon, M.E. van Leerdam, A.J. van Vuuren, M. van Ballegooijen, I. Lansdorp-Vogelaar, W. Spijker, K. Izelaar, L. Hol, E.J. Kuipers, Attendance and diagnostic yield of repeated two-sample faecal immunochemical test screening for colorectal cancer, Gut 66 (1) (2017) 118–123.

[6] L. van Dam, T.R. de Wijkerslooth, M.C. de Haan, E.M. Stoop, P.M. Bossuyt,

P. Fockens, M. Thomeer, E.J. Kuipers, M.E. van Leerdam, M. van Ballegooijen, J. Stoker, E. Dekker, E.W. Steyerberg, Time requirements and health effects of participation in colorectal cancer screening with colonoscopy or computed tomography colonography in a randomized controlled trial, Endoscopy 45 (3) (2013) 182–188.
[7] M. Pioche, C. Ganne, R. Gincul, A. De Leusse, J. Marsot, J. Balique, A. Fond, M. Bretagnolle, L. Henry, Y. Billaud, R. Malezieux, M.G. Lapalus, C. Chambon-Augoyard, E. Del Tedesco, O. Scalone, J.C. Montoy, B. Russias, A. Detry, F. Veniat, J. Qiu, P.J. Valette, A. Taillandier, J.C. Saurin, J. Tomczyk-Ferrero, C. Gandilhon, L. Vecchiato, P. Soler-Michel, T. Ponchon, Colon capsule versus computed tomography colonography for colorectal cancer screening in patients with positive fecal occult blood test who refuse colonoscopy: a randomized trial, Endoscopy 50 (8) (2018) 761–769.
[8] N. Wild, H. Andres, W. Rollinger, F. Krause, P. Dilba, M. Tacke, J. Karl, A combination of serum markers for the early detection of colorectal cancer, Clin. Cancer Res. 16 (24) (2010) 6111–6121.
[9] S. Bouatra, F. Aziat, R. Mandal, A.C. Guo, M.R. Wilson, C. Knox, T.C. Bjorndahl, R. Krishnamurthy, F. Saleem, P. Liu, Z.T. Dame, J. Poelzer, J. Huynh, F.S. Yallou, N. Psychogios, E. Dong, R. Bogumil, C. Roehring, D.S. Wishart, The human urine metabolome, PLoS ONE 8 (9) (2013) e73076.
[10] S. Li, Y. Jin, Z. Tang, S. Lin, H. Liu, Y. Jiang, Z. Cai, A novel method of liquid chromatography-tandem mass spectrometry combined with chemical derivatization for the determination of ribonucleosides in urine, Anal. Chim. Acta 864 (2015) 30–38.
[11] T. Shimura, A. Dagher, M. Sachdev, M. Ebi, T. Yamada, T. Yamada, T. Joh, M.A. Moses, Urinary ADAM12 and MMP-9/NGAL complex detect the presence of gastric cancer, Cancer Prev. Res. (Phila.) 8 (3) (2015) 240–248.
[12] T. Shimura, M. Ebi, T. Yamada, T. Yamada, T. Katano, Y. Nojiri, H. Iwasaki, S. Nomura, N. Hayashi, Y. Mori, H. Kataoka, M.A. Moses, T. Joh, Urinary kallikrein 10 predicts the incurability of gastric cancer, Oncotarget 8 (17) (2017) 29247–29257.
[13] T. Shimura, H. Iwasaki, M. Kitagawa, M. Ebi, T. Yamada, T. Yamada, T. Katano, H. Nisie, Y. Okamoto, K. Ozeki, T. Mizoshita, H. Kataoka, Urinary cysteine-rich protein 61 and trefoil factor 3 as diagnostic biomarkers for colorectal cancer, Transl. Oncol. 12 (3) (2019) 539–544.
[14] H. Iwasaki, T. Shimura, T. Yamada, Y. Okuda, M. Natsume, M. Kitagawa, S.I. Horike, H. Kataoka, A novel urinary microRNA biomarker panel for detecting gastric cancer, J. Gastroenterol. (2019), https://doi.org/10.1007/s00535-019-01601-w.
[15] N. Carty, I. Taylor, O.S. Roath, K. el-Baruni, J.L. Francis, Urinary tissue factor activity in colorectal disease, Br. J. Surg. 77 (10) (1990) 1091–1094.
[16] M. Tobi, C.E. Darmon, P. Rozen, N. Harpaz, A. Fink, B. Maliakkal, A. Halline, S. Mobarhan, Z. Bentwich, Urinary organ specific neoantigen. A potentially diagnostic test for colorectal cancer, Dig. Dis. Sci. 40 (7) (1995) 1531–1537.
[17] S. El-Masry, I.H. El-Sayed, M. Lotfy, L. Mahmoud, M. El-Naggar, Utility of slot-blot-ELISA as a new, fast, and sensitive immunoassay for detection of carcinoembryonic antigen in the urine samples of patients with various gastrointestinal malignancies, J. Immunoass. Immunochem. 28 (2) (2007) 91–105.
[18] J. Wang, X. Wang, S. Lin, C. Chen, C. Wang, Q. Ma, B. Jiang, Identification of kininogen-1 as a serum biomarker for the early detection of advanced colorectal adenoma and colorectal cancer, PLoS ONE 8 (7) (2013) e70519.
[19] H.N. Liu, T.T. Liu, H. Wu, Y.J. Chen, Y.J. Tseng, C. Yao, S.Q. Weng, L. Dong, X.Z. Shen, Serum microRNA signatures and metabolomics have high diagnostic value in colorectal cancer using two novel methods, Cancer Sci. 109 (4) (2018) 1185–1194.
[20] Y.F. Song, Z.B. Xu, X.J. Zhu, X. Tao, J.L. Liu, F.L. Gao, C.L. Wu, B. Song, Q. Lin, Serum Cyr61 as a potential biomarker for diagnosis of colorectal cancer, Clin. Transl. Oncol. 19 (4) (2017) 519–524.
[21] H. Xie, J.H. Guo, W.M. An, S.T. Tian, H.P. Yu, X.L. Yang, H.M. Wang, Z. Guo, Diagnostic value evaluation of trefoil factors family 3 for the early detection of colorectal cancer, World J. Gastroenterol. 23 (12) (2017) 2159–2167.
[22] Q. Li, K. Wang, C. Su, J. Fang, Serum trefoil factor 3 as a protein biomarker for the diagnosis of colorectal cancer, Technol. Cancer Res. Treat. 16 (4) (2017) 440–445.
[23] D.G. Ward, S. Nyangoma, H. Joy, E. Hamilton, W. Wei, C. Tselepis, N. Steven, M.J. Wakelam, P.J. Johnson, T. Ismail, A. Martin, Proteomic profiling of urine for the detection of colon cancer, Proteome Sci. 6 (2008) 19.
[24] D.J. Robertson, T.F. Imperiale, Stool testing for colorectal cancer screening, Gastroenterology 149 (5) (2015) 1286–1293.
[25] S.C. Glockner, M. Dhir, J.M. Yi, K.E. McGarvey, L. Van Neste, J. Louwagie, T.A. Chan, W. Kleeberger, A.P. de Bruine, K.M. Smits, C.A. Khalid-de Bakker, D.M. Jonkers, R.W. Stockbrugger, G.A. Meijer, F.A. Oort, C. Iacobuzio-Donahue, K. Bierau, J.G. Herman, S.B. Baylin, M. Van Engeland, K.E. Schuebel, N. Ahuja, Methylation of TFPI2 in stool DNA: a potential novel biomarker for the detection of colorectal cancer, Cancer Res. 69 (11) (2009) 4691–4699.
[26] B.B. Lee, E.J. Lee, E.H. Jung, H.K. Chun, D.K. Chang, S.Y. Song, J. Park, D.H. Kim, Aberrant methylation of APC, MGMT, RASSF2A, and Wif-1 genes in plasma as a biomarker for early detection of colorectal cancer, Clin. Cancer Res. 15 (19) (2009) 6185–6191.
[27] D.J. Weisenberger, G. Liang, H.J. Lenz, DNA methylation aberrancies delineate clinically distinct subsets of colorectal cancer and provide novel targets for epigenetic therapies, Oncogene 37 (5) (2018) 566–577.
[28] Y.H. Su, M. Wang, D.E. Brenner, A. Ng, H. Melkonyan, S. Umansky, S. Syngal, T.M. Block, Human urine contains small, 150 to 250 nucleotide-sized, soluble DNA derived from the circulation and may be useful in the detection of colorectal cancer, J. Mol. Diagn. 6 (2) (2004) 101–107.
[29] M. van der Vaart, P.J. Pretorius, The origin of circulating free DNA, Clin. Chem. 53 (12) (2007) 2215.
[30] B.P. Song, S. Jain, S.Y. Lin, Q. Chen, T.M. Block, W. Song, D.E. Brenner, Y.H. Su, Detection of hypermethylated vimentin in urine of patients with colorectal cancer, J. Mol. Diagn. 14 (2) (2012) 112–119.
[31] A. Amiot, H. Mansour, I. Baumgaertner, J.C. Delchier, C. Tournigand, J.P. Furet, J.P. Carrau, F. Canoui-Poitrine, I. Sobhani, C.R.C.G.O.V.D. Marne, The detection of the methylated Wif-1 gene is more accurate than a fecal occult blood test for colorectal cancer screening, PLoS ONE 9 (7) (2014) e99233.
[32] W. Xiao, H. Zhao, W. Dong, Q. Li, J. Zhu, G. Li, S. Zhang, M. Ye, Quantitative detection of methylated NDRG4 gene as a candidate biomarker for diagnosis of colorectal cancer, Oncol. Lett. 9 (3) (2015) 1383–1387.
[33] F. Tian, Y. Liao, Y. Zhang, Variations in transrenal DNA and comparison with plasma DNA as a diagnostic marker for colorectal cancer, Int. J. Biol. Markers 32 (4) (2017) e434–e440.
[34] A. Shirahata, K. Sakuraba, T. Goto, M. Saito, K. Ishibashi, G. Kigawa, H. Nemoto, K. Hibi, Detection of vimentin (VIM) methylation in the serum of colorectal cancer patients, Anticancer Res. 30 (12) (2010) 5015–5018.
[35] A. Shirahata, K. Hibi, Serum vimentin methylation as a potential marker for colorectal cancer, Anticancer Res. 34 (8) (2014) 4121–4125.
[36] D.H. Johnson, J.B. Kisiel, K.N. Burger, D.W. Mahoney, M.E. Devens, D.A. Ahlquist, S. Sweetser, Multitarget stool DNA test: clinical performance and impact on yield and quality of colonoscopy for colorectal cancer screening, Gastrointest. Endosc. 85 (3) (2017) 657–665 (e1).
[37] R. Goodacre, S. Vaidyanathan, W.B. Dunn, G.G. Harrigan, D.B. Kell, Metabolomics by numbers: acquiring and understanding global metabolite data, Trends Biotechnol. 22 (5) (2004) 245–252.
[38] J.L. Markley, R. Bruschweiler, A.S. Edison, H.R. Eghbalnia, R. Powers, D. Raftery, D.S. Wishart, The future of NMR-based metabolomics, Curr. Opin. Biotechnol. 43 (2017) 34–40.
[39] C.L. Silva, M. Passos, J.S. Camara, Investigation of urinary volatile organic metabolites as potential cancer biomarkers by solid-phase microextraction in combination with gas chromatography-mass spectrometry, Br. J. Cancer 105 (12) (2011) 1894–1904.
[40] Y. Cheng, G. Xie, T. Chen, Y. Qiu, X. Zou, M. Zheng, B. Tan, B. Feng, T. Dong, P. He, L. Zhao, A. Zhao, L.X. Xu, Y. Zhang, W. Jia, Distinct urinary metabolic profile of human colorectal cancer, J. Proteome Res. 11 (2) (2012) 1354–1363.
[41] L. Deng, K. Ismond, Z. Liu, J. Constable, H. Wang, O.I. Alatise, M.R. Weiser, T.P. Kingham, D. Chang, Urinary metabolomics to identify a unique biomarker panel for detecting colorectal cancer: a multicentre study, Cancer Epidemiol. Biomark. Prev. 28 (8) (2019) 1283–1291.
[42] Y. Kamada, Y. Murayama, U. Ota, K. Takahashi, T. Arita, T. Kosuga, H. Konishi, R. Morimura, S. Komatsu, A. Shiozaki, Y. Kuriu, H. Ikoma, M. Nakanishi, D. Ichikawa, H. Fujiwara, K. Okamoto, T. Tanaka, E. Otsuji, Urinary 5-Aminolevulinic acid concentrations as a potential tumor marker for colorectal cancer screening and recurrence, Anticancer Res. 36 (5) (2016) 2445–2450.
[43] Z. Wang, Y. Lin, J. Liang, Y. Huang, C. Ma, X. Liu, J. Yang, NMR-based metabolomic techniques identify potential urinary biomarkers for early colorectal cancer detection, Oncotarget 8 (62) (2017) 105819–105831.
[44] E.R. Kim, H.N. Kwon, H. Nam, J.J. Kim, S. Park, Y.H. Kim, Urine-NMR metabolomics for screening of advanced colorectal adenoma and early stage colorectal cancer, Sci. Rep. 9 (1) (2019) 4786.
[45] Y.F. Zheng, J. Yang, X.J. Zhao, B. Feng, H.W. Kong, Y.J. Chen, S. Lv, M.H. Zheng, G.W. Xu, Urinary nucleosides as biological markers for patients with colorectal cancer, World J. Gastroenterol. 11 (25) (2005) 3871–3876.
[46] B. Feng, M.H. Zheng, Y.F. Zheng, A.G. Lu, J.W. Li, M.L. Wang, J.J. Ma, G.W. Xu, B.Y. Liu, Z.G. Zhu, Normal and modified urinary nucleosides represent novel biomarkers for colorectal cancer diagnosis and surgery monitoring, J. Gastroenterol. Hepatol. 20 (12) (2005) 1913–1919.
[47] W.Y. Hsu, C.J. Chen, Y.C. Huang, F.J. Tsai, L.B. Jeng, C.C. Lai, Urinary nucleosides as biomarkers of breast, colon, lung, and gastric cancer in Taiwanese, PLoS ONE 8 (12) (2013) e81701.
[48] R. Rozalski, D. Gackowski, A. Siomek-Gorecka, M. Starczak, M. Modrzejewska, Z. Banaszkiewicz, R. Olinski, Urinary 5-hydroxymethyluracil and 8-oxo-7,8-dihydroguanine as potential biomarkers in patients with colorectal cancer, Biomarkers (2015) 1–5.
[49] D. Monleon, J.M. Morales, A. Barrasa, J.A. Lopez, C. Vazquez, B. Celda, Metabolite profiling of fecal water extracts from human colorectal cancer, NMR Biomed. 22 (3) (2009) 342–348.
[50] T.L. Weir, D.K. Manter, A.M. Sheflin, B.A. Barnett, A.L. Heuberger, E.P. Ryan, Stool microbiome and metabolome differences between colorectal cancer patients and healthy adults, PLoS ONE 8 (8) (2013) e70803.
[51] J.J. Goedert, J.N. Sampson, S.C. Moore, Q. Xiao, X. Xiong, R.B. Hayes, J. Ahn, J. Shi, R. Sinha, Fecal metabolomics: assay performance and association with colorectal cancer, Carcinogenesis 35 (9) (2014) 2089–2096.
[52] D.B. Liesenfeld, N. Habermann, R. Toth, R.W. Owen, E. Frei, J. Staffa, P. Schrotz-King, K.D. Klika, C.M. Ulrich, Changes in urinary metabolic profiles of colorectal cancer patients enrolled in a prospective cohort study (ColoCare), Metabolomics 11 (4) (2015) 998–1012.
[53] S.H. Kim, Y.Y. Park, S.W. Kim, J.S. Lee, D. Wang, R.N. DuBois, ANGPTL4 induction by prostaglandin E2 under hypoxic conditions promotes colorectal cancer progression, Cancer Res. 71 (22) (2011) 7010–7020.
[54] D. Wang, L. Fu, H. Sun, L. Guo, R.N. DuBois, Prostaglandin E2 promotes colorectal cancer stem cell expansion and metastasis in mice, Gastroenterology 149 (7) (2015) 1884–1895 (e4).
[55] D.H. Kang, Oxidative stress, DNA damage, and breast cancer, AACN Clin. Issues 13 (4) (2002) 540–549.

[56] M. Valko, C.J. Rhodes, J. Moncol, M. Izakovic, M. Mazur, Free radicals, metals and antioxidants in oxidative stress-induced cancer, Chem. Biol. Interact. 160 (1) (2006) 1–40.
[57] N. Babbar, R.A. Casero Jr., Tumor necrosis factor-alpha increases reactive oxygen species by inducing spermine oxidase in human lung epithelial cells: a potential mechanism for inflammation-induced carcinogenesis, Cancer Res. 66 (23) (2006) 11125–11130.
[58] J.C. Johnson, C.R. Schmidt, M.J. Shrubsole, D.D. Billheimer, P.R. Joshi, J.D. Morrow, M.J. Heslin, M.K. Washington, R.M. Ness, W. Zheng, D.A. Schwartz, R.J. Coffey, R.D. Beauchamp, N.B. Merchant, Urine PGE-M: a metabolite of prostaglandin E2 as a potential biomarker of advanced colorectal neoplasia, Clin. Gastroenterol. Hepatol. 4 (11) (2006) 1358–1365.
[59] Q. Cai, Y.T. Gao, W.H. Chow, X.O. Shu, G. Yang, B.T. Ji, W. Wen, N. Rothman, H.L. Li, J.D. Morrow, W. Zheng, Prospective study of urinary prostaglandin E2 metabolite and colorectal cancer risk, J. Clin. Oncol. 24 (31) (2006) 5010–5016.
[60] S. Chandramathi, K. Suresh, Z.B. Anita, U.R. Kuppusamy, Comparative assessment of urinary oxidative indices in breast and colorectal cancer patients, J. Cancer Res. Clin. Oncol. 135 (2) (2009) 319–323.
[61] T. Hirotsu, H. Sonoda, T. Uozumi, Y. Shinden, K. Mimori, Y. Maehara, N. Ueda, M. Hamakawa, A highly accurate inclusive cancer screening test using Caenorhabditis elegans scent detection, PLoS ONE 10 (3) (2015) e0118699.
[62] H. Sonoda, S. Kohnoe, T. Yamazato, Y. Satoh, G. Morizono, K. Shikata, M. Morita, A. Watanabe, M. Morita, Y. Kakeji, F. Inoue, Y. Maehara, Colorectal cancer screening with odour material by canine scent detection, Gut 60 (6) (2011) 814–819.
[63] S.D. Szajda, M. Borzym-Kluczyk, J. Snarska, Z. Puchalski, K. Zwierz, N-acetyl-beta-D-hexosaminidase and its isoenzymes A and B in blood serum and urine, as a potential colon cancer markers, Hepatogastroenterology 56 (94–95) (2009) 1287–1298.
[64] R. Niu, H. Jing, Z. Chen, J. Xu, J. Dai, Z. Yan, Differentiating malignant colorectal tumor patients from benign colorectal tumor patients by assaying morning urinary arylsulfatase activity, Asia Pac. J. Clin. Oncol. 8 (4) (2012) 362–367.
[65] P.D. Hardt, S. Mazurek, M. Toepler, P. Schlierbach, R.G. Bretzel, E. Eigenbrodt, H.U. Kloer, Faecal tumour M2 pyruvate kinase: a new, sensitive screening tool for colorectal cancer, Br. J. Cancer 91 (5) (2004) 980–984.
[66] Y.M. Shastri, M. Naumann, G.M. Oremek, E. Hanisch, W. Rosch, J. Mossner, W.F. Caspary, J.M. Stein, Prospective multicenter evaluation of fecal tumor pyruvate kinase type M2 (M2-PK) as a screening biomarker for colorectal neoplasia, Int. J. Cancer 119 (11) (2006) 2651–2656.
[67] Y.M. Shastri, S. Loitsch, N. Hoepffner, N. Povse, E. Hanisch, W. Rosch, J. Mossner, J.M. Stein, Comparison of an established simple office-based immunological FOBT with fecal tumor pyruvate kinase type M2 (M2-PK) for colorectal cancer screening: prospective multicenter study, Am. J. Gastroenterol. 103 (6) (2008) 1496–1504.
[68] H.K. Dabbous, Y.A.E. Mohamed, R.F. El-Folly, M.D. El-Talkawy, H.E. Seddik, D. Johar, M.A. Sarhan, Evaluation of Fecal M2PK as a diagnostic marker in colorectal cancer, J. Gastrointest. Cancer 50 (3) (2019) 442–450.
[69] V. Quemener, Y. Blanchard, D. Lescoat, R. Havouis, J.P. Moulinoux, Depletion in nuclear spermine during human spermatogenesis, a natural process of cell differentiation, Am. J. Phys. 263 (2 Pt 1) (1992) C343–C347.
[70] T. Thomas, T.J. Thomas, Polyamines in cell growth and cell death: molecular mechanisms and therapeutic applications, Cell. Mol. Life Sci. 58 (2) (2001) 244–258.
[71] K. Hiramatsu, K. Takahashi, T. Yamaguchi, H. Matsumoto, H. Miyamoto, S. Tanaka, C. Tanaka, Y. Tamamori, M. Imajo, M. Kawaguchi, M. Toi, T. Mori, M. Kawakita, N(1),N(12)-Diacetylspermine as a sensitive and specific novel marker for early- and late-stage colorectal and breast cancers, Clin. Cancer Res. 11 (8) (2005) 2986–2990.
[72] Y. Umemori, Y. Ohe, K. Kuribayashi, N. Tsuji, T. Nishidate, H. Kameshima, K. Hirata, N. Watanabe, Evaluating the utility of N1,N12-diacetylspermine and N1,N8-diacetylspermidine in urine as tumor markers for breast and colorectal cancers, Clin. Chim. Acta 411 (23–24) (2010) 1894–1899.
[73] G. Kuwata, K. Hiramatsu, K. Samejima, K. Iwasaki, K. Takahashi, K. Koizumi, S. Horiguchi, S.S. Moriya, M. Kobayashi, M. Kawakita, Increase of N1, N12-diacetylspermine in tissues from colorectal cancer and its liver metastasis, J. Cancer Res. Clin. Oncol. 139 (6) (2013) 925–932.
[74] M. Linsalata, R. Giannini, M. Notarnicola, A. Cavallini, Peroxisome proliferator-activated receptor gamma and spermidine/spermine N1-acetyltransferase gene expressions are significantly correlated in human colorectal cancer, BMC Cancer 6 (2006) 191.
[75] H. Sun, B. Liu, W. Wang, G.S. Jiang, W. Li, Y.P. Yang, C.X. Xu, Y.F. Yan, X.X. Liu, Adenovirus-mediated expression of spermidine/spermine N1-acetyltransferase gene induces S-phase arrest in human colorectal cancer cells, Oncol. Rep. 20 (5) (2008) 1229–1235.