Clinica Chimica Acta 428 (2014) 57–62

Contents lists available at ScienceDirect

Clinica Chimica Acta

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

Urinary cytidine as an adjunct biomarker to improve the diagnostic ratio for gastric cancer in Taiwanese patients

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ARTICLE INFO

Article history:
Received 17 June 2013
Received in revised form 9 October 2013
Accepted 9 October 2013
Available online 29 October 2013

Keywords:
Cytidine
Gastric cancer
Urinary nucleoside
AFP
CA19-9

ABSTRACT

Background: Gastric cancer is a major public health concern as the fourth most common cancer, and it is of particular relevance as the second most common cause of cancer death worldwide. We caparisoned the urinary nucleoside concentrations between the gastric patients and healthy volunteers that try to evaluate the diagnostic value in the gastric cancer.
Method: Urinary nucleosides from 49 gastric patients and 40 healthy volunteers were evaluated by high-performance liquid chromatography/electrospray ionization–tandem mass spectrometry (HPLC/ESI–MS/MS) under optimized conditions as determined in our previous study.
Results: The mean concentrations of 5 urinary nucleosides, cytidine, 3-methylcytidine (m3C), 1-methyladenosine (m1A), adenosine, and inosine, were found to be elevated in cancer patients, but only cytidine showed a significant elevation. Moreover, cytidine concentrations were significantly elevated by an average of 1.42-fold in patients with late stage (S3 + 4) disease. Combining the determined concentrations of preoperative serum alpha-fetoprotein (AFP, cutoff of 20 μg/l) or carbohydrate antigen 19-9 (CA19-9, cutoff of 37 U/ml) with the mean urinary cytidine concentration was shown to improve the diagnostic ratio (sensitivity) for gastric cancer from 16.3% (8/49 patients) to 38.8% (8 + 11/49 patients) or from 28.6% (14/49 patients) to 51.0% (14 + 11/49 patients), respectively.
Conclusions: Urinary cytidine may be an important adjunct biomarker for gastric cancer.
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1. Introduction

Gastric cancer is a major public health concern as the fourth most common cancer, and it is of particular relevance as the second most common cause of cancer death worldwide [1]. The highest incidence rates are found in East Asia, East Europe, and South America [2]. Unfortunately, most gastric cancer patients are diagnosed at an advanced stage of the disease at which point tumor resection may not be an option. A lot of patients with advanced or recurrent gastric cancer, it is clear that the discovery of biomarkers and their application to traditional diagnostic methods would be of value to prevention and treatment strategies.

Over the last decade, systems biology has developed into a new research platform, which currently occupies a prominent position in biomedical research. Other branches of systems biology, for example, transcriptomics, proteomics, and metabolomics have gained prominence as discovery tools since the completion of the genome sequencing project. The power of metabolomics has been applied to toxicological studies [3], the diagnosis of inborn metabolic errors [4], and to biomedicine for the diagnosis of amyotrophic lateral sclerosis [5]. Moreover, metabolomics is becoming an increasingly

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0009-8981/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.cca.2013.10.008

important tool for cancer diagnosis [6] and for prediction of cancer progression in response to particular therapeutic approach [7].

Nucleosides are the primary constituents of ribonucleic acids (RNAs). When RNAs are biotransformed, normal nucleosides can either be metabolized or reutilized to synthesize nucleic acids. However, in particular cases, some RNAs are transformed to modified nucleosides, which can neither be further degraded nor reutilized. These nucleosides are excreted intact in urine as end products because of a lack of specific phosphorylases [8]. Modified nucleosides are regarded as indicators of the whole-body turnover of RNA. In cancer, which are characterized by unregulated cell proliferation, RNA metabolism increases dramatically and higher concentrations of urinary excreted modified nucleosides are observed. Consequently, the urinary concentrations of modified nucleosides can reflect RNA degradation in the organism; thus, they can be used as potential cancer biomarkers [9–11]. Nevertheless, to date, no specific pattern has been discovered. The search for specific biomarkers for specific cancers is crucial for early cancer diagnosis. In many cases, efficient separation and detection techniques are required to assess the concentrations of these biomarkers.

Although the concentrations of these compounds have been studied over a number of decades, it is only recently that mass spectrometric means have been employed for the diagnosis of diseases [12]. During the last decade, a number of analytical methods for measuring and monitoring nucleosides in biological fluid have been reported. Some of these methods include the following: enzyme-linked immunoassay [13], capillary electrophoresis (CE) [14], cathodic stripping [15], voltammetry, gas chromatography–mass spectrometry (GC–MS) [16–18], and high-performance liquid chromatography–mass spectrometry (HPLC–MS) [17,19,20]. Although the sensitivity and specificity of these methods are high, they involve complex preparation processes for extraction, hydrolysis, and derivatization. Thus, to simplify the process, liquid chromatography–tandem mass spectrometry (LC–MS/MS) was developed as a method of directly determining urinary nucleosides. The co-eluted nucleosides from HPLC were detected by MS, and the selective reaction monitoring (SRM) mode used in this study improved the specificity and sensitivity for quantitation.

In this study, we used HPLC/electrospray ionization–MS/MS (HPLC/ESI–MS/MS) to detect the concentrations of 5 urinary nucleosides [cytidine, 3-methylcytidine (m3C), 1-methyladenosine (m1A), adenosine, and inosine] in urine samples from patients with gastric cancer and from healthy control subjects. The variable urinary nucleoside concentrations were determined and evaluated for diagnosis of primary gastric cancer.

2. Materials and methods

2.1. Patient details

From January to December 2008, 49 patients with primary gastric cancer, which had been treated by resection at the Department of Surgery, China Medical University Hospital, were evaluated in this study. None of the patients had undergone treatment with medication or radiotherapy prior to this study. The control group (40 healthy volunteers) had undergone a routine annual health examination and was recruited from our Health Examination Center. Patients and healthy volunteers were asked to provide single, early-morning urine samples (preoperative samples). The samples were immediately sent to the laboratory and stored at −80 °C until analysis. Use of the urine samples for research purposes complied with the regulations set by the Institutional Review Board (DMR-IRB 97-029). The study was approved by the Ethical Committee of the China Medical University Hospital. The main characteristics of the cancer and control groups are reported in Supplemental data 1. There were no significant differences in body weight, body mass index (BMI), smoking, age, or sex between the patients and control subjects.

2.2. Urine samples and purification

Each urine sample was acidified by the addition of 2 mol/l HCl (adjusted to 0.01 mol/l HCl). The acidified urine was centrifuged, one milliliter of the supernatant was added to 100 μl of the internal standard (ISTD; 2 μg/ml tubercidin), and purified using an Oasis® MCX column (Waters) that had been conditioned and equilibrated with 1 ml methanol and water. The sample was directly loaded onto the MCX columns, washed with H_2O (0.1% formic acid in H_2O), eluted with 2.8% NH_4OH in methanol, dried under a nitrogen stream, and dissolved in the mobile phase (100 μl).

2.3. Chemicals

The nucleosides under analysis in this study were obtained from Sigma-Aldrich (St. Louis, MO, USA): adenosine, cytidine, inosine, m3C, m1A, and tubercidin. Each nucleoside stock solution was prepared at a concentration of 100–1000 μg/l in a mixture of methanol and H_2O according to solubility. Standard solutions of these five nucleosides were prepared as a mixed solution for the calibration, and the ISTD solution was prepared at a concentration of 2 μg/l. All stock solutions were stored in the dark at −20 °C until required. Water from a Milli-Q water system (Millipore) was used.

2.4. Nucleoside determination

Chromatography was performed using a Finnigan™ Surveyor™ HPLC system. HPLC analysis was performed on an Atlantis® dC18 column (2.1 × 100 mm, 3 μm) (Waters). The mobile phases used were (A) 2 mmol/l ammonium acetate (pH 5.0) in H_2O and (B) 2 mmol/l ammonium acetate in 50% MeOH at a flow rate of 0.2 ml/min. The gradient conditions were as follows: isocratic elution (95% A) for 5 min, followed by a 2-min gradient to 20% B, then a 3-min gradient to 30% B, and a final 10-min gradient to 40% B. A Finnigan LCQ DECA XP^{PLUS} quadrupole ion trap mass spectrometer (Thermo Finnigan) equipped with an electrospray ionization source was used. The mass spectrometer was operated in the positive ion mode by applying a voltage of 3.5 K to the ESI needle. The temperature of the heated capillary in the ESI source was set at 295 °C. The flow rate of the sheath gas (nitrogen) was set at 30 (arbitrary units). Selected-reaction mode (SRM) was used during the quantification experiment: the protonated ion was chosen as the precursor ion and isolated in the ion trap. The collision energy, represented as a percentage of a maximum possible energy sufficient to fragment the precursor ion, was used to produce product ion spectra (Table 1). We used SRM transitions for the individual quantification. The oven program and analyses were performed using the software package Xcalibur (Finnigan).

2.5. Creatinine analysis

Urinary creatinine concentration was determined by the SYNCHRON LX System (SYNCHRON LX®, Beckman Coulter Creatinine) from the urine sample combined with the reagent to produce a red complex. Absorbance readings were taken at 520 nm. The absorbance has been shown to be a direct measure of the concentration in urine samples.

2.6. Quantification of urinary nucleosides

To compensate for variations in urine concentration, all nucleoside concentrations were indexed against creatinine and expressed as μmol nucleoside/mmol creatinine [7,21]. Urinary creatinine concentrations were determined by a modified Jaffe method and according our previous study [22,29].

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