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Evaluation of urinary nucleosides in breast cancer patients before and after tumor removal

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Abstract

Objective: Evaluation of altered urinary nucleosides before and after tumor removal of breast cancer (BCa).

Design and methods: Targeted metabolite profiling of 14 urinary nucleosides was conducted with both pre- and post-operative female patients with BCa ($n=150$, age: 46.6±7.7 years), and female controls ($n=150$, age: 46.8±7.7 years) by liquid chromatography-tandem mass spectrometry coupled to on-line extraction.

Results: Levels of modified nucleosides (5-hydroxymethyl-2′-deoxyuridine, $P<0.001$; 8-hydroxy-2′-deoxyguanosine, $P<0.001$; 1-methyladenosine, $P<0.02$; N^2,N^2-dimethylguanosine, $P<0.001$) were significantly higher in pre-operative patients than in both normal controls and post-operative patients.

Conclusions: This approach could be used to further understand the pathogenesis of BCa as well as to evaluate the effects of medical treatment.

Keywords: Nucleosides; Breast cancer; DNA damage; Liquid chromatography-mass spectrometry

Background

Nucleosides are modified by reactive oxygen species (ROS) derived oxidative DNA damage [1] and by post-transcriptional processes related to RNA turnover [2]. ROS are generated continuously as a consequence of normal cellular metabolic reactions and by external environment factors and ultraviolet radiation. As a result of oxidative DNA damage, modified nucleosides are generated by DNA repair. The modified nucleosides generated are then circulated in blood and excreted in urine. Therefore, levels of modified nucleosides in urine reflect oxidative DNA damage [1] and RNA turnover [3].

Alteration of modified nucleosides in cancer is clinically important to reveal oxidative DNA damage [1] and RNA turnover [3,4]. In general, the levels of urinary modified nucleosides were elevated in breast cancer (BCa) [4]. However, previous studies have evaluated urinary nucleosides generated from source of either oxidative DNA damage or RNA turnover. In this study, the level of nucleosides generated from these two major sources was therefore quantified simultaneously for evaluation in BCa patients before and after tumor removal.

Due to overestimation of urinary nucleosides, immunoassay approaches are not sufficient in quantitative analysis [5]. The chromatographic separation combined with mass spectrometry (MS) have been therefore widely used, but it requires a sample preparation step such as extraction or chemical derivatization [6] or could not detect 8-hydroxy-2′-deoxyguanosine [7]. Here, the targeted metabolite profiling based on on-line extraction and MS detection, which was verified in nucleoside analysis [8], was introduced to evaluate the urinary levels of nucleosides in BCa patients before and after surgery against those of normal controls.

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Materials and methods

Chemicals

Fourteen nucleosides (pseudouridine, cytidine, uridine, 1-methyladenosine, 5-methylcytidine, 5-methyl-2′-deoxycytidine, 5-hydroxymethyl-2′-deoxyuridine, guanosine, 3-methyluridine, 2′-deoxyguanosine, 8-hydroxy-2′-deoxyguanosine, adenosine, N^2,N^2-dimethylguanosine, 5-deoxyadenosine) and an internal standard (ISTD; 1-hydroxy-isoguanine) were purchased from Sigma (St. Louis, MO, USA). Each stock solution of nucleosides was prepared at a concentration of 10 μmol/mL with 5 mM ammonium acetate and working solutions were diluted with water at varied concentrations in the range of 0.2–100 nmol/mL. The ISTD was prepared at a concentration of 10 μmol/mL with 5 mM ammonium acetate. All standard solutions were stored at −20 °C until being used. Other chemical reagents and solvents used as the analytical and HPLC grade were purchased from Sigma and Burdic & Jackson (Muskegan, MI, USA). Deionized water was prepared by a Milli-Q water-purification system (Millipore; Milford, MA, USA).

Subjects and sample-collection

Urine samples were collected from female BCa patients (n=150, age: 46.6±7.7 years) and healthy subjects as normal controls (n=150, age: 46.8±7.7 years) at the Samsung and Hanyang University Medical Centers (Seoul, Korea). All subjects underwent the same diagnostic procedures, i.e., a breast physical examination, mammography, and ultrasonography as detailed by the American Joint Committee on Cancer staging. The BCa patients underwent surgery of the modified radical mastectomy (MRM) or lumpectomy with an auxiliary lymph node dissection. Pre- and postoperative samples were collected just before or 2-weeks after surgery. The sex- and age-matched normal controls had no evidence of benign or malignant breast disease. All urine samples were collected in the first morning after a 12 hour fast. The subjects were not treated with, or exposed to, any drugs or chemicals for a defined period, before the urine was collected. Urinary creatinine values were determined using the Jaffé method.

On-line extraction

Urine samples were prepared based on our previous report [8]. Briefly, a 5 μL aliquot of ISTD was added to 50 μL of filtered urine through a PVDF filter (Millipore, pore size 0.2 μm), transferred to a vial. Each aliquot of samples was then injected directly into the column-switching LC-MS/MS system. Column-switching LC was performed using a Shiseido Nanospace SI-2 HPLC system model 3001 (Shiseido Co., Tokyo) equipped with an auto injector (200 μL loop; model 3023) and a dual six-way switching-valve unit (model 3012). Both pre- and analytical columns were connected via a six-way switching valve. The pre-column used was a Shodex MS Pak PK-2A (n-vinylacetamide copolymer, 2.0 mm i.d. ×10 mm length, Showa Denko K. K., Tokyo) and the analytical column was a Capcell Pak C_{18}, AQ (1.5 mm i.d. ×150 mm length, 5 μm particle size, Shiseido Fine Chemical Co., Tokyo). An isocratic eluent consisting of 5 mM ammonium acetate (pH 7.5: eluant A) was used to introduce nucleosides to the pre-column at 500 μL/min. This configuration allowed the proteins and polar interference on the pre-column to be eluted first. 10 minutes after injection of sample, the switching valve was turned to bring the two columns in-line. Separation was achieved using a gradient eluent, which was started at 20% B (pH 4, 5 mM ammonium acetate in 50% methanol) at 100 μL/min and increased to

Table 1
Concentrations of urinary nucleosides for pre- (Pre-OP., BC) and post- (Post-OP., BC) operative breast cancer patients and for normal controls (NC)

Nucleosides	Concentration [a] (mean±SD)			*P*-value		
	Pre-OP., BC (n=150)	Post-OP., BC (n=150)	NC (n=150)	Pre-OP., BC : post-OP., BC	Pre-OP., BC : NC	Post-OP., BC : NC
Cytidine	0.13±0.13	0.11±0.09	0.12±0.08	NS [b]	NS	NS
Uridine	0.20±0.10	0.17±0.11	0.18±0.11	NS	NS	NS
Adenosine	0.27±0.37	0.23±0.19	0.21±0.30	NS	NS	NS
Guanosine	0.10±0.13	0.09±0.06	0.07±0.04	NS	NS	NS
Pseudouridine	11.14±7.02	10.36±6.41	13.72±7.53	NS	NS	NS
1-Methyladenosine	2.83±1.59	2.44±1.68	1.66±0.98	<0.02	<0.02	<0.02
5-Methylcytidine	10.50±5.17	9.22±6.39	8.53±5.94	NS	NS	NS
3-Methyluridine	0.22±0.24	0.26±0.17	0.21±0.15	NS	NS	NS
N^2,N^2-Dimethylguanosine	0.55±0.22	0.46±0.28	0.29±0.17	<0.001	<0.001	<0.001
2′-Deoxyguanosine	0.03±0.02	0.02±0.02	0.02±0.01	NS	NS	NS
5-Deoxyadenosine	0.07±0.11	0.05±0.04	0.06±0.06	NS	NS	NS
5-Methyl-2′-deoxycytidine	0.15±0.09	0.12±0.08	0.13±0.12	NS	NS	NS
5-Hydroxymethyl-2′-deoxyuridine	0.05±0.02	0.01±0.01	0.01±0.01	<0.001	<0.001	<0.001
8-Hydroxy-2-deoxyguanosine	0.02±0.01	0.01±0.01	0.01±0.01	<0.001	<0.001	<0.001

[a] Concentration was expressed as nmol/μmol creatinine (mean±SD).
[b] NS, not significant.