Original Article

Predicting bone marrow suppression from urinal 8-hydroxy-2'-deoxyguanosine level during the treatment with radium-223 in patients with cancer bone metastasis

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Background: Cancer bone metastasis (BM) from castration-resistant prostate cancer (CRPC) is the terminal stage of cancer, and it demonstrates a decreasing quality of life (QOL) due to skeletal-related events such as pain and bone fracture. Radium-223 dichloride administration is frequently selected as an internal radionuclide target therapy. This radioactive molecule has a potency of accumulation in bone minerals and emits alpha particles by decaying radium-223. These physical properties cause cellular damage to bone metastatic CRPC cells. However, some poor outcomes of patients are occasionally observed, such as bone marrow suppression. Therefore, in order to understand the status of deep BM, it is necessary to discover biomarkers that effectively reflect bone metabolism. In this study, we investigated whether urinal 8-hydroxy-2'-deoxyguanosine (8-OHdG), a one of oxidative stress marker, could be a predictive biomarker to identify whether radium-223 administration causes bone marrow suppression in patients.

Methods: The urine and blood serum from four cancer patients with BM were collected and stored at –80 ℃ deep freezer until analysis. Following radiotherapeutic guidelines, three to six radium-223 internal radiotherapy doses were prescribed based on the patient, and it was terminated due to decreased therapeutic reserve.

Results: The patients who were administered six radium-223 doses demonstrated upregulation of urinal 8-OHdG, serum C-terminal telopeptide of type I collagen (1CTP), and type I collagen cross-linked N-telopeptide (NTX) concentrations. Conversely, serum bone alkaline phosphatase (BAP) was downregulated. The patients who were administered less than five radium-223 doses exhibited urinal 8-OHdG downregulation and similar serum 1CTP, NTX, and BAP levels compared to before administration. In the patient who had bone marrow suppression, a negative correlation between time after first administration of radium-223 and urinal 8-OHdG was observed.

Conclusions: These results suggest that a urinal 8-OHdG concentration has a potency of biomarker for bone marrow suppression under the administration of radium-223 in the patient with BM from CRPC.

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Introduction

Cancer bone metastasis (BM) from castration-resistant prostate cancer (CRPC) is a terminal stage of cancer and a prevalent cancer complication (1,2). Skeletal-related events, such as pain and bone fracture, frequently occur in these patients, decrease the quality of life (QOL), and increase the risk of death (3,4). These metastatic cancer cells are characterized by metastatic lesion growth mainly due to mutual information transmission between osteoblasts and osteoclasts, such as cytokines and metabolic factors, which induce pain, spinal cord compression, fractures, and other QOL deterioration. Drug therapy is used as BM management (5). Non-invasive radiotherapy is applied in elderly patients with low reserve capacity due to surgical limitations (6). The administration of radium-223 dichloride, which is an internal radionuclide therapy [radioimmunotherapy (RIT)], is selected as one of the treatment options (7). This drug was approved as the first RIT of an alpha-emitting molecule after a clinical trial, ALSYMPCA (8). This radioactive molecule has a potency of accumulation in BMs and emits alpha particles by decaying radium-223. These physical properties cause cellular damage to bone metastatic CRPC cells and result in life prolongation of patients. This treatment used various blood markers and image biomarkers obtained by scintigraphy as references to implement the treatment and monitor progress (9). However, some poor outcomes of patients are occasionally observed, such as bone marrow suppression, which causes RIT interruption. Therefore, in order to understand the status of deep BM, it is necessary to discover biomarkers that effectively reflect bone metabolism. Early identification of optimal patients for RIT with radium-223 would enable treatment optimization by selectively prescribing patients with a low risk of myelosuppression. Ionizing radiation (IR) is a factor that imparts external oxidative stress to cells, and bone marrow hematopoietic tissue, which is highly sensitive, is severely damaged (10,11). Urinal 8-hydroxy-2'-deoxyguanosine (8-OHdG), that is one of oxidative stress marker, responds to IR but is not analyzed in detail in this patient with RIT. Treatment effects and side effects can be predicted for patients in advance, if individual differences in RIT can be monitored using 8-OHdG, causing optimization. In the present study, urinal 8-OHdG concentration was investigated to determine its effectiveness as a biomarker of side effects (such as bone marrow suppression) by radium-223 internal radiotherapy. We present this article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-812/rc).

Highlight box

Key findings

- The concentration of urinal 8-hydroxy-2'-deoxyguanosine (8-OHdG) is a convenient biomarker that complements hotspot volume and existing markers of bone metastasis from castration-resistant prostate cancer (CRPC) during radium-223 internal radiotherapy.

What is known and what is new?

- The known things are the administration of radium-223 therapy has a potency of accumulation in bone marrow and it gives anti-tumor effect by emitting alpha particles. These physical properties cause cellular damage to bone metastatic CRPC cells and result in life prolongation of patients. In this treatment, some poor outcomes of patients are observed, such as bone marrow suppression, which causes this therapy interruption. Clear biomarkers remain unavailable.
- What's new in this study is that 8-OHdG in the urine of patients with bone metastases was quantified and compared to existing blood markers while receiving radium-223.

What is the implication, and what should change now?

- The findings of this study enable to complement existing blood biomarkers of bone metastases from CRPC during internal beam radiotherapy by quantifying 8-OHdG in urine, predicting side effects and treatment efficacy.

Methods

Study subject and ethical approval

Four patients with CRPC (76.5±3.1 years old) with BM were followed up after radium-223 administration at the

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Table 1 Patient characteristics

Pt. No.	TNM classification (Gleason score; age)	Chemotherapy before RT	External RT (adjuvant drugs)	Dose of radium-223 (adjuvant drugs)	Chemotherapy after RIT	Survival after diagnosis
1	T4N1M1c (GS3+4; 75 years old)	Degarelix acetate, denosumab, bicalutamide	64 Gy/32 fractions (adjuvant: degarelix acetate, denosumab, abiraterone acetate)	5 doses (adjuvant: degarelix acetate, denosumab, abiraterone acetate)	Degarelix acetate, denosumab, abiraterone acetate, DPZ therapy	1,333 days (44.4 months)
2	T4N1M1 (GS5+4; 81 years old)	Degarelix acetate, denosumab, bicalutamide, 6 doses of taxon	–	5 doses (adjuvant: degarelix acetate, denosumab, zoledronic acid hydrate)	Zoledronic acid hydrate, degarelix acetate, CBZ therapy (2 doses)	1,181 days (39.4 months)
3	cTxxN1M1b (GS4+5; 74 years old)	Bicalutamide, zoledronic acid hydrate, flutamide, abiraterone acetate, enzalutamide, G-CSF, paraplatin, docetaxel (7 doses), estramustine phosphate sodium hydrate, cabazitaxel (3 doses)	–	3 doses (adjuvant: ethinylestradiol, prednisone)	Concentrated PLT infusion (several doses)	1,138 days (37.9 months)
4	cT4N1M1b (unknown; 76 years old)	Degarelix acetate, denosumab, bicalutamide, docetaxel (1 dose), G-CSF	60 Gy/30 fractions [adjuvant: docetaxel (2 doses), degarelix acetate, denosumab, G-CSF]	6 doses (adjuvant: degarelix acetate, denosumab, ethinylestradiol, enzalutamide)	Degarelix, acetate, denosumab, enzalutamide	Alive until December 2022 (>1,300 days)

CBZ, cabazitaxel; DPZ, docetaxel + prednisolone + zoledronic acid; G-CSF, granulocyte colony stimulating factor; GS, Gleason score; No., number; PLT, platelet; Pt., patient; RIT, radioisotope therapy; RT, radiotherapy; TNM, tumor-node-metastasis.

Mutsu General Hospital (Aomori, Japan) from October 2016 to December 2022. *Table 1* summarizes each patient's characteristics. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Committee of Medical Ethics of the Hirosaki University Graduate School of Health Sciences (No. 2016-051). Following a detailed verbal explanation regarding the content of this study, a written informed consent was obtained. All patients and their families agreed for publication as scientific research. Serum and urine samples were stored in the deep freezer at Mutsu General Hospital until analysis, and were transported to Hirosaki University for analysis after 2017 and continued until 2020. The description was included consent of examination images. Before RIT, the focused patients were administered external radiotherapy to the region of prostate primary cancer (photon beam, 50 Gy per fraction, 25 fractions). Three to six RIT doses were prescribed based on the patient, and RIT was terminated due to decreased therapeutic reserve (*Figure 1*).

Analysis of BM

Follow-up of BM was identified by hotspot analysis using ^{99m}Tc-HMDP (Nihon Medi-Physics Co., Ltd., Tokyo, Japan) and bone scan index (BSI).

Follow-up by a blood test: the concentration of bone metabolite markers [bone alkaline phosphatase (BAP), C-terminal telopeptide of type I collagen (1CTP), and type I collagen cross-linked N-telopeptide (NTX)] were quantified to observe the patient's condition during treatment using BioMajesty JCA-BM6070 (JEOL Ltd., Tokyo, Japan). Peripheral blood (PB) was collected directly into separation tubes for serum (BD Biosciences, Franklin Lakes, NJ, USA). The serum was separated and stored at –80 ℃.

Analysis of urinal 8-OHdG in patients with BM

We collected urine samples immediately after storage at –80 ℃. We used 0.5-L thermos flasks (Primus AB, Stockholm, Sweden) to maintain the sample temperature. The 8-OHdG in urine was analyzed using an analyzer of

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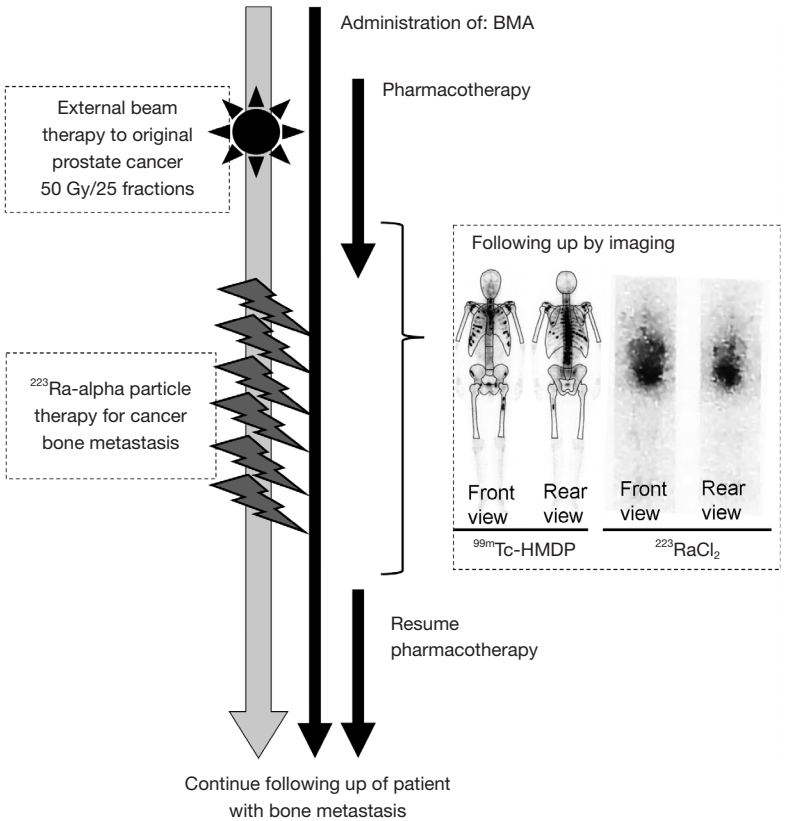

Figure 1 Treatment flow of this study focused on radium-223 administration. Before radium-223 administration, external beam therapy to original prostate cancer was performed using a method of volumetric modulated arc therapy (50 Gy/25 fractions). The following up for patients was used scintigraphy imaging (^{99m}Tc-HMDP and $^{223}RaCl_2$) and blood collections. BMA, bone modifying agent.

Immunochromatography (ICR001; Selista Inc., Tokyo, Japan), and Jaffe's methods were used for 8-OHdG and creatinine (CRE) measurements, respectively. A urine sample of 100 µL and pure water of 100 µL were mixed in a vial tube according to the analyzer protocol and then measured. The quantitated 8-OHdG in urine was corrected by the concentration of urinary CRE, and the value was expressed as the corrected value (ng/mg CRE). The 8-OHdG and CRE detection limits were estimated to be 1.0 ng/mL and 10 mg/dL, respectively.

Cell line

The mice osteoblastic cell line MC3T3-E1 cell was prepared from RIKEN BioResource Center. This cell was maintained in the medium of RPMI-1640 (Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% heat-inactivated fetal bovine serum (Japan-Bioserum, Co., Ltd., Fukuyama, Japan) and 1% antibiotics (penicillin and streptomycin; Thermo Fisher Scientific, Inc.) in a humidified atmosphere at 37 ℃ and 5% CO_2/95% air.

Experiment of radiation exposure

Radiation exposure for *in vitro* experiments was used X-ray generator (MBR-1520R-3; Hitachi Power Solutions Co., Ltd., Hitachi, Japan), X-irradiation (150 kVp, 20 mA with 0.5-mm aluminum and 0.3-mm copper filters) to culture cells was performed with a distance of 45 cm between the focus and target (1 Gy per minutes). The dose was monitored with a thimble ionization chamber placed next to the sample during irradiation.

Quantitation of 8-OHdG in cell culture

The quantitation of 8-OHdG secreted from X-irradiated MC3T3-E1 cell (1 to 8 Gy) was performed using 8-OHdG enzyme-linked immunosorbent assay (ELISA) quantitation

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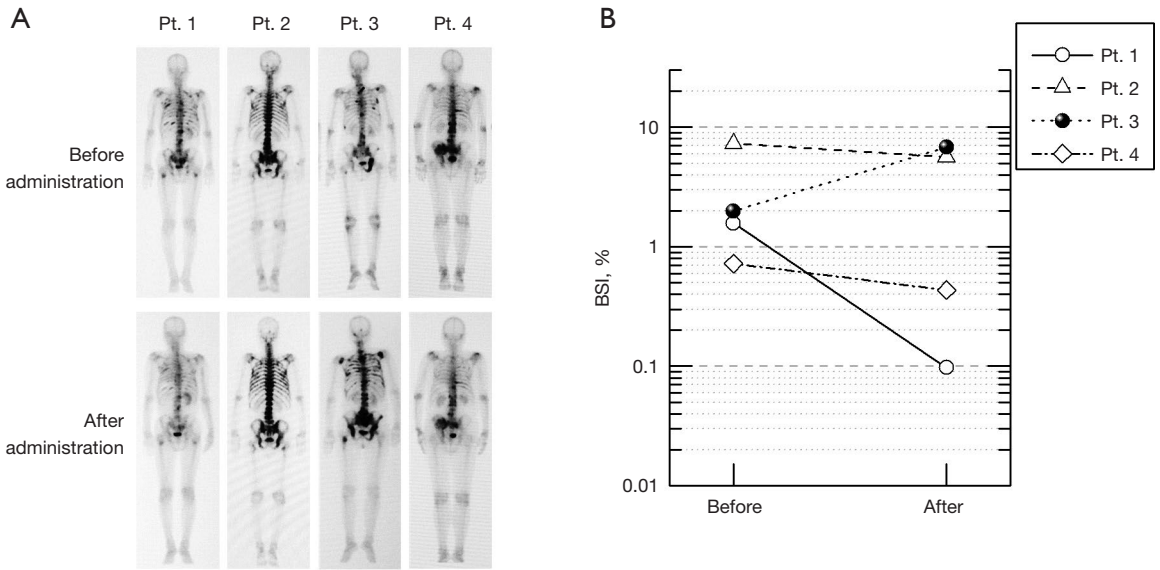

Figure 2 Clinical estimation of cancer BM using bone scintigraphy in patients with CRPC. The four patients who received radium-223 were analyzed by (A) ^{99m}Tc-HMDP scintigraphy and (B) BSI calculated by the software "BONENAVI". BM, bone metastasis; BSI, bone scan index; CRPC, castration-resistant prostate cancer; Pt., patient.

kits (JaICA, Fukuroi, Shizuoka, Japan). The cell culture supernatant was collected and stored –80 ℃ until ELISA analysis. Each sample was filtered through an ultrafiltration membrane in order to remove high-molecular-weight proteins, which interfered with the analysis (molecular weight cut-off, 10,000; Merck, Darmstadt, Germany). The obtained filtrate was concentrated by a SpeedVac® centrifugal evaporator (Savant SPD1010; Thermo Fisher Scientific, Inc.).

Analysis of gamma-H2AX expression

MC3T3-E1 cells exposed to X-irradiation (post 2 h) were fixed in ice-cold 75% ethanol and stored at –20 ℃ for 8 h. The fixed cells were washed with phosphate buffered saline containing 1% bovine serum albumin (PBS-BSA; Sigma Inc., St. Louis, MO, USA) and treated with PBS-BSA containing 0.2% Triton-X 100 on ice for 5 min. After washing, the cell pellets were incubated with anti-gamma-H2AX Ab [mouse, monoclonal immunoglobulin G1 (IgG1); Upstate, Temecula, CA, USA] in PBS-BSA containing 0.2% Triton-X (Thermo Fisher Scientific, Inc.) at room temperature for 1 h. After washing, the cells were treated with a donkey anti-mouse IgG-fluorescein isothiocyanate (FITC) (Santa Cruz Biotechnology Inc., Dallas, TX, USA) in PBS-BSA containing 0.2% Triton X-100 for a half hour in the dark. Thereafter, the stained cells were analyzed using fluorescence plate reader (TriStar 3; Berthold Technologies, Bad Wildbad, Germany).

Statistical analysis

Statistical analysis was performed using the Origin software package (Pro version 9.0; OriginLab Corporation, Northampton, MA, USA) for Windows. Each serum marker data (BAP, 1CTP, NTX, and 8-OHdG) were compared to day 0. The correlation between the ratio of 8-OHdG and gamma-H2AX expression was estimated by Pearson's correlation coefficient. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Analysis of patients with BM

Scintigraphy images and BSI were analyzed to estimate four patients with distant multiple bone metastases (*Figure 2*). These patients demonstrated 0.68–7.31% BSI before RIT, and hot spots centered on the spine were confirmed in all cases. The BSI of 3 patients (75%) was decreased after RIT treatment, indicating the effectiveness of RIT. These patients were also administered hormone

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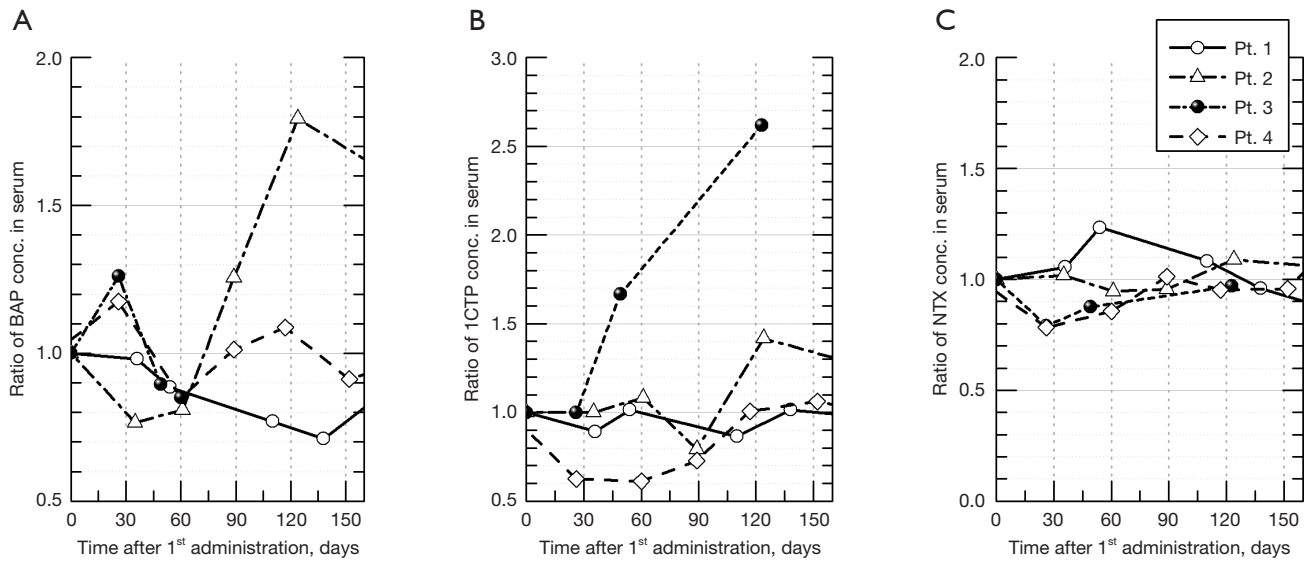

Figure 3 The alterations of bone-related markers in serum. (A) BAP, (B) 1CTP, and (C) NTX were analyzed during radium-223 treatment. Each marker was shown as a ratio of day 0. 1CTP, C-terminal telopeptide of type I collagen; conc., concentration; BAP, bone alkaline phosphatase; NTX, type I collagen cross-linked N-telopeptide; Pt., patient.

therapy [degarelix acetate, ethinylestradiol, abiraterone acetate, and enzalutamide in three, two, one, and one case(s), respectively] and bone-modifying agent treatment (denosumab in three cases and zoledronic acid hydrate in one case) (*Table 1*). The number of RIT doses ranged from 3 to 6 times, and only one patient completed the maximum number at six doses, two patients completed five doses, and one patient completed three doses. The side effects observed during treatment included anemia [patient (Pt.) 1 and Pt. 3], herpes zoster (Pt. 2), fatigue (Pt. 3) and thrombocytopenia (Pt. 3), respectively. A concentrated platelet (PLT) infusion was performed on Pt. 3 that caused severe thrombocytopenia.

Analysis of blood biomarkers

Serum BAP in each patient remained high from day 90 on Pt. 2, whereas it remained at the same level as on day 0 in other patients (*Figure 3A*). An increase in serum 1CTP was observed in Pt. 3 over time (*Figure 3B*). Additionally, serum NTX was at the same level as baseline on day 0 in all patients (*Figure 3C*).

Quantification of urinal 8-OHdG in the patients with BM

The urinary 8-OHdG was analyzed for four patients with bone metastases who were administrated radium-223. The concentration of the urinary component was corrected by the CRE concentration. The pre-treatment (day 0) 8-OHdG concentrations in four patients were different (Pt. 1: 13.1 ng/mL; Pt. 2: 38.6 ng/mL; Pt. 3: 45.4 ng/mL; Pt. 4: 25.2 ng/mL). The similar ratio level with day 0 in Pt. 1 (range, 0.68–1.12 folds) and 4 (range, 0.78–1.06 folds) were observed; however, the level decreased from day 30 in Pt. 2 (range, 0.39–1.00 folds) and 3 (range, 0.52–1.00 folds) was observed (*Figure 4*). In the difference of urinal 8-OHdG concentration (ng/mg CRE) between each period, a large decrease was observed on day 55 of Pt. 3, and a large increase on day 55 of Pt. 4 (*Table 2*). In addition, the correlation of white blood cell (WBC), red blood cell (RBC), and PLT was compared with 8-OHdG concentration (*Figure 5* and *Table 3*). In variance of 8-OHdG, a negative correlation in Pt. 1–3 was observed (less than –0.57). On the other hand, the weak of negative correlation in 8-OHdG of Pt. 4 was observed. In the WBC and PLT, a negative correlation in all patients was observed. On the other hand, RBC fluctuations that did not change significantly were observed.

The relationship between 8-OHdG and gamma-H2AX expression

To clarify the relationship between increased 8-OHdG

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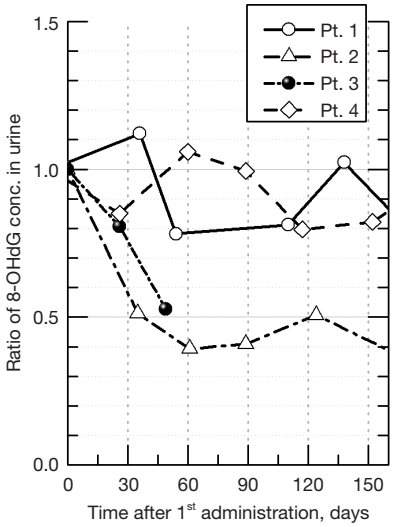

Figure 4 Urinal 8-OHdG concentration. The patient's urine was collected during the therapy and analyzed as a trial biomarker of side effects. 8-OHdG, 8-hydroxy-2'-deoxyguanosine; conc., concentration; Pt., patient.

Table 2 The difference of urinal 8-OHdG concentration (ng/mg CRE) between each period

Periods	Pt. 1	Pt. 2	Pt. 3	Pt. 4
A (Δd30)	1.59	−18.87	−4.73	−11.33
B (Δd55)	−4.47	−4.58	−16.73	12.82
C (Δd100)	0.40	0.64	–	−1.65
D (Δd125)	2.78	3.78	–	−4.96
E (Δd170)	−4.53	−9.08	–	0.64

The difference was defined period A [d26–30 (d30) *vs.* baseline (d0)], B [d49–61 (d55) *vs.* d30], C [d89–110 (d100) *vs.* d55], D [d117–138 (d125) *vs.* d100], and E [d152–195 (d170) *vs.* d125]. Δ, differential of a function; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; CRE, creatinine; d, day; Pt., patient.

concentrations and increased gamma-H2AX expression that mean DNA damage/repair, it was validated by cell culture using the MC3T3-E1 cell exposed to higher dose rate of IR *in vitro* model. In the expression of gamma-H2AX, exposure of 1 to 8 Gy showed a significant up-regulation of 1.24±0.15 to 9.33±1.83 folds compared to 0 Gy (*Figure 6A*). In addition, 8-OHdG into cell culture supernatant secreted from the cell exposed to 1 to 8 Gy also showed a significant up-regulation of 1.10±0.11 to 13.00±0.56 folds compared to 0 Gy. A significant positive correlation was shown between gamma-H2AX and 8-OHdG (R^2=0.86) (*Figure 6B*).

Discussion

We focused on RIT with radium-223 and investigated whether blood sampling data and changes in the urinary oxidative stress marker 8-OHdG of patients with CRPC with bone metastases could predict its treatment efficacy and side effects. It was suggested that this object of the study could predict bone marrow suppression and the associated weakened immune system. All of the target patients received hormone/chemotherapy concomitantly, but patients who received radium-223 5–6 times (Pt. 1, 2, and 4) also received bone-modifying drugs. Pt. 3, who discontinued administration after three doses, received no bone-modifying drugs concomitantly. McKay *et al.* revealed that the proportion of patients who received radium-223 5–6 times appeared higher in the group in which radium-223 was combined with hormone therapy using the antiandrogens abiraterone acetate or enzalutamide than in the group without combination therapy. However, the concomitant use of bone-modifying drugs is not associated with the number of radium-223 administrations (12). In four cases of our study, they were administered the bone-modifying drugs (*Table 1*), such as abiraterone acetate, enzalutamide, and zoledronic acid hydrate. In general, the treatment guideline for BM in each country, such as the United States and Japan, recommends administering this drug to reduce skeletal-related events (13,14). In the comparison analysis, the authors did not consider it to be biased as in previous reports. Additionally, complex patient characteristics, such as disease stage and BSI, are likely to affect the number of radium-223 doses. The analysis of bone-related markers revealed that BAP and NTX demonstrated no adverse events; however, 1CTP in Pt. 3, which exhibited upregulation, became a bone marrow suppression marker (*Figure 3*). The phase II study of RIT by radium-223 reported that bone metabolism markers, including BAP and BSI, decreased from baseline (15), but this study revealed that 1CTP and NTX increased after 5–6 radium-223 doses compared with day 0. Nakashima *et al.* reported that 1-CTP tended to increase, regardless of radium-223 administration in CRPC with BM (16). In addition, Li *et al.* reported that NTX combining with other markers can become a feasible biomarker for the diagnosis and prognosis prediction for BM in Asian people (17). Similar to these reports, it has been suggested that increased alpha irradiation with frequent administration of radium-223 upregulates 1CTP and NTX markers in serum, however BAP has individual variance.

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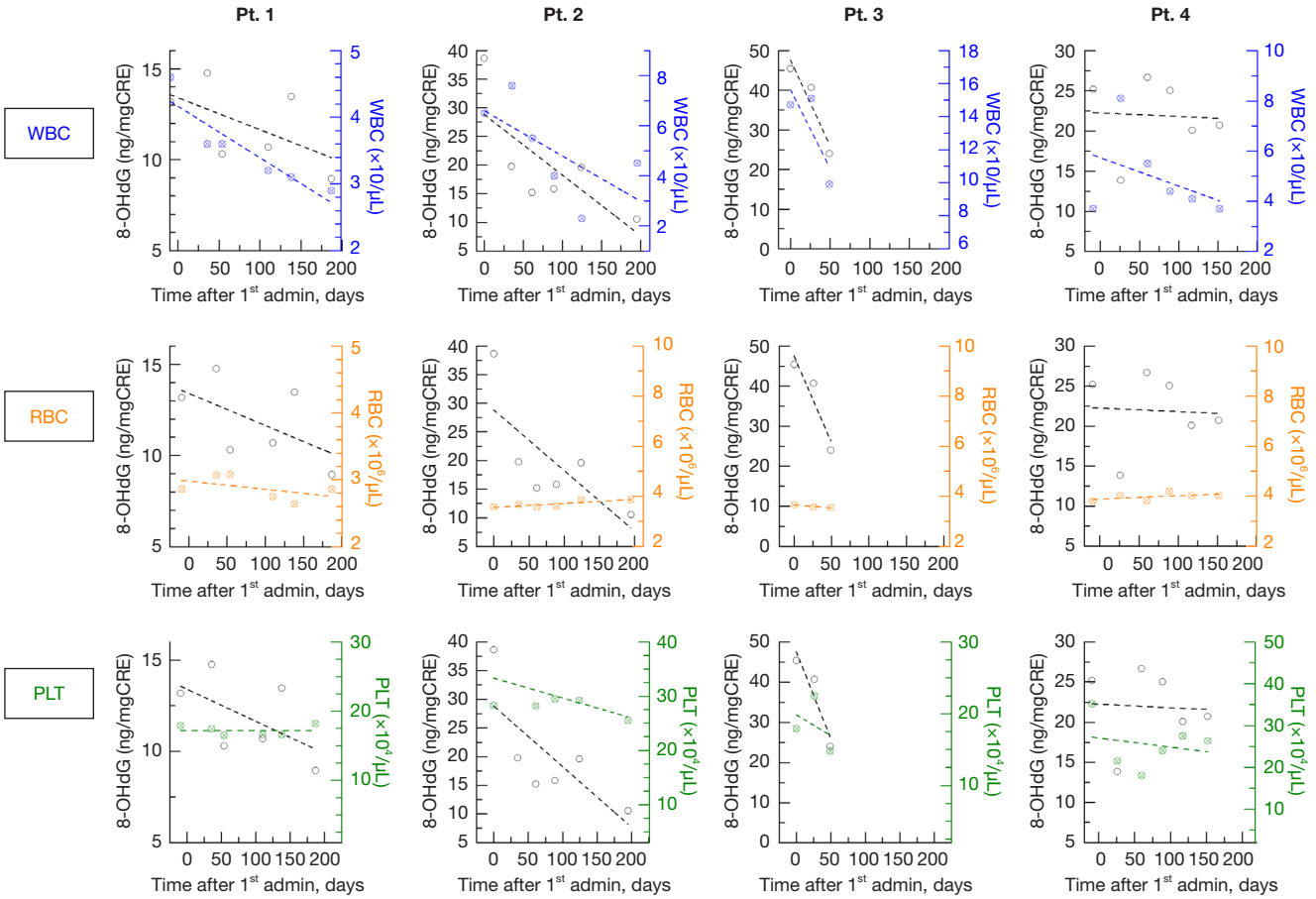

Figure 5 Relationship between 8-OHdG and hemocyte markers. The number of WBC, RBC, and PLT were compared with urinal 8-OHdG concentration during 200 days after first administration of radium-223. 8-OHdG, 8-hydroxy-2'-deoxyguanosine; CRE, creatinine; PLT, platelet; Pt., patient; RBC, red blood cell; WBC, white blood cell.

Table 3 Correlation coefficient between period and 8-OHdG or hematocytes

Correlation [ref. time after 1st admin (days)]	Correlation parameters	Pt. 1	Pt. 2	Pt. 3	Pt. 4
8-OHdG	R	−0.57	−0.75	−0.94	−0.056
	Slope	−0.17±0.013	−0.11±0.047	−0.43±0.16	−0.0045±0.040
WBC	R	−0.92	−0.66	−0.81	−0.39
	Slope	−0.0077±0.0017	−0.018±0.010	−0.096±0.069	−0.011±0.013
RBC	R	−0.50	0.83	−0.98	0.51
	Slope	−0.0012±0.0010	0.0016±0.00056	−0.0023±0.00051	0.0013±0.0011
PLT	R	−0.57	−0.47	−0.37	−0.22
	Slope	−0.000050±0.0051	−0.037±0.034	−0.058±0.15	−0.022±0.048

The values of R show Pearson's correlation coefficient between days 0 and 200. The slope means a linear function (mean ± SD). 8-OHdG, 8-hydroxy-2'-deoxyguanosine; PLT, platelet; Pt., patient; RBC, red blood cell; SD, standard deviation; WBC, white blood cell.

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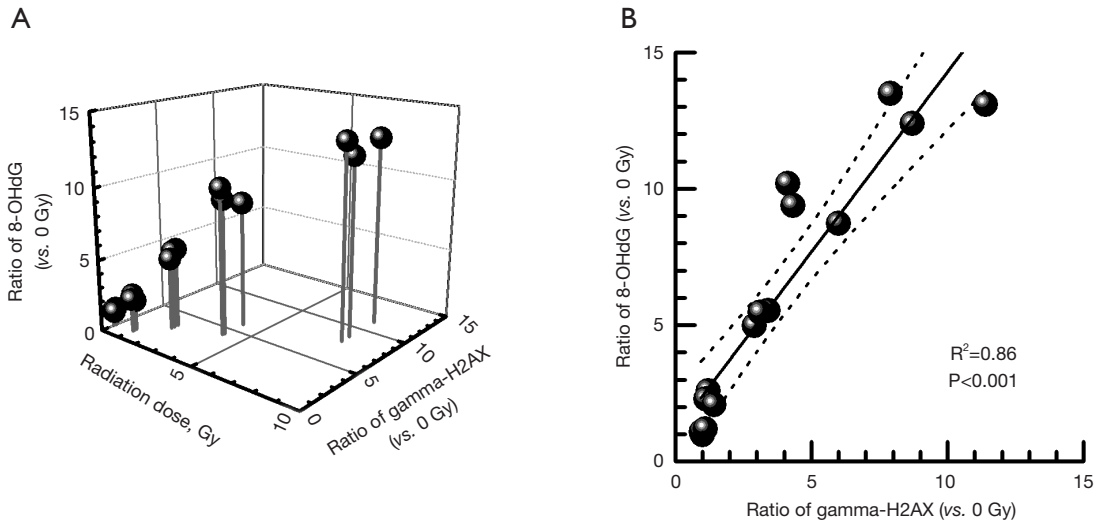

Figure 6 Relationship between radiation dose, 8-OHdG and gamma-H2AX using osteoblastic cell line (MC3T3-E1 cell). (A) The secretion of 8-OHdG from cell and expression of gamma-H2AX (DNA repair marker) were quantified using ELISA method and fluorescent plate reader, respectively. These data were shown as three-dimensional plots. (B) The correlation between 8-OHdG and gamma-H2AX was estimated by Pearson's correlation coefficient. 8-OHdG, 8-hydroxy-2'-deoxyguanosine; ELISA, enzyme-linked immunosorbent assay.

Conversely, markers of osteoclasts, which show contrasting metabolism in osteoblasts and bone turnover, were confirmed to not show sensitive fluctuations. Furthermore, biomarkers that better reflect the environment of bone metastases are required to optimize treatment. The investigated urinary 8-OHdG levels tended to decrease from the initial measurement values in the hotspot group or in Pt. 2 and 3 that responded to BAP or 1CTP (*Figure 4*). An increase in urinary 8-OHdG concentration indicates an increase in oxidative damage to DNA due to radiation stress, as well as the function of repair enzymes, thus it may include the body's reserve ability to resist oxidative stress (18). Two previous studies that investigated the relationship between serum or urinal 8-OHdG levels and the prognosis and incidence of side effects during chemo/radiotherapy revealed that the lower the 8-OHdG level before treatment or the higher the 8-OHdG level after treatment, the better the prognosis and the fewer side effects caused by chemo/radiotherapy (19,20). However, because the urinary baseline in this study was very similar between non-myelosuppression (Pt. 4) and myelosuppression (Pt. 1, 2, 3), it could not be used to predict the development of myelosuppression. On the other hand, bone marrow suppression may be able to predict by the paralleled relationship between a negatively correlated changing of 8-OHdG concentration and a negative correlation in the number of WBC or PLT. Additionally, another report on urinal 8-OHdG has demonstrated that the 8-OHdG level is not easily affected by diet or cell death, is not formed from deoxyguanosine due to the presence of hydrogen peroxide and other components in urine, and is mostly caused by DNA repair (21,22). Our present *in vitro* data also showed that expression of gamma-H2AX which is one of DNA repair marker is positive corelated to 8-OHdG secretion from osteoblastic cell line (*Figure 6*). Moreover, urinal 8-OHdG can suppress the effects of diurnal fluctuations in excretion by correcting CRE levels (23). We compared urinal 8-OHdG with existing bone metabolism markers for the first time and demonstrated its usefulness to evaluate individual differences in the effects and side effects of RIT. Urinary 8-OHdG can originate from both normal and tumor cells. The data of this study were derived from indirect detection of urinary 8-OHdG concentrations released from tumor or normal tissues locally exposed to alpha particles and represent a comprehensive biomarker. However, the low number of patients is a limitation of the present study, and that a larger cohort study is required in the future to validate these findings.

Conclusions

In conclusion, our results suggest that urinal 8-OHdG

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concentration has a potency of biomarker for bone marrow suppression under the administration of radium-223 in the patient with BM.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Committee of Medical Ethics of the Hirosaki University Graduate School of Health Sciences (No. 2016-051) to ensure the welfare and privacy of the donors. Following a detailed verbal explanation regarding the content of this study, a written informed consent was obtained. All patients and their families agreed for publication as scientific research.

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