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Inhibiting and Enhancing Effects on DNA Repair of Rare Metal Elements in Cultured Human Lymphoblastoid Cells

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Along with increasing relevance of rare earth (RE) elements in industrial technology, the risk of their environmental release and occupational exposure on human health is of concern. Although many toxicological studies were reported for REs, it is not known how they affect DNA repair. In this study, the effects on DNA repair of all RE ions except radioactive promethium (Pm) were studied. Human lymphoblastoid WTK1cells were irradiated to UV followed by 2h exposure to each RE with and without DNA repair inhibitor cytosine-1β-D- arabinofuranoside (araC), and then single

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strand breaks (SSBs) were detected by the comet assay. UV-induced pyrimidine dimers are removed by nucleotide excision repair (NER) which consists of recognition of the DNA lesion, excision of a 24–32 nucleotide stretch containing the lesion by dual incision of the damaged DNA strand on both sides, and re-synthesis of the resulting gap by DNA polymerase, and ligation of the nick. SSBs are generated in the incision step of nucleotide excision repair (NER) and disappear in the re-synthesis step of NER. Seven REs, Yb, Lu, Dy, Er, Sc, Pr, and Ce, enhanced comet positive response without araC but not with araC, suggesting that araC is antagonistic to the 7 REs. Since araC inhibits re-synthesis of NER, these seven REs would inhibit the re-synthesis step of NER. Six REs, Tm, Sm, Tb, Gd, Eu, and Y suppressed comet positive responses with and without araC, suggesting that they decreased comet assay detectable SSBs. Therefore, these 6 REs are considered to inhibit the incision step of NER. Only La decreased tail length without araC but increased with araC, suggesting that La increased comet assay detectable SSBs and that only La would enhance the incision step of NER. Neither Nd nor Ho affected tail length with or without araC.

Keywords: Co-genotoxic potential; incision step;re-synthesis step; inhibition of nucleotide excision repair; rare earth element.

1. INTRODUCTION

Rare earth (RE) elements involve scandium, yttrium and 15 lanthanides and are indispensable materials for improving the performance of electronic products such as storage batteries, light emitting diodes, and magnets. RE elements, especially lanthanoids, exhibit physically unique properties because their electron configurations are different from those of ordinary elements. It is used as a material for hydrogen storage alloys, secondary battery raw materials, optical glass, strong rare earth magnets, phosphors, and abrasives. Mechanical properties are improved by adding a small amount to the magnesium alloy [1]. Along with their increasing relevance in industrial technology, the risk of environmental release and occupational exposure of REs on human health is of concern [2–4]. Considering that we are always exposed to various kinds of environmental mutagens, interactions between them might present a serious problem to our health. β-Carbolines and heterocyclic amines, such as harman, 3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1), and 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2), show co-genotoxic activity in human cells by inhibiting DNA repair [5]. Not only those organic compounds but an inorganic cadmium ion (Cd^{2+}) also shows co-genotoxic activity by inhibiting nucleotide excision repair (NER) [6]. Although many toxicological studies using animal models and cultured cell lines were reported for REs [7], it is not known whether they can show co-genotoxic activity by inhibiting NER. In this study, we examined how all RE ions, except radioactive promethium, affect NER.

2. MATERIALS AND METHODS

2.1 Reagents

The rare earth metal salts used were cesium chloride (CeCl MW168.36, 99.0+%), dysprosium chloride hexahydrate ($DyCl_3 \cdot 6H_2O$, MW376.95, 99.5%), erbium chloride hexahydrate ($ErCl_3 \cdot 6H_2O$, MW381.71, 99.9%), europium (III) nitrate hexahydrate ($Eu(NO_3)_3 \cdot 6H_2O$, MW337.98, 99.9%), gadolinium chloride ($GdCl_3$, MW263.61, 99.9%), holmium chloride hexahydrate ($HoCl_3 \cdot 6H_2O$, MW379.38, 99.9%), lanthanum(III) nitrate hexahydrate ($La(NO_3)_3 \cdot 6H_2O$, MW433.01), lutetium (III) chloride hexahydrate（$LuCl_3 \cdot 6H_2O$, MW281.33, 99.9%), neodymium nitrate hexahydrate ($Nd(NO_3)_3 \cdot 6H_2O$, MW438.35, 99.5%), praseodymium(III) chloride heptahydrate ($PrCl_3 \cdot 7H_2O$, MW373.35, 99.95%), samarium(III) chloride hexahydrate ($SmCl_3 \cdot 6H_2O$, MW364.81, 99.5%,scandium(III) chloride hexahydrate ($ScCl_3 \cdot 6H_2O$, MW259.41, 99.9%), terbium(III) chloride hexahydrate ($TbCl_3 \cdot 6H_2O$, MW265.29, 99.9%), thulium(III) chloride heptahydrate ($TmCl_3 \cdot 7H_2O$, MW401.4, 99.9%), Ytterbium(III) Chloride hexahydrate ($YbCl_3 \cdot 6H_2O$, MW387.49, 99.9%), and Yttrium(III) nitrate hexahydrate ($Y(NO_3)_3 \cdot 6H_2O$, MW383.01, 99.9%). All rare earth metal salts were obtained from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan).

Cells: *$TK^{+/-}$* heterozygotes of WTK1 human lymphoblastoid cells exhibiting mutant-type *p53* (kindly provided by Dr. Honma, National Institute of Health Sciences, Tokyo) were used. WTK1

cells were maintained using RPMI 1640 medium (Nissui Pharmaceutical Co., Ltd.) supplemented with 10% horse serum (SAFC Biosciences), 200 µg/mL sodium pyruvate and 200 µg/mL streptomycin at 37°C under a 5% CO_2 atmosphere.

2.2 Comet Assay

One mL of cell suspension in saline (5 x 10^5 cells/mL) in a 6-cm dish was irradiated to UVC using a germicide lamp (National GL15, 15 W, Matsushita Electric Industrial Co., Japan) at 25 µW/cm2. Cells exposed to UVC were post-treated with each RE for 2 h in the presence or absence of the DNA repair inhibitors cytosine-1-β-D-arabinofuranoside (araC, 1.8 mM). AraC (Fujifilm Wako Pure Chemical Industries, Ltd.) was dissolved in physiological saline. Exposed cells were sampled immediately after chemical treatment and the percentage of viable cells was measured by the trypan blue exclusion test. Relative survivals (survivals of treated cells compared with that of an untreated control cells) were obtained. The experimental design described above was same to that in our previous studies [8,9].

Sampled cells were suspended in 1% agarose-LGT at 5×10^5 cells/75 µL, and 75 µL of cell suspension was immediately deposited on a fully frosted slide (Matsunami Glass Ind., Ltd., Osaka, Japan), which was coated with 1% agarose GP-42 and then covered with another slide glass. The slides were placed so as to allow the agarose to gel. The samples on the slides were then immediately exposed to a lysing solution (pH 10) of 2.5 M NaCl, 100 mM EDTA disodium (Na_2EDTA), 10 mM Trizma, 1% sarkosyl, 10% DMSO and 1% Triton X-100, and left at 4°C for 1 h. The slides were then placed on a horizontal gel electrophoresis platform and covered with pH >13 alkaline solution composed of 300 mM NaOH and 1 mM Na_2EDTA. The slides were left in solution at 0 oC for 20 min to allow unwinding of the DNA and expression of alkali-labile sites to occur. The power supply was set at 25 V and 250 mA. The DNA was subjected to electrophoresis at 0°C for 20 min and the slides were rinsed with 400 mM Trizma (pH 7.5) to neutralize the excess alkalinity. Each slide was stained with 50 µL of 20 µg/mL ethidium bromide (Wako Pure Chemical Industries, Ltd.) and covered with a cover slip. Fifty cells on one slide per dose (one slide was prepared for each dose) were examined and photographed (ASA 400 Fuji film) at 200× magnification using a fluorescence microscope (Olympus) equipped with a G filter. The tail length of the comet image was measured (Fig 1). The effect of chemical treatment on tail length was analyzed by ANOVA and Dunnett multiple comparison using statistic software Statcel 4.

3. RESULTS

The effects of REs on the tail length in UV-irradiated and non-irradiated WTK1 cells are shown in Fig. 2 and summarized in Table 1. Relative survivals in WTK1 cells irradiated to UV followed by RE exposure are also shown in Fig. 2. Sc, Ce, Pr, Dy, Er, Yb, and Lu increased tail length significantly in the absence of araC but did not affect tail length in the presence of araC. Y, La, Sm, Eu, Gd, Tb, and Tm decreased tail length significantly in the absence of araC. In the presence of araC, they decreased tail length significantly except for La, but La on the contrary, increased tail length. Neither Nd nor Ho affected tail length with or without araC. Relative survival was ≧70% with and without DNA repair inhibitors, showing that observed decrease and increase in tail length were not due to cytotoxic effect of REs. REs did not affect tail length in UV-unexposed WTK1cells, suggesting that they do not have genotoxic potential in WTK1 cells.

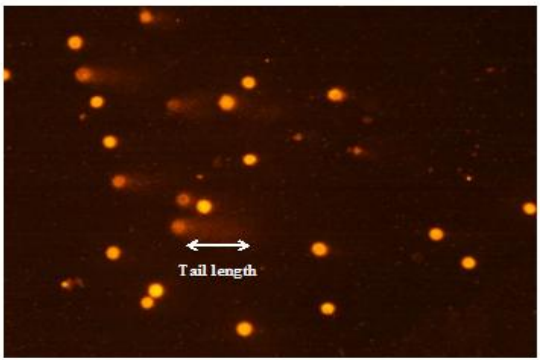
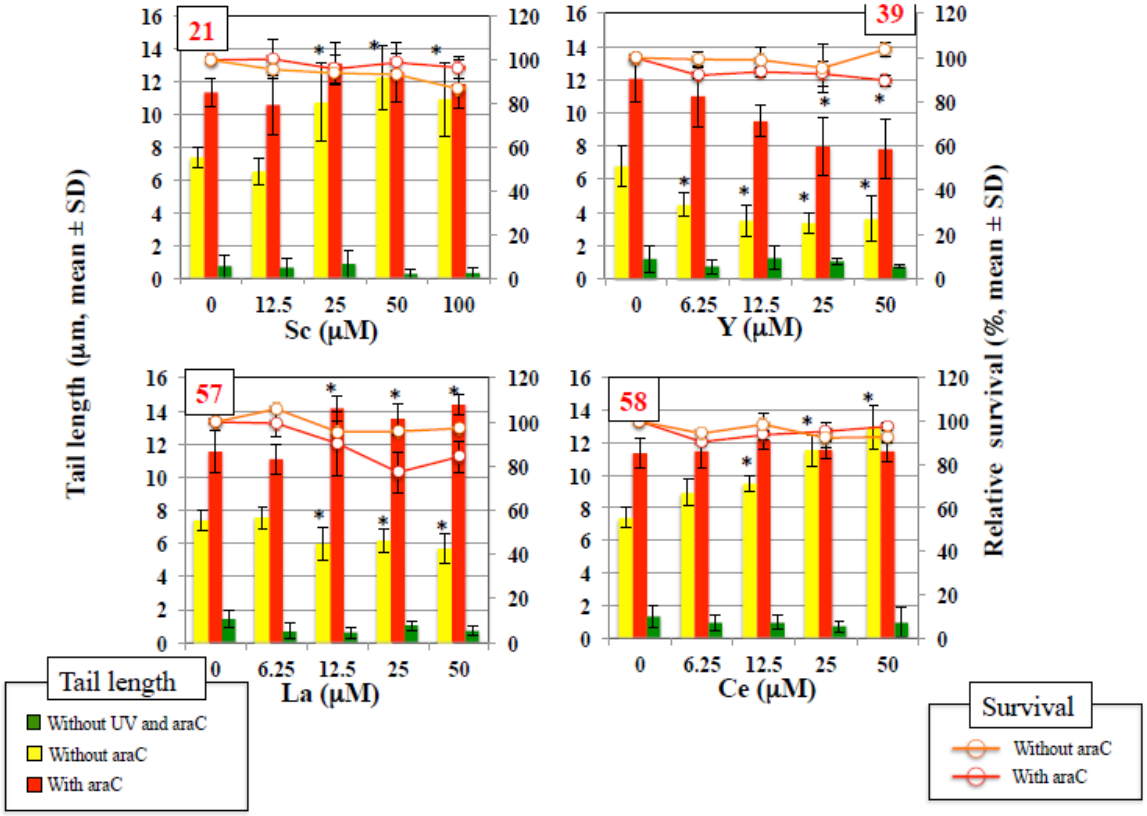
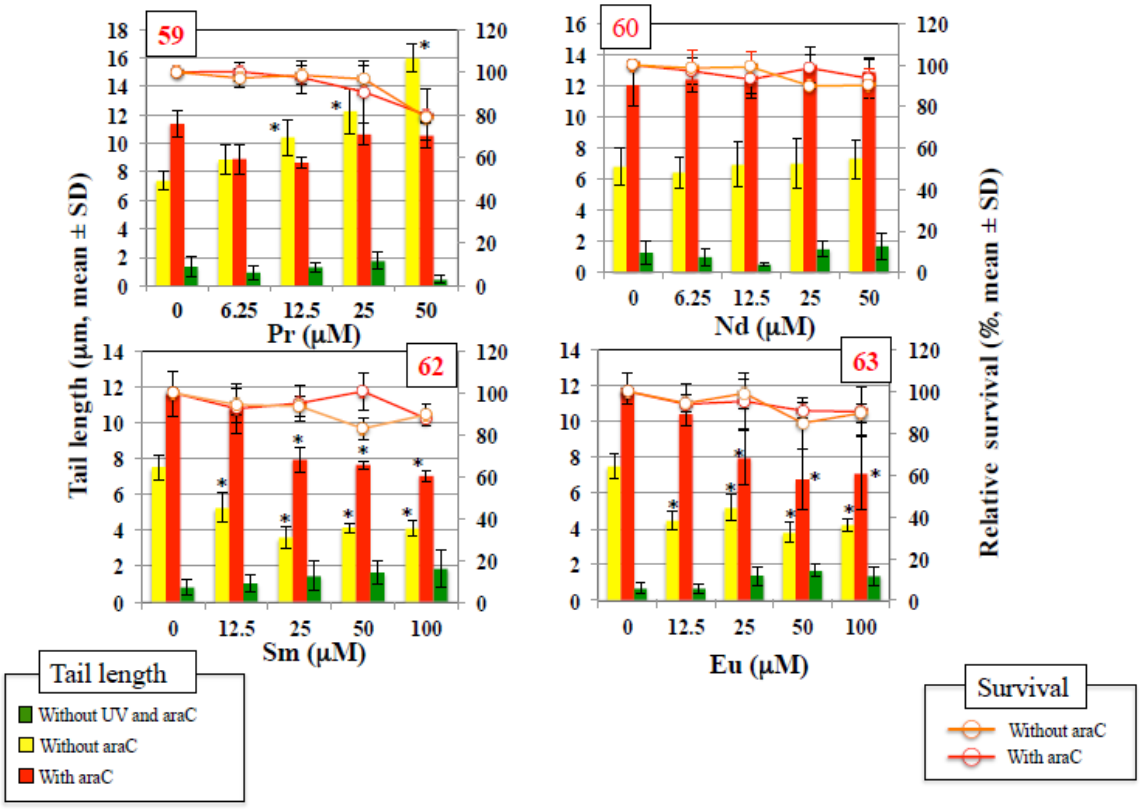


Fig. 1. Comet images in UV-irradiated WTK1 cells


21
Sc (μM)
39
Y (μM)
57
La (μM)
58
Ce (μM)
Tail length (μm, mean ± SD)
Relative survival (%, mean ± SD)
Tail length
Without UV and araC
Without araC
With araC
Survival
Without araC
With araC


59
Pr (μM)
60
Nd (μM)
62
Sm (μM)
63
Eu (μM)
Tail length (μm, mean ± SD)
Relative survival (%, mean ± SD)
Tail length
Without UV and araC
Without araC
With araC
Survival
Without araC
With araC

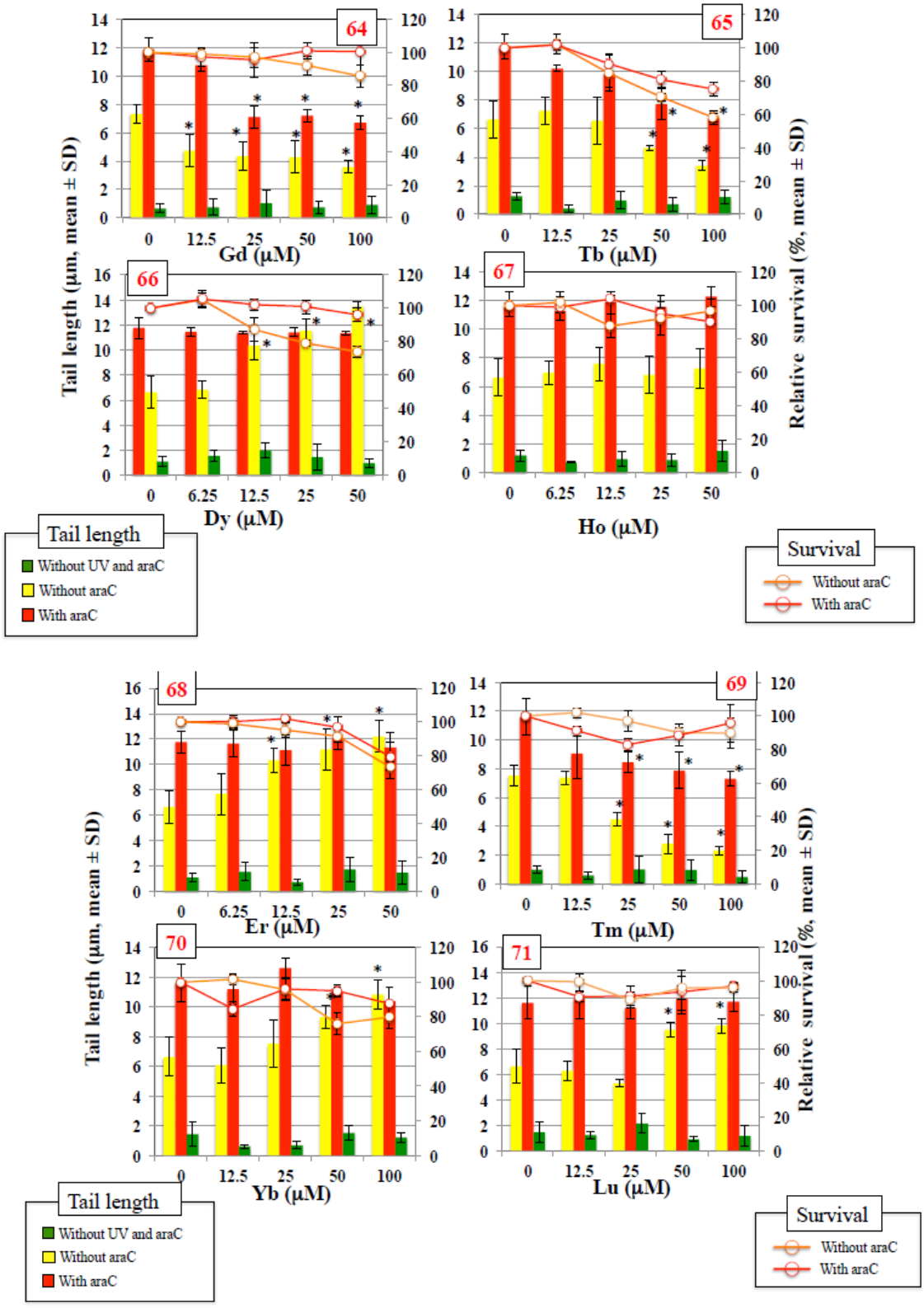


Fig. 2. Effects of REs on the tail length and relative survival in WTK1 cells

*UV-irradiated and non-irradiated WTK1 cells were treated with REs for 2 h. The error bars indicate standard deviation of the mean of three independent trials. Numbers in red are atomic numbers of REs. *Significant difference from RE-untreated control: p<0.05*

Table 1. Summary of effects of REs on comet positive response by UV

Atomic No.	REs	-araC*	+araC*	Category**
21	Sc	E	—	1
39	Y	S	S	2
57	La	S	E	3
58	Ce	E	—	1
59	Pr	E	—	1
60	Nd	—	—	4
62	Sm	S	S	2
63	Eu	S	S	2
64	Gd	S	S	2
65	Tb	S	S	2
66	Dy	E	—	1
67	Ho	—	—	4
68	Er	E	—	1
69	Tm	S	S	2
70	Yb	E	—	1
71	Lu	E	—	1

**E, enhance; S, suppress; —, no effect **see discussion for the category*

4. DISCUSSION

Based on the obtained results, studied REs are classified into four categories (Table 1). Seven REs, Yb, Lu, Dy, Er, Sc, Pr, and Ce, are classified into category 1. They enhanced comet positive response without araC but not with araC. Six REs, Tm, Sm, Tb, Gd, Eu, and Y, are classified into category 2. They suppressed comet positive responses with and without araC. The comet assay detects single strand breaks (SSBs) produced as initial lesions and also those that are generated during the repair of initial lesions such as alkylated bases, bulky base adducts and pyrimidine dimers [10]. It is known that pyrimidine dimers are repaired by NER, which consists of the following four steps: recognition of the DNA lesion, excision of a 24–32 nucleotide stretch containing the lesion by dual incision of the damaged DNA strand on both sides, filling in of the resulting gap by DNA polymerase, and ligation of the nick. During the process, SSBs are produced as intermediates, which can be visualized as a comet tail in the comet assay. Therefore, the inhibition of SSB formation in the incision step and promotion of SSB disappearance in re-synthesis step would result in the suppression of comet positive responses [11]. In the case of REs in category 2, their effects with araC are the same to those without araC, suggesting that their effects are independent to the re-synthesis step. Therefore, the suppression of comet responses by them would reflect the decrease in comet-detectable SSB by the inhibition of initiation step. The DNA re-synthesis inhibition by araC is due to either direct inhibition of DNA polymerase when araCTP is bound to the dCTP binding site of the enzyme, or indirect inhibition through araCMP incorporation into a repaired region of DNA rendering it unsuitable for further polymerase action [12]. In the case of REs in category 1, their effects on comet responses were canceled by araC, suggesting that araC is antagonistic to them. Therefore, they are considered to inhibit re-synthesis step to increase tail length. Only La decreased tail length without araC but increased with araC. La in category 3 enhanced comet responses when re-synthesis step is cancelled by araC. Since produced SSBs disappear in the re-synthesis step following to the incision step, the enhancement of comet positive response by araC inhibiting the re-synthesis step would suggest that La promotes the production of SSBs in the incision step. Considering that we have already shown and discussed that enhanced SSB-formation in the incision step result in more rapid disappearance of SSB and the suppression of comet positive response [11,13], La could be considered to enhance the incision of NER, from which only La has an anti-genotoxic but not co-genotoxic effect. RE ions also have nematicidal activities as they strongly perturb the embryonic development of the nematode [14]. Although nematicidal activity increased with increasing atomic number of lanthanide ions [14], the correlation between their effects on NER and atomic number was not observed. Inorganic

metal ions, such as $Fe2^{+}$, Cu^{+} and $Zn2^{+}$, act co-factors of various proteins including respiratory enzymes, transcription factors, etc. that are essential for cellular metabolism. Metal complexes with biological activity are of increasing importance in medicine as potential alternatives for biologically active organic compounds, which often show severe side effects [15]. It has been reported that xanthine derivatives, such as caffeine, an inhibitor of post-replication repair, enhance the antitumor actions of cisplatin, UV, and X-rays [16,17]. Although there are still many unclear points about this mechanism, the inhibition of DNA repair is considered as one possible mechanism [18]. Therefore, RE ions are presently shown to inhibit DNA repair, which might suggest the possibility of their medicinal applications as antitumor drug enhancers.

5. CONCLUSION

Except for Nd and Ho, REs affect NER by the different mode of mechanisms. Seven REs inhibit the re-synthesis step of NER and 6 Res inhibit the incision step of NER. Although those 13 REs inhibit NER, only La enhances he incision step of NER. To our knowledge, this is the first report that shows REs have inhibitory effects on NER by different modes of mechanisms.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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