Extracellular dGMP Enhances *Deinococcus radiodurans* Tolerance to Oxidative Stress

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Abstract

Free extracellular DNA provides nutrition to bacteria and promotes bacterial evolution by inducing excessive mutagenesis of the genome. To understand the influence of extracellular DNA fragments on *D. radiodurans*, we investigated cell growth and survival after extracellular DNA or dNMPs treatment. The results showed that the extracellular DNA fragments inhibited the growth of *D. radiodurans*. Interestingly, dGMP, a DNA component, enhanced *D. radiodurans* tolerance to H2O2 and gamma-radiation significantly. Further experiments indicated that extracellular dGMP stimulated the activity of one catalase (KatA, DR1998), and induced gene transcription including the extracellular nuclease (drb0067). When this only extracellular nuclease gene (drb0067) in *D. radiodurans* was deleted, the mutant strain showed more sensitive to H2O2 and gamma-radiation than the wild type strain. These results suggest that DRB0067 plays an important role in oxidative stress resistance. Taken together, we proposed a new anti-oxidation mechanism in *D. radiodurans*. This mechanism acts to increase expression levels of DRB0067 which then secretes active nuclease to degrade extracellular DNA fragments. The extracellular nuclease has a two-fold benefit, creating more free dNTPs for further cell protection and the removal of extracellular DNA fragments.

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Introduction

Bacteria cell death releases cytoplasmic contents, including DNA components into the microenvironment [1]. In addition, many living bacteria such as *Acinetobacter, Azotobacter, Bacillus, Deinococcus, Neisseria* and *Pseudomonas* release DNA into the surrounding environment during cell growth [1–6]. These bacteria benefit in several ways from free extracellular DNA and its degradation product [7–11]. For instance, the uptake of extracellular DNA, from the same or different organisms, promotes the evolution of bacteria. This occurs via horizontal gene transfer, such as transformation, transduction, or conjugation between bacteria [7,8]. Extracellular DNA is also required for the initial establishment of bacterial biofilms, such as in *Pseudomonas aeruginosa*. The degradation of extracellular DNA by DNase I can strongly inhibit biofilm formation [9,10]. Extracellular DNA, both homospecific and heterospecific, is known as an important nutrient source for organisms [11]. However, if extracellular DNA is not degraded immediately, it can threaten the survival of organisms by reincorporating damaged bases into the genome [12]. In most case, extracellular DNA components are degraded by extracellular nucleases secreted by many kinds of bacteria [13–16]. As a result, a threat to the organism is removed, and its by-products, dNMPs, are a nutrient source for bacteria [11,12,16].

*Deinococcus radiodurans* are extremely resistant to ionizing radiation, UV radiation, hydrogen peroxide and desiccation [12,17–21]. The high resistance of this bacterium to reactive oxygen species (ROS) results from the strong ability of oxidative resistance [22] and an efficient DNA repair mechanism [23,24]. Ionizing radiation [17] or UV [25] radiation attacks intracellular DNA producing large amounts of damaged oligonucleotides within the nucleotide pool [26]. These damaged oligos are exported into the surrounding medium and finally degraded [27]. However, it is not known if *D. radiodurans*, the most ionizing radiation resistant bacteria, has the ability to reuse these damaged extracellular DNA fragments.

Recently, Daly et al. demonstrated that *D. radiodurans* ultrafilter, which was enriched in Mn, phosphate, peptides, nucleosides and bases, could protect proteins from ionizing radiation-induced ROS damage [28,29]. These findings implied that degradation and re-absorption of damaged DNA components might contribute to this organism’s extreme ROS resistance. Here, we investigated the effects of extracellular DNA fragments and dNMPs on cell
growth, \( \text{H}_2\text{O}_2 \) resistance, as well as UV and gamma-radiation in both \( \text{D. radiodurans} \) and \( \text{E. coli} \). Our results indicated that the uptake of extracellular DNA fragments represented a new mechanism of protection from oxidative damage.

**Results**

Extracellular DNA fragments inhibit the growth of \( \text{D. radiodurans} \) but not \( \text{E. coli} \)

Free extracellular DNA is abundant in the environment, and its existence may have an effect on the growth of bacteria. To understand this effect, we grew \( \text{D. radiodurans} \) and \( \text{E. coli} \) cells in the presence or absence of 3.6 mg/ml DNA fragments or dNMPs. We observed large amounts of DNA fragments resulted in a distinct growth inhibition of \( \text{D. radiodurans} \). Absorption reading (\( \text{OD}_{600} \)) was 0.26 after 10 hours in the presence of DNA fragments, while control cells \( \text{OD}_{600} \) reading was 2.38 and that with dNMPs treatment was 2.30 and that with dNMPs treatment was 2.19 (Fig. 1A). In \( \text{E. coli} \), the \( \text{OD}_{600} \) values of control, DNA fragments and dNMPs treated groups were almost the same (\( \text{OD}_{600} \approx 2.3 \)) after 4 hours, which indicates neither DNA fragments nor dNMPs affect the growth of \( \text{E. coli} \) (Fig. 1B). These data suggested that DNA fragments and dNMPs have different effects on \( \text{D. radiodurans} \) and \( \text{E. coli} \) growth rates. Extracellular DNA fragments instead of dNMPs were harmful to \( \text{D. radiodurans} \) cells growth.

Extracellular dGMP greatly enhances \( \text{D. radiodurans} \) tolerance to \( \text{H}_2\text{O}_2 \)

The influence of extracellular DNA fragments or dNMPs on \( \text{D. radiodurans} \) and \( \text{E. coli} \) cell survival under oxidative stress was evaluated. The presence of DNA fragments caused a modest decrease in \( \text{H}_2\text{O}_2 \) resistance in \( \text{D. radiodurans} \) (Fig. 2A). Similarly, the presence of DNA fragments did not have an obvious effect on \( \text{E. coli} \) resistance to \( \text{H}_2\text{O}_2 \) as well (Fig. 2B). However, the survival rate of \( \text{D. radiodurans} \) was dramatically increased when 10 mM dNMPs was present (Fig. 2A). There was a 33-fold increase in survival when compared to samples without dNMPs treatment. In \( \text{E. coli} \), no distinct difference was observed between either the DNA fragments or dNMPs treatment groups (Fig. 2B). To understand which dNMPs accounted for this effect, dAMP, dTMP, dCMP and dGMP were separately added to \( \text{D. radiodurans} \) growths. Here we observed only dGMP had an effect, which dramatically increased \( \text{H}_2\text{O}_2 \) resistance by approximately 57-fold (Fig. 3). In addition, dGMP enhanced the resistance to gamma-radiation, but not UV (Fig. S1A). In sum, extracellular dGMP has an important role in \( \text{D. radiodurans} \) anti-oxidation, but not in \( \text{E. coli} \).

Extracellular dGMP induces KatA activity

In \( \text{D. radiodurans} \), catalases and SODs protect proteins from ROS-mediated damage \( \text{in vivo} \) [30]. PAGE activity-staining assay reveals that \( \text{D. radiodurans} \) stain possesses activity corresponding to two catalases and one SOD, and stains that carry mutations in these genes (\( \text{katA} \) and \( \text{sodA} \)) are more sensitive to ionizing radiation than wild type [30]. In order to understand how dGMP increases \( \text{D. radiodurans} \) tolerance to \( \text{H}_2\text{O}_2 \), the activity change in catalases and SODs was measured after 2.5 mM dGMP was added to growth medium by PAGE activity-staining assay. The additional dGMP enhanced whole cell KatA activity by \( \approx 50\% \), but had no effect on KatB or any of the SODs assayed (Fig. 4A/4B). It is possible that extracellular dGMP increases \( \text{D. radiodurans} \) tolerance to \( \text{H}_2\text{O}_2 \) by inducing KatA catalase activity \( \text{in vivo} \).

Extracellular dGMP stimulates transcription of anti-oxidation related genes

To better understand extracellular dGMP’s involvement in anti-oxidation, the expression patterns of ROS response genes were investigated using Real-time quantitative PCR (Table 1). The addition of dGMP increased the transcriptional level of \( \text{katA} \) gene (\( \text{dr}1998 \)) about 2.8-fold, but not any other catalase or SOD genes, which agrees with the results from the PAGE activity-staining assay. In other words, extracellular dGMP induces the activity of KatA by increasing the expression level of KatA \( \text{in vivo} \).

The high intracellular Mn/Fe ratio in \( \text{D. radiodurans} \) could contribute to its remarkable resistance to environmental stresses [28,29]. Here, the extracellular dGMP also increased the transcription levels of genes regulating the intracellular Mn/Fe ratio. DR2244, a sensory transduction histidine kinase, was induced by extracellular dGMP, which might be a signaling response to oxidative stress. Considering the role of extracellular nuclease in degrading extracellular DNA, the transcription of \( \text{drb0067} \), the extracellular nuclease gene in \( \text{D. radiodurans} \), was also investigated. When extracellular dGMP was present, transcription

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**Figure 1.** \( \text{D. radiodurans} \) and \( \text{E. coli} \) cell growth after DNA fragments or dNMPs treatment. (A) Growth of \( \text{D. radiodurans} \) after the addition of 3.6 mg/ml DNA fragments or dNMPs. (B) Growth of \( \text{E. coli} \) K-12 after the addition of 3.6 mg/ml DNA fragments or dNMPs. Values are the mean ± standard deviation of three independent experiments. R1, \( \text{D. radiodurans} \) wild type strain.

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of *drb0067* was stimulated (2-fold), thus enhancing the degradation of extracellular DNA fragments and increasing the pool of dNMPs.

**Drb0067 encodes the only extracellular nuclease in *D. radiodurans***

Upon investigation of the *D. radiodurans* genome, we found that *drb0067* is the only extracellular nuclease gene. To further explore the role of DRB0067 in *D. radiodurans*, a *drb0067* null mutant (Δdrb0067) was constructed and verified by PCR (Fig. 5A). To test the mutant and wild type strains ability to degrade extracellular DNA, each strain was inoculated onto DNase test agars plates. A distinct clear zone was observed surrounding the wild type cells, but not Δdrb0067 (Fig. 5B). Moreover, there was no nuclease activity detected in the culture medium of Δdrb0067 (Fig. 5C).

Bases on these two assays, we demonstrated DRB0067 is the only extracellular nuclease in *D. radiodurans*. And this extracellular nuclease is secreted through the secretory pathway since the deletion of *secD/secF* gene (Δdr1822) inhibited the secretion of DRB0067 (data not shown).

The extracellular nucleases act as a modulator for natural transformation in some bacteria, such as *Vibrio cholerae* [16]. Next, we investigated the transformation frequency for the Δdrb0067 strain and found the transformation efficiency was 4.5-fold higher than the wild type strain (Fig. 5D). These results suggest that the DRB0067 protein is also an important modulator for natural transformation in *D. radiodurans*.

We next investigated the effect of DNA fragments on growth in the wild type and Δdrb0067 strains. In the absence of extracellular DNA fragments, wild type and Δdrb0067 strains had similar growth patterns except that the mutant strain (OD 600 < 4.61) exhibited slightly lower OD readings than wild type (OD 600 < 5.75) at the stationary phase. However, in the presence of DNA fragments, Δdrb0067 growths were more sensitive than the wild type growths (Fig. 5E). Under these conditions, the wild type strain entered stationary phase after 29 hours (OD 600 < 4.60), whereas Δdrb0067 was still in logarithmic phase (OD 600 < 2.45) at that time. Therefore, the presence of DNA fragments resulted in a severe cell growth decline in the Δdrb0067 strain when compared to wild type.

**Deletion of *drb0067* impairs H2O2 resistance of *D. radiodurans***

Our experiments indicated that the extracellular dGMP, not extracellular DNA, enhanced the resistance of *D. radiodurans* to H2O2 and gamma-radiation. To understand the role of DRB0067 in this process, the H2O2 resistance of wild type and Δdrb0067 strains were measured. These results, which were expected, revealed the *drb0067* mutation to have a decreased resistance to H2O2. The survival rate of the Δdrb0067 strain was 6 times lower than that of the wild type strain under 30 mM H2O2 treatment (Fig. 6A/6B). Furthermore, the Δdrb0067 was more sensitive to gamma-radiation, though not to UV radiation (Fig. S1B). Next, the effects of oligo(dG)50 and dGMP on Δdrb0067 resistance were investigated with varying concentrations of H2O2. Here, we found that the addition of dGMP (2.5 mM) restored the mutant strain’s resistance to H2O2. The survival fraction with dGMP treatment...
Figure 4. Addition of extracellular dGMP increases the activity of KatA in *D. radiodurans*. (A) Extracellular dGMP (2.5 mM) increased the activity of KatA, but not KatB. (B) Extracellular dGMP (2.5 mM) had no effect on the activity of SOD. (C) and (D) Quantification of the intensity of bands was performed using ImageJ. Each sample contains 80 μg of total protein. Values are the mean ± standard deviation of three independent measurements. R1, *D. radiodurans* wild type strain; KatA, catalase A; KatB, catalase B.

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Table 1. Influence of dGMP (2.5 mM) on *D. radiodurans* transcription levels.

| ORF   | Annotation                                      | Fold   | p value    |
|-------|-------------------------------------------------|--------|------------|
|       | catalase and sod genes                          |        |            |
| DR1998| catalase                                        | 2.80   | 5.16E-06   |
| DRA0146| catalase                                      | 1.01   | 0.85       |
| DRA0259| catalase                                      | 0.65   | 2.12E-05   |
| DR1279| Mn family superoxide dismutase                 | 0.88   | 0.18       |
| DR1546| Cu/Zn superoxide dismutase                    | 0.95   | 0.29       |
| DRA0202| Cu/Zn superoxide dismutase                    | 0.73   | 0.78       |
|       | other genes                                     |        |            |
| DR2283| manganese ABC transporter permease             | 2.98   | 1.79E-05   |
| DR2523| Manganese/iron transport system substrate-binding protein | 1.36 | 0.0003 |
| DR2539| Mn-dependent transcriptional regulator         | 2.25   | 0.00014    |
| DRB0016| iron complex transport system ATP-binding protein | 2.79 | 0.0047 |
| DRB0092| starvation-inducible DNA-binding protein       | 2.49   | 8.73E-05   |
| DRB0121| iron ABC transporter, ATP-binding protein      | 1.62   | 1.70E-05   |
| DRB0124| iron-chelator utilization protein, putative    | 9.71   | 2.43E-06   |
| DRB0067| extracellular nuclease                         | 2.00   | 0.0005     |
| DR2244| sensory transduction histidine kinase          | 2.01   | 0.0032     |

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was about 4 times higher than that with oligo(dG)50 treatment, and 5 times higher than control under the stress of 30 mM H$_2$O$_2$ (Fig. 6C/6D). In addition, D. radiodurans secreted more active extracellular nuclease after gamma-radiation treatment (Fig. S2). These results suggested that DRB0067 might be involved in ROS resistance through degradation of extracellular DNA to dNMPs, which increases the pool of dGMP. This pool then aids in enhancing the D. radiodurans tolerance to oxidative stress.

Discussion

Here, we report extracellular dGMP enhanced the resistance of D. radiodurans to H$_2$O$_2$ and gamma-radiation. These findings suggest extracellular dGMP plays an important role in the organism’s anti-oxidation pathway. Interestingly, we observed extracellular dGMP enhances the expression levels of KatA (DRJ1998) in D. radiodurans. These findings have yielded clues that may reveal the underlying mechanism of extracellular dGMP in anti-oxidation. Moreover, we found extracellular dGMP modulated expression of other genes, including one sensory transduction histidine kinase gene (dr2244), and genes involved in the regulation of manganese/iron. The up-regulation of these genes may enhance the tolerance of H$_2$O$_2$ and gamma-radiation as well.

Both cAMP and cGMP, as second messengers, have been widely studied in eukaryotes. It has been reported that cGMP can protect eukaryotic cells from oxidative stress, as in endothelial progenitors [31]. In bacteria, the production of cGMP has also been demonstrated. However, the physiological role of cGMP is still not well defined [32]. Recently Misra et al. reported that a DNA damage-induced signaling mechanism including secondary messengers and signaling enzymes exist in D. radiodurans [33]. Considering that the GC content (66.6%) in D. radiodurans is higher than most of other bacteria [27], we hypothesize that guanine base, obtained from the breakdown of extracellular dGMP [1], could be converted into cGMP after absorbed, and protect the cells from oxidative damage. However, further experiments are required to fully characterize how dGMP enhances D. radiodurans tolerance to oxidative stress.

In D. radiodurans, DRB0067, encoded on the mega-plasmid, is an extracellular nuclease [27]. We demonstrated the absence of DRB0067 completely abolished nuclease activity from medium (Fig. 5B/5D). These results indicate DRB0067 is the only extracellular nuclease in this bacterium. Further experiments suggested this extracellular nuclease is secreted through the secretory pathway. Under normal growth conditions, DRB0067 is bound to the carotenoid-containing hexagonal layer [27].
Interestingly, the nuclease is released into the medium after ionizing radiation [34–36], indicating that this nuclease participates in *D. radiodurans* post-irradiation recovery. This hypothesis was indirectly supported by a transcriptome study reporting that the expression level of DRB0067 was induced after ionizing radiation [37]. Here, we have found direct evidence that deletion of *drb0067* decreases the survival ability of *D. radiodurans* after H2O2 or gamma-radiation treatment. Moreover, we report gamma-radiation enhances the secretion of DRB0067, indicating an important role for this protein in anti-oxidation. It is quite possible DRB0067 is required to degrade damaged DNA fragments that are exported after radiation damage to avoid genome mutagenesis. This mechanism also provides essential nutrition for cells’ recovery. Interestingly, dGMP, one product from DNA degradation, dramatically stimulates *D. radiodurans* resistance to oxidative stress, which may indicate another purpose for DRB0067 induction after DNA damage stress.

Materials and Methods

Strains, media, and growth conditions

The bacterial strains and plasmids used in this study are listed in Table S1. *D. radiodurans* (ATCC 13939) was used as the wild-type strain and for construction of mutants. All cells were cultured at 30°C in TGY medium (0.5% Bacto tryptone, 0.3% Bacto yeast extract, 0.1% glucose) or on TGY plates containing 1.5% Bacto agar powder. *E. coli* strain DH5α was used for propagation of plasmids and was grown at 37°C on LB media with appropriate antibiotics.

Disruption of the *drb0067* gene in *D. radiodurans*

Disruption of *D. radiodurans* *drb0067* gene was performed using the double crossover recombination method [38]. In brief, the 0067upF and 0067upR primers (Table S1) were used for the upstream fragment and 0067downF and 0067downR primers (Table S1) for the downstream fragment. The upstream and downstream were digested by *Hind*III and *Bam*HI respectively, extracellular DNA had no effect on its growth. These findings support the importance of extracellular nuclease in this organism. Taken together, the degradation of extracellular DNA into dNMPs by extracellular nuclease DRB0067 serves many purposes in *D. radiodurans*. First by converting extracellular DNA into nutrients, thus reversing cell growth inhibition, and finally by enhancing *D. radiodurans* tolerance to oxidative stress.

Figure 6. Sensitivity of Δ*drb0067* strain subjected to H2O2. (A) and (B) Sensitivity of R1 and Δ*drb0067* to different concentration of H2O2. (C) and (D) Sensitivity of Δ*drb0067* to different concentration of H2O2 with the addition of 0.05 mM oligo(dG)50 or 2.5 mM dGMP. Data represent the means ± deviations of three independent experiments. R1, *D. radiodurans* wild type strain; Δ*drb0067*, the *drb0067* null mutant. doi:10.1371/journal.pone.0054420.g006
and ligated to the BsmHI-HindIII fragment of the kanamycin resistance cassette containing the groEL promoter. The kanamycin resistance cassette was obtained from pRADK, a shuttle plasmid modified from pRADZ3 [39]. The fragment was then transformed into D. radiodurans R1 with CaCl2 as described previously [40]. The mutant strain was obtained on TGY agar with 30 μg/ml kanamycin, and was confirmed by PCR with the primers 0067qPF and 0067downR primers.

**Growth curve and survival fraction tests**

Bacteria growth was determined using optical density data (OD) at 600 nm. The strains were cultured in 20 ml liquid TGY or LB medium until an OD<sub>600</sub>=0.15 was reached, and DNA fragments (Herring sperm DNA from Sigma-Aldrich Company) or dNMPs, at a final concentration of 3.6 mg/ml, were added. The cultures were incubated with 230 rpm at 30°C or 37°C and samples were taken to measure the OD<sub>600</sub> value at different time. All experiments were repeated in triplicate.

For the sensitivity assay, the strains were cultured in 5 ml liquid TGY medium until an OD of OD<sub>600</sub>=0.75 was reached. Then DNA fragments, dNMPs, dGMP, or oligo(dG)50 were added to the growths (Table S1). Cultures were grown for another 3 hours. As a negative control autoclaved distilled water was added to a culture of each strain. After washed and diluted to an appropriate concentration with PBS solution, the cultures were treated with different concentrations of H<sub>2</sub>O<sub>2</sub> (25 mM, 50 mM or 15 mM, 30 mM) for 30 min at 4°C. After treatment, the cells were plated on TGY plates and incubated at 30°C for 3 days before colonies were enumerated. The H<sub>2</sub>O<sub>2</sub> survival assays on E. coli were performed as described above, except the cells were treated with 20 mM or 40 mM H<sub>2</sub>O<sub>2</sub> plated on LB agar, and incubated at 37°C for 15 hours. Survival fraction (%) was calculated using the following equation: Survival fraction (%) = N<sub>sample</sub>/N<sub>control</sub>×100%, where N<sub>control</sub> is the number of control colonies and N<sub>sample</sub> is the number of H<sub>2</sub>O<sub>2</sub> treated colonies. For the dripping test, the cultures were washed and serially diluted 1:10 with PBS solution, and then treated with H<sub>2</sub>O<sub>2</sub> (10 mM, 20 mM or 30 mM), gamma-radiation (2.5 h for 2 kGy) or UV (408 J/m<sup>2</sup>) separately [41]. 20 μl of cells were dripped onto TGY plates.

**Transformation test**

The plasmid pRADK was used to test the effect of the extracellular nuclease DRB0067 on the natural transformation. Here, 1 μg plasmid was used for each transformation. The pRADK was then transformed into D. radiodurans R1 with CaCl<sub>2</sub> as described previously [40]. Natural transformation frequencies were determined using the following equation: Natural transformation frequencies = N<sub>TK</sub>/N<sub>TYG</sub>, where N<sub>TK</sub> is the number of clones on the TGY plates with 30 μg/ml kanamycin and N<sub>TYG</sub> is the number of clones on the TGY plates.

**Extracellular nuclease activity assay**

DNase test agar plates were used to test the extracellular nuclease activity. The cells were cultured in TGY until an OD of OD<sub>600</sub>=1.0 was reached, then 20 μl of cells were dripped onto DNase test agar plates (plates contain 42.0 g of DNase Agar Base (Qingdao Hope Bio-Technology Co., Ltd, China), 0.05 g of methyl green and 2 g of glucose per liter of distilled water). Cultures were incubated at 30°C for 3 days. We used pRADK plasmid to test the activity of nuclease outside of the cells. The cells were cultured in TGY, and then centrifuged to collect the supernatant for enzyme reaction, during which 10 mM MgCl<sub>2</sub> was added.

**Activity measurement of Catalase and SOD**

Cells were treated with dGMP (2.5 mM) when they reached OD=0.8, incubated for 3 hours and then disrupted with an ultrasonicator. The protein concentration of the supernatant was measured by the Bradford’s method [42]. The catalase activity was assayed by the horseradish peroxidase-diaminobenzidine method [43]. In detail, samples were separated, using electrophoresis, in an 8% non-denaturing polyacrylamide gel matrix at 4°C for 4–5 hours (15 mA). The gel matrix was then washed with distilled water for 3 times. Next, it was incubated with a 0.06% H<sub>2</sub>O<sub>2</sub> solution for 20 min under slow shaking. The gel was washed again and then incubated with FeCl<sub>3</sub> (2%) and Fe(CN)<sub>6</sub> (2%) (V/V = 1:2) until bright strips appeared on the gel. For the assay of SOD activities, a 10% non-denaturing polyacrylamide gel was used according to the nitroblue tetrazolium-riboflavin method [44]. This method was nearly the same to the one described above except for the gel-running time (2–2.5 h) and the staining solution (2.43 mM nitroblue tetrazolium chloride (NBT), 28 mM TEMED, 28 μM riboflavin, and 100 mM EDTA, pH = 7.8). The gel was stained for 30 min and then exposed under lamplight until the bright strips appeared. For the activity measurement experiments 80 μg proteins were used per lane.

**Real-time quantitative PCR**

Real-time quantitative PCR was used to determine the influence of the extracellular dGMP on the expression levels of Catalases, SODs and other genes of interest in D. radiodurans. In short, cells were grown to an OD<sub>600</sub>=0.2 and then 2.5 mM dGMP was added. Cells were harvested by centrifugation at 4000 rpm for 5 min at 4°C when an OD<sub>600</sub> of 0.4–0.45 was reached. The extraction of total RNA and cDNA synthesis were performed as described previously [45]. SYBR Premix Ex Taq™ (TaKaRa Biotechnology (Dalian) Co, Ltd, China) was used for amplification, and all assays were performed using the STRATA-GENE Mx3005P™ Real-time detection system.

**Supporting Information**

**Figure S1 UV and gamma-ray sensitivity in R1 and Δdrb0067 strains.** (A) Sensitivity of R1 to UV (408 J/m<sup>2</sup>) and gamma-radiation (2 kGy) with the addition of 10 mM dNMPs or 10 mM dGMP. (B) Sensitivity of R1 and Δdrb0067 to UV (408 J/m<sup>2</sup>) and gamma-radiation (2 kGy). R1, D. radiodurans wild type strain; Δdrb0067, the dbb0067 null mutant. (TIF)

**Figure S2** Gamma-radiation enhances the secretion of active extracellular nuclease. The cells were cultured in TGY until the OD<sub>600</sub>=1.0, treated with 2 kGy or 4 kGy gamma-radiation, and then centrifuged to collect the supernatant for enzyme reaction. The extracellular nuclease is secreted more from D. radiodurans after treatment. But no obvious extracellular nuclease activity change is observed from Δdrb0067 after gamma-radiation treatment. M denotes molecular standards. R1, D. radiodurans wild type strain; Δdrb0067, the dbb0067 null mutant. (TIF)

**Table S1 Strains, plasmids and primers used in this study.**

(DOC)

**Author Contributions**

Conceived and designed the experiments: YH LW ML HS. Performed the experiments: ML HS. Analyzed the data: ML HS QF HL YZ. Contributed
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