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To Investigate the Functional Interplay Between TDR1 and the DNA Repair Pathway in Tardigrades Following Exposure to Ionizing Radiation

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Abstract. Tardigrades are renowned for their remarkable tolerance to extreme conditions. Their resistance relies not only on some protective proteins such as DSUP, but also on highly efficient DNA repair mechanisms. A novel tardigrade-specific gene, Tardigrade DNA Damage Response1 (TDR1), was identified, and it plays an important role in DNA repair through non-canonical mechanisms, while the classical repair factor XRCC5 (Ku80) is significant for tardigrades to respond radiation. Do TDR1 and XRCC5 merely co-express or do they function synergistically? This question is still unexplored. Does the loss of TDR1 affect the expression of XRCC5 and then impact the efficiency of DNA repair? Does the loss of XRCC5 cause the compensatory expression of TDR1? We hypothesize TDR1 and XRCC5 have functional interactions and work synergistically to enhance the DNA repair capacity of tardigrades after radiation exposure. Tardigrade Hypsibius exemplaris works as a model organism. Establishing of radiation gradient (100 Gy and 2000 Gy) and analyzing different time points (4 h and 24 h) can help to determine the optimal radiation dose and time point for subsequent experiments. Then, CRISPR/Cas9 technology can help construct knockout mutant strains of TDR1 and XRCC5, and set wild type and empty vector control groups. Mutant and control groups are exposed to 2000 Gy of radiation, and their DNA damage and repair response is evaluated using the following parameters: the intensity and persistence of γ-H2AX foci, and changes in the mRNA and protein expression levels of XRCC5 (Ku80) and TDR1. After knocking out TDR1, there is more persistent and stronger γ-H2AX signal (suggesting repair defects), and XRCC5 compensatory mechanism for upregulation of expression. After knocking out XRCC5, γ-H2AX signal is stronger, with an obvious upregulation of TDR1, which demonstrates TDR1 may serve as an alternative or auxiliary repair pathway to be activated. Therefore, this study functionally demonstrates that TDR1 and XRCC5 play a critical, synergistic, and complementary role in the exceptional radiation resistance of tardigrades.

Keywords: tardigrades, DNA repair, radiation resistance, TDR1 (Tardigrade DNA Damage Response1), XRCC5 (Ku80), CRISPR/Cas9, γ-H2AX, gene knockout

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1. Introduction

1.1. Extreme environmental adaptability of tardigrades

Tardigrades are microscopic organisms widely distributed in marine, freshwater, and semi-terrestrial habitats (such as mosses and lichens), renowned for their remarkable tolerance to extreme conditions including desiccation, freezing, osmotic stress, and infrared radiation [1,2]. This unique ability to withstand various environmental extremes has earned them the status of the "toughest animals on Earth" [3]. Different species exhibit significant variations in resistance to extreme conditions. For example, Ramazzottius oberhaeuseri can tolerate rapid dehydration, whereas Hypsibius dujardini only adapts to gradual water loss [4]. The freshwater species Thulinius ruffoi completely lacks desiccation tolerance [5]. In terms of radiation resistance, most species can tolerate doses exceeding 4,000 Gy [6]. However, certain species (e.g., Echiniscoides sigismundi) exhibit a median lethal dose (LD50) of only 1,000 Gy [7], and their tolerance thresholds for maintaining reproductive capacity are even lower (e.g., merely 100 Gy in Hypsibius exemplaris) [8]. Whole-genome analyses reveal that tardigrades exhibit the highest ratio of gene gain to loss among metazoans [9], and multiple stress-resistance-related gene families (such as CAHS, MAHS, SAHS, AMNP) have been identified [10,11]. However, functional validation of these genes remains constrained by limitations in gene-editing techniques [12].

1.2. Radiation damage-related proteins and genes

A novel tardigrade-specific protein Damage suppressor (Dsup) was identified by Hashimoto and Kunieda [6]. Dsup in human cells can increase resistance to DNA damage induced by ionizing radiation (IR) or hydrogen peroxide treatment, whereas in tardigrade Ramazzottius varieornatus it binds to nucleosomes and protects chromatin from hydroxyl radicals, demonstrating a function similar to that of a Dsup ortholog found in tardigrade H. exemplaris [13]. A conserved region within Dsup proteins exhibits sequence similarity to the nucleosome-binding domain of vertebrate HMGN proteins, and this region is functionally important for facilitating nucleosome binding and providing protection from hydroxyl radicals [13]. In conclusion, Dsup promotes the survival of tardigrades under diverse conditions by a direct mechanism that involves binding to nucleosomes and protecting chromosomal DNA from hydroxyl radicals [13]. However, subsequent studies suggest that tardigrade radiation resistance depends not solely on protective proteins like Dsup, but also on highly efficient DNA repair mechanisms [14]. In support of this, a novel tardigrade-specific gene, Tardigrade DNA Damage Response1 (TDR1), was identified by Marwan Anoud and his team, which exhibited significant upregulation post-irradiation, and validated its functions of radiation-induced expression, binding DNA and facilitating repair through aggregation, and DNA protection in heterologous systems [14]. TDR1 is a critical factor enabling tardigrade radiation resistance through unique DNA-binding properties, and its non-canonical repair mechanism opens new research avenues [14].

1.3. Rationale

Some evidence indicates that the classical repair factor XRCC5 (Ku80) in tardigrades is strongly upregulated after radiation, which is directly associated with their survival advantage [15]. Recent studies have revealed that intrinsically disordered proteins (IDPs) finely regulate DNA repair through phase separation and dynamic assembly. Particularly in extremophiles, IDPs have been

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confirmed to coordinate DNA repair response networks (e.g., the tardigrade TRID1 protein). This reveals that IDPs may be a potent contributing factor to the robust radiation resistance of tardigrades [16]. The novel IDP factor TDR1 in tardigrades exhibits characteristics highly associated with radiation repair: it can be synchronously and significantly upregulated with classical repair genes such as XRCC5, co-localizes with DNA damage foci in vivo, and directly binds to DNA in vitro. However, the potential functional relationship between the novel TDR1 and the canonical XRCC5-mediated repair pathway remains entirely unexplored. Therefore, we hypothesize that TDR1 functionally interacts with the classical repair machinery (XRCC5) [14].

2. Project description

2.1. Research question

Do TDR1 and XRCC5 merely co-express or do they function synergistically? Does the loss of TDR1 affect the expression of XRCC5, and does it impact the efficiency of DNA repair?

2.2. Hypothesis

We hypothesize that TDR1 and XRCC5 cooperate in tardigrades to enhance DNA repair following radiation-induced damage. To test this, we will use CRISPR/Cas9 technology to knock out TDR1 and XRCC5, and then measure the changes in γ-H2AX signal, XRCC5 mRNA, and protein levels after exposure to IR.

3. Method

3.1. Establish radiation gradient treatment in model organisms

We expose tardigrades H. exemplaris to varying levels of radiation, 100 Gy and 2000 Gy, and measure the mRNA levels in their bodies. Then we compare mRNA levels under different radiation gradients at the same time point, 4 hours and 24 hours, respectively. The reason why we expose H. exemplaris to 100 Gy and 2000 Gy is that it is based on the research of Anoud M's team [14], and the LD50 of H. exemplaris is 4360 Gy.

3.2. Crispr/Cas 9 technology

3.2.1. Knock out TDR1

We use CRISPR/Cas9 technology to knock out TDR1 in H. exemplaris and set up wild-type and Cas9 control (with intact CRISPR/Cas9 but no gene knockout) [17]. We expose the control and experimental groups to a certain radiation dose, then recordthe γ-H2AX signal along with XRCC5 mRNA and protein levels post-irradiation.

3.2.2. Knock out XRCC5

We utilize CRISPR/Cas9 technology to knock out XRCC5 and set up wild-type and Cas9 control (with intact CRISPR/Cas9 but no gene knockout). Then we expose the control and experimental groups to a certain radiation dose, and then record the γ-H2AX signal along with XRCC5 mRNA and protein levels post-irradiation.

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4. Expected results

4.1. Different responses of radiation gradients and time points

Table 1. mRNA levels under 100 Gy at 4 h and 24 h post-irradiation

	XRCC5 mRNA	TDR1 mRNA
4 h	++	-
24 h	-	-

-: no up-regulation. ++: an obvious up-regulation.

In Table 1, at 4 h post-irradiation, XRCC5 mRNA is obviously up-regulated, but TDR1 mRNA shows stable. Because XRCC5 responds to DNA damage rapidly and TDR1 may not be activated or to have a delayed response. At 24 h post-irradiation, XRCC5 mRNA returns to normal level as the preliminary repair mechanism is completed, while there is no significant change or slight increase of TDR1 mRNA.

Table 2. mRNA levels under 2000 Gy at 4 h and 24 h post-irradiation

	XRCC5 mRNA	TDR1 mRNA
4 h	++	++
24 h	++/+	++

+: up-regulation. ++: an obvious up-regulation.

In Table 2, at 4 h post-irradiation, both of TDR1 and XRCC5 are evidently up-regulated as they respond strongly to DNA damage. At 24 h post-irradiation, XRCC5 mRNA might keep a high level or start to decrease, which depends on the repair process. TDR1 mRNA may continue to be up-regulate or reach even higher levels.

It is concluded that both TDR1 and XRCC5 are involved in DNA repair. Based on the responses above, we decide to expose H. exemplaris under 2000 Gy and record the γ-H2AX signal, XRCC5 mRNA, and protein levels at 4 h post-irradiation.

4.2. Interaction between TDR1 and XRCC5

Table 3. The γ-H2AX signal, XRCC5 mRNA, and protein levels under 2000 Gy at 4 h post-irradiation

Experimental Group	γ-H2AX signal	XRCC5 mRNA	TDR1 mRNA	Ku80	TDR1
TDR1 Knockout	+	+	-	+	-
XRCC5 Knockout	++	-	+	-	+

-: no up-regulation. +: up-regulation. ++: an obvious up-regulation

In Table 3, the results show that after knocking out TDR1, γ-H2AX signal is stronger than that of the wild type and maintains longer time, which suggests a decrease of DNA repair efficiency.

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XRCC5 mRNA might be up-regulated more significantly because of compensatory mechanisms attempt to repair. Ku80 may express more but its function is not enough for complete DNA repair.

After knocking out XRCC5, γ-H2AX signal is significantly enhanced and sustains. TDR1 mRNA might be obviously up-regulated as TDR1 may serve as an alternative or auxiliary repair pathway to be activated. TDR1 increased because it may try to compensate for the repair defects caused by XRCC5 deficiency.

5. Conclusion

Based on the experiments above, we have some conclusions. First, TDR1 and XRCC5 are co-upregulated under IR. Our experimental design contains radiation gradient and CRISPR/Cas 9 to knock out TDR1 or XRCC5. And then we detect the γ-H2AX signal, XRCC5 mRNA, and protein levels to analyze the interplay between TDR1 and XRCC5. They have dose-dependent co-expression. Under low dose of 100 Gy, XRCC5 responds at 4 h but TDR1 not. Under high dose of 2000 Gy, both of them respond. When TDR1 is knocked out, Ku80 may express more, and γ-H2AX signal increases. After knocking out XRCC5, TDR1 increased because it may try to compensate for the repair defects caused by XRCC5 deficiency, and γ-H2AX signal persists. Consequently, we conclude that TDR1 and XRCC5 are co up-regulated under IR and both of them play a key role in DNA repair.

References

[1] Jönsson K. I. (2019). Radiation Tolerance in Tardigrades: Current Knowledge and Potential Applications in Medicine. Cancers, 11(9), 1333. https: //doi.org/10.3390/cancers11091333
[2] Guidetti, R., Altiero, T., & Rebecchi, L. (2011). On dormancy strategies in tardigrades. Journal of insect physiology, 57(5), 567–576. https: //doi.org/10.1016/j.jinsphys.2011.03.003
[3] J. Copley, Indestructible, New Sci. (1999). https: //www.newscientist.com/ article/mg16422095–100-indestructible/ (accessed September 5, 2022).
[4] Wright JC. 1989. Desiccation tolerance and water- retentive mechanisms in tardigrades. J Exp Biol 142: 267–292. DOI: https: //doi.org/10.1242/jeb.142.1.267
[5] Kondo K, Mori M, Tomita M, Arakawa K. Pre-treatment with D942, a furancarboxylic acid derivative, increases desiccation tolerance in an anhydrobiotic tardigrade Hypsibius exemplaris. FEBS Open Bio. 2020; 10(9): 1774-1781. doi: 10.1002/2211-5463.12926
[6] Hashimoto T, Kunieda T. DNA Protection Protein, a Novel Mechanism of Radiation Tolerance: Lessons from Tardigrades. Life (Basel). 2017; 7(2): 26. Published 2017 Jun 15. doi: 10.3390/life7020026
[7] Jönsson KI, Hygum TL, Andersen KN, Clausen LK, Møbjerg N. Tolerance to Gamma Radiation in the Marine Heterotardigrade, Echiniscoides sigismundi. PLoS One. 2016; 11(12): e0168884. Published 2016 Dec 20. doi: 10.1371/journal.pone.0168884
[8] Beltrán-Pardo E, Jönsson KI, Harms-Ringdahl M, Haghdoost S, Wojcik A. Tolerance to Gamma Radiation in the Tardigrade Hypsibius dujardini from Embryo to Adult Correlate Inversely with Cellular Proliferation. PLoS One. 2015; 10(7): e0133658. Published 2015 Jul 24. doi: 10.1371/journal.pone.0133658
[9] Guijarro-Clarke C, Holland PWH, Paps J. Widespread patterns of gene loss in the evolution of the animal kingdom. Nat Ecol Evol. 2020; 4(4): 519-523. doi: 10.1038/s41559-020-1129-2
[10] Hesgrove C, Boothby TC. The biology of tardigrade disordered proteins in extreme stress tolerance. Cell Commun Signal. 2020; 18(1): 178. Published 2020 Nov 4. doi: 10.1186/s12964-020-00670-2
[11] Arakawa K. Examples of Extreme Survival: Tardigrade Genomics and Molecular Anhydrobiology. Annu Rev Anim Biosci. 2022; 10: 17-37. doi: 10.1146/annurev-animal-021419-083711
[12] Goldstein B. Tardigrades. Nat Methods. 2022; 19(8): 904-905. doi: 10.1038/s41592-022-01573-5
[13] Chavez C, Cruz-Becerra G, Fei J, Kassavetis GA, Kadonaga JT. The tardigrade damage suppressor protein binds to nucleosomes and protects DNA from hydroxyl radicals. Elife. 2019; 8: e47682. Published 2019 Oct 1. doi: 10.7554/eLife.47682

DOI: 10.54254/2753-8818/2026.AU29336

[14] Anoud M, Delagoutte E, Helleu Q, et al. Comparative transcriptomics reveal a novel tardigrade-specific DNA-binding protein induced in response to ionizing radiation. Elife. 2024; 13: RP92621. Published 2024 Jul 9. doi: 10.7554/eLife.92621

[15] Clark-Hachtel CM, Hibshman JD, De Buysscher T, Stair ER, Hicks LM, Goldstein B. The tardigrade Hypsibius exemplaris dramatically upregulates DNA repair pathway genes in response to ionizing radiation. Curr Biol. 2024; 34(9): 1819-1830.e6. doi: 10.1016/j.cub.2024.03.019

[16] Feng LL, Bie SY, Deng ZH, et al. Ubiquitin-induced RNF168 condensation promotes DNA double-strand break repair. Proc Natl Acad Sci U S A. 2024; 121(28): e2322972121. doi: 10.1073/pnas.2322972121

[17] Kondo K, Tanaka A, Kunieda T. Single-step generation of homozygous knockout/knock-in individuals in an extremotolerant parthenogenetic tardigrade using DIPA-CRISPR. PLoS Genet. 2024; 20(6): e1011298. Published 2024 Jun 13. doi: 10.1371/journal.pgen.1011298