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Discovering the importance of anti-oxidant in radiation resistance using the model of Eisenia Fetida

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Abstract:

Current studies have revealed that the stem cells of various animal species exhibit high levels of ionizing radiation, which is a phenomenon that requires further investigation to fully understand its underlying mechanisms. It is well established that antioxidants can protect organisms from radiation damage primarily by inactivating free radicals. Therefore, we propose the bold hypothesis that antioxidants are the primary contributors to the radiation tolerance of animal stem cells. To test this hypothesis, we plan to use *Eisenia Fetida* as a model organism to identify the potential antioxidant enzymes and proteins within its cells, then investigate whether these molecules are crucial factors in the overall radiation resistance of the organism. The conclusions drawn from this hypothesis could contribute to a broader theoretical framework in the field and benefit future research efforts.

Keywords: *Eisenia Fetida;* anti-oxidant; radiation resistance

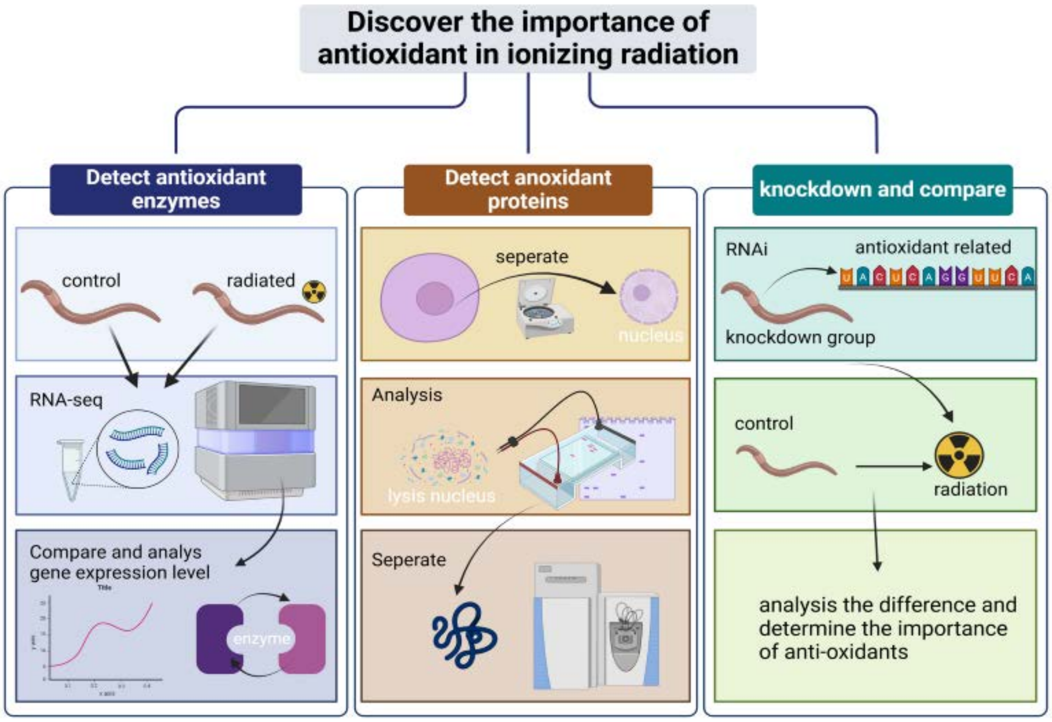

Figure 1: graphical abstract of the proposal

1. Introduction

1.1 . Eisenia Fetida

Eisenia Fetida, commonly known as the red wiggler worm, is a kind of earthworm with a lifespan of 1 to 5 years[1], and a commonly-used organism in bio-monitoring soil pollution[2]. The organism can endure high doses of radiation—there can still be a 50% survival rate for the organism 30 days after being exposed to 65kR (6500Sv) of radiation, and the survival rate under 24kR follows a similar pattern to that of the control group.[3]. They can also have 49 cocoons laid and 10 hatched under conditions of 60Gy of gamma radiation, meaning that its cocoon viability is not affected seriously[4]. While for humans, 10Sv could lead to death in few weeks. The organism has a high regenerative ability, and is a widely-used model for regeneration experiments. *Eisenia Fetida* has been well studied as a model to test for toxicity of chemicals[5] [6] and its ability of regeneration while being cut into pieces[7]. However, we currently lack direct evidence that illustrates the mechanism of its regeneration, and few studies have been conducted to the stem cells of the organism or the mechanisms behind its generation abilities.

1.2 . Effect of radiation to organisms

Ionizing radiation is defined as energy capable of removing electrons from atoms and, thereby, causing ionization. The three main ionizing radiation technologies are gamma radiation technology (based on photons), electron beam (eBeam) technology (based on electrons), and X-rays (based on photons)[8]. The destructive effects of ionizing radiation (IR) are associated with two main factors: direct damage to DNA and indirect damage through the generation of reactive oxygen species (ROS) and free radicals by radiolysis of water[9]. Additionally, superoxide radicals, also produced during water radiolysis, are believed to accumulate within microbial cells, causing severe damage to proteins, particularly enzymes with exposed iron-sulfur clusters[10].

1.3 . Anti-oxidants

All cells contain antioxidant systems that specifically detoxify superoxide and hydrogen peroxide or contribute to the defense against ROS[11]. Antioxidants can act through various mechanisms, including scavenging free radicals, sequestering transition metal ions, decomposing hydrogen peroxide, quenching active pro-oxidants, and enhancing endogenous antioxidant defenses. Additional-

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ly, antioxidants can aid in repairing cellular damage[12]. Consequently, the survival of animal stem cells across several species when exposed to high doses of radiation. Common antioxidants include vitamin C, vitamin E, and tea polyphenols. Recent discoveries have highlighted the significant role of antioxidants in radiation resistance[13], prompting us to investigate their profound effects on the radio-resistance of organisms through this proposal.

1.4 . Purpose for the proposal

Through the proposal, we hope to promote the development of radiation resistance and fill the blank in general conclusion in the field of stem cell radiation resistance, so that cancer cells can be better dealt. For some patients that have cancer, after several months of their radiation therapy, the cancer cells would come back with radiation resistance with undiscovered mechanisms. If the theory is proved to be right, we may be able to explain the phenomenon and prevent it in advance. This can also provide a higher radiation resistance to human cells in the future, reducing the harm radiation resistance brought to normal body cells of humans. We humans can also have a higher radiation resistance to prevent possible nuclear leakages in the future.

2. Project description

The main goal of this project is to identify potential anti-oxidant enzymes or proteins in *Eisenia Fetida* and test if they are the essential factors for the stem cells to resist radiation. We hypothesize that anti-oxidants exist in the stem cells of *Eisenia Fetida*, and would use various ways to detect them. After detection, we would knock off the corresponding protein using RNAi and measure its radiation resistance after protein being knocked out, and compare with stem cells that have not been modified. We expect to see obvious changes in the radiation resistance of the organisms to be a proof of our hypothesis.

2.1 . Objective 1

To detect potential anti-oxidant enzymes in the stem cell

2.1.1 . Rationale

Under radiation conditions, anti-oxidant related genes would be over-expressed to resist radiation.

2.1.2 . Method

The Eisenia Fetida will be divided into two groups: a control group and a radiated group. The radiated group will be further subdivided to expose it to different doses of ionizing radiation, while the control group will be maintained under the same conditions without radiation. To prevent damage to the organisms caused by excessive radiation, which could bias the experimental results, the radiated group will include tests with doses of 20kR, 25kR, 30kR. The mRNA from the stem cells will be extracted using extraction kits at appropriate time points (before radiation, 12 hours after, and 24 hours after). To ensure the concentration and purity of the RNA, NanoDrop and Agilent Bioanalyzer will be utilized. Next, the RNA population intended for sequencing will be converted into cDNA fragments through reverse transcription. The cDNA will then be fragmented, and adapters will be added to each end. Polymerase chain reaction (PCR) will be employed to amplify the cDNA library. Subsequently, the cDNA library will be loaded onto a sequencing platform (e.g., Illumina) to generate reads. To identify the genes encoding antioxidant enzymes, the aligned sequences will be annotated. The final step will involve comparing the gene expression levels between the radiated and control groups to identify differential expressions.

2.1.3 . Expected outcome

The expected outcome of the experiment is to identify a series of antioxidant enzyme genes and determine the changes in their expression levels. The following step is to further analyze the impact on stem cells after knocking down the antioxidant-related genes.

2.2 . Objective 2

To detect potential anti-oxidant proteins in the stem cell

2.2.1 . Rationale

The essay 'Chromatin analysis of adult pluripotent stem cells reveals a unique stemness maintenance strategy' [14] shows that chromatin proteins can be the potential anti-oxidant proteins in *Schmidtea mediterranea*, so we decided to try similar strategies in our model.

2.2.2 . Method

First, we would centrifuge the stem cell to get its nucleus as the nucleus is the heaviest organelle in a cell. This is to separate the chromatin from the unwanted cells. Then, we would break the nucleus and release the genetic material inside, following by a mass spectrometry to the protein to get its sequencing. In the sequencing, I would find the non-histone proteins that exists in the chromatin. Furthermore, I knock the proteins down for one group of *Eisenia Fetida*, the other group remains unchanged. At last, compare the number of free radicals inside the cells including DPPH, hydroxyl and superoxide anions to observe changes due to the lack of this protein.

2.2.3 . Expected outcome

We expect a significant rise of free radical amount if the anti-oxidant proteins have been knocked down. If the situation happens, we can be sure that the proteins knocked down are the anti-oxidant proteins. This is to prepare for the knock-down of the anti-oxidants in objective 3.

2.3 . Objective 3

To knock down the proteins using RNAi and observe the changes of radiation resistance

2.3.1 . Rationale

The importance of anti-oxidants in radiation resistance in the organism can be proved by knocking out the corresponding protein or enzyme that has been detected using RNAi, and compare its radiation resistance with organisms that has not been gene-edited.

2.3.2 . Method

First, we will knock down the potential anti-oxidant enzymes and proteins by using RNAi, which is to induce double-strand RNA into the stem cells that is complementary to the anti-oxidant related RNA. Then, we will compare the group with another group of unmodified *Eisenia Fetida* by putting them into same condition of irradiation, then observe their survival curve through time, and by testing their survival time after being irradiated. This is able to prove the importance of anti-oxidants in Eisenia Fetida in the field of radiation resistance. If there is a significant drop of highest doses endured and a downward shift of the survival curve, then we can conclude that anti-oxidants are indeed crucial factors in radiation resistance in *Eisenia Fetida*, vice versa. If the organisms almost lose all their radiation resistance, we can conclude that the anti-oxidants are one of the main factors for animals to resist radiation.

2.3.3 . Expected outcome

According to our hypothesis, we hypothesize that the ability to resist radiation for the gene-modified stem cells is going to drop significantly comparing to the original group. We expect the surviving rate and the highest doses the stem cells are able to resist would both be decreasing significantly. If the results are the same as our hypothesis, then our hypothesis of anti-oxidant being a main factor of radiation resistance would gain one more evidence, and similar methods could be used to other organisms in the area.

3. Conclusion

Overall, the proposal provides a hypothesis that anti-oxidants play a crucial role in radiation resistance of stem cells, and is trying to prove it with the model of *Eisenia Fetida*. Through the proposal, the ability of anti-oxidants to resist radiation damage in *Eisenia Fetida* would be proved. In further studies, other similar species will be tested using similar procedures to find more proofs for the hypothesis

Tianyin Zhao, Yuhan Li and Xingshuaihao Chen contributed equally to this work and should be considered co-first authors.

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