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Macrostomum Lignano Quiescent Stem Cells (QNs) Orchestrate Distinct Mechanisms Following Gamma Ray Exposure

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Abstract. This research proposal aims to investigate the marine flatworm Macrostomum lignano, an ocean flatworm, which has a particularly powerful regenerative capacity, mainly relying on a cell called resting stem cells (QNs) in the body to maintain the balance of the body's tissues, and can also repair damage in the event of injury. Gamma rays can cause great harm to cells, such as destroying the double-stranded structure of DNA, causing oxidative stress reactions, and disrupting the cell cycle, which can seriously affect the function of stem cells. And how to coordinate the regeneration process. We plan to irradiate worms with 150 Gy gamma rays, which is expected to cause sufficient damage while preserving QN viability. We use transgenic report lines, single-cell RNA sequencing technology, HCR RNA-FISH technology, RNA interference technology, and long-term live imaging to observe the activation process of QN cells after exposure, and find out the key genes and signalling pathways that regulate QN cells, such as the DDR signal pathway, the Hippo/YAP signalling pathway, and the Wnt signalling pathway. How radiation works, such as whether they regulate the accuracy of DNA repair, whether they change the way they metabolize, or if they secrete some signalling molecules to help restore recovery. This study could help us better understand the role of QN cells in responding to radiation damage, and also give us more insight into the repair mechanisms of stem cell-mediated in other organisms with regenerative abilities.

Keywords: Macrostomum lignano, Quiescent Stem Cells (QNs), Gamma Rays, DNA Damage Response, Tissue Regeneration, Signal Pathways

1. Introduction

These stem cells are like backup systems that help organisms cope with the stresses of the environment and repair damaged tissue. The reason for this dormant stem cell may be to reduce the risk of genetic mutations and ensure that the tissue is healthy for a long time. When studying the law of stem cell activity, the Macrostom lignano is particularly suitable for the study of the stem cell system [1]. This is called dormancy. Some studies have found that these stem cells are activated when organisms encounter external pressure, but it is unclear what kind of repair role they will play after radiation damage [2].

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This suggests that QN may function similarly to different species. Unlike some other flatworms (e.g., planarians) that can regenerate a head, M. lignano has a different regenerative capacity and a unique stem cell control system, which necessitates separate study of its QN mechanism.

Recent studies have developed experimental methods for M. lignano, such as transgenic techniques that can label specific tissues, as well as single-cell RNA sequencing and long-term live observations [1]. Experiments have found that the proliferation stem cells of this organism temporarily stop growing after being exposed to gamma rays, but can begin to proliferate again within a few weeks. But now there are several important questions that are not clear: first, it is not known whether 150 Gy gamma rays will activate QN cells and help the regeneration process; and secondary to study which molecular pathways the QN cells will affect after exposure, such as DDR.

Previous studies have focused on the role of QN in other organisms and found that it could influence the DNA repair process through the ATM/ATR-p53 pathway [3]. In addition, QN is involved in metabolic reprogramming, such as converting cells from oxidative phosphorylation to glycation metabolism. In addition, QN also has some effect on paracrine signaling [4].

2. Methods

2.1. Animal culture and gamma ray irradiation

In this paper, we did an irradiation experiment, using gamma rays from a 60Co radioactive source, and the irradiated 1-2 mm long adult worms. The radiation dose received in the experimental group was 150 Gy, and the control group did not really radiate. We collected samples at different points in time, including just after the photo, and 6 hours, 24 hours, 7 days, 14 days, and 21 days later, so that we could see the changes in different time periods after the irradiation.

2.2. Transgenic reporter line construction

The main point of this paper is to use the Gibson Assembly to make QN-specific reporters. The specific approach is to clone QN-specific promoters such as piwi-1 and soxB, and then use them to control the expression of the fusion protein GeNL. GeNL is composed of mNeonGreen and NanoLuciferase, so that fluorescence and bioluminescence signals can be seen at the same time. Validate the line via confocal microscopy to ensure QN-specific expression [5].

2.3. scRNA-seq and bioinformatics analysis

We will focus on bioinformatics, and we used the CellRanger tool to deal with read alignment, and then used Seurat software for cell clustering and DEG analysis. To figure out which pathways are related to QN, we also did a condensed analysis, including GO and KEGG.

2.4. HCR RNA-FISH validation

We will focus on several important genes, including candidate genes such as p53, YAP, Wnt2, and Rad51, as well as the QN-labeled piwi-1. To study these genes, we specifically designed probes for the molecular instrument HCR kit [6].

We did a three-channel imaging experiment with a ZEISS LSM800 confocal microscope, mainly observing three different markers, of which the QN nucleus uses mScarlet red fluorescent markers, the candidate mRNA is marked with green fluorescence, and piwi-1 mRNA is displayed with far-red

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fluorescent markers. Analyze co-localization using Fiji/ImageJ to confirm candidate gene expression in QNs.

2.5. RNAi-mediated functional validation

We will focus on several important genes, such as p53, YAP, and Wnt2, that play a key role in cell growth. We also focus on a special type of double-stranded RNA, ccdB, that controls the activity of other double-stranded RNAs [1].

The first experiment was to soak the worms in a double-stranded RNA solution of 2 micrograms per millilitre, and let them stay in it for three weeks [7]. The second experiment irradiated the RNA-disrupting worms with a radiation dose of 150 Gy, and then observed their regeneration over the next 21 days to see what would change.

2.6. Quantitative analysis of regeneration and behavior

Images of free-moving transgenic worms for up to 3 weeks using a custom tracking microscope (squid platform) [5]. Track QN migration, division, and differentiation dynamics; quantifying regenerative rates (body length recovery) and behavioral activity.

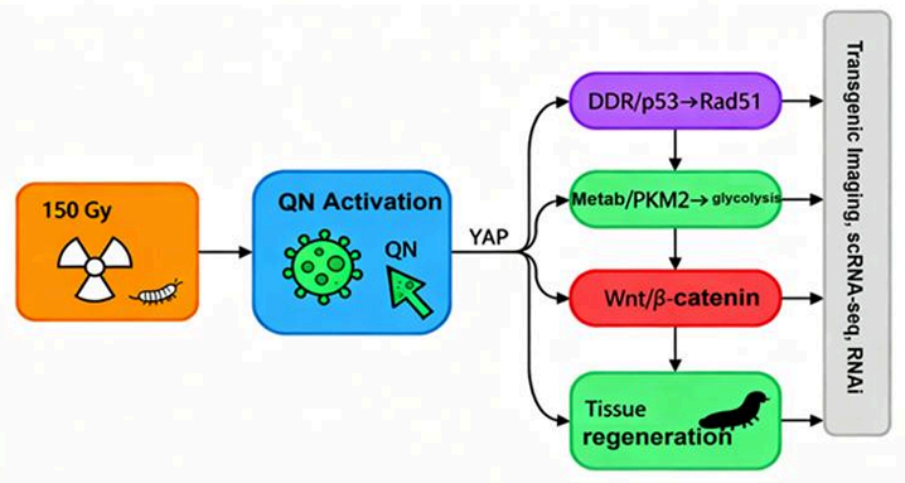


Figure 1. The diagram summarizes a mechanism where 150 Gy radiation activates quiescent stem cells (QN), which then, via the YAP hub, coordinate key pathways to drive tissue regeneration

3. Results

3.1. QN activation dynamics post-irradiation

In the experiment, we expect to found that QN cells exited from the G0 period between 24 and 48 hours after radiation exposure, a phenomenon that could be detected by the use of both EdU and BrdU markers, and the expression of GeNL increased. It was also seen that these cells moved to the wound position in about 7 days.

In the second case, the QNs cells themselves do not move, but they release paracrine factors, such as the Wnt ligand. Although these factors do not spread directly around them, they make it easier for nearby neo-oblasts to spread. In this case, QNs do not move, but what they secrete has an effect on the activity of the new mother cells, allowing these cells to spread better.

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In Scenario C, only a small fraction of QN cells is activated, at which point other types of stem cells may be helping to repair damaged tissue. Given the reserve function of QN cells themselves, this is unlikely to happen, as the body should prioritize the use of QN cells to cope with damage.

3.2. Key genes and pathways regulated by QNs

We will use single-cell RNA sequencing to find differential expression genes in QN, some of which have significantly increased expression, such as the Rad51 and BRCA1 genes involved in DNA repair, and some proteins involved in the DNA damage response pathway, such as ATM, ATR, Chk1, and Chk2, and also found changes in the expression of signalling molecules such as YAP and β-catenin [8, 9].

The paper, through GO and KEGG analysis, identified important biological pathways, mainly related to the process by which cells repair DNA, as well as the mechanisms that control cell division, and the way cells respond to oxidative stress, such as the NRF2 signalling pathway, which is a prime example of this. These results help us better understand how cells respond to various stresses.

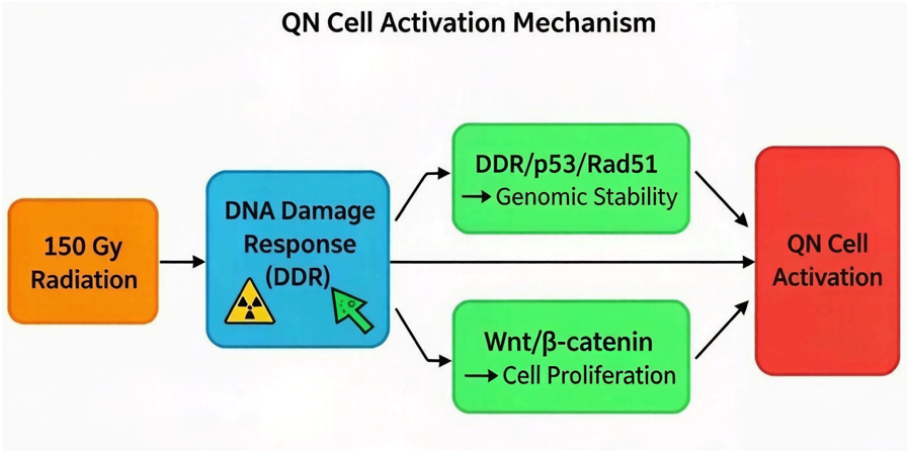


Figure 2. The schematic illustrates the core signalling pathways: radiation triggers DDR, which activates the DDR/p53/Rad51 axis (genomic stability) and Wnt/β-catenin pathway (cell proliferation), collectively leading to QN activation

3.3. Functional validation of candidate genes

RNAi of p53: Impaired DNA repair, increased QN apoptosis, and delayed regeneration [7].

RNAi of YAP: Reduced QN activation and proliferation, leading to incomplete tissue recovery.

RNAi of Wnt2: Disrupted QN-neoblast crosstalk, compromising regenerative coordination [10].

3.4. QN-mediated regeneration outcomes

Long-term imaging will show QNs contribute to ~30–50% of regenerated tissue (via lineage tracing).

Under normal circumstances, the behavior of the worms takes about two to three weeks to return to their original level, but for worms whose QN function is knocked out, their ability to move is still affected and cannot be fully restored like ordinary worms.

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4. Discussion

The proposed study will address the following key points:

(1) This proposal will apply new experimental methods, such as single-cell RNA sequencing, real-time imaging, and RNA interference techniques, to study what exactly the QN gene does when M. lignano is exposed to radiation. None of these methods have been used to study this biological model before. We deliberately selected a relatively low gamma-ray dose to do experiments, so that the repair process in which the QN gene is involved in being different from other stress responses, and it is clearer how the QN gene regulates this repair process [10, 11].

5. Conclusion

The main purpose of this paper is to find out how the QNs cells of M. lignano, which help the body to repair and regenerate after being exposed to gamma rays. We especially want to know how these cells work, which may be to directly repair DNA damage, or to regulate the transmission of signals in cells, or to help us to understand the surrounding cells by secreting some substances.

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