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The Investigation on the Function of Circular RNA in Adult Zebrafish Caudal Fin Regeneration

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Abstract. Zebrafish is an important model organism that is widely used in regenerative biology research due to its strong regenerative ability. The fin regeneration of zebrafish is a classic research model, and its regeneration mechanism includes cell migration, proliferation, differentiation, and precise regulation of gene expression. Nowadays, many researchers use zebrafish for experiments in the field of biological regeneration. Similarly, I will study the association between zebrafish tail regeneration and its intracellular circular RNAs. First, through high-throughput sequencing techniques (such as RNA-Seq), obtain the transcriptome data of zebrafish at different time points during fin regeneration. Secondly, use qPCR technology to quantitatively analyze the expression levels of specific circular RNAs at different time points during fin regeneration to evaluate their relationship with the regeneration process. (The observation days should be 1, 3, 5, and 7, because zebrafish tail regeneration is very rapid within 30 hours, so the observation days should not be too far apart, and the subsequent changes are small, and the observation is not very meaningful.) Finally, use Bioinformatics analysis and Luciferase Reporter assay techniques to determine specific circular RNAs and study their effects on the zebrafish tail regeneration process.

Keywords: Zebrafish, Circular RNA (circRNA), tail regeneration, fins regeneration

1. Introduction

Biological tissue regeneration is a biological process by which damaged or lost tissues completely recover to their original structure and function [1,2]. This phenomenon is widely observed in nature. The regenerative abilities of different organisms vary greatly [3,4]. From simple cell repair to complex organ regeneration, the mechanisms and processes of biological regeneration are an important field in biological research. There are many types of regeneration as well [5,6]. For instance, there are cell regeneration, tissue regeneration, organ regeneration, and regenerative individuals [7]. Among them, cell regeneration indicates that after the skin epidermal cells are damaged, they will rapidly divide to repair the wound [1]. Tissue regeneration can be exemplified by the liver's regenerative ability. Organ regeneration is like the fact that zebrafish can regenerate their fins and hearts, and Salamanders can regenerate lost limbs, tails, and even parts of organs such as the heart and eyes [2]. Examples of sea stars and certain types of snails not only can regenerate parts of their bodies but also can form new individuals through regeneration [8].

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The zebrafish (Danio rerio) is a widely used model organism in biomedical and developmental biology research [9]. Its unique regenerative ability makes it an important model for studying the mechanisms of regeneration. Zebrafish are capable of effectively regenerating their fins, tails and other tissues after injury [10]. This process involves complex biological mechanisms and has attracted significant attention from numerous researchers [11]. In the laboratory, researchers would surgically remove the fins of zebrafish to observe and document the regeneration process. Generally, techniques such as RNA sequencing and real-time quantitative PCR are employed to identify the role of circular RNAs in the regeneration process and their contribution to regeneration [12].

Circular RNA (circRNA) is a special type of non-coding RNA, characterized by its circular structure [13]. Unlike traditional linear RNA, circRNA is formed through reverse splicing into a closed loop, thus possessing high stability and tolerance [9,15]. Based on their sources and functions, circRNA can be classified into exon-based circRNA, which is typically expressed in specific cell types or under specific physiological conditions; intron-based circRNA, which is related to gene regulatory functions; and mixed-type circRNA (containing both exons and introns) [16].

2. Methods 1

Based on the strong tissue regeneration ability of zebrafish and the ability of its intracellular circular RNAs to promote tissue regeneration, a research direction can be provided regarding the connection between zebrafish tail regeneration and its intracellular circRNAs [17]. For instance, based on some other research experiments, it can be found that the circRNA that helps heart regeneration in zebrafish is ciRS-7, and the main circRNA that aids limb regeneration is circRNA_001033 [17]. Since it is impossible to clearly determine whether circular RNAs in zebrafish bodies play an important role in their tissue regeneration ability, I attempted to propose conducting experiments to investigate the functions of circular RNAs in the tail regeneration process of adult zebrafish.

Objective 1: Identify and characterize a certain circular RNA that are expressed differently in adult zebrafish after caudal fin injury

Rationale: After the tail of a zebrafish is injured, the expression of circRNAs in its body may change.

Methods1.1:

Models: In this experiment, adult zebrafish will be used as the experimental subjects. The experiment will be divided into two groups: a control group without tail removal and an experimental group with tail injury.

Tail severance experiment: The tail length of adult zebrafish typically accounts for approximately 25% to 30% of their total body length [9]. This experiment mainly involved using a scalpel to vertically remove half of the tail fin of the zebrafish. The exact length of the removed part was approximately between 0.5 and 1 centimeter [9,18].

circRNA purification: Within 3 days after the experiment, I will extract all the RNA from the tail of the zebrafish using the Total RNA extraction technique [17], and then use the RNase R technique to process some linear RNAs that are not needed for the study to reduce interference from RNA samples other than the research subjects [5]. Then, the remaining circRNAs were sequenced, and the sequencing results were subjected to bioinformatics analysis [17]. Finally, the luciferase reporter gene detection technique was used to obtain the experimental results, and it was examined whether there were indeed any changes in the expression of circRNAs in the zebrafish's tail regeneration compared to the normal condition [9,19].

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Results and Discussion 1.1: Through experiments, it was found that there are indeed some circular RNAs (labeled as A) in the tail of zebrafish, and their expression patterns vary during the regeneration process.

Objective 2: investigate the role of certain circular RNA

Rationale: To gain a deeper understanding of which specific circRNAs are involved in the tail regeneration experiment of zebrafish.

Methods 1.2:

Model: adult zebrafish

Search for functional circRNAs: Use the method in object1 to identify which circRNAs were specifically expressed in the experiment. The qRT-PCR technique was then used to confirm that A circRNA plays a significant role in the zebrafish tail regeneration experiment [14,20]. This is because we can observe the interaction between A circRNA and specific proteins related to regeneration in the zebrafish body, thereby inferring that the effect of A circRNA is considerable. The distribution of A circRNA in the zebrafish tail was determined using in situ hybridization technology [12].

Results and Discussion: Experiment to determine the effect of A circRNA on zebrafish tail regeneration.

3. Conclusion

Summary: The main purpose of this project is to further elucidate the role of circRNA in the tail regeneration experiment of zebrafish. The specific contents include determining whether circRNAs do indeed have an effect on regeneration and identifying the circRNAs that have the greatest impact on regeneration.

Limitation: This project only observed data related to regeneration, lacking research on other regeneration mechanisms.

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