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Circular RNA: Identifying the Synergistic Effects of Circular RNA and miRNA in the Regeneration of Muscle Tissue in an Axolotl

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Abstract:

Circular RNAs are unique endogenous genes. They maintain various functions, especially in regeneration, including miRNA sponges, interaction with RBPs, etc. Among them, the miRNA sponge is spotlighted. However, Our current understanding of the functions of circular RNAs, especially miRNA sponges, mainly focuses on the development and treatment of diseases such as cardiovascular disease, cancers, Alzheimer's disease, and so on. The knowledge about the role it plays in the regeneration of animals is limited nowadays.To overcome intellectual barriers, I will select axolotl as an ideal model to study the functions of circular RNAs in animal regeneration due to their high regerative capacity. Although circular RNAs have been proven to play a significant role in the regeneration of axolotl's limbs, the accurate mechanism in the regeneration of muscle tissue deserves to be studied. In this paper, I aim to discover the connections between miRNA sponge and the regeneration of muscle tissue in axolotl by locating the miRNA in axolotl and comparing the abundance of specific miRNA and relevant circular RNAs in the normal stage and regeneration stage. Ultimately, the purpose of this paper is to assess if the circular RNAs will influence the regeneration of muscle tissue in axolotl and its pathway. The result of this paper may provide a shred of evidence about the regulation of circular RNAs in the regeneration of animals

Keywords: circular RNA, regeneration, miRNA sponge, muscle tissue, axolotl.

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1. Introduction

1.1 Circular RNA

Circular RNAs are a kind of endogenous genes, which does not code any class of protein. In 1976, circular RNAs were detected in human HeLa cells [1]. Circular RNAs form as the result of back-splicing events. These normally consist of a reaction between the 3' and 5' ends of the first and last exon, respectively. This gives a closed-loop structure with circular RNA [2]. Owing to this, circular RNAs possess more stability than linear RNA structures. Functions of circular RNA have been evaluated with the development of continuous research in recent years, including miRNA sponges, regulation of gene expression, and interaction with RBPs [3] Although vital roles played by circular RNA, especially the miRNA sponge in regenerations, have been noticed, there are limitations when we recognize their specific functions during the regeneration of particular species and tissues, especially muscle tissues.

1.2 miRNA sponge

MiRNAs (microRNAs) are powerful endogenous genes that can regulate gene expression [4]. They participate in several bioprocesses, including regeneration of cells, apoptosis, and myoblast differentiation. At the same time, repairing and the regeneration of tissues also depend on the functions of miRNAs [5].

MiRNA sponge is one of the most significant functions of circular RNA to regulate the expression of genes. Circular RNAs impact the process of translation, circularity of mRNA, and transcription through sponging miRNA [6] According to several types of research, a part of specific circular RNAs, such as circular FGFR4, those that lead to the miRNA sponges, have been proven to lead relevant miRNAs to be inactive [6]. However, similar circular RNAs and their functions are not ensured in the muscle tissue of axolotl during regeneration. Therefore, advanced research should be accomplished to evaluate it as scientists study their functions.

2. Specific Stem Cells During the Regeneration of Muscle Tissues and Model Used for the Experiment

Stem cells are specific cells that have the self-capability to differentiate into a variety of functional cells that serve in different tissues and organs. Based on various stages of development, stem cells can be divided into two types---embryonic stem cells, which can differentiate into all kinds of cells, and adult stem cells, which only work on cells in a specific tissue. In skeletal muscle regeneration, satellite cells (SCs) are adult stem cells, working as a prerequisite, which will be activated, expended, and differentiated [7]. However, the recognition of functions of circular RNA in muscle tissue is limited. To observe functions of circular RNA in stem cells and animal regeneration in muscle tissues, axolotl is an ideal model.

Axolotl is a kind of amphibian that mainly lives in several lakes in Mexico [8]. Axolotl have a highly ability of regeneration. They maintain features of laver for a long period even in the stage of adulthood [9]. This means that adult axolotl can regenerate various organs or tissues, differing from most species. On the one hand, axolotl can highly regenerate, originating from the formation of regeneration-specific blastema with activation of heterogeneous progenitor cells, especially in limbs, the brain, the heart, and so on. On the other hand, comparing the genome of axolotl and humans, 5331 unique circular RNAs exhibited orthology with human circular RNAs among 35,956 putative circular RNAs in axolotl [10]. these are also the reasons why it can be regarded as a model organism used in research on regeneration.

3. Methods

RNA sequence is a tool, which is applied for analyzing expressions of genes and discovering new RNAs, including circular RNA [11]. Differing from linear RNA, circular RNA can be sequenced by degrading extra linear RNA through exonuclease R, followed by RNA sequence.

In addition, to identify the miRNA, which can be potentially sponged by relative circular RNA. Based on the similarity between the genome of humans and axolotl, the method, which is related to RNA target prediction, enrichment analyses, and visualization, can be utilized to compare the lists of circular RNA coming from RNA sequence done for axolotl with the final data frame of circular RNA responding with miRNA, which will be obtained through merging lists of annotated circular RNA and miRNA–circular RNA interactions through matching CircbaseID [12]. Biotin-Coupled miRNA and circular RNA Capture are methods, which can test the interrelationship between circular RNA and miRNA. this method describes the combination of biotin-coupled probes and the targeting RNA and capturing relevant circular RNA or miRNA by streptavidin-coated magnetic beads. To observe change of abundance of circular RNA or miRNA during regeneration, qPCR will work to locate and present a visual result [12].

Objective 1: locations of circular RNA existing in axolotl and linear relationship between circular RNA and miRNA sponge.

Rationale: circular RNA is a unique material undergoing back-splicing, which prevents circular RNA away from digesting with the addition of specific RNA enzymes. This provides independent individuals for sequence, avoiding interrupting by linear RNA. At the same time, the similarity of the genome existing between axolotl and humans offers the sample for us to figure out the relationship between circular RNA and miRNA in the muscle tissue of axolotl.

Method: Exonuclease R deposal and RNA sequence: To investigate the location and identify circular RNA presenting in muscle tissues of axolotl, I will extract cells coming from muscle tissue in limbs of axolotl. Dispose the cells with exonuclease R to digest all linear RNA and restore circular RNA. Sequence circular RNAs which exist in the muscle tissue of axolotl to obtain specific lists of them.

Circular RNA target prediction, enrichment analyses, and visualization. According to the circular RNA lists, I will download the annotated circular RNA and miRNA-circular RNA interactions from the CricBank database and human database to acquire a circular RNA frame by merging them. Specific circular RNA that caused the miRNA sponge will be observed by matching the CircbaseID between frame and circular RNA lists [12].

Objective 2: The functions of miRNA sponge in muscle tissue regeneration of axolotl.

Rationale: Abondance of circular RNA and miRNA captured during regeneration differ from normal stages presenting in axolotl. According to this, by comparing the change in abundance of circular RNA or miRNA captured during stages of regeneration and non-regeneration, the relationship between circular RNA and miRNA can be determined.

Method: Biotin-coupled miRNA and circular RNA Capture. During this experiment, amounts of axolotl will be divided into two groups- the control group and the experimental group. I plan to narcotize all axolotl and cut down the limbs of experimental axolotl. For the first step, to ensure that circular RNA can bind with the miRNA, according to the specific sequence tested by RNA sequence, a mimic of a particular miRNA will be created and inserted into the muscle tissue of all axolotl through incision by electroporation, which induces nucleic acid to enter cellular cytoplasm through transient pores created by electrical pulses in plasma membrane [13]. After cultivation with a piece of time, streptavidin-coated magnetic beads will be utilized to capture circular RNA. At the end of the experiment, an abundance of circular RNA will be presented by qPCR. By comparing the changes in the abundance of circular RNA between the control group and the experimental group, we can prove that circular RNA will regulate the regeneration of muscle tissues in axolotl by miRNA sponge [12].

4. Conclusion

In conclusion, circular RNAs can influence the process during regeneration through various functions, especially miRNA sponges. Through these methods, we can assess and evaluate the functions of circular RNAs, which work as miRNA sponges in regeneration in muscle tissue of axolotl. Therefore, an initial identification will be made to clarify that there is an innegligible bond between circular RNAs and regeneration in animals. The current study is in its infancy. Thus, further work is needed to ensure a degree of relationship between circular RNAs and regeneration of animals. However, it is worth mentioning that this will provide thought and evidence for future study in this field.

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