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Identifying the role of circRNAs in the interaction with miRNAs during the limb regeneration in axolotl

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Abstract:

The current literature lacks a comprehensive assessment of circular RNA's role in the limb regeneration in the axolotl model, which has become the foundation for recent investigations into this topic. This paper aims to address this important gap by presenting a detailed overview and reassessment of circular RNA's potential implications in the context of axolotl limb regeneration, particularly in relation to the interaction with miRNAs. Moreover, this paper shows. the utility and significance of the axolotl model organism as a valuable resource for the study of limb regeneration mechanisms. Furthermore, the study delves into the methodology and theoretical underpinnings behind the identification and mapping of circular functional genes, which is an integral step in understanding their specific contributions to the process of limb regeneration. The outcomes of this research could probably provide a solid foundation for future studies on limb regeneration, as well as a deeper understanding of the complex regulatory mechanisms that underlie this biological process.

Keywords: circRNAs, miRNAs, limb regeneration, axolotl

1. Introduction

Stem cells are central to various physiological and pathological processes, including tissue repair and regeneration, due to their remarkable self-renewal and differentiation capabilities. Circular RNAs (circRNAs), widely distributed in eukaryotes, have been implicated in stem cell differentiation, yet their roles in muscle regeneration remain poorly understood. The axolotl, with its unparalleled ability to regenerate limbs, provides a unique model for studying regeneration mechanisms. Despite the sequencing of the axolotl genome, there is a lack of comprehensive data on circRNAs. As during limb regeneration, hindering our understanding of their potential roles. This study aims to bridge this gap by investigating circRNAs as miRNA sponges in the axolotl limb regeneration process. Utilizing the Biotin-Coupled miRNA and circRNA Capture method, we will identify and analyze circRNAs that may regulate gene expression during regeneration. In the work, we will try to explore a multi-faceted approach to uncover the molecular

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mechanisms behind axolotl limb regeneration, potentially revealing new targets for regenerative medicine.

2. Circular RNA

Stem cells, renowned for their extraordinary self-renewal and differentiation abilities, play a significant role in a lot of physiological and pathological processes, including tissue repair regeneration and homeostasis [1]. It is important to highlight that circRNAs are found widely distributed among eukaryotes [1]. However, the expression levels of numerous circRNAs may change during stem cell differentiation, and some of these RNAs have been implicated in the regulation of stem cell differentiation [2]. Since the discovery of these circRNAs, extensive research has been conducted to elucidate their significant biological roles, which have highlighted their vital regulatory roles in various biological and health conditions. Despite these insights, the field of muscle regeneration has not received a significant amount of comprehensive and in-depth research, which is an area where more research is needed to improve the comprehension of key regulatory principles.

3. The importance of axolotl as a model for limb regeneration

Axolotls are an invaluable model organism for the study of regeneration, given their unique ability to effectively regenerate body parts, organs, and various tissues such as limbs [3]. This remarkable capacity has made them a fundamental model organism for the investigation of regeneration, and their use has provided valuable insights into the mechanisms of tissue and organ regeneration. Additionally, axolotls are relatively easy to breed and share numerous functional and structural proteins and signaling pathways with mammals [3], making them an invaluable tool for the study of human diseases and disorders. The axolotl genome has been sequenced [4], which provides future insights into the mechanisms of molecular and cellular regeneration, and the study of this organism has the potential to provide significant advances in our understanding of the mechanisms of regeneration. Despite its notable contributions to our regeneration knowledge, certain data sets are lacking, including circRNA info during the complete limb genesis period. This hampers the in-depth exploration of their role in regeneration [4], and the availability of these datasets could provide valuable insights into the mechanisms of such regeneration.

4. Circular RNAs Act as miRNA Sponges

MicroRNA is a small, naturally occurring RNA produced in the cells of living organisms that can regulate the activity of specific mRNA molecules. This is accomplished by bonding with the 3' UTR on the targeted RNA, effectively silencing it. [5]. MicroRNAs process a critical role in various biological processes such as development, differentiation, and apoptosis, and their imbalance might be linked to many illnesses. One of the methods used to inhibit microRNA activity is the use of microRNA sponges, which are synthetic constructs designed to bind to specific microRNA molecules. By providing an excess amount of target mRNA, microRNA sponges can effectively inhibit the activity of the microRNA and probably restore normal gene expression. Emerging evidence indicates that circRNAs play an integral role in regulating gene expression by functioning as miRNA sponges, thereby inhibiting miRNA activity. Although certain circular RNA has been detected as a typical miRNA sponge, which has been observed in various cancers such as astrocytoma and lung cancer, the precise role of circRNAs in the interaction with miRNAs during regeneration, particularly in limb regeneration, remains to be unclear. [6-7].

5. Biotin-Coupled miRNA and circRNA Capture

The Biotin-Coupled miRNA and circRNA Capture method involves biotinylating the 3' end of a specific miRNA or circRNA to generate biotinylated miRNA (Bi-miRNA) or biotinylated circRNA (Bi-circRNA) [8]. These labeled molecules are then transfected into cells, allowing them to bind to their respective target mRNA molecules [9]. To isolate these mRNA molecules, streptavidin-coated magnetic beads are used to pull down the mRNA bound to Bi-miRNA or Bi-circRNA. The captured mRNA is subsequently extracted and purified using Trizol or other suitable reagents. To identify the direct targets of the miRNA or circRNA, the enriched mRNA is analyzed using techniques such as microarrays or qRT-PCR and compared to a control sample (cells transfected with unmarked miRNA or circRNA) [10]. This comparison allows for the identification of genes whose mRNA levels are significantly altered, indicating that they are likely direct targets of the miRNA or circRNA [9]. The method can directly identify the miRNA or circRNA directly target genes and indirectly target genes, avoiding the occurrence of the experimental results of false positives [8].

6. Objective

6.1 Objective 1: Identify and locate the specific circular gene that functions as miRNA sponges during limb regeneration in the axolotl.

6.1.1 Rationale

Based on the interaction between circRNA and miRNA, the position of circRNA in cell function regulation was revealed by analyzing the expression changes of circRNA and the biological processes and signaling pathways involved in its target genes

6.1.2 Methods

circRNA target prediction, enrichment analyses, and visualization: Firstly, I will collect RNA samples from different stages of axolotl limb regeneration and treat total RNA with RNase R to remove linear RNA [11]. Then, I will perform high-throughput sequencing to obtain circRNA and miRNA expression profiles. Next, I will use bioinformatics tools such as TargetScan to predict the interaction between circrnas and mirnas and screen for potential target genes. Then, I will perform GO, KEGG and Reactome pathway enrichment analyses to reveal the biological processes and pathways that circRNAs may be involved in [3]. To validate the prediction results, I will use qPCR to detect the expression levels of circRNAs and miRNAs, and construct a PPI network to demonstrate the signaling pathways regulated by circRNAs. Finally, I will use graphs and PPI networks to visualize the interaction between circRNAs and miRNAs and their regulatory mechanism on the regeneration process, trying to provide new insights into the molecular mechanism of axolotl limb regeneration.

6.2 Objective 2: Analysis of circular RNA expression and determine their role as a miRNA sponge.

6.2.1 Rationale

Biotin is attached to miRNA or circular RNA, and then they are separated from cell extracts using streptavidin, a protein that can bind specifically to biotin.

6.2.2 Methods

Biotin-Coupled miRNA and circRNA Capture: I will start by constructing a biotin-labeled mimic of potential miRNA, adding the biotin-label mimic. Next, I will transfect the constructed biotin-labeled miRNA mimic into the limb of axolotl. I will then collect and lyse the cells to release the RNA and proteins within them. Using streptavidin-coated magnetic beads, I can specifically capture the biotin-labeled miRNA mimic. Finally, I will detect the level of circRNA captured by qPCR and observe whether the specific circRNA will be enriched in the miRNA-captured fraction or not, while the negative control group may not show significant enrichment.

7. Conclusion

This study underlines the vital role of circular RNAs (circRNAs) in stem cell differentiation and limb regeneration, particularly in the axolotl model. The highlight is the potential of circRNAs to act as miRNA sponges, influencing gene expression during regeneration. The axolotl's unique regenerative abilities and its sequenced genome provide a promising platform for uncovering the mechanisms behind circRNA function. The proposed methods for circRNA target prediction and the innovative Biotin-Coupled miRNA and circRNA Capture technique offer a direct approach to identifying and validating circRNA-miRNA interactions. Future research in this direction will not only enhance the understanding of limb regeneration but also pave the way for novel regenerative medicine strategies.

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