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TURRITOPSIS SP. (TSP.) COULD BE USED TO EXPLORE SYSTEM REJUVENATION, SELF-REPAIR AND REGENERATION HUMAN

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ABSTRACT

Objective

Given the remarkable ability to avoid inevitable harm and death, Turritopsis sp. (T. Sp.) therefore consider scientists a way to create a tool for studying aging. This study presents a method to create a research model of anti-aging and functional diversity of T. Sp.

Methods

T. sp. Jellyfish collected in the Pacific Ocean near Japan were grown under general laboratory conditions. Tissues from part of the gastrovascular cavity (GP) and part of the nerve ring (NP) were collected and total RNA was extracted. To compare the different transcriptome landscapes between GP and NP, mass RNA sequencing was performed.

Results

The GP fragment can be used to research and create anti-aging drugs, while the NP fragment can be used to research and improve systemic rejuvenation, self-renewal and regeneration.

Conclusions

A conventional system for studying aging, using the latest tools and methods of the genomic revolution, comprehensive elucidation of the composition, development and functions of T. Sp. Allowed us to investigate the basic mechanisms of renewal and rejuvenation.

Library preparation. Whole jellyfish were anesthetized in 3.5% MgCl2 (w/v in seawater). Tissues were frozen on dry ice and stored at -80 °C for subsequent RNA extraction..

Keywords: Aging, Immortal jellyfish, Regeneration, RNA-seq, Turritopsis sp.

1. Introduction

As a member of the hydrozoa within the phylum Turritopsis, including Turritopsis dohrnii and Turritopsis sp. (T. Sp.), it is commonly known as the “immortal jellyfish”. They exhibit the greatest response to environmental stress and their anti-aging system. When senescence occurs, the injured medusa form shrinks, loses its swimming ability and undergoes a retrograde transformation into a protective, poorly differentiated cyst-like structure that eventually gives rise to a previous juvenile morph, the polyp. This results in an efficient regeneration system and allows it to restart its life cycle, which is known as “rejuvenation”.[1]

The basic life cycle of Turritopsis proceeds from an adult medusa to a planula larva and a polyp, which is an asexual and often colonial stage. The structure of the adult jellyfish includes a gastrovascular cavity with a set of mandibles, mouthparts, root canal, and gonads inside, as well as a velum surrounded by a nerve ring with tentacles. The body wall of the gastrovascular cavity consists of an outer epidermis, outer mesoglea, endoderm,

intermesoglea forming the gastric cavity, radial canals, and an annular canal. A layer of mononuclear striated muscle cells and a smooth muscle layer are also observed in the mature jellyfish.

Using the latest systems and tools and methods of the genomic revolution, such as next-generation sequencing, single-cell/molecular sequencing, spatial transcriptomics, multiomics integration, and CRISPR-Cas9 genome editing, it has become possible to comprehensively establish the system, development, and functions of Turritopsis, allowing us to study rejuvenation. The current study presents a method for comparative analysis of jellyfish. It revealed structural and functional diversity among different fragments of T. Sp., Turritopsis medusa, which was collected in the Pacific Ocean near Japan, for the study of aging.[2]

2. Methods

2.1. Cultivation

About 100 T. Sp. Jellyfish were collected in August 2023 in Japan (and cultured in the laboratory at 25 °C in 500-ml beaker.

Long-term culture for more than 6 months can be difficult and requires additional specialized equipment for water circulation. A combination of sponge filters, protein skimmers and filters was used for the culture systems. Lighting is crucial for the development of T. Sp. And rejuvenates the study object. Blue-dominated light was set to mimic natural sunlight, and the lighting cycle mimicked day and night periods at regular intervals.

2.2. Total RNA extraction and library preparation

Whole jellyfish were anesthetized in 3.5% MgCl2 (w/v in seawater). Tissues were frozen on dry ice and stored at -80 °C for subsequent RNA extraction.

Total RNA was isolated by using the NucleoSpin RNA Plus XS kit and quantified on a Qubit 4 ThermoFisher Scientific fluorometer. After assessing the quality of RNA, samples with a maximum value of . Nanopore sequencing was performed using a ligation-based cDNA-PCR sequencing kit (Oxford Nanopore Technologies (ONT), Didcot, UK) according to the manufacturer's protocol. LongAmp Taq 2x Master Mix was used to amplify full-length transcripts, and PCR products were purified using Agencourt AMPure XP magnetic beads (Beckman Coulter, USA). Adaptor-ligated libraries.

2.3. Data collection and bioinformatics analysis

Base calling was performed using base calling, by translating ion signals into nucleotide sequence. FASTQ files were aligned with SAM files with a reference genome generated from the 1000 Years genome assembly.

The SAM files were then converted to BAM files, sorted and indexed using Samtools software. Bioinformatic procedures were computed using SHIROKANE, a high-performance computing facility located at the Human Genome Center.

A heat map of all DEGs was then generated, in which the numerical values of the points were represented by a range of colors. Pathway analysis of all DEGs was performed using parametric gene set enrichment analysis (PGSEA) with gene sets from the Gene Ontology Molecular Function.

3. Results

3.1. Requires the detection of tissue and functional diversity, which can now be predicted by group sequencing. Using comprehensive transcriptome sequencing, heat map analysis extracted the top 10,000 genes with up- and down-regulation compared between GP and NP. By exploring the database Co.Expression.Tissue.Protein.Expression.from.Human.Proteome.Map, the PGSEA results preliminarily showed. These genes were functionally defined as belonging to four clusters. Functional enrichment revealed that genes in cluster 2 are related to metabolic processes, while genes in cluster 3 are initially associated with negative regulation of metabolic processes and transferase activity, cell projection assembly, rhythmic processes, and phagocytosis. These results systematically indicate the potential structural and functional diversity of T. Sp. Medusa.

3.2. GO pathway enrichment analysis of differentially expressed genes (DEGs) showed

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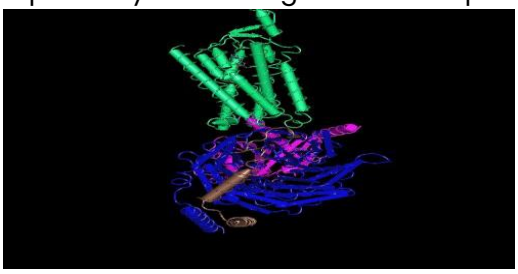
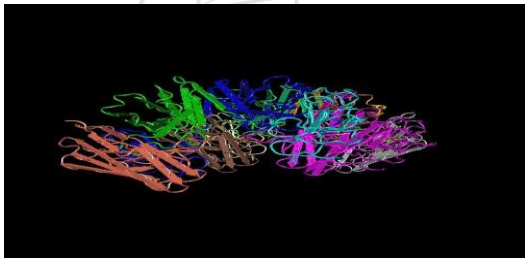
that pathways including cellular responses to



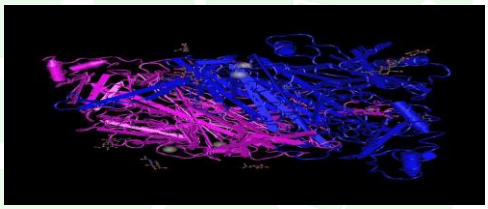
external or internal stress, such as DNA damage, abiotic stimulus, radiation, and reactive oxygen species, were dominant in the GP fragment compared to the NP fragment. Functions related to the regulation of sensory function, central nervous system development, and hypoxia response were significantly activated in the NP fragment. Overexpression of stress response-related genes including TPO, GSS, BTK, SLOX5, PKD2, and PLA2R1 was observed in the GP fragment. In DEGs collected from the hypoxia response pathway, which was considered a major factor in stem cell activation, higher activation was confirmed in the GP fragment compared to the NP fragment. Genes encoding proteins involved in transcription-related genes (TGFB1, ERCC3, and FAXO3), fertilization and development-related genes (SRC, EP300, ADORA1, and ADAM17), cell cycle-related genes (ATM and NPEPPS), and structure-related genes (ANGPT4, OPRD1, MMP2, THBS1, and HSP90B1) Results of GO biological process pathway analysis in GP and NP fragments. Functional prediction of differentially expressed genes was performed using PGSEA analysis by examining the GO biological process database.

The results suggest that the GP fragment is functionally dominant in regulating cellular responses to external or internal stress, while the NP fragment may contribute to developmental processes in T. Sp.



3.3. We then explored all available databases equipped with the PGSEA module. Contributing to the rejuvenation response, our attention was drawn to partial DEGs involved in HMGIY target genes. The expression of genes encoding regulatory factors involved in the initiation of development and proliferation, such as PPP1R15B, ATF5, HEATR4, ROR1, FUT8, and EIF4H, was significantly higher in the NP fragment compared with the GP fragment. Increased expression of PEF1 and TM7SF3, which encode factors associated with programmed cell death, was observed.

In line with observations from previous studies, genes encoding proteins expected to be participants in the neural network, including AOC3, ZFYVE26, RAB39B, CLCN3, and GABRD, were enriched in the NP fragment. Expression of HMGIY target genes in the GP and NP fragments.



Downregulated pathways are highlighted in blue; upregulated pathways are highlighted in red. The normalized expression of genes involved in the HMGIY pathway was visualized in both GP and NP samples using a green-black-red heat map.

Together, these results demonstrate that the NP fragment indeed serves a central nervous system role in T. Sp. The existence of stem cells as a placental-like tissue contained in the NP fragments, the enrichment of factors involved in the process of developmental initiation and programmed cell death, strongly suggest that the NP fragment may play a crucial role in the fate of the rejuvenation process in T. Sp.

4. Discussion

Death as a cause has been mainly investigated in a limited number of experimental systems, including mammals (Mus musculus, Homo sapiens). In fact, the spectrum of valuable systems for studying aging is much broader. In

a study, with a special focus on one of the permutations of aging, immortality (eternal existence), the contribution of tissue diversity to the response to environmental stress and rejuvenation was dissected in a less traditional hydrozoan system, T. Sp., by comparing tissue fragments.

In the current study, a retina-like structure was also confirmed in the NP fragment. As a characteristic signaling pathway, but not limited to this, the upregulated HMGIY genes may be one of the factors initiating transcriptional activation of stem cells in the NP fragment, due to the existence of breakpoints outside the genome. On the other hand, the GP fragment is more responsible for the systemic response to various types of stimuli, such as DNA damage, oxidative stress, and radiation. Therefore, we suggest that the GP fragment may be mainly responsible for systemic aging, while the NP fragment may be fundamental for the rejuvenation process. The results showed that it is crucial for the destabilization and stolon formation in the polyp. To study aging using the T. Sp. System, the GP fragment can be used for studies of systemic aging, while the NP fragment can be investigated for self-renewal and regeneration approaches. This knowledge may contribute to the development of pharmaceutical interventions aimed at modulating the promotion of regenerative processes in human tissues and the creation of immortality drugs.

First of all, the mature immortal jellyfish T. Sp. Has amazing regenerative abilities, its biological mechanisms can be directly translated to other organisms, including humans. Extension of broad conclusions or generalized conclusions from T. Sp. To other species requires caution and further comparative studies of different organisms. Finally, the collection and manipulation of T. Sp. Specimens should be carried out responsibly and in accordance with ethical principles. Ensuring the welfare and humane treatment of T. Sp. During research is of great importance.

Is it possible to apply the process of rejuvenation, the exceptional ability to return to an earlier stage of development through transdifferentiation. Or equip homo sapiens with this fantastic ability? Perhaps the extraordinary progress in the technological problems of preserving and reanimating the body of a deceased person, while preserving its structural integrity and the viability of stem cells, can help us answer these questions. In any case, achieving true rejuvenation and reversing the effects of aging in humans, as demonstrated in T. Sp., requires further research to significantly elucidate the fundamental biological differences between T. Sp. And humans.

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