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Single-cell transcriptome sequencing reveals the cell populations and functions associated with stony coral *Pocillopora damicornis*

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Introduction: *Pocillopora damicornis*, a key species of stony corals, has been the subject of considerable scientific study. However, the cellular composition of *P. damicornis* and the roles of these cells in endosymbiosis and biomineralization remain elusive. The development of single-cell technology has provided new opportunities for researching the cellular and molecular mechanisms underlying symbiosis and mineralization. Nevertheless, the stringent environmental requirements, the complexity of the cellular components, and the paucity of high-quality reference genomes of *P. damicornis* have posed significant challenges for single-cell transcriptome research.

Methods: In this study, we quantified the transcriptomic expression of *P. damicornis* by aligning its single-cell transcriptome (scRNA-seq) data to multiple species, including *Stylophora pistillata*, *P. damicornis*, and *Pocillopora verrucosa*. We determined the cell types of *P. damicornis* by comparing its cluster-specific genes with the published cell type-specific genes of *S.pistillata* and conducted gene function and enrichment analyses.

Results: Unsupervised clustering analysis yielded the identification of ten distinct cell populations, including epidermis cells, gastrodermis cells, algae-hosting cells, calicoblast, cnidocytes, and immune cells. In addition, we identified 53 genes that were highly similar to known sequences in the symbiotic zooxanthellae. These genes were mainly expressed in four different cell populations, corresponding to active symbiotic populations.

Conclusion: This study identified cell types closely associated with symbiosis and calcification in *P. damicornis*, along with their marker genes, which are consistent with the findings in *S. pistillata*. These results offer insights into the cellular functions and symbiotic mechanisms of *P. damicornis*.

KEYWORDS

Pocillopora damicornis, ScRNA-seq, endosymbiosis, gastrodermis, epidermis, cnidocyte, calicoblast

Introduction

Coral reefs have been likened to tropical rainforests in the context of the oceans. These ecosystems provide a habitat for fish and play a role in the biodiversity of the ocean (Hughes et al., 2017). Coral reefs are primarily formed by the accumulation of calcium carbonate secreted by reef-building corals over extended periods of time. The survival and reef-building activities of stony corals are inseparable from the important contributions of the algae that live in symbiosis with their cells, known as *Symbiodinium* (Wilson and Hilferty, 1931). In the event of a disruption to the external environment, such as an increase in temperature, the symbiont undergoes disintegration, a process referred to as coral bleaching (Odum and Odum, 1955; Muscatine, 1967; Moberg and Folke, 1999; Costanza et al., 2014; Huang et al., 2019; Reimer et al., 2019). The ongoing threat to the coral ecosystem, precipitated by global warming and marine environmental pollution, has led to coral bleaching as a primary factor in the degradation of coral reefs (Swain et al., 2016). The study of the physiological characteristics and symbiotic mechanisms of corals is of great significance to the preservation of coral ecosystems. The primary methodologies employed in coral research encompass the use of isotope labeling (Clayton and Lasker, 1984), and metabolite chromatography analysis (Biel et al., 2007; Gates et al., 1995). As coral genome data continues to improve (Shinzato et al., 2011; Polato et al., 2011; Levy et al., 2021), transcriptomic analysis has become a staple of coral research. In comparison with conventional transcriptional analysis, single-cell RNA sequencing (scRNA-seq) has been shown to identify the transcriptional profile of individual cells and to identify multiple types of cells in *Stylophora pistillata*, including immune cells, gastrodermis cells, neurons cells, alga-hosting cells, and calicoblasts (Mansour et al., 2016; Hayward et al., 2011; Reyes-Bermudez et al., 2009; Bay et al., 2009). Nevertheless, corals have evolved over 500 million years, and there are substantial disparities between their genomes. Consequently, the analysis of single-celled organisms belonging to other stony coral families remains a significant area of research (Reimer et al., 2019).

To this end, the present study employs *P. damicornis* to elucidate its cell specificity. Both *P. damicornis* and *S. pistillata* are classified under the monophyletic group of the Pocilloporidae family. The family Pocilloporidae, belonging to the order Scheractinia(stony corals),is predominantly distributed in the Indo-Pacific Ocean. The family includes several genera, notably Pocillopra and Stylophora (Schmidt-Roach et al., 2014). *P. damicornis* exhibits a broad distributed across tropical and subtropical reefs, with notable populations in the Indo-Pacific and Eastern regions (Schmidt-Roach et al., 2014) (Barbosa et al., 2016). This study sampled cells from different parts of adult *P. damicornis* while retaining a small number of symbiotic algae genes to facilitate the identification of symbiotic cells.

Materials and methods

Collection and cultivation of samples

The biological samples utilized in this study were obtained from the Xisha Islands, situated in the South China Sea (15°40'–17°10' N, 111°–113°E). The coral samples were cultivated in a coral tank specifically designed to replicate their natural habitat conditions. All *P. damicornis* specimens were maintained in a RedSea® tank (redsea575, Red Sea Aquatics Ltd., London, UK) at 26°C and 1.025 salinity (Red Sea Aquatics Ltd). The temperature was maintained at 26°C and the salinity at 1.025 (as per Red Sea Aquatics Ltd. specifications). The coral cultivation system was equipped with three coral lamps (AI®, Red Sea Aquatics Ltd), a protein skimmer (regal250s, Reef Octopus), a water chiller (tk1000, TECO Ltd, Port Louis, Mauritius), two wave-making devices (VorTechTM MP40, EcoTech Marine Ltd), and a calcium reactor (Calreact 200, Reef Octopus).

Single-cell suspension preparation, library construction, and sequencing

To prepare the single -cell suspension, we first diluted a 0.02 M PBS (Solarbio, P1010-2L, Beijing, China)to obtain a 0.035 M PBS solution with a pH of 8.0. The sample was thoroughly rinsed with this solution, and the rinsing process was repeated three times.

Next, we prepared a digestion solution by mixing a 0.1% trypsin (Sigma-Aldrich, T1426-500MG, St. Louis, MO, USA) with the 0.035 M PBS solution. The sample was then placed in this digestion solution at room temperature for 2 - 3 hours. Subsequently, the solution was filtered through a filter with a pore size of 40 μm Fisherbrand™ Sterile Cell Strainers (Thermo Fisher Scientific, 22-363-547, Waltham, MA, USA) and centrifuged at a centrifugal force of 350 G for 5-6 minutes. The enzymatic reaction was terminated by adding a stop solution containing 0.4 mg of BSA.

We employed the trypan blue staining method. An automatic cell counter (TC20™, Bio-Rad Laboratories, Inc, Hercules, CA, USA) was used to add a 0.4% trypan blue (Invitrogen, T10282, Carlsbad, CA, USA) to the cell suspension at a 1:1 ratio. To ensure the optimal single - cell capture effect, we defined the qualified criteria as a cell survival rate greater than 60% and a cell concentration ranging from 1×10^5 to 1×10^7 cells/mL. After washing and resuspension, the cell suspension was diluted to a concentration of 800 - 1200 cells/μL. The prepared cell suspension was then sent to Tianjin Novogene Company for library construction(10x Genomics, Inc, Pleasanton, CA, USA) and quality control. Finally, sequencing was carried out on the HiSeq PE150 platform(Illumina, Inc, San Diego, CA, USA).

Bioinformatics analysis

Reference genome construction and quantification

Due to the current paucity of high-quality coral genome data and the presence of numerous gap regions, the species with relatively high-quality genome data in the family Pocilloporidae, suborder Astrocoeniina, order Scleractinia on NCBI currently include *S. pistillata*, *P. damicornis*, and *Pocillopora verrucosa*. This study utilizes these three species as references, in conjunction with

our laboratory's *P. damicornis* full-length transcriptome data (Han et al., 2022), employing the CellRanger (V8.0.1) mkref function to construct a reference genome and the count function for gene expression quantification.

Identification of symbiotic zooxanthellae genes

To identify symbiotic zooxanthellae genes, we utilized the genome of *S. pilosum* (NCBI accession: GCA_964212085.1) as a reference. Through CellRanger count analysis to detect symbiodinium homologous genes in coral host cell.

Gene function annotation

The gene functions were annotated based on the previously published *P. damicornis* full-length transcriptome (Han et al., 2022) using Pfam (protein family; Finn et al., 2016); KOG/COG (EuKaryotic Orthologous Groups; Clusters of Orthologous Groups of proteins; Tatusov et al., 2003); KEGG (Kyoto Encyclopedia of Genes and Genomes; Kanehisa et al., 2004); and GO (Gene Ontology; Ashburner et al., 2000).

Cluster and marker gene identification

An unsupervised clustering algorithm with a resolution parameter set at 0.1 was employed for cell clustering. For the analysis of differential expression, we established thresholds of a minimum expression percentage (min.pct) of ≥ 0.01 and a log fold change (logfc.threshold) of > 0.25. Dimensionality reduction was conducted utilizing the Uniform Manifold Approximation and Projection (UMAP) algorithm, which facilitates the low-dimensional visualization of high-dimensional data.

Cell type identification

Presently, the extant literature on marker genes for coral cell types is scant, with the study on *S. pistillata* constituting a rare resource. The present study employs a three-step approach to address this paucity of knowledge. First, the significantly upregulated genes that are uniquely expressed in each cluster identified in the *P. damicornis* dataset will be determined. Second, these cluster-specific marker genes are then cross-referenced with the cell type-specific markers reported for *S. pistillata*. Finally, combining the enrichment analysis result of the overlapping marker genes will allow for the inference of the corresponding cell types present in *P. damicornis*.

Function enrichment analysis

Functional enrichment analysis was conducted using the Molecular Signatures Database (MSigDB) framework (Liberzon et al., 2015). The analysis pipeline incorporated multiple resources, including GO terms for biological processes, molecular functions, and cellular components; KEGG pathways for metabolic and signaling networks; KOG for evolutionary relationships and Pfam for protein domain information. The statistical significance of the enrichment patterns was assessed using Fisher's exact test, with a significance threshold of $P < 0.05$. Enriched terms were prioritized based on their statistical significance ($-\log10$[P-value]). The resulting functional categories were then visualized using the ggplot2 package, with particular emphasis on biological processes and molecular pathways relevant to coral biology.

Result

Single-cell genome mapping

In this study, single-cell RNA sequencing (scRNA-seq) analysis was performed on five reference genomesfrom the family Pocilloporidae: *S. pistillata*, *P. damicornis*, *P. verrucosa*, and *P. damicornis* full-length transcriptome data, and the full-length transcriptome data of S. *pistillata* and *P. damicornis*. Among the species examined, *P. verrucosa* exhibited the highest number of expressed genes (32,915), followed by *P. damicornis* (23,077), *S. pistillata* (22,408), and *P. damicornis* (14,402) (see Table 1 for details).

We observed samples-specific variations in the average number of genes expressed per cell. *P. verrucosa* demonstrated the highest average expression levels, with 87 genes per cell. In contrast, *P. damicornis*, *S. pistillata*, and *P. damicornis* exhibited average expressions of 75, 74, and 74 genes per cell, respectively. Integration of transcriptomic data from all five species yielded a comprehensive dataset comprising 53,661 genes, with an average of 260 genes expressed per cell across the integrated dataset (Table 1).

Clustering and differential expression analysis

UMAP visualization revealed ten distinct clusters in two-dimensional space (Figure 1A). Differential expression analysis was performed to characterize the molecular signatures of these clusters, with significance criteria set as: Absolute value of log2 fold change > 0.25, expression in > 1% of cells, and $P < 0.05$. The differential expression results of each cluster are shown in Table 2. The specific differentially expressed genes for each cluster are in Supplementary Table S1.

Symbiotic cell identification

Among the 53 identified Symbiodinium genes, 25 were functionally annotated (Supplementary Tables S3, S4). These genes were primarily highly expressed in clusters 4, 5, 8, and 9 (Figure 1C). These genes are mainly involved in energy metabolism, biosynthesis, stress response (including HSP family), cytoskeleton regulation, and protein synthesis and modification.

Cell type identification

Cross-species comparison of cell-type markers between *S. pistillata* and *P. damicornis* revealed distinct cell population correspondences. Based on shared marker genes, we identified six major cell types: cluster 0 as epidermis cells, cluster 1 as

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TABLE 1 Comparative analysis of reference genomes and integrated datasets used for single-cell transcriptome alignment.

Reference Genomes	Annotated Genes	Mapped Cells	Total UMIs	Mean Genes per Cell
P. damicornis	23,077	2,434	183,122	75
P. damicornis[2]	14,402	2,480	158,817	64
P. verrucosa	32,915	2,303	201,030	87
S. pistillata	22,408	2,122	156,897	74
S. pistillata[2]	28,498	1,553	57,758	37
Merge	53,661	2,921	759,095	260

Summary of single-cell RNA sequencing data from three coral species: *P. damicornis* (including full-length transcriptome data, *P. damicornis*[2]), *P. verrucosa* and *S. pistillata* (including NCBI dataset GCF_002571385.2, *S. pistillata*[2]). The "Merge" row displays the consolidated information for all the above data.

gastrodermis cells, cluster 2 as algal host cells, cluster 3 as calicoblasts, cluster 6 as cnidocytes, and cluster 7 as immune cells (Figure 1B; Supplementary Table S2). This cell-type assignment was supported by overlapping marker genes between the two species (Supplementary Table S1, S8), providing molecular evidence for conserved cell populations across coral species.

The ectoderm predominantly comprises of epidermis cells (Cluster 0), which form the outermost protective barrier and contain cnidocytes (Cluster 6) for defense and predation. The endoderm, on the other hand, contains gastrodermis cells (Cluster 1) and various symbiotic cell subpopulations (Clusters 2, 4, 5, 8, and 9), which are responsible for nutrient absorption and maintaining the symbiotic relationship with zooxanthellae. The calicoblast of the basal ectoderm (Cluster 3) is responsible for bone formation, while the immune cells distributed in the mesoderm (Cluster 7) are responsible for immune defense (Figures 1B, D; Supplementary Table S3).

Differentially expressed gene enrichment analysis

Gene set enrichment analysis was performed using MSigDB against KEGG and GO databases. Additionally, transcripts were

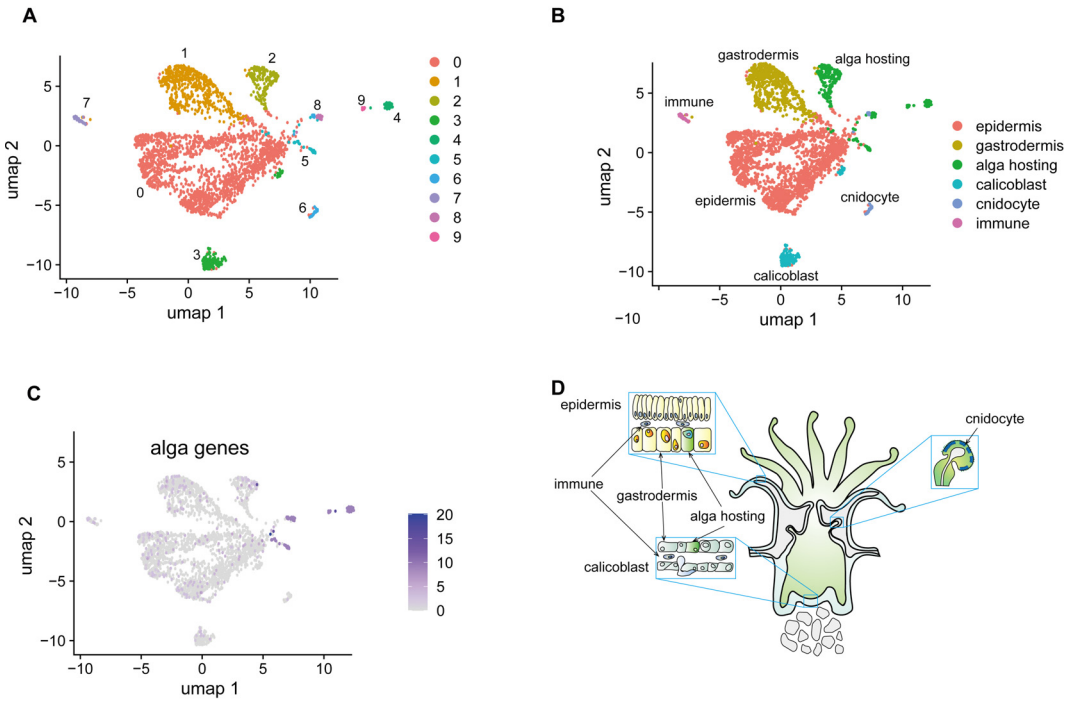

FIGURE 1
A study of the coral symbiotic system via single-cell transcriptome analysis. **(A)** Utilizing UMAP dimensionality reduction of single-cell transcriptome data, ten transcriptionally distinct cell subpopulations were identified. The utilization of distinct colors and numerical identifiers enables the clear representation of these diverse cell clusters. **(B)** Annotation of major cell types on the UMAP plot, including epidermis cells, gastrodermis cells, alga hosting cells, calicoblast, cnidocytes, and immune cells. **(C)** The expression distribution of algal symbiont genes on the UMAP plot is represented by different colors, with different colors representing different expression levels. **(D)** A schematic diagram of the main cell types identified is shown, with their spatial relationships within the coral symbiotic system illustrated.

TABLE 2 Cell cluster statistics and marker gene identification.

Cluster	Cells	Genes	Markers
0	1,736	44,408	1,586
1	571	28,659	2,237
2	182	16,974	2,139
3	138	13,757	1,898
4	89	2	1,152
5	57	165	265
6	49	3,873	1,753
7	40	6,201	3,402
8	37	77	473
9	22	1	51

Cells: Number of cells in a cluster; Genes: Number of expressed genes; Markers: Number of cluster-specific genes selected by differential expression analysis (Wilcoxon rank-sum test; threshold: min.pct ≥ 0.01, logfc > 0.25, $p < 0.05$).

categorized based on GO, KEGG, KOG/COG, and Pfam annotations, followed by Fisher's exact test ($P < 0.05$ considered significant; Supplementary Table S4).

Epidermis cells

GO analysis of upregulated genes in coral epidermis cells revealed enrichment in transcription elongation, DNA binding, and ribosome biogenesis, suggesting active gene expression and protein synthesis regulation (Mayfield et al., 2014). KEGG analysis identified enriched pathways in metabolism and signal transduction (Rädecker et al., 2021). KOG and Pfam analyses highlighted specific regulatory elements, including BTG1/TOB anti-proliferative factors, Mafk bZIP transcription factor, and basic leucine zipper domains, which may modulate epidermal cell functions (Li et al., 2018; Supplementary Figure S1).

Gastrodermis cells

Functional enrichment analysis of upregulated genes in gastrodermis cells revealed multiple digestive-related pathways (Figure 2). These included catalytic activity regulation and cysteine peptidase activity, indicating active enzyme secretion (e.g., pepsinogen); lipid transport and lipoprotein pathways; and N-acetyltransferase activity for substrate modification. Signal transduction analysis showed enrichment in the PI3K-Akt pathway, while structural features were highlighted by enrichment in cell adhesion and actin cytoskeleton components, reflecting their epithelial barrier function (Raz-Bahat et al., 2017). The enrichment of lysosomal and protein digestion pathways further supports their role in protein processing (Coffey and De Duve, 1968; Moya et al., 2012).

Calicoblast

GO functional enrichment analysis of coral calicoblast revealed significant pathways in energy metabolism, ion transport, and cytoskeletal regulation. Biological processes showed enrichment in ATP synthesis coupled proton transport, mitochondrial electron transport, and anion transport. Molecular functions highlighted cytochrome c oxidase activity, NADH dehydrogenase activity, and ion transport mechanisms. Cellular component analysis identified enrichment in mitochondrial structures and ion transport complexes. KEGG pathway analysis revealed enrichment in oxidative phosphorylation and related metabolic pathways (Supplementary Figure S2). KOG and Pfam analyses identified key components including carbonic anhydrases, cytoskeletal proteins, ion transporters (Na+/K+-ATPase), and regulatory domains (FerB, calponin homology, tRNA synthetase classes).

Cnidocyte

GO functional enrichment analysis of coral cnidocytes revealed significant enrichment in protein deubiquitination, calcium ion homeostasis, and protein processing pathways. Molecular function analysis identified enrichment in various enzymatic activities (metallocarboxypeptidase, ubiquitin-specific protease), calcium channels, and neurotransmitter transport. Cellular component analysis highlighted the DNA-dependent RNA polymerase III complex, suggesting active transcriptional regulation.

KEGG pathway analysis showed enrichment in protein export, endoplasmic reticulum processing, and mucin-type O-glycosylation biosynthesis, potentially involved in toxin modification (Supplementary Figure S3). KOG classification revealed enrichment in signal transduction, cytoskeleton organization, and protein modification. Pfam analysis identified domains crucial for cnidocyte function, including EF-hand calcium-binding, dynein heavy chain, myosin head, and toxin-related domains.

These findings indicate that cnidocytes possess specialized features for toxin synthesis and secretion, including protein modification systems, calcium signaling pathways, and dynamic cytoskeletal regulation (Jouiaei et al., 2015; Özbek, 2011; Beckmann and Özbek, 2012; Rachamim et al., 2015).

Immune cells

GO analysis revealed enrichment in protein deubiquitination, catalytic activity regulation, DNA binding, and exosome complex functions. KEGG pathways highlighted immune-related processes, including lysosomal and platelet activation pathways. KOG and Pfam analyses identified regulatory proteins such as E3 ubiquitin ligases and interferon factors, suggesting roles in coral immune regulation (Supplementary Figure S4).

Discussion

As the cornerstone of the marine ecosystem, the survival of coral depends on the functional collaboration between cell groups (Levy et al., 2021). We revealed the molecular characteristics of certain cell populations in *P. damicornis* and their synergistic mechanisms in symbiosis, calcification and immunity through single-cell transcriptome analysis.

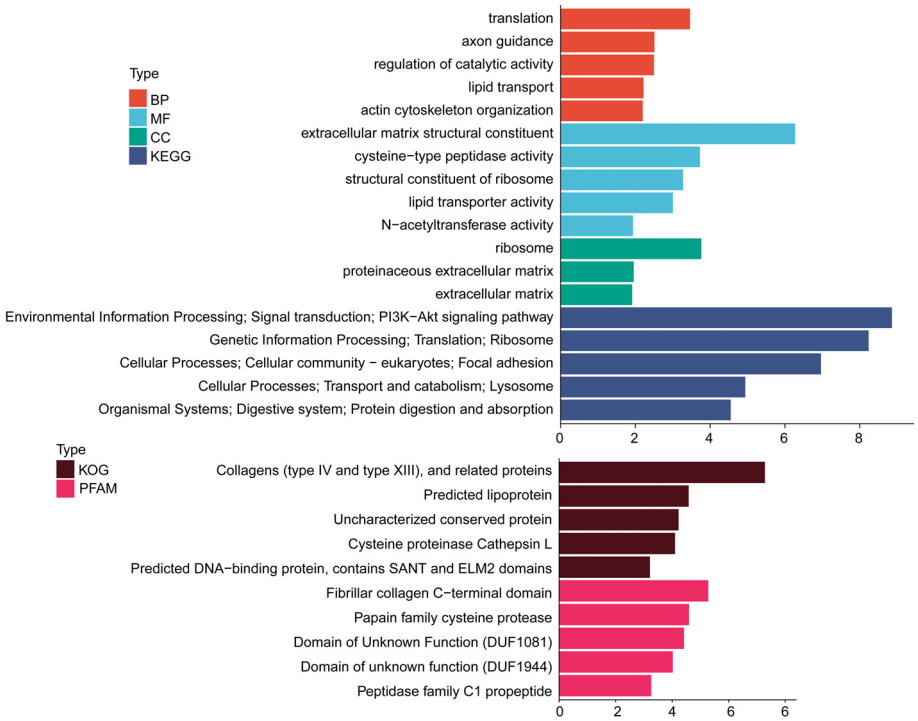

FIGURE 2
Enrichment analysis of genes and proteins in coral gastrodermis cells. The figure shows the functional enrichment analysis results of genes and proteins categorized by biological process (BP), molecular function (MF), cellular component (CC), and KEGG pathways, as well as the enrichment fold changes of specific genes and proteins. The x-axis represents the negative logarithm transformation of the p-value (-logPV), with higher values indicating higher enrichment levels. "-logPV" represents the negative logarithm (base 10) transformation of the p-value.

Functional collaboration network between cell populations

Epithelial cells act as the first barrier to the external environment, and they quickly respond to stress by highly expressing HSP90AB1 and Jun genes (Cziesielski et al., 2018).The cytokines they secrete may regulate the metabolic activity of adjacent gastric epithelial cells through paracrine signals. The significant upregulation of ribosomal proteins (RPL13, RPS20) and collagen (COL1A2, COL5A1) in gastric epithelial cells indicates that they are not only responsible for nutrient absorption and transport, but may also provide structural support for the symbiotic algae through extracellular matrix (ECM) remodeling (Mass et al., 2014). It is worth noting that the genes for lipid metabolism (ALDH3A2, ACSL4) in symbiotic cells are highly expressed together with the mitochondrial oxidative phosphorylation pathway (such as COX6B1, NDUFA4) in calicoblast (Drake et al., 2020). This may suggest that photosynthetic products are directly supplied via lipid carriers to ATP required for calcification.

The role of multiple cell types in symbiosis

The maintenance of the coral-alga symbiosis depends on the coordinated action of multiple cell types (Levy et al., 2021). A lipid metabolism network comprising ALDH3A2, APOD and ACSL4 in the symbiotic cells supports nutrient exchange, while the expression of ATP6V1G1 maintains an acidic pH suitable for algal photosynthesis (Barott et al., 2015). Gastric epithelial cells play a key role in regulating nutrient transport and cell proliferation by upregulating ribosomal proteins and signaling molecules such as MST1R, YAP1 and JAG2 (Rosic et al., 2014).

Multicellular population synergies for environmental adaptation

We found high co-expression of mesoheat shock protein (HSP90AB1) in epidermal cells with oxidative stress genes (e.g. SOD2) in symbiotic cells (Cziesielski et al., 2018). This may suggest the activation of our transcellular antioxidant network. Meanwhile the high expression of thioredoxin (Trx) in calcified cells not only supports mitochondrial energy metabolism, but may also protect calcified frontiers from oxidative damage by scavenging reactive oxygen species (ROS) (Drake et al., 2020; Ramos-Silva et al., 2013; Guo et al., 2021).This multilayered defense mechanism may be the molecular basis of coral response to climate change (DeSalvo et al., 2010; Barshis et al., 2013).

Co-regulation of the calcification process

High expression of thioredoxin in calcified cells provides redox balance and energy support for biomineralization (Drake et al.,

2020). Gastrodermal cells produce ATP through ETS-mediated aerobic respiration to provide energy support for the calcification process (Tambutté et al., 2011). Meanwhile, the expression of collagen proteins such as COL1A2, COL3A1, and COL5A1 in gastrodermal cells provides an extracellular matrix scaffold for the calcification process (Mass et al., 2014), ARHGEF17 and ACTB-mediated cytoskeleton promotes transport of calcified material (Zoccola et al., 2015).

Multi-layered immune defense system

The immune cells and the cnidocyte together form the defense line of the coral (Palmer, 2018; Sun et al., 2024). Immune cells express a variety of stress-related proteins that are involved in the basal defense (Rachamim et al., 2015). Cnidocyte regulate the synthesis of nematocystin through the expression of PDIA3 and RCN1 In terms of tissue defense, both immune cells and cnidocyte express specific functional genes such as FGF1 and IRF transcription factors in immune cells and ADAMTS9 and CMH1 in cnidocytes (Libro et al., 2013). The toxin and release mechanisms of cnidocyte exhibit dynamic regulatory mechanisms (Columbus-Shenkar et al., 2018).

Conclusions

In this study, we revealed the division of labor among multiple cell populations in corals in the maintenance of physiological symbiosis through single-cell transcriptome analysis. Epidermal, gastrodermal, and calicoblast collectively maintain coral symbiosis, calcification processes, and environmental adaptation through the synergy of metabolic networks, signaling pathways, and structural support. These findings provide new perspectives for understanding the cellular molecular basis of coral physiological functions, as well as a theoretical basis for predicting and improving coral adaptations to the environment.

Limitations and prospects

Although the present study reveals the functional collaboration network among coral cell populations, there are still some shortcomings: firstly, the single-cell transcriptome analysis reflects the gene expression profiles at a specific point in time, which makes it difficult to capture the spatial and temporal characteristics of the dynamic cell-cell interactions; secondly, many of the hypothesized intercellular signaling pathways still lack functional validation. In the future, we can: 1. analyze and elucidate the dynamics of cellular interactions by combining with time series; 2. validate the key signaling pathways by combining with *in vitro* culture and other experiments; 3. explore the plasticity of cellular collaborative networks in response to environmental stresses; 4. verify the spatial location of coral cells by *in situ* hybridization experiments.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

WD: Data curation, Methodology, Software, Supervision, Validation, Writing – original draft. SZ: Data curation, Methodology, Software, Writing – original draft, Writing – review & editing. XZ: Methodology, Software, Visualization, Writing – review & editing. PW: Data curation, Visualization, Writing – review & editing. BD: Software, Visualization, Writing – review & editing. ZH: Data curation, Funding acquisition, Writing – review & editing. YL: Methodology, Software, Validation, Writing – original draft. HC: Conceptualization, Formal Analysis, Investigation, Project administration, Resources, Supervision, Writing – review & editing. ZL: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2025.1551763/full#supplementary-material

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