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Metabolic and immune costs balance during natural acclimation of corals in fluctuating environments

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ABSTRACT

Epigenetic modifications based on DNA methylation can rapidly improve the potential of corals to adapt to environmental pressures by increasing their phenotypic plasticity, a factor important for scleractinian corals to adapt to future global warming. However, the extent to which corals develop similar adaptive mechanisms and their specific adaptation processes remain unclear. Here, to reveal the regulatory mechanism by which DNA methylation improves thermal tolerance in *Pocillopora damicornis* under fluctuating environments, we analyzed genome-wide DNA methylation signatures in *P. damicornis* and compared the differences in the methylation and transcriptional responses of *P. damicornis* from fluctuating and stable environments using whole-genome bisulfite sequencing and nanopore-based RNA sequencingtranscriptome sequencing. We discovered low methylation levels in *P. damicornis* (average methylation 4.14%), with CpG accounting for 74.88%, CHH for 13.27%, and CHG for 11.85% of this methylation. However, methylation levels did not change between coral samples from the fluctuating and stable environments. The varied methylation levels in different regions of the gene revealed that the overall methylation level of the gene body was relatively high and showed a bimodal methylation pattern. Methylation occurs primarily in exons rather than introns within the gene body In *P. damicornis*, there was only a weak correlation between methylation and transcriptional changes at the individual gene level, and the methylation and gene expression levels generally exhibited a bell-shaped relationship, which we speculate may be due to the specificity of cnidarian species. Correlation analysis between methylation levels and the transcriptome revealed that the highest proportion of the top 20 enriched KEGG pathways was related to immunity. Additionally, *P. damicornis* collected from a high-temperature pool had a lower metabolic rate than those collected from a low-temperature pool. We hypothesize that the dynamic balance of energy–expenditure costs between immunity and metabolism is an important strategy for increasing *P. damicornis* tolerance. The fluctuating environment of high-temperature pools may increase the heat tolerance in corals by increasing their immunity and thus lowering their metabolism.

1. Introduction

In the past half century, coral reef ecosystems have been under severe threat owing to the combined influence of climate change and human activities (Hughes et al., 2003; Hughes et al., 2018; Hughes et al., 2017a,b). Specifically, in the El Niño climate cycle, the global seawater temperature is expected to rise over the next 50 years, due to the large amount of greenhouse gas emissions. Previous field ecological studies have shown that high-frequency temperature variability reduces the risk of coral bleaching (Ainsworth et al., 2016; Barshis et al., 2013; Bay et al., 2017; Grottoli et al., 2014; Oliver and Palumbi, 2011; Rogers et al., 2016; Safaie et al., 2018; Schoepf et al., 2015, 2020; Swain et al., 2016; Yu et al., 2021), which may improve the outlook for coral reefs in the future (van Oppen et al., 2015; Yu et al., 2020a,b). Studies have suggested that corals also possess an adaptive-like immunological repertoire, which may play a critical role in the corals' ability to combat future external interference. The extent to which corals have developed such an adaptive-like immune repertoire will determine whether corals will survive climate change and other anthropogenic disturbances. However, despite increasing efforts to study the mechanisms underlying

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this interesting ecological phenomenon (Kenkel et al., 2013; Kenkel and Matz, 2016; Kirk et al., 2018; Matz et al., 2019; Yu et al., 2020a,b; Zhou et al., 2017), we still lack knowledge regarding the basic molecular processes involved. Specifically, the role of epigenetic mechanisms in the regulation of this phenomenon and their potential to contribute to increased resilience in response to environmental stress—as reported in other organisms (Mirouze and Paszkowski, 2011; Sahu et al., 2013)—remains unstudied. Under changing environmental conditions, it is predicted that acquiring phenotypic plasticity through epigenetic mechanisms may mitigate the loss of fitness (Chevin et al., 2013) and facilitate evolutionary adaptation (Price et al., 2003; Yeh and Price, 2004). For sessile organisms, such as plants and corals, plasticity through epigenetic mechanisms is predicted to be of particular importance as these organisms cannot migrate from suboptimal environments (Barshis et al., 2013; Nicotra et al., 2010).

DNA methylation is a crucial epigenetic modification of the genome (Robertson, 2005); previous studies are increasingly highlighting the continued role of DNA methylation across an organisms' lifespan (Baker-Andresen et al., 2013; Dimond, 2019). As DNA methylation is affected by the environment, it may contribute to the ability of basal metazoans to adapt to environmental changes (Dimond and Roberts, 2016; Dixon et al., 2018; Liew et al., 2020). In a previous study, DNA methylation was established as a key link between the environment and phenotype (Dimond and Roberts, 2016). Previous findings have shown that DNA methylation is involved in mediating stress-induced transgenerational responses of *Arabidopsis* within and between generations (Boyko et al., 2010; Dubin et al., 2015). Epigenetic changes are involved in the adaptation of eukaryotic Scots pine (*Pinus silvestris*) to chronic radiation exposure. Meanwhile, DNA methylation-mediated stress memory in plants growing under stress conditions has been reported (Feng et al., 2012; Hauser et al., 2011). This inherent epigenetic plasticity plays an important role in the immediate response of organisms and the establishment of long-term adaptation under conditions of stress (Mirouze and Paszkowski, 2011). This interesting phenomenon has also been observed in aquatic organisms along with enhanced epigenetic plasticity in freshwater sticklebacks, this may serve as a regulatory mechanism that compensates for the lack of genetic variation in freshwater populations (Artemov et al., 2017). To date, only a few studies have evaluated the effects of DNA methylation in corals under conditions of environmental stress. DNA methylation is involved in symbiotic homeostasis and induces a response to thermal stress, ocean acidification, and nutrient stress in cnidarians (Dimond et al., 2017; Dimond and Roberts, 2016; Dixon et al., 2014; Li et al., 2018; Liew et al., 2018, 2020; Marsh et al., 2016; Putnam et al., 2016; Rodriguez Casariego et al., 2018). A field transplant experiment revealed that DNA methylation in the scleractinian coral *Porites astreoides* is responsive to environmental changes and isa part of the potential transgenerational acclimatization responses (Dimond and Roberts, 2020). Thus, deciphering the mechanism by which the methylation-based epigenetic machinery responds to different tolerances to environmental stresses in scleractinian corals is important. However, there is a lack of research with respect to the role of methylation in mediating the adaptive response of scleractinian corals in thermally variable reef environments.

Pocillopora damicornis, one of the most well-studied and widely distributed scleractinian corals, is a branching coral; however, it has a high tolerance similar to that of a massive coral, which makes it an excellent model species for studying the mechanisms (e.g., epigenetic regulation) underlying stress adaptation (Tang et al., 2018). A high-quality *P. damicornis* genome sequence has been published (Cunning et al., 2018), which provides the basis for whole-genome bisulfite sequencing (WGBS) and transcriptome sequencing. In this study, to reveal whether epigenetic mechanisms are involved in regulating the thermal acclimation of scleractinian corals in response to fluctuating reef habitats, we used *P. damicornis* to perform WGBS and evaluate DNA methylation. Understanding the exposure–response relationships of these molecular mechanisms could potentially allow the quantification of the effects of the environment on phenotypic variation, thereby increasing our capacity to predict population responses after environmental changes.

2. Materials and methods

2.1. Coral sampling and study site

Our study was conducted in the Luhuitou fringing reef in Sanya Bay, Hainan, China (18° 12.7′ N, 109° 28.5′ E). It is a typical fringing reef, which is host to the highest level of research on Hainan Island. As described in our previous study (Yu et al., 2021), with the influence of tides and coastal topography, there are two different growth environments in this sea area: a high-temperature pool (HP) and a low-temperature pool (LP), HP has shallower depth, higher thermal variability, and higher water flow than LP, which has a more stable environment (Fig. 1). It offers a natural laboratory model that is suitable for studying the impact of a fluctuating environment on the tolerance of reef-building corals and for assessing the adaptive potential of reef-building corals under future global warming scenarios. Ten *P. damicornis* colonies in good condition were collected from HPs and LPs. In the HP and LP groups, any two samples were selected from ten samples and mixed them in equal molar amounts to form a new mixed sample, so that each group had five biological replicates for subsequent analysis. All samples were stored in liquid nitrogen for subsequent bisulfite sequencing (WGBS) and transcriptome sequencing.

2.2. DNA extraction and bisulfite sequencing

The experiment was performed as per the standard protocol provided by Illumina (Smith et al., 2009). The DNA of each coral sample was extracted using a Universal Genomic DNA Extraction Kit (DV811A; TaKaRa, Kusatsu, Japan). After the genomic DNA of the sample was tested for qualification, the DNA was fragmented using the Covaris ultrasonicator method (He et al., 2017). The fragments were purified using AMPure XP beads and end-repaired, and a single "A" nucleotide was added to the 3′ ends of the blunt fragments followed by ligation to the methylated adapter with a T overhang. Agarose gel electrophoresis was performed for fragment size selection, followed by bisulfite treatment using a ZYMO EZ DNA Methylation-Gold™ Kit (ZYMO, Irvine, CA, USA). Subsequently, PCR amplification was performed to generate a sequencing library; the constructed library was inspected using a 2100 Bioanalyzer (Agilent Technology, Santa Clara, CA, USA) and quantified using qPCR (Agilent qPCR NGS Library quantification kit; Agilent Technology). The qualified library was used for WGBS using the Illumina platform at the Biomarker Technology Company (Beijing, China).

To ensure quality analysis, raw reads were filtered to obtain clean reads for subsequent information analysis. First, the reads with adapters were removed. Next, reads with a N content exceeding 10% and reads with a base mass value below 10 exceeding 50% were filtered. The sequenced reads obtained from whole-genome methylation sequencing were relocated to the reference genome using Bowtie 2 for subsequent methylation analysis (Langmead and Salzberg, 2012). Bismark software was used to compare short methylated sequences obtained from second-generation high-throughput sequencing with the reference genome (Krueger and Andrews, 2011). Bowtie 2 was employed on the Bismark platform. For the results obtained from the Bowtie comparison, the best unique comparison result is selected for subsequent analysis. The 5-methylcytosine (5 mC) modifications were divided into CpG, CHG, and CHH, based on different bases. CpG O/E is an indicator used to measure the ratio between the observed and expected values of the CpG dinucleotide symmetry in a DNA sequence, where O represents the observed value and E represents the expected value. CpG dinucleotide symmetry is the presence of an inorganic phosphodiester bond between adjacent C and G bases in the DNA sequence. During the evolution of DNA, the CpG dinucleotide symmetry changes due to factors such as

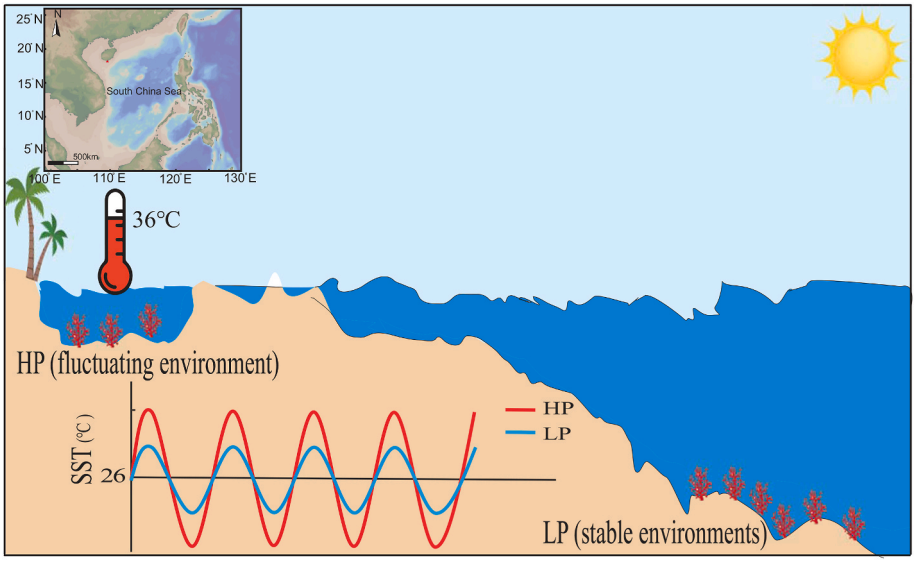

Fig. 1. Study area and schematic diagram of environmental characteristics of sampling locations.

methylation. CpG islands are regions showing enriched CpG dinucleotides in DNA sequences, often associated with the promoters of genes. CpG O/E can assess the effects of methylation levels and evolutionary pressure on DNA sequences by comparing the difference between the observed CpG dinucleotide symmetry and the desired CpG dinucleotide symmetry. If the value of CpG O/E is greater than 1, the observed CpG dinucleotide symmetry is more than the expected value, which may indicate that excessive methylation exists in this region, while if the value of CpG O/E is less than 1, the observed CpG dinucleotide symmetry is less than the expected value, which may indicate that there is less methylation present in this region. After comparison, the position of unique reads on the reference genome was located, the sequencing depth and genome coverage of each sample were counted, and the methylation sites were detected. Based on the location of the methylation sites and the gene location information in the reference genome, we obtained the region or regions where the site occurs in the genome (upstream, gene body, downstream, etc.). Each region was divided into 20 bins to calculate the methylation levels of different regions of the whole genome (Schultz et al., 2012). The area methylation levels were calculated using the weight methylation level method (Schultz et al., 2012).

2.3. Transcriptome analysis

To understand the molecular basis of natural acclimation within *P. damicornis* in fluctuating environments, we performed transcriptome sequencing (Oxford Nanopore Technologies, Oxford, UK) on the PromethION platform. The total RNA (1 μg) of frozen coral samples was used to prepare cDNA libraries. The raw reads of transcriptome sequencing in this study have been submitted to the NCBI under the accession number: RJNA647655 (Yu et al., 2021). The sequencing and analysis methods have been described in our previous study (Yu et al., 2021). The raw reads of transcriptome sequencing were first filtered according to the standard, with a minimum read length of 500 bp and a minimum average read quality score of 7 (Chen et al., 2020). Ribosomal RNA was discarded after mapping it to the rRNA database. Full-length non--chimeric (FLNC) transcripts were determined by searching for primers at both ends of the reads. For each sample, clusters of FLNC transcripts were obtained after the reads were aligned to the *P. damicornis* genome using minimap2 (Cunning et al., 2018; Li, 2018). Pinfish was used to obtain consensus isoforms after polishing each cluster (Chen et al., 2020). To remove redundant sequences, minimap2 was used to map the obtained sequences onto the reference genome (Cunning et al., 2018; Li, 2018). Sequence alignments and subsequent analyses were performed using the full-length sequencing transcriptomes and known genome transcripts as references, and sequence alignment was performed between clean reads and the reference transcriptome using minimap2 to obtain corresponding information regarding the transcriptome and reference transcriptome.

2.4. Correlation analysis between methylation and transcriptome

To analyze the association between gene expression and methylation levels, we examined the association between differentially expressed genes (DEGs) and differentially methylated genes (DMGs). The counts per million (CPM) values were used as a measure of transcripts or gene expression (Zhou et al., 2014). DESeq was used for analyzing the DEGs among sample groups (Anders and Huber, 2010). Fold change ≥ 2 and false discovery rate (FDR) < 0.05 were used as the screening criteria. DMGs were detected between samples based on the cytosine mapping results of the unique mapped reads of the samples on the reference genome. MOABS (Sun et al., 2014) was used to screen the differentially methylated regions, which required a coverage depth of no less than 10 $\times$, the presence of at least three differentially methylated sites, and a minimum difference in methylation level of 0.2 (0.3 for CG type), using Fisher's exact test with a p-value <0.05. As distinct C types (CG, CHG, and CHH) are methylated by different methods in different species and even within the same species, we investigated DMRs of different types of MCS separately. The assembled unigenes were annotated against the KEGG metabolic pathway database (Kanehisa and Goto, 2000). To annotate the different methylated regions (DMR) and obtain the associated methylation differential genes (DMG), use the following methods: Gene annotation: associate the DMR with known genes to determine which DMR is located in the promoter, intron, or exonic regions of the gene. This can be done by using the gene annotation database (such as Ensembl, UCSC Genome Browser) and the corresponding genome annotation files (such as GTF files). Functional annotation: For genes located near the DMR, further functional annotation can be performed to determine the biological processes or functions in which these genes may be involved. Gene functional annotation databases (e. g., Gene Ontology, KEGG Pathway) can be used to perform functional enrichment analysis of genes to identify biological processes, molecular functions and pathways associated with DMR. Co-expression network analysis: Genes near the DMR are compared to known co-expression networks to determine whether there are genes with correlated expression patterns. Gene co-expression databases (e. g. STRING,

CoExpedia) can be used to build and analyze co-expression networks. Transcription factor binding site prediction: Regions found in the DMR may contain transcription factor binding sites, and these sites may be potential mechanisms of gene regulation. Transcription factor binding site prediction tools (e. g., JASPAR, MEME Suite) can be used to predict potential transcription factor binding sites in DMR and further analyze whether these sites are related to related transcription factors and regulation of gene expression. These methods can help annotate DMR and provide DMG related to DMR. Comprehensive use of gene annotation, functional annotation, co-expression network analysis and transcription factor binding site prediction provides a thorough understanding of the biological functions and regulatory mechanisms of DMR. Overrepresentation analysis using Fisher's exact test (Benjamini–Hochberg adjusted p-value <0.05) was used for KEGG enrichment analysis (Backes et al., 2007; Désert et al., 2008). The obtained p-value was corrected using the Benjamini–Hochberg method for multiple test correction to control the probability or frequency of errors in the overall inference results (Benjamini and Hochberg, 1995; Désert et al., 2008).

3. Result

3.1. Differential transcriptional response between different groups

We generated high-quality sequencing reads via third-generation sequencing using the ONT. Each sample was sequenced to produce 2.70 GB of clean data. In total, 10,634,905 and 11,167,173 raw reads were generated from the HP and LP groups, with average read lengths of 1380 and 1352 bp and N50 of 1653 and 1624 bp, respectively. After filtering the short- and low-quality reads, 10,595,305 and 11,124,365 clean reads were obtained, of which 80.87% and 79.29% were identified as being full-length, respectively.

3.2. Changes in genomic methylation patterns

To further understand the role of DNA methylation in mediating the adaptive response of *P. damicornis* in fluctuating environments, we performed WGBS with an average coverage of 30 × per individual on ten colonies, with five biological replicates per group. In total, 344,340,043 clean reads were obtained for each group. The Q30 ranged from 92.11% to 96.12%. The GC% ranged from 18.95% to 19.30%, Q30 refers to the base error rate; GC% refers to the percentage of base GC content (Supplementary table 1). The conversion ratio of these groups of mapped reads was 99.52% (Supplementary table 2), with 44.71–52.03% of clean reads uniquely mapped (Supplementary table 3). Conversion rate in methylation usually refers to the conversion efficiency during DNA methylation. DNA methylation is an important biologically modifying process in which methyl groups on DNA molecules are added to the bases of DNA. Such a modification can affect the gene expression and cellular function.

The coverage of bases on the reference genome was counted after the reads were mapped to the reference genome. The mean depth of coverage for each sample and the corresponding genome coverage ratios at each depth are listed in Supplementary table 4. The distribution of methylated C bases across the genome consisted of three forms (CG, CHG, and CHH, where H represents A, T, or C bases). The coverage of the C bases of the three different distribution types was determined, and the statistics are presented in Supplementary table 5.

Based on the best comparison result of clean reads in the reference genome, the base information of the whole-genome cytosine (C) site was extracted (Supplementary table 6). The *P. damicornis* genome was sparsely methylated, with an average of 4.14% of all CpGs, of which CpG accounted for 74.88%, CHH for 13.27%, and CHG for 11.85%, and there was no difference between the two groups ($p > 0.05$) (Supplementary table 6; Supplementary table 7). We used the binomial distribution test principle to detect the 5 mC of each C site. The screening conditions were coverage ≥ 4x and FDR <0.05. There was no difference in methylation levels between *P. damicornis* samples from the HP and LP groups ($p < 0.05$), which were 4.08% and 4.2%, respectively.

On each chromosome, the methylation level of each window was calculated by considering 100 k as a window, describing the distribution of methylated C bases from the chromosomal level, and drawing the whole-genome chromosome level methylation map (Supplementary figure 1). Based on the location of the methylation sites in the reference genome and the gene location information in the reference genome, we can obtain the region or the region where the site occurs in the genome (upstream, gene body, downstream, etc.). Each region is divided into 20 bins to calculate the methylation level of different regions of the whole genome (Fig. 2). To characterize the genome-wide methylation model of *P. damicornis*, we analyzed the methylation levels in different regions of the gene (Fig. 3). Collectively, the methylation levels in the gene body were relatively high.

3.3. Correlation analysis between expression and methylation

To further analyze the relationship between gene expression and methylation, we analyzed the methylation rates of genes with different expression levels (Fig. 4). The results showed limited correlation between differentially expressed and differentially methylated genes (Fig. 5).

3.4. DMGs and DEGs are involved in mediating the adaptive response in the scleractinian coral P. damicornis

The regulatory mechanism by which DNA methylation is involved in enhancing thermal tolerance in *P. damicornis* in fluctuating environments was investigated. Upon performing an association analysis of the DEGs and DMGs between the two groups (Supplementary table 8). We performed KEGG functional enrichment analysis on the filtered target genes (comprehensively considered the targeted genes of DMGs and overlapping ones between DEGs and DMGs) (Fig. 6), and the top 20 enriched pathways were as follows: focal adhesion, endocytosis, NOD receptor signaling pathway, p53 signaling pathway, RIG−I−like receptor signaling pathway, toll-like receptor signaling pathway, tight junction, herpes simplex infection, apoptosis, nitrogen metabolism, vascular smooth muscle contraction, homologous recombination, beta-alanine metabolism, regulation of actin cytoskeleton, RNA transport, metabolism of xenobiotics by cytochrome P450, base excision repair, AGE−RAGE signaling pathway in diabetic complications, amino sugar and nucleotide sugar metabolism, and Fanconi anemia pathway. The DE-lncRNA-targeted mRNAs were significantly enriched in ko00531 glycosaminoglycan degradation, ko00603 glycosphingolipid biosynthesis - globo series, ko00511 other glycan degradation, ko04142 lysosome, ko00604 glycosphingolipid biosynthesis - ganglio series, ko03013 RNA transport, and ko00520 amino sugar and nucleotide sugar metabolism.

4. Discussion

To assess the role of DNA methylation in the adaptability of scleractinian corals, we analyzed changes in the DNA methylomes and transcriptomes of *P. damicornis* in fluctuating and stable environments.

4.1. Low methylation levels in P. damicornis

Interestingly, we found low methylation levels in *P. damicornis*. However, similar to our findings, other studies have found that some cnidarians, such as *Acropora hyacinthus*, *A. millepora*, *A. palmata*, *Porites astreoides*, and *Stylophora pistillata* have relatively low methylation levels (Dimond and Roberts, 2016). As with most other invertebrate taxa surveyed, a bimodal distribution was observed in coral transcriptomes. All the distributions were dominated by relatively high CpG O/E scores, suggesting relatively low methylation levels for most genes in the reefs.

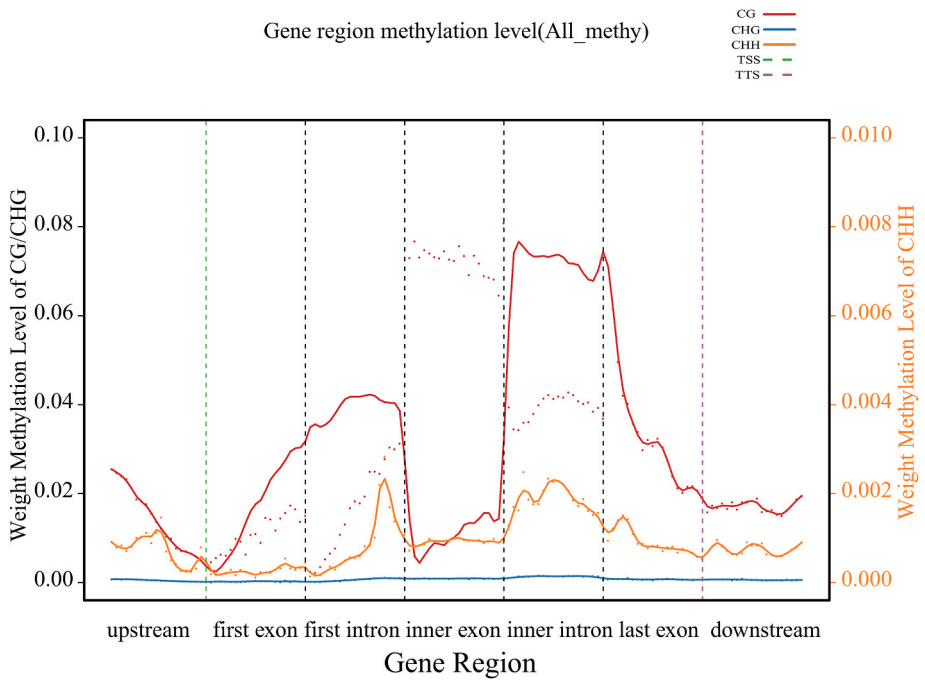

Fig. 2. The methylation level of each sample was mapped to the distribution of each functional element in the gene region.

A similar pattern was observed in the whole-genome analysis of the sea anemone *Nematostella vectensis* (Sarda et al., 2012; Zemach et al., 2010) and analysis of exons in *A. millepora* (Dixon et al., 2014). In contrast, CpG O/E curves of other invertebrates, such as the oyster *Crassostrea gigas* and ascidian *Ciona intestinalis*, indicate low CpG O/E fractions (Dixon et al., 2014; Gavery and Roberts, 2010). DNA methylation levels broadly reflect evolutionary relationships (Tweedie et al., 1997; Zemach et al., 2010), and coral methylation is similar to that of *N. vectensis* (Dimond and Roberts, 2016). Previous studies have also reported that methylation of genomic cytosines occurs in humans at a rate of approximately 4–6%, whereas in insects, the methylation rate is only 0.1–0.2% (Bonasio et al., 2012; Li et al., 2010; Lister et al., 2009; Lyko et al., 2010; Wang et al., 2014; Xiang et al., 2010).

In contrast to the almost ubiquitous methylation in mammalian genomes, genomic methylation in numerous invertebrates occurs at CpG dinucleotides within the gene bodies (Chapman et al., 2010; Suzuki et al., 2007; Zemach et al., 2010). Within gene bodies, methylation occurs predominantly in exons rather than in introns (Falckenhayn et al., 2013; Wang et al., 2013). The density of gene body methylation was not equal across genes. Studies on multiple invertebrate taxa have reported bimodal patterns of gene body methylation, with genes classified into two categories: hyper- and hypomethylation (Falckenhayn et al., 2013; Sarda et al., 2012). Bimodal methylation was consistent in different invertebrate taxa, including Hymenoptera (Park et al., 2011), Hemiptera (Hunt et al., 2010), Lepidoptera (Xiang et al., 2010), Orthoptera (Falckenhayn et al., 2013), mollusks (Gavery and Roberts, 2010), and cnidarians (Sarda et al., 2012). Evidence for bimodal methylation in Cnidaria, as well as in other diverse taxa, suggests that this ancient mechanism has been conserved for over 500 million years of evolution (Chapman et al., 2010). Thus, gene body methylation may promote the consistent expression of genes that perform essential functions, for which aberrations can be lethal (Roberts and Gavery, 2012). Studies have argued that vertebrate genomes have much higher levels of methylation than invertebrate genomes and that methylation may have evolved as an essential function to reduce transcriptional noise (i.e., pseudotranscription) in more complex vertebrates with a higher number of genes (Bird, 1995). There is experimental evidence for this hypothesis; for example, Huh's study demonstrated a negative correlation between gene body methylation and transcriptional noise (Huh et al., 2013), and a study showed that methylation protects gene bodies from spurious entry by RNA polymerase II (Neri et al., 2017). Invertebrates may benefit to some extent from transcriptional noise, owing to their greater potential for transcriptional (phenotypic) plasticity compared to that of vertebrates, which may have greater benefits for less complex organisms owing to the lower buffering capacity of these organisms (Roberts and Gavery, 2012). There is substantial evidence that gene body methylation plays a role in alternative splicing in both vertebrates and invertebrates (Flores et al., 2012; Maunakea et al., 2013). Not all exons are included during the transcription of a specific gene. Alternative splicing is a phenomenon in which transcript variants are generated through exon splicing. For example, Flores et al. (2012) found that the DNA methylation level of exons included in transcripts was higher than that of the excluded exons in honeybees, and the incidence of alternative splicing was higher in methylated genes than in unmethylated genes. Another hypothesis for the role of gene body methylation is that it may facilitate the consistent expression of ubiquitously expressed core genes required for basic biological functions (Roberts and Gavery, 2012).

4.2. *Limited correlations between DEGs and DMGs in P. damicornis*

The analysis of gene expression–methylation relationships revealed limited correlations between the differentially expressed and DMGs. In KEGG enrichment analysis, we found that the number of genes in numerous pathways is very small, which may be attributed to the fewer target genes screened. A similar phenomenon was discovered in two recent cnidarian studies: Li et al. discovered several differentially methylated DEGs in their study on *E. pallida* symbiosis, but little overlap between the two sets of genes (Dixon et al., 2018; Li et al., 2018); similarly, Dixon and Li found only a weak correlation between methylation and transcriptional changes at the gene level in the model system *Aiptasia,* and *Acropora millepora* (Dixon et al., 2018; Li et al., 2018). This finding supports the hypothesis that hypomethylation is associated with transcriptional plasticity (Roberts and Gavery, 2012). However, it is interesting to note that both studies discovered an association between methylation and transcriptional changes when gene functional groupings or pathways were considered rather than

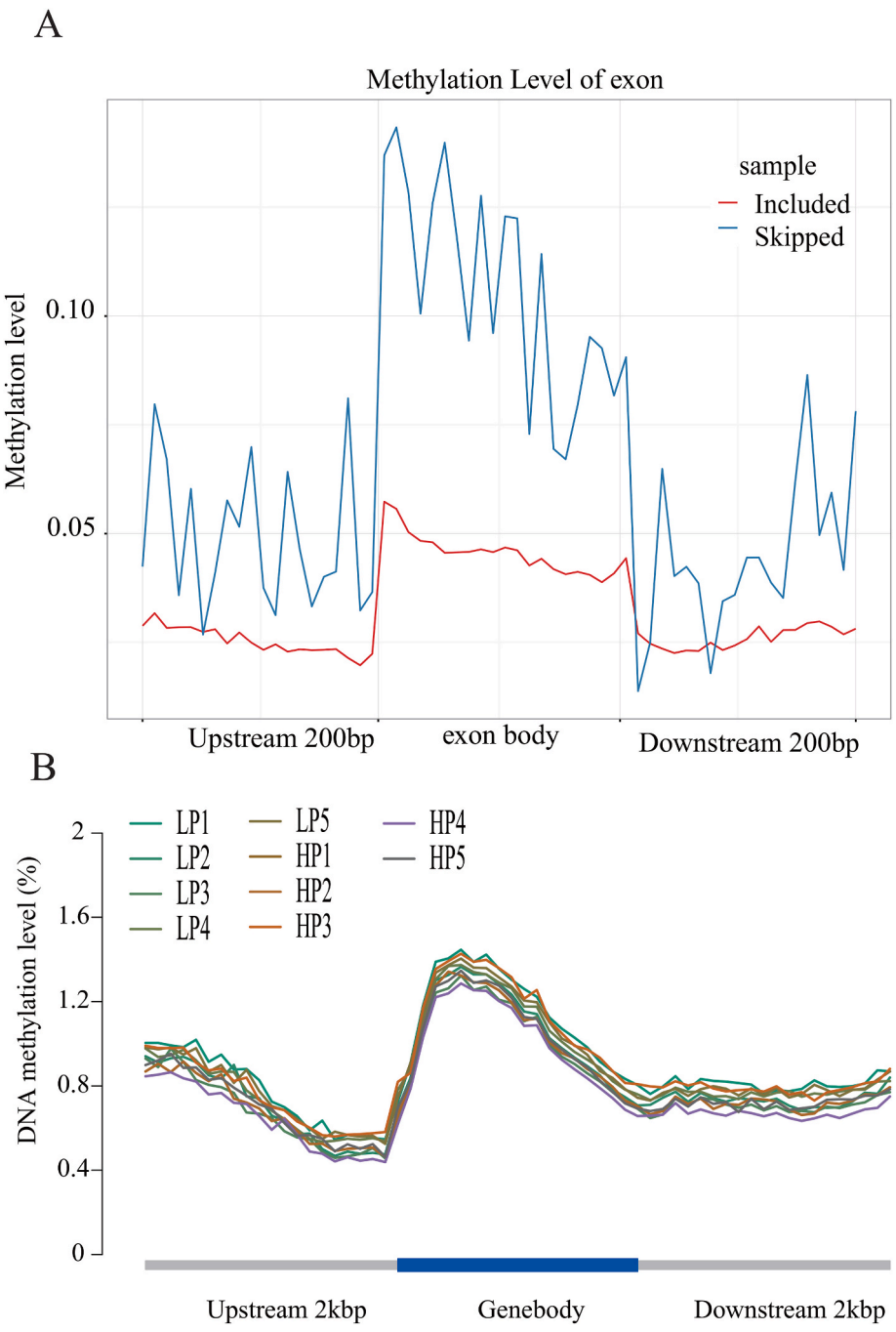

Fig. 3. Methylation levels in different regions of the gene.

individual genes. Both studies concluded that there is some complementarity between methylation and transcription at a wide range of functional levels, but the precise mechanisms remain the subject of speculation (Dixon et al., 2018; Li et al., 2018). Jjingo et al. observed that methylation and gene expression levels often exhibit a bell-shaped relationship (Jjingo, 2012). Both the highest and lowest levels of gene body methylation were correlated with the lowest levels of expression, whereas high levels of expression were correlated with moderate levels of methylation. Based on this phenomenon, Jjingo et al. proposed a model in which methylation levels are influenced by expression levels, as opposed to the more general notion that methylation affects expression (Jjingo, 2012). Their model suggests that low levels of expression limit the access of DNA methyltransferases to DNA because of dense nucleosome packing, whereas high levels of expression limit the access of methyltransferases to DNA because of intense RNA polymerase trafficking. Moderate levels of expression allow sufficient access to DNA to allow methylation to occur (Jjingo, 2012). An issue with this hypothesis may arise when such a relationship between methylation and expression levels does not exist (Lokk et al., 2014). All the hypotheses proposed above are likely to have some validity, and none are mutually exclusive. Indeed, all hypotheses can be applied to a conceptual model in which the overall effect of gene body methylation provides direction for various aspects of transcription. In other words, gene body methylation appears to help determine where transcription should start, how quickly it should proceed, which exons should be included, and which parts of the genome this should be accomplished in. The part of the genome in which this direction seems to be the most prevalent is precisely where we expect them to be, highly conserved genes that perform essential

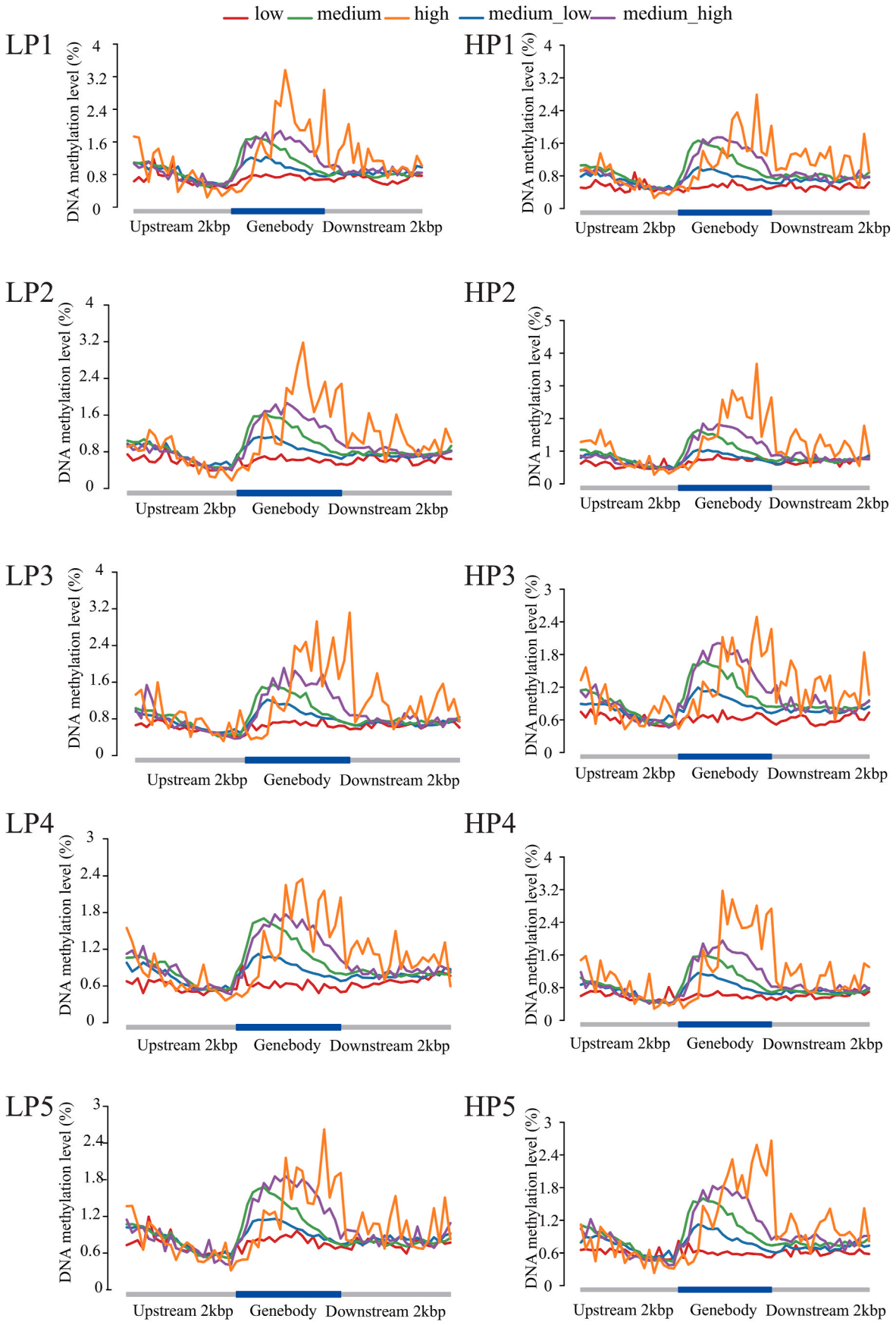

Fig. 4. Methylation rates of genes with different expression levels.

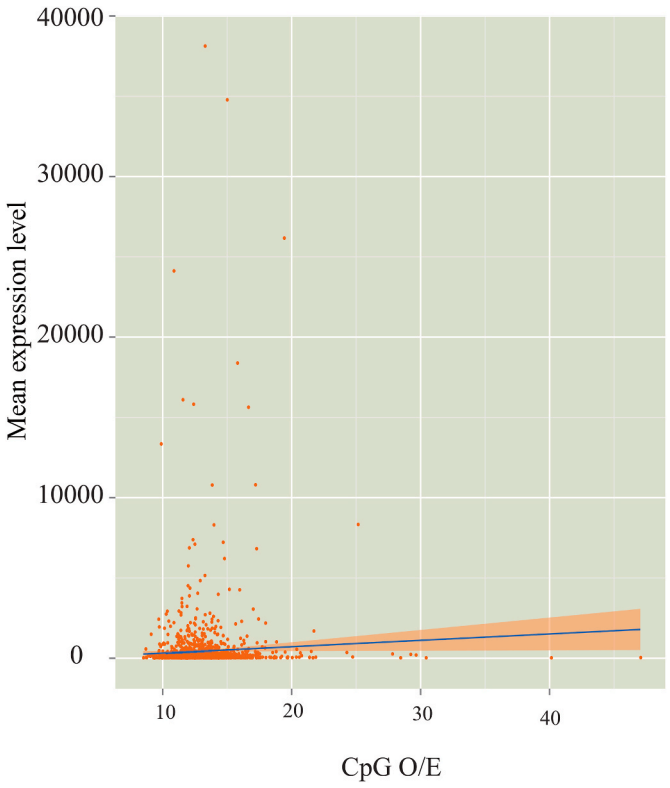

Fig. 5. Correlation between methylation and gene expression.

processes, leaving little room for error (Dimond, 2019). Thus, when considered more comprehensively, the function of gene body methylation may not be as ambiguous as it appears (Dimond, 2019). However, some studies have demonstrated a positive linear correlation between intragenic DNA methylation and gene expression in *N. vectensis* and the silkworm *Bombyx mori* (Xiang et al., 2010; Zemach et al., 2010). In other eukaryotes, including honeybees, a parabolic relationship has been reported in which most moderately transcribed genes are more hypermethylated than lowly or highly expressed genes, implying a lack of positive correlation between DNA methylation and transcription at the gene level in these species (Zemach et al., 2010). It has been suggested that intragenic DNA methylation—more precisely exonic DNA methylation—may be associated with alternative splicing events (Lyko et al., 2010; Park et al., 2011). However, whether this relationship can be observed on a genome-wide scale remains to be determined. In addition, it has been reported that gene body methylation in *Arabidopsis* and honeybees is positively correlated with gene length and gene conservation (Takuno and Gaut, 2011; Zeng and Yi, 2010) and that gene length is highly correlated between honeybees and *Drosophila* (Takuno and Gaut, 2011; Zeng and Yi, 2010). Moreover, Mendoza found that Symbiodinium have evolved cytosine methylation patterns unlike any other eukaryote, with most of the genome methylated at CG dinucleotides (de Mendoza et al., 2018). The retrotransposons could self-methylate retrotranscribed DNA (de Mendoza et al., 2018). This is an example of how retrotransposons incorporate host-derived genes involved in DNA methylation. In some cases, this event could have implications for the composition and regulation of the host epigenomic environment.

4.3. Resource reallocation between immunity and metabolism is the strategy of P. damicornis thermal acclimation

The highest proportion of the top 20 enriched KEGG pathways were associated with organismal immunity. For example, the NOD receptor, p53, RIG-I-like receptor, toll-like receptor, apoptosis, and AGE-RAGE signaling pathways are involved in diabetic complications. The immune system is the provider of balance in coral holobionts and is also the coordinator of the relationship between the hosts and the symbiotic microbiome (Palmer, 2018). The immune system provides health and balance to coral holobionts and maintains symbiosis upon receipt of external biotic and abiotic disturbances (Palmer, 2018). Different manifestations of immunity and support for resistance are driven by physiological trade-offs under evolutionary constraints (Palmer, 2018). Therefore, changes in immunity may have a significant impact on coral survival under climate change (Palmer, 2018). The hypothesis of susceptibility damage threshold for coral holobionts suggests that holobionts with higher immune components and greater tolerance may be better able to maintain homeostasis by perturbation than holobionts using resistance strategies, which may be more susceptible to submersion (Palmer et al., 2010). Life history theory argues that, at any given moment, an organism's immune strategy occurs within predetermined limits of evolutionary trade-offs, dependent on the environment, phenotypic plasticity, and physiological trade-offs (Sheldon and Verhulst, 1996). Physiological costs (determining plasticity) may arise during the maintenance and implementation of immunity, including energy expenditure and potential self-harm (autoimmunity) (Schmid--Hempel, 2003). For numerous organisms, including corals, this physiological cost may manifest as a temporary reduction in fecundity or growth owing to the reallocation of resources and/or destruction by immune responses (Schmid-Hempel, 2003; Sheridan et al., 2014). Therefore, the body balance of the whole organism of corals maintained by immunity may be directly related to the availability of energy and the ability to compensate for costs, such as through increased heterotrophy and reduced metabolism (Palmer, 2018; Yu et al., 2020a,b). Our previous results of transcriptomics results found that *P. damicornis* from the HP had a lower metabolic rate than that from the LP (Yu et al., 2021). Therefore, we speculate that the dynamic balance of energy–expenditure costs between immunity and metabolism is an important strategy for increasing *P. damicornis* tolerance. The fluctuating environment of high-temperature pools may increase the heat tolerance of corals by increasing their immunity and lowing their metabolism.

Data statement

Sequencing raw reads in this study were deposited into the NCBI sequence reads archive (SRA) database: RJNA647655 and PRJNA841961.

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CRediT authorship contribution statement

Xiaopeng Yu: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing, Resources, Validation, Visualization. **Kefu Yu:** Conceptualization, Funding acquisition, Methodology, Resources, Validation. **Biao Chen:** Formal analysis, Software, Visualization. **Zhiheng Liao:** Investigation, Resources, Writing – review & editing. **Jiayuan Liang:** Formal analysis, Project administration, Writing – review & editing. **Zhenjun Qin:** Data curation, Formal analysis, Validation, Writing – review & editing. **Xu Gao:** Data curation, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

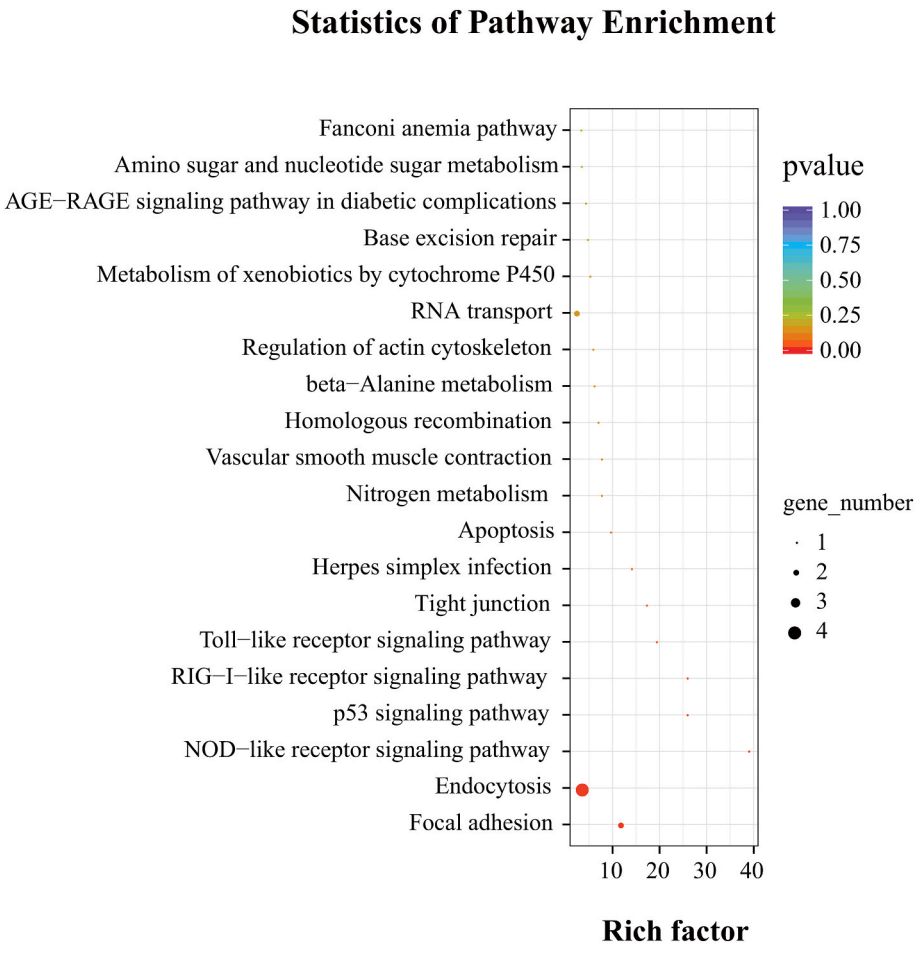

Fig. 6. KEGG pathway enrichment analysis of targeted genes. The size of the point indicates the number of DEGs enriched in the pathway, and the color indicates the significance of enrichment, FDR<0.05.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2023.106284.

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