Comparative transcriptome analysis of papilla and skin in the sea cucumber, *Apostichopus japonicus*

Xiaoxu Zhou, Jun Cui, Shikai Liu, Derong Kong, He Sun, Chenlei Gu, Hongdi Wang, Xuemei Qiu, Yaqing Chang, Zhanjiang Liu, Xiuli Wang

Papilla and skin are two important organs of the sea cucumber. Both tissues have ectodermic origin, but they are morphologically and functionally very different. In the present study, we performed comparative transcriptome analysis of the papilla and skin from the sea cucumber (*Apostichopus japonicus*) in order to identify and characterize gene expression profiles by using RNA-Seq technology. We generated 30.6 and 36.4 million clean reads from the papilla and skin and *de novo* assembled in 156,501 transcripts. The gene ontology (GO) analysis indicated that cell part, metabolic process and catalytic activity were the most abundant GO category in cell component, biological process and molecular funcation, respectively. Comparative transcriptome analysis between the papilla and skin allowed the identification of 1,059 differentially expressed genes, of which739 genes were expressed at higher levels in papilla, while 320 were expressed at higher levels in skin. In addition, 236 differentially expressed unigenes were not annotated with any database, 160 of which were apparently expressed at higher levels in papilla, 76 were expressed at higher levels in skin. We identified a total of 288 papilla-specific genes, 171 skin-specific genes and 600 co-expressed genes. And 40 genes in papilla-specific were not annotated with any database, 2 in skin-specific. Development-related genes were also enriched, such as *fibroblast growth factor*, *transforming growth factor-β*, *collagen-α2* and *Integrin-α2*, which may be related to the formation of the papilla and skin in sea cucumber. Further pathway analysis identified ten KEGG pathways that were differently enriched between the papilla and skin. The findings on expression profiles between two key organs of the sea cucumber should be valuable to reveal molecular mechanisms involved in the development of organs that are related but with morphological differences in the sea cucumber.

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ABSTRACT

Papilla and skin are two important organs of the sea cucumber. Both tissues have ectodermic origin, but they are morphologically and functionally very different. In the present study, we performed comparative transcriptome analysis of the papilla and skin from the sea cucumber (*Apostichopus japonicus*) in order to identify and characterize gene expression profiles by using RNA-Seq technology. We generated 30.6 and 36.4 million clean reads from the papilla and skin and *de novo* assembled in 156,501 transcripts. The gene ontology (GO) analysis indicated that cell part, metabolic process and catalytic activity were the most abundant GO category in cell component, biological process and molecular function, respectively. Comparative transcriptome analysis between the papilla and skin allowed the identification of 1,059 differentially expressed genes, of which 739 genes were expressed at higher levels in papilla, while 320 were expressed at higher levels in skin. In addition, 236 differentially expressed unigenes were not annotated with any database, 160 of which were apparently expressed at higher levels in papilla, 76 were expressed at higher levels in skin. We identified a total of 288 papilla-specific genes, 171 skin-specific genes and 600 co-expressed genes. And 40 genes in papilla-specific were not annotated with any database, 2 in skin-specific. Development-related genes were also enriched, such as *fibroblast growth factor*, *transforming growth factor-β*, *collagen-α2* and *Integrin-α2*, which may be related to the formation of the papilla and skin in sea cucumber. Further pathway analysis identified ten KEGG pathways that were differently enriched between the papilla and skin. The findings on expression profiles between two key organs of the sea cucumber should be valuable to reveal molecular mechanisms involved in the development of organs that are related but with morphological differences in the sea cucumber.

KEYWORDS

Comparative transcriptome, Skin, Gene expression, High-throughput sequencing, Papilla, Sea

cucumber (*Apostichopus japonicus*)

INTRODUCTION

Sea cucumbers group (Echinodermata, Holothuroidea) comprise of approximately 1,250 species (Du et al. 2012). Sea cucumbers are mostly processed into a dry product called trepang, bêche-de-mer or hai-san, which is widely recognized as a delicate food with medicinal effect for human consumption. Sea cucumbers have been harvested commercial use since a thousand years ago, and they are now widely cultured in more than 70 countries (Steven, Purcell & Chantal, 2012). The sea cucumber *Apostichopus japonicus* (Holothuroidea, Aspidochirotida) is intensively cultured in many East Asian countries and is naturally found along the coasts of China, Japan, Korea and Russia of Northeast Asia (Sloan, 1984; Chang et al., 2009). It is intensively cultured as an important aquaculture species in many countries of East Asia.

The pentamerous radial symmetry is considered as one of the characteristics of echinodermata. In sea cucumber, pentamerous symmetry is usually determined based on the presence of five meridional ambulacra bearing podia (Steven, Purcell & Chantal, 2012). Papillae represent the podia on the dorsal surface, and generally have no locomotive function. With *A. japonicus*, fleshy and conical papillae, with a sensory spina at its apex, are present in two loose rows on the dorsal surface and two rows at the lateral margins of the ventral surface (Steven, Purcell & Chantal, 2012). Previous studies have investigated the morphological characteristics of papilla in the *A. japonicus* (Vanden et al., 1995; Chang et al., 2011; Steven, Purcell & Chantal, 2012). In the papillae, the ciliated cells and histamine-like immunoreactivity neurons are in contact with the nerve plexus (Hyman, 1955; Luke et al., 2012). Therefore, the dorsal papillae have long been associated to a sensory role, which may involve chemoreception and mechanoreception (Vanden et al., 1995).

The thicker body wall of *A. japonicus* consists of a thin cuticle over the epidermis and a thick dermis underneath. The cuticle and epidermis as the outer tissues of the *A. japonicus* are represented by skin (Steven, Purcell & Chantal, 2012). The skin forms a protective barrier, forming the first line of defence against the environment. Previous studies have been conducted

on skin with main focused on the intrinsic mechanisms underlying immune response to skin ulceration and peristome tumescence (Liu et al., 2010; Zhang et al., 2013).

The papillae are closely associated with the skin in sea cucumber. Both organs are mainly composed of collagen (up to 70%), and are the major component of the body wall. Moreover, the papillae and skin are formed by similar elements and homologous cell types, such as keratinocytes, epidermis and dermis, all derived from the ectoderm (Chang et al., 2004; Lowdon et al., 2014). Despite the common embryonic origin of the two organs, they exhibit clear morphological differences and play distinct functions. The molecular mechanisms underlying differentiation between the papilla and skin remain largely unknown. The lack of reference genome and the limited genetic resources of *A. japonicus* represent a major obstacle to better understand the function of these two organs.

In this study, we conducted RNA-Seq of these two organs to determine global changes in gene expression between the papilla and skin in the *A. japonicus*. RNA-Seq technology has been widely used for the generation of genetic resources in echinoderms (Anisimov, 2008; Wang, Gerstein &Snyde**,** 2009). Recently, several RNA-Seq based transcriptome analyses have been conducted in the *A. japonicus*, including studies on histology (Sun et al., 2011, 2013), immunology (Li et al., 2012), physiology (Zhao et al., 2014a, 2014b), embryonic development and gene marker discovery (Du et al. 2012; Zhou et al. 2014). The first transcriptome sequencing of the *A. japonicus* intestine and body wall was performed by Sun et al (Sun et al., 2011). Thereafter, the global dynamic changes during all stages of intestine regeneration were further investigated (Sun et al., 2013). To identify candidate transcripts potentially involved in aestivation and generate a wide coverage of transcripts involved in a broad range of biological processes, eight cDNA libraries were constructed and sequenced by Du et al. (Du et al., 2012). Immune-related genes and pathways in response to pathogen infection were identified (Zhou et al., 2014; Gao et al., 2015). Moreover, many physiological networks were identified and characterized in the *A. japonicus* on the basis of transcriptomic resources (Wang et al., 2015; Yang et al., 2015).

Here, in this work, we report comparative transcriptome analysis of the papilla and skin. A relatively large number of genes that displayed distinct expression profiles between the papillae and skin were identified. Further enrichment analysis identified pathways such as tight junction and p53 signaling pathway could be involved in the development of the papilla and skin. This work provided the essential genomic resources for further investigations into the molecular interactions and multiple biological process of appendages such as the papilla and skin in the *A. japonicus*.

MATERIALS AND METHODS

Sample collection

A total of 45 sea cucumbers (average weight of 25g) provided by the Key Laboratory of Mariculture in North China (Dalian, Liaoning) were used in the present study. In order to have a good reference transcriptome, the skin around the papillae, papilla and tube foot tissues were collected for RNA-Seq. We randomly group these 45 sea cucumbers into three groups as replicates. Within each group, ~1g tissue was dissected from each individual, respectively. Tissues collected from each group were of every individual were pooled (one pool per tissue) and placed in 2ml of RNAlater®Solution (Ambion) for overnight at 4^{o}C followed by transferring to -80^{o}C until RNA extraction.

RNA-Seq

Total RNA was extracted from the pooled samples using the TRIzol Reagent (Invitrogen, CA, USA) following the manufacturer's recommendations. The quantity and integrity of total RNA were assessed using an Agilent 2100 Bioanalyzer and 1% agarose gel electrophoresis. High quality RNA was used for the construction of cDNA. Library construction and sequencing was performed in the Biomarker Biotechnology Corporation (Beijing, China). Paired-end sequencing was conducted on an Illumina HiSeq 2500 platform to generate 125bp paired-end (PE) reads.

Transcriptome assembly and annotation

Low quality reads and adaptors were trimmed before assembly. Trimed reads were *de novo* assembled by Trinity software using default parameters (Grabherr et al., 2011) and used as a

reference for gene expression analysis. Transcirptome was annotated using Basic Local Alignment Search Tool (BLAST) searches against the NCBI non-redundant (NR) database, Swiss-Prot, KEGG (the Kyoto Encyclopedia of Genes and Genomes) and GO (Gene ontology), Clusters of Orthologous Groups (COG) and eukaryotic Orthologous Groups (KOG) with e-value cutoff of 1e-5.

Differentially Expressed Gene (DEG) Analysis

Gene expression was determined by the FPKM (Fragments Per kb of transcript per Million mapped fragments) method. The gene expression differences between the papilla and skin tissues were identified following the formula:

$$\text{Fold change} = Log_2 \frac{FPKM_{Papilla}}{FPKM_{Skin}}$$

DEGs were determined with the absolute fold change values greater than 2.0, and FDR (false discovery rate) lesser than 0.01 (Cui et al. 2014, 2015).

To further investigate DEGs identified between papilla and skin, genes were compared to those identified from the *A. japonicus* intestine RNA-Seq dataset (accession NO. GSE44995) from a previous study by Sun et al. (2013). Intestine is responsible for the metabolic rate depression under deep aestivating conditions (Chen et al., 2013) and play a role for organ regeneration (Sun et al., 2013). In the present study, we uesd intestine as major site in the internal environment of *A. japonicus* for further investigate DEGs identified between papilla and skin. All assembled sequences of *A. japonicus* published in Sun et al. (Sun et al., 2013) were downloaded as database to blast the DEGs, the differential expression of DEGs among the papilla, skin, and intestine was estimated using the formula:

$$\text{Score} = Log_2 \frac{FPKM_{Tissues}}{RPKM_{Intestines}}$$

Where $FPKM_{Tissues}$ indicates the FPKM of papilla or skin; $RPKM_{Intestines}$ indicates the RPKM of intestine. Significant candidates were determined as the absolute score greater than 4.0.

qRT-PCR validation

158 RNA-Seq results were validated by qRT-PCR analysis of 16 randomly selected DEGs.
159 Primers were designed following the manufacturer's recommendations of SYBR Premix Ex
160 TaqTM Ⅱ kit (Takara, Dalian). The β-actin was used as housekeeping. All the primers are
161 shown in supplementary TableS1. Briefly, the amplificaation was performed in a total volume of
162 16 μL, containing 8μL 2× SYBR Premix Ex Taq Ⅱ, 1μL of cDNA, and 0.3μL of 10μM of each
163 gene-specific primer. The qRT-PCR reactions were performed on ABI stepone plus platform and
164 replicated in three pools. And three technical replications were performed for each qRT-PCR
165 validation. PCR was conducted as follows: 94 °C for 30 s, 45 cycles of 94 °C for 5s, annealing
166 temperature (showed in TableS1) for 15s, and 72 °C for 15s.

167 **Gene enrichment analysis**

168 The gene enrichment analysis was conducted using KEGG database. The over-presentation
169 of the DEGs was determined in the specific pathways. The level of enrichment was indicated by
170 enrichment factor, and p-value was used to calculate the significance of enrichment. The top 10
171 KEGG enrichments were selected to carry out further analysis.

172 **RESULTS**

173 **Sample sequencing**

174 RNA-Seq of the papilla and skin samples yielded over 70 million pair-end reads with
175 average length of 125bp (Table 1). Similar number of reads was obtained from both tissues, with
176 over 33 million reads from papilla and over 37 million from skin. After trimming, 30.6 and 36.4
177 million high-quality reads were retained from papilla and skin, respectively. Totally, 7.7 billion
178 bases generated from the papilla, 9.2 billion bases generated for the skin and 8.6 billion bases
179 generated for the tube foot were used for down-stream analysis of *de novo* assembly and
180 mapping. Data obtained from papilla, skin and tube foot were deposited to the sequence read
181 archive (SRA) with the accession numbers of SRA275705 and SRA275706.

182 **Transcriptome assembly and annotation**

183 The *de novo* assembly resulted in a total of 156,501 transcripts, with the average length of

910.77bp and N50 length of 1,694bp (Table 2). The length distribution of transcripts and unigenes are shown in Fig. 1. Over 84% of reads from both tissues were successfully mapped back to the *de novo* transcriptome assembly.

The transcriptome assembly was annotated by BLASTX against NCBI NR, Pfam, Swiss-Prot, KEGG, COG and KOG databases with E-value threshold of 1e-5. Annotation resulted in the identification of 92,343 unigenes (unique transcripts matched with known proteins). From all the 92,343 unigenes, 30,706 were found to have homologs in NR database, 22,261 found to posses functional domains in Pfam database; 18,944 unigenes showed significant matches to Swiss-Prot database, 22,361 to KOG, 11,190 to KEGG, 10,876 to COG and 12,410 unigenes were associated with GO terms (Table 2). Taken together, a total of 33,584 unigenes had at least one significant matches to these databases (Table 2). The unigenes annotated with NR database accounted for the largest proportion (91.4%), followed by Pfam and Swiss-Prot (Fig. 2).

Distribution of the 12,140 unique proteins in different GO categories is shown Fig. 3. The transcriptome was enriched in cell component GO categories related to cell part (22.8%) and cell (22.6%). For biological process, metabolic process (28.1%) was the most abundant GO categories. Regarding to molecular function, catalytic activity (45.5%) and binding (39.0%) were the most abundant GO categories. In the correlational study, Du et al. found that membrane-bounded organelle was the most represented GO term in cell component; the major category in biological process was the primary metabolic process; and genes involved in hydrolase activity accounted for major proportion in molecular function. To be noted, due to the samples used in Du et al. study were collected from different developmental stages and adult tissues (intestines, respiratory trees and coelomic fluid), there may be some biases.

Identification of DEGs

A total of 1,059 DEGs were identified between the papilla and skin. The MA plot showed significant DGE (blue) against all non-significant DEG (red) (Fig. 4A). Among identified DEGs, 739 were expressed at significantly higher in papilla, while 320 genes were expressed at significantly higher levels in skin (TableS2). The number of genes with higher expression levels in papilla was over twice than the number of that in skin. Papilla, as the projections of body wall, included more unique contents than skin, such as the calcareous ossicles, which are hidden in the

dermis of body wall, papillae and tentacles (Steven, Purcell & Chantal, 2012). We also analysed the expression profiles of 1,059 DEGs in each tissue. Papilla-specific genes represent the DEGs that there is no expression in the skin, and that goes for skin-specific. A total of 288 papilla-specific DEGs were expressed only in papilla, while 171 DEGs were found to be only expressed in skin (skin-specific). A total of 600 DEGs were expressed in both papilla and skin (Fig. 4B). Apparently, the number of skin-specific (53.44%) genes is higher than papilla-specific genes (38.97%).

Of the 1,059 DEGs, 61 DEGs were annotated to homologous genes in *strongylocentrotus purpuratus*, a model species that is closely related to *A. japonicus*. *Hsp gp96*, *Hsp26*, *ALDOA* (aldolase class-1 protein) and *tenasxin* were annotated with *A. japonicus*. Our results revealed that *Hsp gp96* and *ALDOA* were 3.93- and 4.45-Fold up-regulated in papillae, respectively. In constrast, *Hsp26* and *tenascin* were -2.39- and -3.62-Fold down-regulated in skin, respectively. In addition, 236 differentially expressed genes were not annotated with any database, 160 of which were apparently higher in papilla. Further analysis revealed that 40 of which were papilla-specific and two were skin-specific.

Putative genes related to development that may be associated with the formation of the papilla were identified (Table 3). Detailed information of develop-related genes was provided in Table S3. Our results revealed that *cuticle collagen 2* and *alpha-2 collagen* were highly expressed in papilla with 5.76 and 2.55, respectively. Several genes that know to be related to the collagen development (Hinz et al., 2003, 2009; Leask& Abraham, 2004), such as *fibroblast growth factor* (*FGF*), *transforming growth factor-β* (*TGF-β*) and *integrin-α2* (*ITGA2*) were found to be significantly expressed. Several *Ras*-related genes such as *Ran*, *Rab1a*, *Arf3*, *Ran1,Ras, RhoA, Rho Guanine nucleotide exchange factors* (*RhoGEF*), Rho GTPase, Rho GTPase activation protein (RhoGAP) and Ran-binding protein 1 (RanBP1), which play key roles in the development by regulating growth and morphogenesis, were also identified in our study (Table S4). All Ras-related DEGs were expressed at lower levels in skin except for *RhoGEFs* that were reported to be associated with cancer, pathogen infection or neural system related

240 diseases and development (Reichman et al., 2015). Understanding of the function of *Ras*-related
241 genes will facilitate to unravel the mechanisms of some physiological and pathological process
242 in the skin of *A. japonicus.*

243 To further verify DEGs data, we compared our results with those DEGs identified in the
244 intestine of *A. japonicus* from a previous study (Sun et al., 2013) (results are shown in Table 4).
245 7 DEGs showed the same score trend as that of fold change in papilla. The reason for this
246 observation could be due to the lack of a complete RPKM data (Sun et al., 2013).

247 **qRT-PCR validation**

248 To validate the DEGs results obtained, we randomly selected 16 DEGs for validation using
249 qRT-PCR. As shown in Fig. 5, the DGEs identified from qRT-PCR analysis were correlated well
250 with those obtained from qRT-PCR, indicating the reliability and accuracy of the RNA-Seq
251 method used in the present study.

252 **Enrichment analysis of DEGs**

253 A total of 296 DEGs identified were mapped to 133 pathways. KEGG enrichment pathway
254 analysis was also carried out to investigate their potential functional roles. The top 10 enrichment
255 pathways were selected by a hypergeometric test ($p<0.05$) (Table 5). One of which is the
256 ribosome pathway, which was related to the protein biogenesis and was observed to be involved
257 in intestine regeneration (Sun et al., 2013) and aestivation (Chen et al., 2013; Zhao et al., 2014)
258 in the *A. japonicus*. In addition, tight junction and p53 signaling pathway were also detected in
259 enrichment pathways analysis (detailed information is provided in Table S5).

260 **DISCUSSION**

261 In this study, we conducted comparative transcriptome analysis between papilla and skin,
262 two important organs of sea cucumber. A total of 1,059 differentially expressed genes were
263 identified between the two organs. This result lay the foundation to identify genes that were
264 potentially involved in the development of the papilla and skin. The generated genomic resources
265 should be valuable for other genetic and genomic studies in the *A. japonicus*.

266 As previously reported, excessive deposition resulting from abnormal balance of growth

267 factors and cell proliferation can improve local hyperplastic collagen production in skin in
268 response to injury in mammalians (Tuan &Nichte, 1998). Keloids (Seifert &Mrowietz, 2009;
269 Shih &Bayat, 2010) and hypertrophic scar (HS) (O'Leary, Wood &Guillou, 2002), are
270 characterized by fibroblastic proliferation and accumulation of extracellular matrix (ECM),
271 especially excessive deposition of collagen. However, such prominences are regarded as benign
272 tumors (Diao et al., 2011). It has been suggested that factors such as *FGF*, *ITGA2*, *TGF* and *S-*
273 *adenosylmethionine* (*a-SMA*) can cause those lesions (Hinz et al., 2003, 2009; Leask& Abraham,
274 2004). The FGF activity was first identified from bovine pituitary in 1974 (Gospodarowicz&
275 Moran, 1974). *FGF* signaling is required for different developmental stages during
276 embryogenesis (Sun et al. 1999; Naiche et al., 2011; Niwa et al., 2011; Vega-Hernández et al.,
277 2011). Compared with normal dermal fibroblast, *TGF-β* is believed to induce collagen
278 production and increase the contractile activity in keloid fibroblasts (Bran et al., 2010;
279 Sandulache, Parekh & Li-Korotky, 2007). In additon, *TGF-β* associated with *connective tissue*
280 *growth factor* (*CCN2*) has been revealed to stimulate *a-SMA*, *collagen* expression (Jiang et al.,
281 2008). *ITGA2* is the main cell adhesion molecule that takes part in the modulation of collagen
282 contraction and the activity of myofibroblast in HS (Cooke, Sakai & Mosher, 2000). The
283 expression levels of *ITGA2* were also found up-regulated in hypertrophic scar fibroblasts,
284 compared with normal skin tissues in human. In our study, we found that *col-α2* was expressed
285 at a higher level in the papilla, and we also observed differential expression patterns of genes
286 involved in collagen synthesis as the major differences between the papilla and skin. The
287 expression of *FGF*, *TGF-β* and *ITGA2*, associated with collagen development, were all
288 expressed at higher levels in the papilla. Compare to previous studies of local hyperplastic
289 collagen in mammals, we speculate that these collagen-related genes may play critical roles in
290 stimulating the production of collagen in papilla and might be involve in the morphological
291 differentiation between the two organs.

292 Besides the development-related genes as discussed above, some immune-related genes are

293 also identified to be differentially expressed between papilla and skin in this study such as
294 *Hspgp96*, *Hsp26*, *ALDOA* and *tenascin*. Many lines of evidences support that Hsps act as natural
295 immunoregulatory agents, increasing the awareness of innate immune cells to pathogens
296 (Ciancio&Chang, 2008; Prohaszka&Fust, 2004; Zugel& Kaufmann, 1999). *ALDOA* plays a role
297 in glycolysis pathway (Oparina et al., 2013). Further investigation is required to explore the
298 pathological researches for papilla and skin. Through analysis of the DEGs against intestine
299 transcriptome data from a previous study (Sun et al., 2013). *Fibrinogen-like protein A* (*fglA*), a
300 member of the fibrinogen-related protein superfamily, plays crucial roles including innate
301 immune response, regeneration and blood clotting (Yamamoto et al., 1993). Previous studies
302 have demonstrated that *fglA* is widely distributed in *A. japonicus* body wall, intestines,
303 longitudinal muscles and respiratory tree of *A. japonicus* (Wu et al., 2014). Our results also show
304 that *fglA* was 3.02 fold change and 4.96 score up-regulated in papilla, respectively. The role of
305 f*glA* in the development of papilla remains unclear and further investigation is required to
306 understand its functional roles.

307 Enrichment KEGG analysis revealed that tight junction and p53 signaling pathway were
308 highlighted in enrichment pathways. In human, the content of G2-M arrested cells in keloid skin
309 was higher than normal skin (Shohreh et al., 2011). Keloid fibroblasts showed a higher rate of
310 senescence and lower proliferative capacity in comparison to normal fibroblasts (Shohreh et al.,
311 2011). In our study, a set of genes, including growth arrest and DNA-damage-inducible protein
312 (*gadd45*), cyclin dependent kinase 1 (*cdk*), cyclin-B (*cyc-B*), cyclin-A (*cyc-A*) and cytochrome C
313 (*cyt-C*), were all expressed at higher levels in the papilla (Table 1). These genes are involved in
314 the p53 signaling pathway. Once p53 signaling pathway is activated, it can induce either cell
315 cycle arrest or apoptosis in the damaged cell. In humans, cyc-B and cdk2 kinase influence a cell's
316 progress through the cell cycle, which is especially important in several skin cancers (Ely et al.,
317 2005; Casimiro et al., 2014). Cyc-B forms the regulatory subunits and cdk2 form the catalytic
318 subunits of an activated heterodimer. The cyc-B has no catalytic activity, and cdk2 is inactive in
319 the absence of a partner cyc-B. Once activated the cdk2/cyc-B complex control cell cycle (Abreu,

Velez & Howard, 2015). Gadd45 is a ubiquitously expressed 21 protein with a key role in response to genotoxic agents, and it is involved in many biological processes related to maintenance of genomic stability and apoptosis. It has been shown that gadd45's inhibits cdk2 kinase activity through alteration of cyc-B subcellular localization, inducing the arrest of the cell cycle in G2-M state (Jin et al., 2000; Smith et al., 1994). These results indicated that the level of cell cycle arrest at the G2-M in the papilla might be higher than in the skin. It's speculated that papilla fibroblasts commit to a higher rate of senescence, which may cause fibroblast-related genes eventually stop expressing and maintain external morphology of the papilla.

Genes involved in tight junction were enriched in papilla. Tight junctions are essential for epithelial morphology, which can form seals between epithelial cells and create a selectively permeable barrier to intercellular diffusion (Zheng et al., 2011). Besides, we also found that the expression of Serine/threonine-protein phosphatase (PP2A) in papilla is higher than that in the skin. Many reports showed that PP2A regulates*ataxia telangiectasia mutated* (*ATM*), *ataxia telangiectasia Rad3 related* (*ATR*), *check point kinase-1* (*CHK1*), and *checkpoint kinase-2* (*CHK2*) after DNA damage, and activate the checkpoint of G2-Massociated with the p53 signaling pathway. The process activated by PP2A may also regulate the external morphological of papilla and skin of *A. japonicus*.

CONCLUSION

In this study, we performed comparative transcriptome analysis of the skin and papailla *A. japonicus* by using RNA-Seq. In total, 156,501 transcripts and 92,343 unigenes were assembled. A total of 1,059 differentially expressed genes were indentified between the two important organs of *A. japonicus.* We identified 236 novel genes (not annotated with any database), 160 of which were expressed at higher levels in papilla. Further tissue-expression analysis identified 288 papilla-specific genes and 171 skin-specific genes. Gene pathway enrichment analysis revealed several gene pathways that were involved in development. In addition, many DEGs involved in the process of p53 signaling pathway and tight junction were also identified, which were ported to be relative to keloid skin in human. This result provided insights into genes and

pathways that may be associated with the formation of the papilla and skin in sea cucumber, laying foundation for further investigation to understand the development of the papilla in *A. japonicus*. Moreover, the generation of larger-scale transcriptomic data presented in this work enriched genetic resources of echinodermata species, which should be valuable to comparative and evolutionary studies in echinoderms.

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551
552

Table 1(on next page)

Summary of the RNA-Seq data.

1 **Table 1. Summary of the RNA-Seq data.**

	Number of reads	Number of reads after trimming	Number of nucleotides after trimming (bp)
Papilla	33,504,127	30,657,027	7,723,425,469
Skin	37,384,685	36,444,908	9,182,161,309
Total	70,888,812	67,101,935	16,905,586,778

2

Table 2(on next page)

Statistics of transcriptome reference assembly and annotation.

Table 2. Statistics of transcriptome reference assembly and annotation.

Assembly	Number of transcripts	156,501
	Maximum transcript length	18,781 bp
	Minimum transcript length	201 bp
	Average transcript length	910.77bp
	N50 length	1,694bp
	Number of mapped reads from the papilla	25,946,333 (84.6%)
	Number of mapped reads from the skin	30,913,283 (84.8%)
Annotation	Unigenes with blast hits to NR	30,706
	Unigenes with blast hits to Pfam	22,261
	Unigeneswith blast hits to Swiss-Prot	18,944
	Unigeneswith blast hits to KOG	22,361
	Unigeneswith blast hits to COG	10,876
	Unigeneswith KEGG terms	11,190
	Unigeneswith GO terms	12,140
	Total	33,584

Table 3(on next page)

Differentially expressed genes between the papilla and skin that are involved in development.

Table 3. Differentially expressed genes between the papilla and skin that are involved in development.

Unigene ID	Gene symbol	Foldchange
c14695.graph_c0	*cdk*	4.96
c40875.graph_c0	*cyc-B*	6.26
c75877.graph_c0	*cyc-A*	6.346
c76406.graph_c0	*cytC*	2.76
c76859.graph_c0	*gadd45a*	4.82
c12901.graph_c0	*ck2bl*	4.40
c14611.graph_c0	*MAGUKs*	4.34
c18023.graph_c0	*PP2A*	4.54
c19039.graph_c0	*claudin*	6.31
c37832.graph_c0	*actin*	6.05
c16255.graph_c0	*FGF*	5.26
c58770.graph_c0	*ITGA2*	2.12
c15897.graph_c0	*TGF-β*	6.21
c54738.graph_c0	*col-α2*	2.55
c54237.graph_c0	*tub-α*	-4.53
c77661.graph_c0	*emmhc*	4.49
c54933.graph_c0	*eef2*	-5.43
c42633.graph_c0	*gtf 8*	4.96
c57892.graph_c0	ubeE2	-4.18
c76626.graph_c0	*ctATPase*	4.62
c38162.graph_c0	*cul-α2*	5.76

Table 4(on next page)

The result of DEGs with significantly different expression by comparison with the intestine.

Table 4. The result of DEGs with significantly different expression by comparison with the intestine.

Unigene ID	Isotig ID	Annotate	Foldchang	Score
c45050.graph_c0	isotig25664	Cell death abnormality protein 1	8.62	14.40
c64723.graph_c0	isotig15743	Sushi domain (SCR repeat)	7.15	4.09
c67657.graph_c0	isotig19241	Fibrinogen-like protein A	3.02	4.96
c66534.graph_c0	isotig15670	hypothetical protein CAPTEDRAFT_211426	2.84	11.35
c73725.graph_c2	isotig27287	Sulfotransferase family	2.56	12.76
c60095.graph_c0	isotig09563	hypothetical protein BRAFLDRAFT_231341	2.54	4.31
c60588.graph_c0	isotig18328	Histone-lysine N-methyltransferase	2.16	13.36

The “Isotig ID” column indicates the gene ID from the data of Sun et al. (Sun et al., 2013).

Table 5(on next page)

Enrichment analysis of genes with significantly differential expression between the papilla and skin.

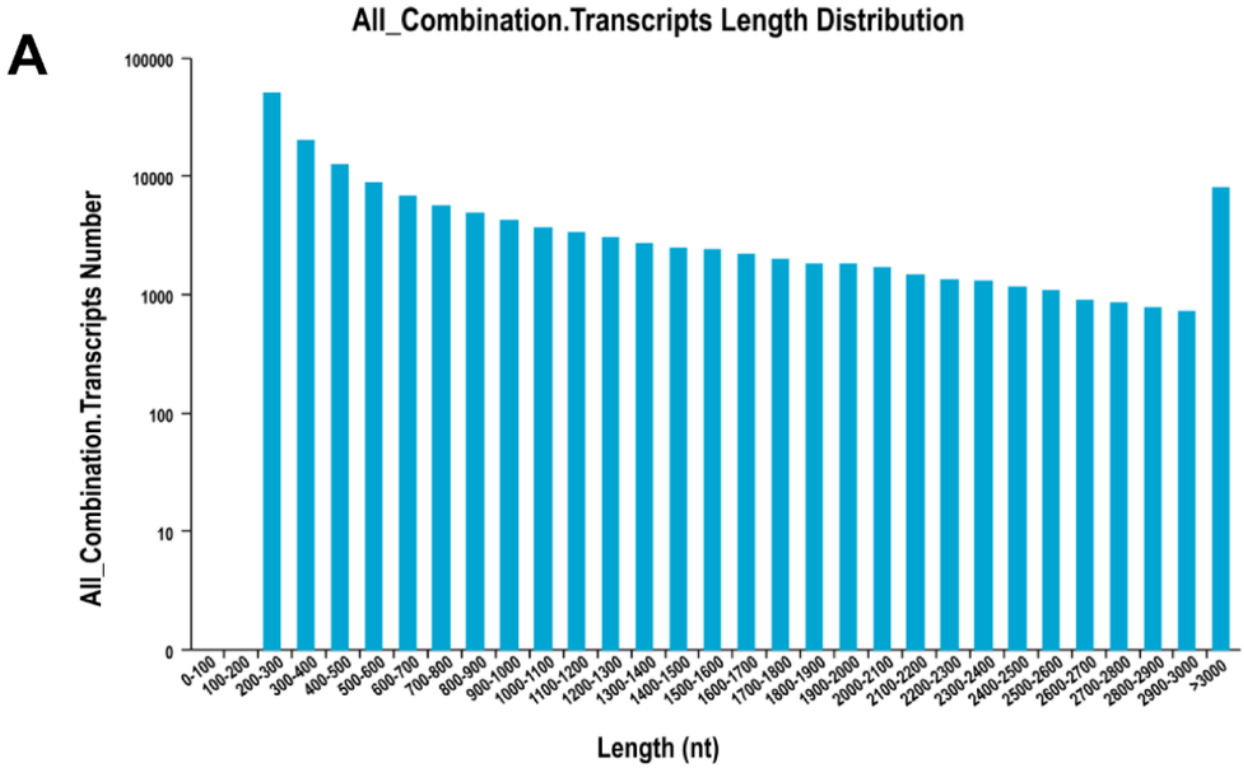
Table 5. Enrichment analysis of genes with significantly differential expression between the papilla and skin.

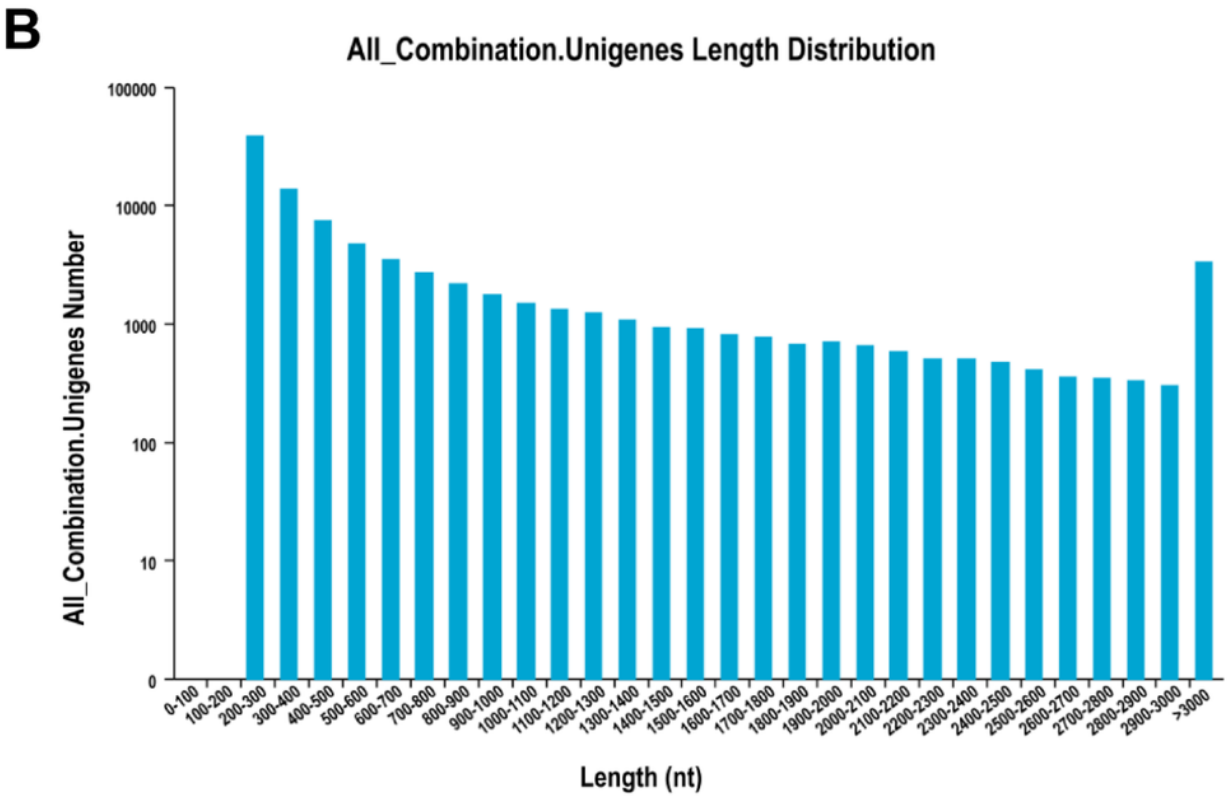
Pathway	KO	Enrichment factor	p-value
Ribosome	ko03010	0.39	1.47E-13
Oocyte meiosis	ko04114	0.27	2.07E-06
Cell cycle	ko04110	0.40	0.000557178
Glycolysis / Gluconeogenesis	ko00010	0.43	0.000610528
p53 signaling pathway	ko04115	0.28	0.003222732
Tight junction	ko04530	0.38	0.004679565
NOD-like receptor signaling pathway	ko04621	0.27	0.010624351
Regulation of actin cytoskeleton	ko04810	0.44	0.017102208
RNA transport	ko03013	0.68	0.029611824
Progesterone-mediated oocyte maturation	ko04914	0.52	0.033017313

1

The distribution of the size of transcripts and unigenes.

Length distribution of assembled transcripts (A) and unigenes (B) of sea cucumber (*Apostichopus japonicus*).

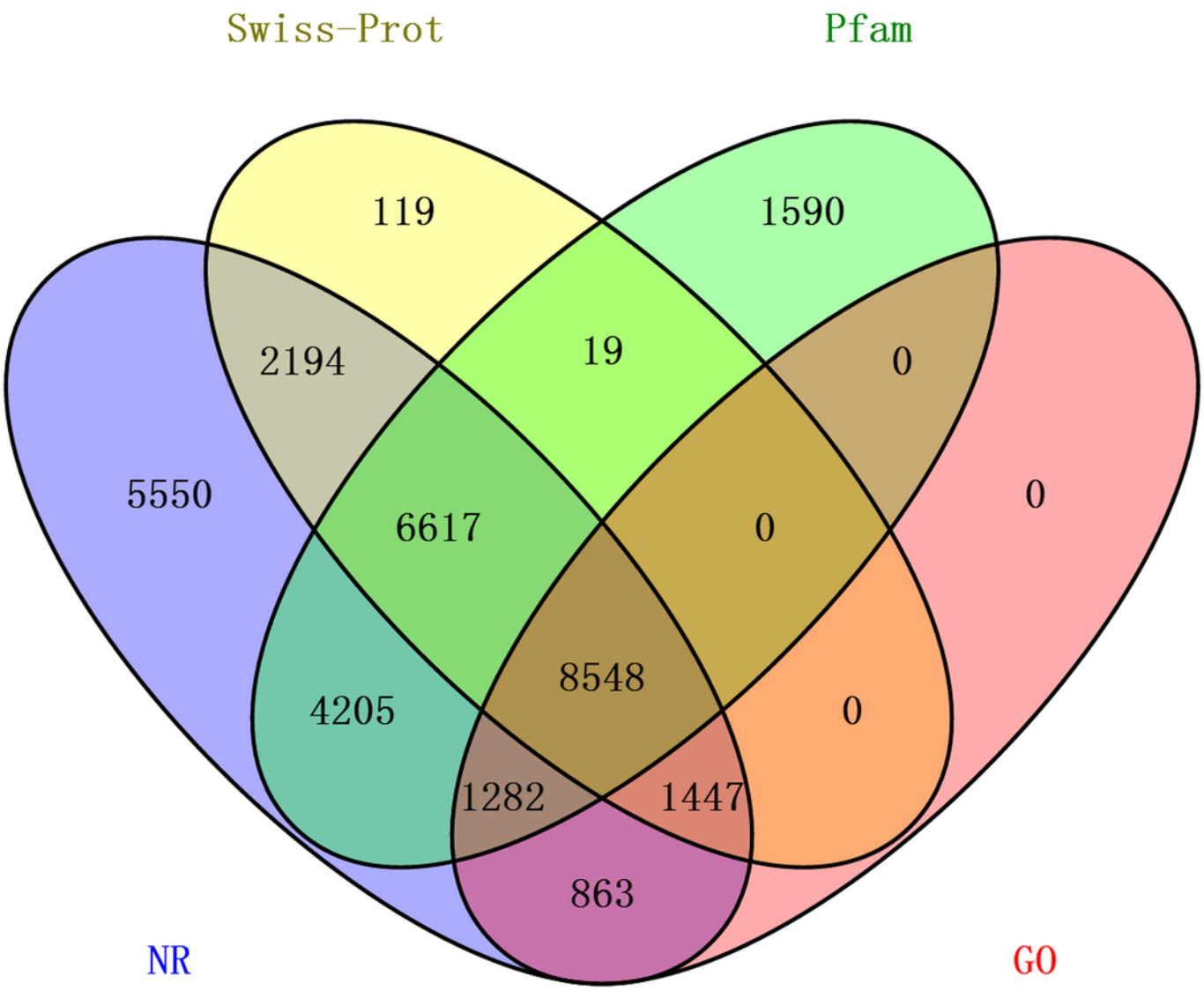

A
All_Combination.Transcripts Length Distribution
All_Combination.Transcripts Number
100000
10000
1000
100
10
0
0-100
100-200
200-300
300-400
400-500
500-600
600-700
700-800
800-900
900-1000
1000-1100
1100-1200
1200-1300
1300-1400
1400-1500
1500-1600
1600-1700
1700-1800
1800-1900
1900-2000
2000-2100
2100-2200
2200-2300
2300-2400
2400-2500
2500-2600
2600-2700
2700-2800
2800-2900
2900-3000
>3000
Length (nt)


B
All_Combination.Unigenes Length Distribution
All_Combination.Unigenes Number
100000
10000
1000
100
10
0
0-100
100-200
200-300
300-400
400-500
500-600
600-700
700-800
800-900
900-1000
1000-1100
1100-1200
1200-1300
1300-1400
1400-1500
1500-1600
1600-1700
1700-1800
1800-1900
1900-2000
2000-2100
2100-2200
2200-2300
2300-2400
2400-2500
2500-2600
2600-2700
2700-2800
2800-2900
2900-3000
>3000
Length (nt)

2

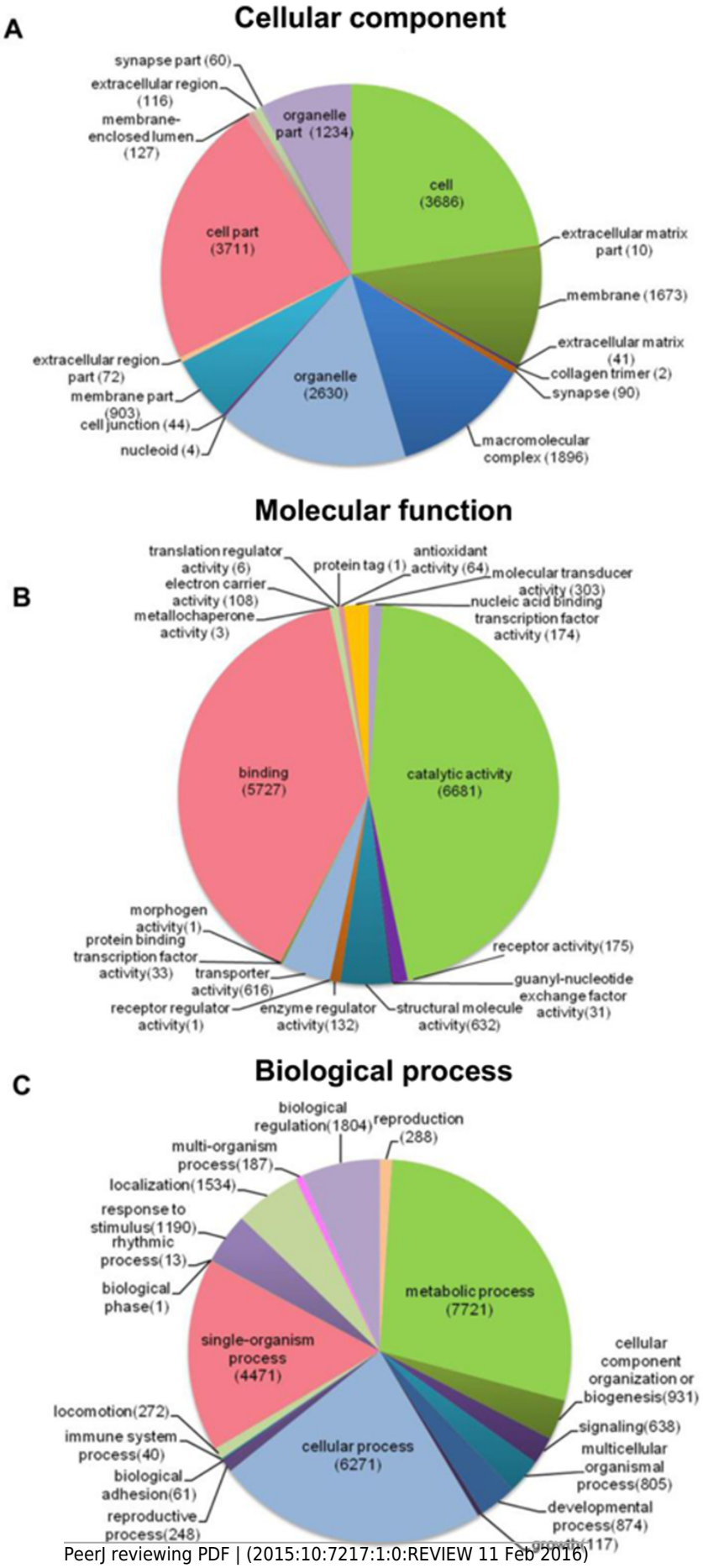
The distribution of annotated unigenes across database.

Venn diagram display of the proportion of annotated unigenes in NR, Pfam, Swiss-Prot and GO.



3

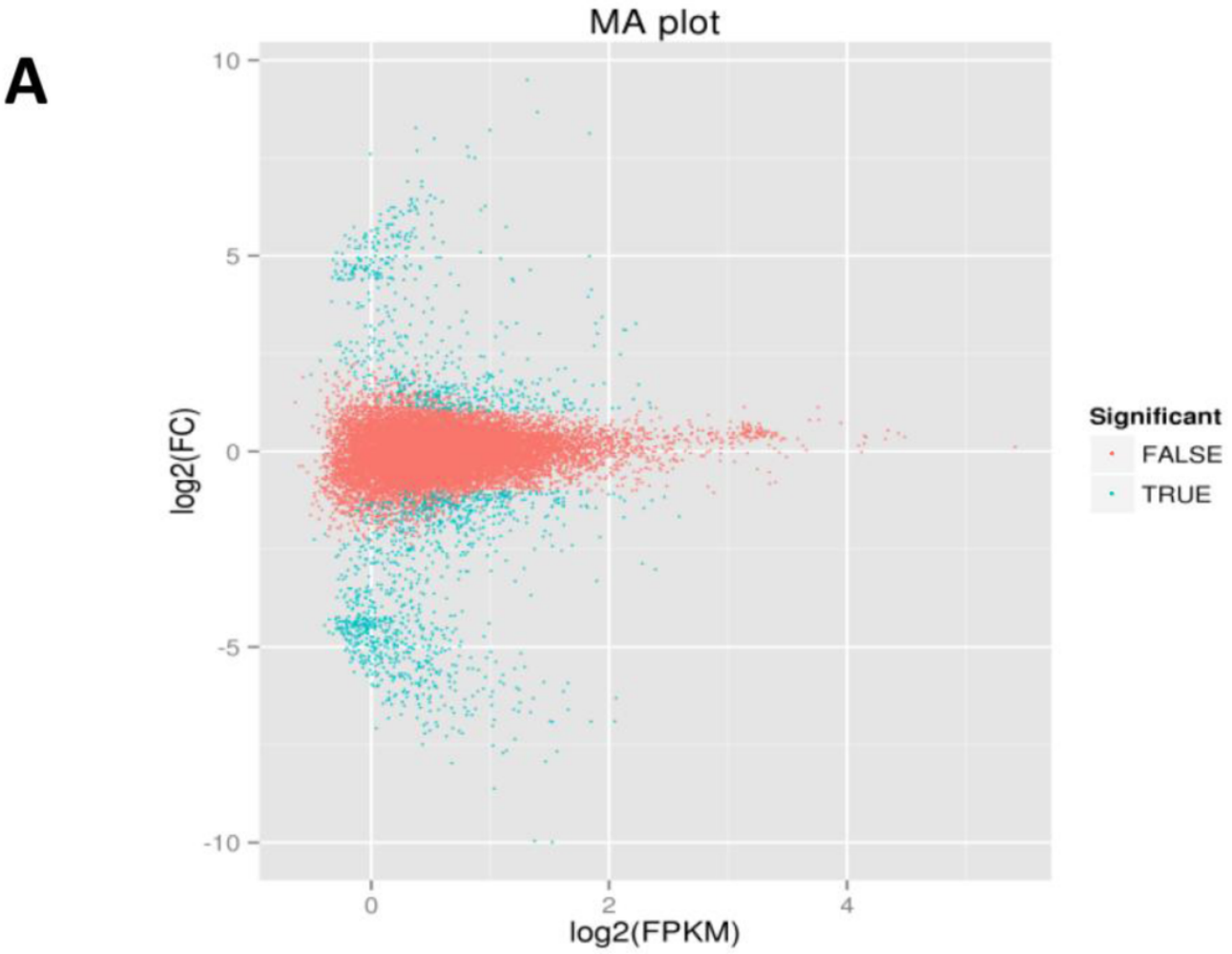
Distribution of the most common GO term categories.

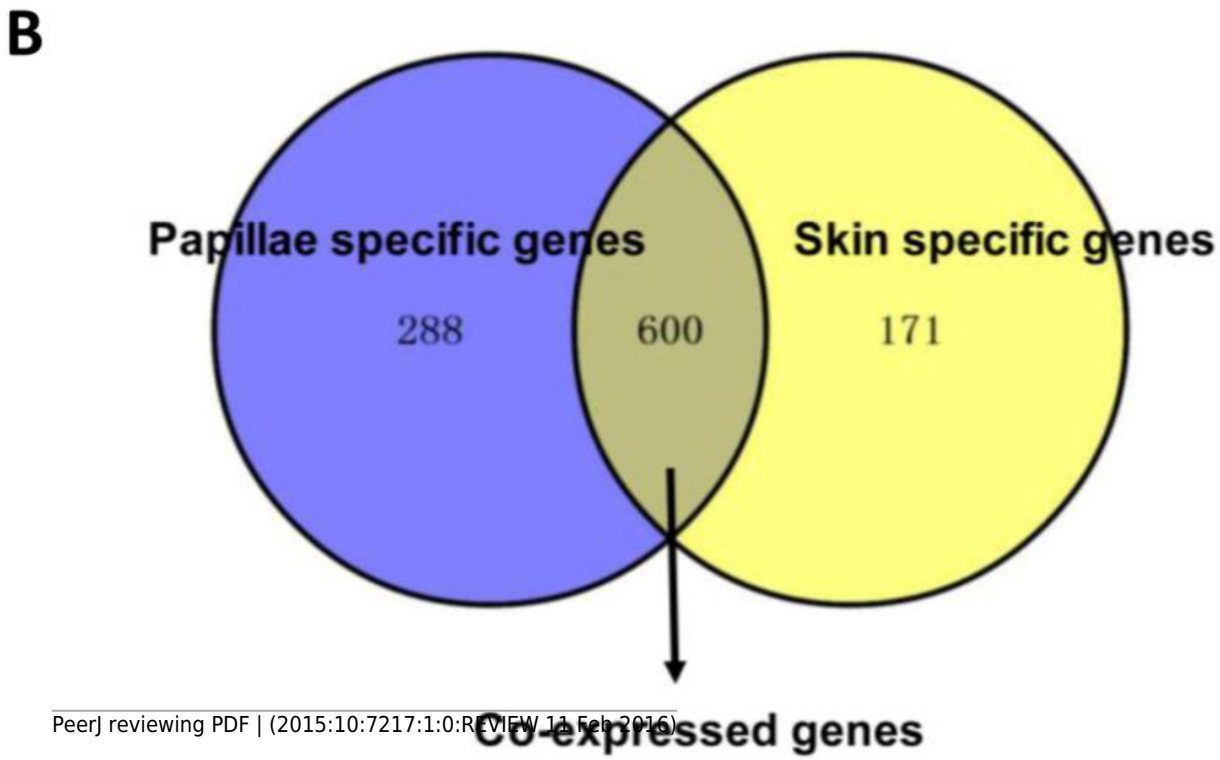

A
Cellular component
synapse part (60)
extracellular region (116)
membrane-enclosed lumen (127)
organelle part (1234)
cell (3686)
cell part (3711)
extracellular matrix part (10)
membrane (1673)
extracellular matrix (41)
collagen trimer (2)
synapse (90)
extracellular region part (72)
membrane part (903)
cell junction (44)
nucleoid (4)
organelle (2630)
macromolecular complex (1896)
B
Molecular function
translation regulator activity (6)
protein tag (1)
antioxidant activity (64)
molecular transducer activity (303)
electron carrier activity (108)
nucleic acid binding transcription factor activity (174)
metallochaperone activity (3)
binding (5727)
catalytic activity (6681)
morphogen activity(1)
protein binding transcription factor activity(33)
transporter activity(616)
receptor activity(175)
guanyl-nucleotide exchange factor activity(31)
receptor regulator activity(1)
enzyme regulator activity(132)
structural molecule activity(632)
C
Biological process
biological regulation(1804)
reproduction (288)
multi-organism process(187)
localization(1534)
response to stimulus(1190)
rhythmic process(13)
biological phase(1)
metabolic process (7721)
single-organism process (4471)
cellular component organization or biogenesis(931)
signaling(638)
multicellular organismal process(805)
developmental process(874)
locomotion(272)
immune system process(40)
biological adhesion(61)
reproductive process(248)
cellular process (6271)

4

The DEGs in the papilla and skin of sea cucumber.

(A) M-A plots showing gene expression in papilla and skin. The x-axis represents the logarithm of FPKM and y-axis represents the logarithm of foldchange; (B) Venn diagram displays the number of papilla-specific, skin-specific, and co-expressed genes.

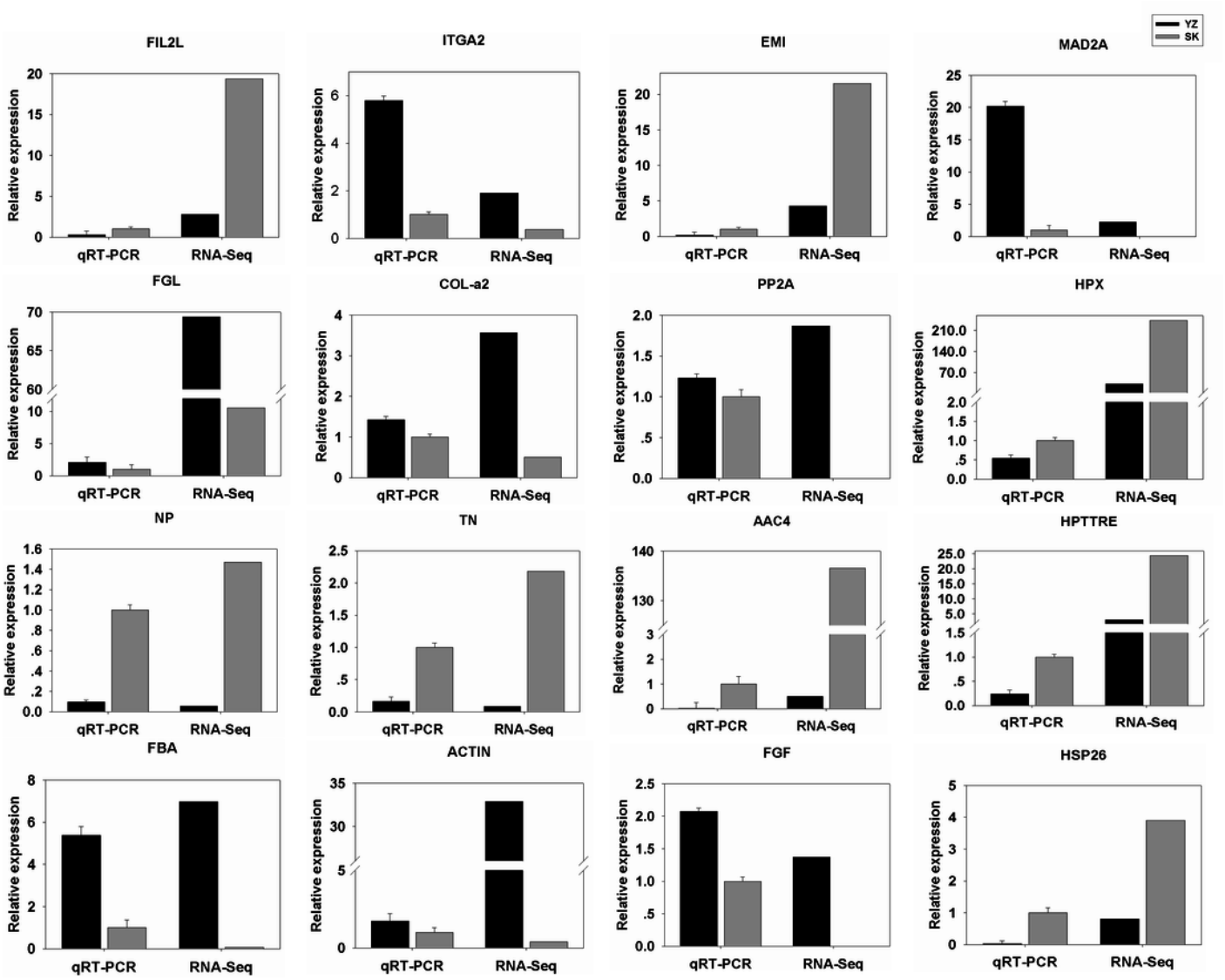

A
MA plot
10
5
0
-5
-10
log2(FC)
0
2
4
log2(FPKM)
Significant
FALSE
TRUE


B
Papillae specific genes
288
600
Skin specific genes
171
Co-expressed genes

5

Comparison between RNA-Seq results and qRT-PCR validation results.

X-axis shows genes in two tissues validated in this study; Y-axis shows Log_2Ratio of expression of SK (skin) versus YZ (papilla). AAC4PL, AAC-rich mRNA clone AAC4 protein-like; Hsp26, heat shock protein 26; NP, novel protein; TN, Tenascin; EMI, EMI domain; Hp TTRE, hypothetical protein TTRE_0000953901;FGL, Fibrinogen-like protein A; HpX975-24482, hypothetical protein X975_24482, partial; PP2A, Serine/threonine-protein phosphatase; FIL2L, Fibrinogen-like protein A; Col-α FiCollagen gen-like protein A; Col-l-ase; FIL2L, Fibrinogen-like proteiphosphatealdolase; IN, Integrin alpha 2; MAD2A, Mitotic spindle assembly checkpoint protein.


YZ
SK
FIL2L
ITGA2
EMI
MAD2A
FGL
COL-a2
PP2A
HPX
NP
TN
AAC4
HPTTRE
FBA
ACTIN
FGF
HSP26
Relative expression
qRT-PCR
RNA-Seq