The trophocytes and oenocytes of worker and queen honey bees (*Apis mellifera*) exhibit distinct age-associated transcriptome profiles

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Received: 21 January 2021 / Accepted: 19 March 2021 / Published online: 31 March 2021
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Abstract Despite the identical genomic context, trophocytes and oenocytes in worker bees exhibit aging-related phenotypes, in contrast to the longevity phenotypes in queen bees. To explore this phenomenon at the molecular level, we evaluated the age-associated transcriptomes of trophocytes and oenocytes in worker bees and queen bees using high-throughput RNA-sequencing technology (RNA-seq). The results showed that (i) while gene expression profiles were different between worker and queen bees, they remained similar between young and old counterparts; (ii) worker bees express a high proportion of low-abundance genes, whereas queen bee transcriptomes display a high proportion of moderate-expression genes; (iii) genes were upregulated to a greater extent in queen bees vs. worker bees; and (iv) distinct aging-related and longevity-

related candidate genes were found in worker and queen bees. These results provide new insights into the cellular aging and longevity of trophocytes and oenocytes in honey bees. Identification of aging-associated biomarker genes also constitutes a basis for translational research of aging in higher organisms.

Keywords Transcriptome · Next-generation sequencing · Aging · Longevity · Honey bee

Introduction

Honey bee (*Apis mellifera*) is a unique model animal for aging and longevity studies because queen bees have a much longer lifespan than worker bees, even though they have the same genome [58–60, 62]. Besides, honey bees live in large colonies, are easily manipulated, and their genome has been sequenced [3, 18, 27, 68].

The difference in lifespan between worker and queen bees is mainly related to food intake [39]. Queen bees feed on royal jelly throughout their lives, whereas worker bees mostly consume glandular secretions, honey, and pollen [69]. Queen bees live in the hive except during mating flights and colony fission and generally lay 1500–2000 eggs per day throughout their lives [7]. By contrast, worker bees spend only the first two weeks of life in the hive and perform tasks such as comb-building, cell-cleaning, and brood-nursing before transitioning to the foraging stage. In the foraging stage, workers fly up to 21 km per day to collect nectar and pollen for the hive [55]. However, while worker and

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queen bees perform distinct tasks, these differences are rather inconsequential in altering the lifespan of honey bees [32, 39, 59].

The trophocytes and oenocytes of honey bees have been targeted as cells for cellular senescence and longevity studies due to their biological relevance as white adipose tissue or liver, the ease of isolation from the abdomen, and convenient manipulation [11, 26, 56, 64]. Trophocytes, which are large and irregularly shaped, and oenocytes, which are small and spherical, attach to form a single layer of cells around each segment of the honey bee abdomen. Most importantly, these cells do not divide during adulthood, which is a requirement for studying aging and longevity [29, 30]. Previous studies have shown that several metabolic features — mitochondrial energy utilization, energy regulation activity, cellular degradation activity, and cellular metabolism — in the trophocytes and oenocytes of worker bees decrease with age [13, 34, 35]. However, these processes are maintained with age in the trophocytes and oenocytes of queen bees [36–38]. These findings thus indicate that the trophocytes and oenocytes of worker bees exhibit aging phenotypes and that those of queen bees show pro-longevity phenotypes.

To further explore this phenomenon, we analyzed transcriptomes in the trophocytes and oenocytes of worker bees and queen bees using RNA-seq [21, 66]. Comparative analysis of the age-associated transcriptomes in this unique insect model may provide a mechanistic understanding of aging and longevity control in worker and queen bees.

Methods

Honey bees

The honeycomb frames containing pupae from the different colonies were transferred to an incubator (34 °C, 95% relative humidity). Seventy newly emerged worker bees were collected in a cage (15 × 10 × 12 cm), put into a 34 °C thermostat (NK system, Nippon, Japan), and fed honey and fresh pollen grains mixed with honey every day [32]. Young (5-day-old) and old (30-day-old) worker bees were collected for the following studies; thus, the difference between them is only age. According to previous studies [13, 29, 32, 35, 48], worker bees reared in a thermostat at 34 °C throughout their lives can replace worker bees reared in field hives for aging

studies. Young (2-month-old) and old (16-month-old) queen bees were collected from different hives for the following experiments. The young and old queen bees were mated with drones and were able to lay eggs. Since queen bees cannot be reared outside hives without altering their normal physiological state, they need to be collected from the field hives. If young queen bees can lay eggs, beekeepers will cut their wings to prevent colony fission. Young queen bees and old queen bees undergo the same behavioral patterns including mating flights, eating royal jelly, the absence of colony fission, and laying eggs. The difference between young and old queen bees is only age [31]. Young and old worker bees and young and old queen bees were collected on the same dates for subsequent experiments.

RNA isolation, library preparation, and sequencing

Trophocytes and oenocytes were isolated from three biological replicates of ten young and ten old worker bees, or five young and five old queen bees. Total RNA was extracted from trophocytes and oenocytes by using TRIzol (15596018; Invitrogen, Carlsbad, CA, USA). The concentration and quality of the RNA were determined by using an ND-1000 spectrophotometer (Nanodrop Technology, Wilmington, DE, USA) and Bioanalyzer 2100 (Agilent Technology, Santa Clara, CA, USA) with the RNA 6000 Labchip Kit (Agilent Technologies, USA). Library preparation and sequencing were performed according to the manufacturer's instructions from Illumina at Welgene Biotech Co., Ltd. (Taipei, Taiwan). Library construction of all samples was done by TruSeq RNA Sample Prep Kits v2 for 50-bp (Single-End) sequencing on the Solexa platform. The sequence was directly determined using sequencing-by-synthesis technology via the TruSeq SBS Kit. Raw sequences, roughly 10 million reads per sample, were generated from the Illumina GA Pipeline software CASAVA v1.8.

Raw data processing and statistical analysis

The original image data generated by the sequencer were transformed into sequence data by base calling, which was defined as raw reads and stored in fastq format. Initially, the sequences generated were subjected to a filtering process to obtain qualified reads. ConDeTri was implemented to trim or remove the reads according to the quality score. Qualified reads after

filtering low-quality data were analyzed using TopHat/Cufflinks for gene expression estimation. The gene expression level was calculated as fragments per kilobase of transcript per million mapped reads (FPKM). For differential expression analysis, CummeRbund (Illumina Inc.) was employed to perform statistical analyses of gene expression profiles. The reference genome and gene annotations were retrieved from the Beebase database. To identify differentially expressed genes (DEGs), the gene dataset of young worker bees was used as the baseline, to which the genes of other samples were compared.

Function and pathway analysis

All of the differential expressed genes were analyzed using Gene Ontology (GO) and then mapped using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

Validation of RNA-sequencing data by quantitative real-time PCR analysis

To verify the RNA-sequencing results, quantitative real-time PCR (qPCR) was used to measure DEGs expression levels. RNA isolation and qPCR were carried out as previously described [35]. Briefly, total RNA was isolated from the trophocytes and oenocytes (isolated from one bee for each experimental group) using TRIzol. Complementary DNA (cDNA) was synthesized by using 1 µg of total RNA and iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). qPCR reaction containing 0.5 µl of 10 µM each primer, 12.5 µl of SYBR Green (170-8882; Bio-Rad, Hercules, CA, USA), 1 µl of diluted cDNA, and 10.5 µl of ddH₂O in a final volume of 25 µl was assayed by using iQ5 RT-PCR detection system (Bio-Rad, Hercules). Primers were designed based on GenBank nucleotide sequences (Supplementary Table 1). The *actin* gene was used as a control housekeeping gene [18, 70]. The relative expression levels of genes were calculated using the $2^{-\Delta\Delta C_t}$ method [46]. All samples were run in three biological replicates.

Statistical analysis

The difference in the mean values among the four groups was determined by one-way ANOVA and by Tukey's HSD for pairwise comparisons. SPSS software

was used for statistical analysis. Statistical significance was set at 0.05.

Results

Raw data processing

The transcriptome library of young worker bees, old worker bees, young queen bees, and old queen bees on average yielded 30,721,785, 22,072,970, 18,267,899, and 17,796,501 usable reads, respectively. After mapping to the reference genome and the junction database, 15,314 uniquely mapped reads of young worker bees, old worker bees, young queen bees, and old queen bees were acquired (Fig. 1). The overall gene expression profile of young worker bees was similar to that of old worker bees. Likewise, the gene expression profile of young queen bees was similar to that of old queen bees. However, the transcriptomes between the two types of bees were quite different, indicating that developmental fate has a greater impact than age on the transcriptome output. To this end, most upregulated genes identified in worker bees were downregulated in queen bees, and vice versa.

Expression level analysis

To evaluate the transcriptional status between worker and queen bees at young and old ages, we classified the gene expression levels into eight grades based on their FPKM and defined low-, moderate-, and high-expression genes according to criteria outlined in previous studies [12, 44]. The proportion of low-expression genes (< 1) was higher in worker bees compared with queen bees. In contrast, the proportion of moderate-expression genes (10~100) was higher in queen bees compared with worker bees. The proportion of high-expression genes (500~ ≥ 1000) was similar between worker and queen bees (Fig. 2).

Screening analysis of DEGs

To screen the genes possibly related to aging and longevity, we analyzed the DEGs between young and old bees. A total of 367 DEGs were found between old vs. young worker bees, of which 224 were upregulated and

Fig. 1 Heat map showing clustering of the 15,314 DEGs between worker and queen bees of young and old ages. The hierarchical clustering of samples was arranged as shown above the heat map. Induced genes are represented in red, whereas suppressed genes are shown in blue. YW, young worker bees; OW, old worker bees; YQ, young queen bees; OQ, old queen bees

143 downregulated. A total of 401 DEGs were found between old vs. young queen bees, of which 356 were upregulated and 45 downregulated. Overall, queen bees were found to express 884–1799 upregulated genes and 405–616 downregulated genes in comparison to worker bees (Fig. 3a). The Venn diagram in Fig. 3b shows the distributions of unique and shared DEGs among different bee groups.

To further associate the DEG candidates with aging and longevity, we identified upregulated and downregulated genes between young and old worker bees as well as between young and old queen bees by using pairwise comparisons. In worker bees, the following genes were highly expressed in old worker bees compared with young worker bees: key lactase metabolism genes encoding *L-lactate dehydrogenase* (LDH) (GB48134) and *L-lactate dehydrogenase-like* (GB48315); genes involved in the sensory perception such as *odorant receptors* (GB52386, GB52385, GB52381, GB52379, GB52387, GB52372, GB52371, GB52388, GB52389); immunity genes such

as *serine protease 1* (GB45701), *serine protease 14* (GB55007), *defensin 2* (GB47618), *beta-1,3-glucan recognition protein 1* (GB42685) and *beta-1,3-glucan recognition protein 2* (GB42981); and dopamine synthesis genes including *aromatic-L-amino-acid decarboxylase-like* (GB45938) and *tyrosine hydroxylase* (GB40967). In contrast, the downregulated genes included candidates implicated in critical metabolic pathways: genes involved in carbohydrate metabolic process such as *glycogen phosphorylase* (GB42835), and *chitinase* (GB48474); genes involved in lipid metabolic process such as *hydroxymethylglutaryl-coenzyme A synthase* (GB44420), *trans-2,3-enoyl-CoA reductase-like* (GB46772), *alkyldihydroxyacetonephosphate synthase* (GB47736), *1-acyl-sn-glycerol-3-phosphate acyltransferase alpha-like* (GB43362), *phospholipase A1 member A-like* (GB50722), *acyl-CoA Delta(11) desaturase-like* (GB42218), and *elongation of very-long-chain fatty acids protein 4-like* (GB54399); *cuticular protein* (GB50453, GB48981, GB50438, GB40566); genes of mitochondrial

Fig. 2 Abundance distribution of unigenes expressed in the indicated samples, as detected by RNA-seq. The expression level ranges are categorized as follows: 0~10, low-expression genes; 10~500, moderate-expression genes; 500~∞, high-expression genes

respiratory chain complex such as *cytochrome b-c1 complex subunit 6, mitochondrial-like* (GB50335), *ATP synthase, H⁺ transporting, mitochondrial F1 complex, and alpha subunit 1* (GB41028); and *pancreatic triacylglycerol lipase-like* (GB43513) (Fig. 4a).

In queen bees, the following genes were highly expressed in the old counterparts: *odorant receptor* (GB40202, GB52376, GB52363, GB42587, GB40201, GB52362, GB50051); *juvenile hormone esterase-like* (GB48046); immunity genes such as *defensin 1* (GB41428), *serine protease 45* (GB43369), *serine protease homolog 57* (GB48080), *serine protease 47* (GB48081), *toll like receptor 8* (GB48399), and *apidaecin* (GB51306); *catalase* (GB41427); *LOC100576135 glutamate receptor 1-like* (GB54881);

LOC409777 glutamate receptor; ionotropic kainate 2-like (GB47549); *corazonin receptor* (GB44824); *LOC100578314 monocarboxylate transporter 10-like* (GB53682); *LOC408646 monocarboxylate transporter 8-like* (GB49280); and *neurotrimin-like* (GB54005, GB44739, GB53527). Conversely, genes downregulated in old vs. young queen bees include *Pangolin* (GB53166); *synaptic vesicle glycoprotein 2B-like* (GB50473); *adenylate cyclase type 6-like* (GB48012); *EF-hand domain-containing protein CG10641-like* (GB51014); *rap guanine nucleotide exchange factor 6-like* (GB50393); *hemicentin-2-like* (GB44248); *protein fork head-like* (GB53404); and *cuticular protein analogous to peritrophins 3-E* (GB52854) (Fig. 4b).

Fig. 3 Overall transcriptome differences between worker and queen bees of young and old ages. **a** Distribution of upregulated and downregulated DEGs across different comparisons. **b** Venn

diagram representation of the number of DEGs in the indicated comparison. YW, young worker bees; OW, old worker bees; YQ, young queen bees; OQ, old queen bees

Fig. 4 Hierarchical clustering analysis of DEGs between young and old ages: **a** 367 DEGs between young and old worker bees; **b** 401 DEGs between young and old queen bees. Induced genes are

represented in red, while downregulated genes are shown in blue. YW, young worker bees; OW, old worker bees; YQ, young queen bees; OQ, old queen bees

By further integrating these comparisons, we defined aging-related candidate genes as those with higher expression in old worker bees than in the other three bee groups. The examples of these genes included *L-lactate dehydrogenase-like (LDH)* (GB48135), *clavesin-1-like* (GB49504), *cytochrome P450 6AS5 (CYP6AS5)* (GB49890), *tyrosine decarboxylase 2* (GB55830), and *β -glucosidase* (GB54486) (Fig. 5). Likewise, longevity-related candidate genes were defined as those showing higher expression in young worker bees and young and old queen bees (vs. old workers) or unique expression in queen bees but not in worker bees. The examples of these genes included *pancreatic triacylglycerol lipase-like* (GB43513), *epoxide hydrolase* (GB42829), *Sir2 histone deacetylase (SirT1)* (GB53035), *major royal jelly protein 1 (MRJP1)* (GB55205), *epidermal growth factor receptor-like (EGFR)* (GB54477), and *vitellogenin (Vg)* (GB49544) (Fig. 6).

Validation of RNA-sequencing data by qPCR analysis of cholesterol-hydroxyecdysone-Vg pathway genes

To confirm the deep sequencing data, eight randomly selected DEGs were validated by using qPCR in old worker bees, young queen bees, and old queen bees

compared with young worker bees. The extents of correlation of old worker bees (0.993179), young queen bees (0.995733), as well as old queen bees (0.996576) in comparison to young worker bees were quite high. The fold changes were also similar among them (Supplementary Table 2). Thus, these qPCR results were consistent with and therefore authenticating the RNA-sequencing findings.

The expression levels of genes involved in the cholesterol-hydroxyecdysone-Vg pathway, such as *Cyp314A1*, *EcR-A*, *EcR-B1*, *USP*, *E74*, *E75*, *BR-C*, *Vg*, and *VgR*, were lower generally in worker bees than in the queen bees [49]. The unique expression patterns of the cholesterol-hydroxyecdysone-Vg pathway observed in this study were consistent with the previous study (Supplementary Fig. 1), demonstrating the reliability of the current RNA-sequencing results and further supporting the link of this pathway to the aging process.

Discussion

Some gene expression studies have previously been conducted to document gene changes associated with

Fig. 5 Expression profiles of the aging-related candidate genes as revealed by RNA-seq. YW, young worker bees; OW, old worker bees; YQ, young queen bees; OQ, old queen bees

queen bee longevity [16], aging [24, 47], reproductive status [22], and flight [53]. However, due to the design of these studies, which are mainly based on gene microarray and real-time PCR analysis of selected genes, they might be limited in the scope of genome-wide changes being detected. In this study, we evaluated the global transcriptomes of trophocytes and oenocytes in young and old worker bees and young and old queen bees

using RNA-sequencing and provided a system-wide and comprehensive profile of transcriptome changes associated with caste-specific aging. The findings indicated that the gene expression of trophocytes and oenocytes was different between worker and queen bees, and showed a distinct association with cellular aging in worker bees and cellular longevity in queen bees.

Fig. 6 Expression profiles of the longevity-related candidate genes as revealed by RNA-seq. YW, young worker bees; OW, old worker bees; YQ, young queen bees; OQ, old queen bees

Raw data processing and expression level analysis

RNA-seq has been used to analyze the transcriptomes of the fat body of nurse bees, foragers, and winter worker bees [64], the ovaries of worker and queen bees [57], the

hypopharyngeal gland of honeybees [44, 45], queen- and worker-destined larvae of honeybees [12, 19], and the kidneys of rat [66]. As shown by our RNA-seq experiments, gene expression patterns of worker bees were different from those of the queen bees.

Importantly, even though they share the same genome, most upregulated genes of worker bees were downregulated in queen bees, and vice versa, indicating that royal jelly reprograms the transcriptome of queen bees [39]. Low-expression genes are mainly expressed in worker bees and moderate-expression genes are mainly expressed in queen bees, suggesting that low-expression genes might be associated with aging and moderate-expression genes might be associated with reproduction and longevity in female honey bees.

Screening analysis of DEGs

The main function of the hypopharyngeal gland is to produce and secrete the protein components of royal jelly. A previous study revealed 614 DEGs in the hypopharyngeal gland between nurse bees and forager, of which 25 were upregulated and 589 downregulated in forager as compared with nurse bees [45]. In trophocytes and oenocytes, DEGs between young and old worker bees were numbered at 314, of which 170 were upregulated and 144 downregulated in old worker bees as compared with young worker bees. The number of DEGs ($n = 314$) between young and old worker bees is close to the results between nurse bees and foragers [64]. Moreover, identified DEGs in the trophocytes and oenocytes between young and old queen bees are numbered at 401, in which 356 upregulated and 45 downregulated when old queen bees compared with young queen bees.

Functional analysis of DEGs

The genes whose expression altered between young and old worker bees might be associated with aging. The functions of upregulated genes in old worker bees compared to young worker bees corresponded to the detection of chemical stimulus, sensory perception of smell, RNA metabolic process, transcription, and cellular amino acid metabolic process. The genes downregulated in old worker bees might imply the maintenance of youth. This set was enriched in genes associated with lipid metabolic process, structural molecule activity, structural constituent of the cuticle, and calcium ion binding.

In contrast, the upregulated genes detected in old queen bees might be associated with longevity. The putative functions of the detected upregulated genes fell in the categories of olfactory receptor activity, sequence-specific DNA binding, ATP binding, response to stress,

oxidation-reduction process, transporter activity, and peptidase activity. The genes downregulated in old queen bees might be associated with youth. In this regard, this set was enriched in genes implicated in the carbohydrate derivative metabolic process and organ nitrogen compound metabolic process. Collectively, these results provided new candidate biomarkers and insights into the molecular mechanisms underlying the aging and longevity of honey bees.

GO annotation of DEGs

All of the DEGs were analyzed using gene ontology (GO). In worker bees, GO terms were generated for 172 items from 314 DEGs. These items were divided into three categories: molecular function, biological process, and cellular component. Binding, metabolic process, and membrane were dominant in the three categories. In terms of molecular functions, GO analysis revealed an over-representation of the genes involved in catalytic activity, ion binding, heterocyclic compound binding, organic cyclic compound binding, and hydrolase activity. In terms of biological processes, GO analysis revealed an over-representation of the genes involved in the single-organism process, cellular process, organic substance metabolic process, single-organism cellular process, and primary metabolic process. In terms of cellular components, GO analysis revealed an over-representation of the genes involved in cell, cell part, membrane part, intracellular, and integral component of membrane.

In queen bees, GO terms were generated for 146 items from 258 DEGs. These items were also divided into three categories: molecular function, biological process, and cellular component. Binding, metabolic process, and membrane were also dominant in the three categories. In terms of molecular functions, GO analysis revealed an over-representation of the genes involved in protein binding, heterocyclic compound binding, organic cyclic compound binding, catalytic activity, and ion binding. In terms of biological processes, GO analysis revealed an over-representation of the genes involved in the cellular process, single-organism process, metabolic process, single-organism cellular process, and biological regulation. In terms of cellular components, GO analysis revealed an over-representation of the genes involved in the intrinsic component of membrane, membrane part, the integral component of membrane, cell, and cell part.

Pathway annotation of DEGs within castes

To further understand the functions of these DEGs, we mapped DEGs by using the Kyoto encyclopedia of genes and genomes (KEGG) database for signaling pathways analysis. In worker bees, 13 pathways were identified and included synthesis and degradation of ketone bodies, oxidative phosphorylation, valine, leucine and isoleucine degradation, tyrosine metabolism; novobiocin biosynthesis, starch and sucrose metabolism, amino sugar and nucleotide sugar metabolism, glycerolipid metabolism, ether lipid metabolism, propanoate metabolism, methane metabolism, folate biosynthesis, and aminoacyl-tRNA biosynthesis. Of those, genes involved in amino sugar and nucleotide sugar metabolism were the most significantly enriched between young worker bees and old worker bees. In queen bees, 4 pathways were identified and included fatty acid elongation, amino sugar, and nucleotide sugar metabolism, glycerolipid metabolism, glyoxylate, and dicarboxylate metabolism.

Aging-related candidate genes

Aging-related candidate genes were defined as higher expression in old worker bees in comparison to young worker bees and young and old queen bees. These genes included *LDH-like* (GB48135), *clavesin-1-like* (GB49504), *CYP6AS5* (GB49890), *tyrosine decarboxylase 2* (GB55830), and *β-glucosidase* (GB54486).

LDH catalyzes the conversion of lactate to pyruvate and back. LDH gene expression increased in old worker bees, which is consistent with previous studies showing that LDH activity and lactate concentration increase with age in the trophocytes and oenocytes of worker bees (*Apis mellifera*) [48], in the brain and cerebrospinal fluid of mice [61, 71], and in the cerebrospinal fluid of human [42]. These studies indicate that LDH is associated with aging. Clavesin-1 was essential for the normal morphology of late endosomes and lysosomes [40]. Lysosomal dysfunction is associated with age-related diseases and a decline in lifespan [10]. Clavesin-1 gene expression increased in old worker bees, which is consistent with previous studies showing that lysosomal efficiency decreased with age in the trophocytes and oenocytes of worker bees [35] but remained similar between young and old queen bees [38]. These studies together indicate that clavesin-1 is associated with aging. CYP6AS5 is involved in insecticide metabolism

and resistance [1, 4, 14, 52]. Low CYP6AS5 expression may cause low detoxification leading to aging or the accumulation of harmful substances. Tyrosine decarboxylase catalyzes tyrosine to tyramine and CO₂. Tyramine can be converted into octopamine, which decreases the number of vitellogenic oocytes and the fecundity in *Drosophila* [23]. Therefore, tyrosine decarboxylase is associated with aging. β-glucosidase catalyzes the hydrolysis of terminal non-reducing residues in β-glucosides and releases glucose. β-glucosidase gene expression increased in old worker bees, which is consistent with a previous study showing that its activity increased with aging in *C. elegans* [15].

Longevity-related candidate genes

Longevity-related candidate genes were defined as higher expression in young worker bees and young and old queen bees or young and old queen bees compared with old worker bees. These genes included *pancreatic triacylglycerol lipase-like* (GB43513), *epoxide hydrolase* (GB42829), *major royal jelly protein 1* (MRJP1) (GB55205), *epidermal growth factor receptor-like* (EGFR) (GB54477), *Sir2 histone deacetylase* (GB53035), and *Vg* (GB49544).

Lipase catalyzes the hydrolysis of triacylglycerol. Lipase gene expression increased in young worker bees and young and old queen bees, which is consistent with previous studies showing that lipase activity decreases with age in the trophocytes and oenocytes of worker bees [48] and rats [5, 9] but remains similar in the trophocytes and oenocytes of young and old queen bees [50]. Epoxide hydrolase cleaves oxirane derivatives to yield the corresponding diols and plays roles in detoxification and metabolism [2, 51]. Epoxide hydrolase gene expression increased in young worker bees and young and old queen bees, which is consistent with previous studies showing that β-oxidation decreases with age in the trophocytes and oenocytes of worker bees [48], human beings [41, 43], rats [65, 67], and mice [28] but remains similar in the trophocytes and oenocytes of young and old queen bees [50]. Besides, epoxide hydrolase participates in lipid catabolism [51].

MRJP1 and EGFR gene expression increased in young and old queen bees, which is consistent with previous studies showing that MRJP1 induces the differentiation of queen and worker bees through

EGFR in the larval stage and increases the lifespan of female fly (*Drosophila melanogaster*) [39] and nematode (*C. elegans*) through EGFR [17]. SirT1 regulates cellular functions, including cellular stress responses and energy metabolism. SirT1 gene expression increased in young worker bees and young and old queen bees, which is consistent with previous studies showing that SirT1 activity decreases with age in the trophocytes and oenocytes of worker bees [34] and rats [6] but remains similar in the trophocytes and oenocytes of young and old queen bees [36]. In addition, SirT1 activity is increased in calorie restriction [8], ambient temperature reduction [33], and exercise [20], which are all beneficial for longevity. Vg is important storage, transport, and longevity-related protein. Vg gene expression increased in young and old queen bees, which is consistent with previous studies showing that Vg protein and gene maintain at high levels in mature queen bees and long-lived worker bees [47, 49, 63] and decrease in worker bees [25]. In addition, knockdown of Vg decreases the lifespan of worker bees [54].

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11357-021-00360-y>.

Author contribution C.Y.H. designed research; C.Y. L. and Y.T.W. performed research; C.Y. L. and Y.T.W. analyzed data; C.Y.H. and B.T. wrote the paper.

Funding This work was supported by grants (CMRPD1G0582, CMRPD1G0583, and CMRPD1K0481) from the Chang Gung Memorial Hospital, Linkou, Taiwan, and a grant (MOST 108-2320-B-182-037-MY3) from the Ministry of Science and Technology, Taiwan.

Declarations

Conflict of interest The authors declare no competing interests.

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