Transcriptomic response of *Caenorhabditis elegans* expressing human Aβ_{42} gene treated with salvianolic acid A

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Alzheimer's disease is associated with the deposition of β-amyloid peptide in the brain. A genome-wide transcriptomic study was performed to determine the response of transgenic *Caenorhabditis elegans* expressing full-length human Aβ_{42} gene towards salvianolic acid A (Sal A). The genes associated with antioxidant response, *gst-4*, *gst-10*, *spr-1* and *trxr-2*, were upregulated. Aβ_{42} caused oxidative stress and the antioxidant response genes possibly provide some sort of protection to the nematode. *trxr-2* gene product was also associated with the defence system and probably has a role in the lifespan of the nematode. Other genes involved in DNA replication, reproduction, immune response and antimicrobial activities were also found to be upregulated. Treatment of Sal A also increased the rate of reproduction in the nematode, and elevated its immunological protection system towards microorganisms. On the other hand, the genes responsible for ligand-gated cation channel, embryonic and postembryonic development, locomotion and neuromodulation of chemosensory neurons were found to be downregulated. As an effector, Sal A might conceivably reduce the movement of the nematode by interfering with neuronal transmission, and embryonic and postembryonic development.

Keywords: β-amyloid peptide, *Caenorhabditis elegans*, salvianolic acid A, transcriptome.

ALZHEIMER'S disease (AD) is a persistent, progressive brain disorder that affects the middle-aged and elderly population. In the long term, AD will eventually cause a loss in thinking ability until it becomes worse over time and interferes with daily activities. The number people with AD was predicted to double every 20 years to a whooping 81.1 million by 2040 worldwide[1].

AD can be illustrated using the amyloid cascade hypothesis. The first evidence of amyloid hypothesis was the discovery of senile plaques (SPs) and neurofibrillary tangles (NFTs) by a physician, Aloi Alzheimer, when autopsy was performed on the brain of an AD patient in 1907 (see ref. 2). The amyloid cascade hypothesis[3] was proposed after the discovery of amyloid precursor protein (APP)[4] as well as mutations of its sequence[5]. In addition, the discovery of amyloid-beta within SPs[6] also contributed significantly to the amyloid cascade hypothesis. The amyloid cascade hypothesis involved chopping of APP by β-secretase and γ-secretase to form Aβ peptides, followed by aggregation of Aβ oligomers to produce SPs and eventually causing toxicity to the brain cells by oxidative stress.

Among the potential drugs that block the production of Aβ are those that contain anti-Aβ aggregation properties as well as antioxidant effects to decrease the oxidative stress caused by Aβ. The days available at present can only delay the onset of AD[7]. However, there are some drawbacks associated with these drugs and thus, there is a need for new potential disease-modifying AD drugs.

Salvianolic acid A (Sal A) which has a polyphenolic structure is one of the major water-soluble compounds in the water Danshen extract, besides salvianolic acid B and Danshensu[8]. Danshen, *Salvia miltorrhiza* is a Chinese. Sal A showed antioxidant properties by inhibiting ROS and significantly scavenging $HO^{\bullet}$ produced in phorbol myristate acetate-stimulated rat neutrophils[9,10]. Previous studies proved that Sal A has the ability to improve memory impairment when the compound was intravenously injected to mice[11]. In addition, a concentration of 10 mg/kg of Sal A can inhibit cerebral lipid peroxidation and clear free $HO^{\cdot}$ radicals in mice. Hence, it can be deduced that there is a relationship between the antioxidant properties of Sal A and its improving effects on memory impairment induced by cerebral ischaemia-reperfusion in mice[11].

Our previous study using the same organism has shown that Sal A has the ability to delay the paralysis of *C. elegans*. The same treatment also showed the inhibition of Aβ fibrils and decreased ROS induced by Aβ (ref. 12). This indicates that Sal A has huge potential as an alternative drug to treat AD. However, the transcriptional response of *C. elegans* towards Sal A is yet to be elucidated.

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In this study, transgenic *C. elegans* that expressed Aβ_{42} gene was treated with Sal A to determine its therapeutics effect. In addition, we conducted a genome-wide transcriptomic analysis to determine the metabolic targets and pathways that were affected as a result of Sal A treatment.

Methods and materials

C. elegans strains and maintenance

Transgenic *C. elegans* strain GMC101(dvIs100[unc-54p::A-beta-1-42::unc-54 3′-UTR + mtl-2p::GFP) and *Escherichia coli* OP50 strain were obtained from the Caenorhabditis Genetics Center (CGC), University of Minnesota, MN, USA. The transgenic *C. elegans* was kept at 16°C on nematode growth medium (NGM) supplemented with *E. coli* OP50. GMC101 is a transgenic strain that expressed the human Aβ_{42} gene.

RNA extraction, quality and quantity evaluation

C. elegans strain GMC101 grown on NGM plates supplemented with 100 µg/ml Sal A was used in the transcriptomic study. Approximately 5000 adult nematodes were collected after 32 h temperature from 16°C to 25°C to induce the expression of Aβ. They were harvested by washing with M9 medium to remove *E. coli* OP50 cells. RNA extraction was performed as described by He[13] using AccuZol (Bioneer, South Korea). For control samples, the nematodes were grown on NGM plates without Sal A. The extracted RNA was then treated with DNase (Macherey-Nagel, Germany) to remove any DNA contaminants and RNA was repurified using NucleoSpin RNA Clean-up XS (Macherey-Nagel). The total RNA was quantified using agarose gel electrophoresis and spectrometric technique (NanoPhotometer® spectrophotometer, IMPLEN, CA, USA). RNA concentration was measured using Qubit® RNA Assay Kit (Qubit® 2.0 Flurometer, Life Technologies, CA, USA) and RNA integrity was assessed using the RNA Nano 6000 Assay Kit (Bioanalyzer 2100 System, Agilent Technologies, CA, USA).

RNA-seq library preparation

Standard illumina protocol was used for library prep involving fragmentation of mRNA, synthesis of double-stranded cDNA, polyadenylation, adapter ligation and library size selection (150–200 bp). The libraries were generated using NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA). Briefly, poly-T oligo-attached magnetic beads were used to isolate mRNA from the total RNA samples. Fragmentation was carried out using NEBNext First Strand Synthesis Reaction Buffer. First-strand cDNA was synthesized using random hexamer primer and M-MuLV Reverse Transcriptase. The second-strand cDNA synthesis was performed using DNA Polymerase I. The remaining overhangs were converted into blunt ends by exonuclease/polymerase activities. Post adenylation of 3′-ends of DNA fragments, NEBNext Adaptor with hairpin loop structure was ligated to prepare for hybridization. The library fragments were purified with AMPure XP system in order to select 150–200 bp cDNA fragments (Beckman Coulter, Beverly, USA) and the adapter was ligated. The library was analysed using Agilent Bioanalyzer 2100 System and subsequently used for RNA-seq. Raw data generated were cleaned and trimmed by the adapter as well as removing the low-quality reads.

Mapping of reads to the reference genome

The reference genome and gene model annotation files were downloaded from the genome websites (ftp://ftp.ensembl.org/pub/release-76/fasta/caenorhabditis_elegans/dna) and (ftp://ftp.ensembl.org/pub/release-76/gtf/caenorhabditis_elegans) respectively. An index of the reference genome was built using Bowtie v2.2.8.0 (refs 14, 15) and paired-end clean reads were aligned to the reference genome using TopHat v2.1.1 (ref. 16). The program HTSeq v0.6.1 was used to count the read numbers mapped to each gene[17]. The Fragments Per Kilobase of transcript per Million mapped reads (FPKM) value of each gene was calculated based on its length and reads count mapped to this gene. FPKM is the expected number of fragments per kilobase of the transcript sequence per million base pairs sequenced. It considers the effect of sequencing depth and gene length for the read counts at the same time, and is currently the most commonly used method for estimating gene expression levels[18].

Differential expression analysis

The DESeq R package version 1.18.0 was used to perform differential expression analysis of the two experimental conditions (with two biological replicates per condition)[19]. The read counts of each sequenced library were adjusted by the edgeR program package through one scaling normalized factor[20]. Differential expression analysis of the two conditions was performed using the DEGSeq R package (1.20.0)[21]. The *P*-values were adjusted using the Benjamini and Hochberg method. Corrected *P*-value of 0.005 and log 2 (fold change) of 1 were set as the threshold for significantly differential expression[22].

GO and KEGG enrichment analysis of differentially expressed genes

Gene ontology (GO), enrichment analysis of differentially expressed genes was implemented by the GOseq R

package, in which gene length bias was corrected. GO terms with corrected *P*-value less than 0.05 were considered significantly enriched by differential expressed genes[23]. The KOBAS software was used to test the statistical enrichment of differential expression genes in the KEGG pathways[24].

Real-time PCR validation of transcriptomic results

To validate the bioinformatics analysis of differentially express patterns, ten genes (five up- and five down-regulated) were chosen. The primers of the selected genes were designed using Primer 3 software (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and synthesized commercially (Integrated DNA Technologies; Singapore) (Supplementary Material 1). Normalization was carried out against two reference genes which were *tba-1* (tubulin, alpha family member gene), and *cdc-42* (cell division cycle related gene)[25]. All reactions were conducted with three biological and technical replicates.

Results

Clean reads mapping

A total of 48,540,212, 47,260,368 and 48,116,402 raw reads were generated from the treated samples for replicate 1 (Treat_S1), replicate 2 (Treat_S2) and replicate 3 (Treat_S3) respectively. For the untreated samples (control), a total of 42,927,272, 40,774,690 and 40,110,332 of raw reads were generated for replicate 1 (CT1), replicate 2 (CT2) and replicate 3 (CT3) respectively. After quality control (QC), clean reads obtained were 46,931,624, 45,680,280 and 46,326,584 for the treated samples, while 41,562,272, 38,370,118 and 38,869,146 reads were obtained from control samples.

The cleaned reads were mapped to the *C. elegans* genome. For control sample CT1, 96.7% of the reads were mapped to exon region, 3.2% intergenic and 0.1% intron. For CT2, 92.4% were mapped to exon, 7.5% intergenic and 0.1% intron. For CT3, 95.4% were mapped to exon, 4.5% intergenic while 0.1% to intron region. On the other hand, for treated sample Treat_S1, 85.3% of the cleaned reads were mapped into exon region, 14.6% intergenic and 0.1% intron. For Treat_S2, 91.6% mapped to exon, 8.3% mapped to intergenic and 0.1% to intron. Finally, for Treat_S3, 91.2% of the cleaned reads were mapped to the exon region, 8.7% to intergenic and 0.1% to intron. Figures 1 and 2 summarize the statistics of mapped cleaned reads to the *C. elegans* genome. Pearson correlation was used to determine the relation of treated and control samples (Figure 3). All the treated samples showed high similarity among replicates and the same correlation was also observed for control samples. After the reads were mapped into the chromosome, they were internally normalized to minimize the effect of sequencing depth and gene length for the read counts at the same time. Figure 4 shows the distribution of control versus treated samples in FPKM.

Identification of differentially expressed genes

Based on transcriptomic studies, Sal A-treated and untreated *C. elegans* GMC101 strain showed differences in global gene expression. The treated *C. elegans* showed down-regulation of 2020 genes (Supplementary Material 2) and upregulation of 1435 genes (Supplementary Material 3) when the variations were at least two-fold. The differentially expressed genes were clustered together (control versus treated) and have been presented in the form of a heat map (Figure 5).

Sal A affected homologues associated with Alzheimer's disease in human

Based on the transcriptomic results, two homologues that are related to AD in humans were affected by Sal A (Figure 6). The gene *trxr-2*, a putative thioredoxin reductase gene, was upregulated while *ptl-1*, a homolog of the MAP2/MAP4/tau family in the nematode, was down-regulated.

Sal A upregulated antioxidant response genes in the transgenic C. elegans GMC101 strain

Among the differentially expressed genes, four antioxidant response genes were identified, namely *sod-1*, *gst-4*, *gst-10* and *spr-1*. These genes were affected when *C. elegans* strain GMC101 was fed with 100 µg/ml Sal A compared to the unfed nematodes. The gene expressions of antioxidant response genes such as *gst-4*, *gst-10* and *spr-1* were enhanced (Figure 7).

GO enrichment analysis

All the 3455 differentially expressed genes (DEGs) were subsequently analysed using GO enrichment analysis. The genes were classified into three broad categories of biological process, cellular component and molecular function (Figure 8).

For down-regulated GO, the sub-categories classified under 'biological process' were neuropeptide signalling pathway, response to external stimulus, nervous system development, cell communication, response to stimulus, single organism signalling, signalling, neuron differentiation, taxis, generation of neurons, locomotion, neurogenesis, multicellular organismal development, chemotaxis, anatomical structure development, collagen and cuticulin-based cuticle development and system development. In

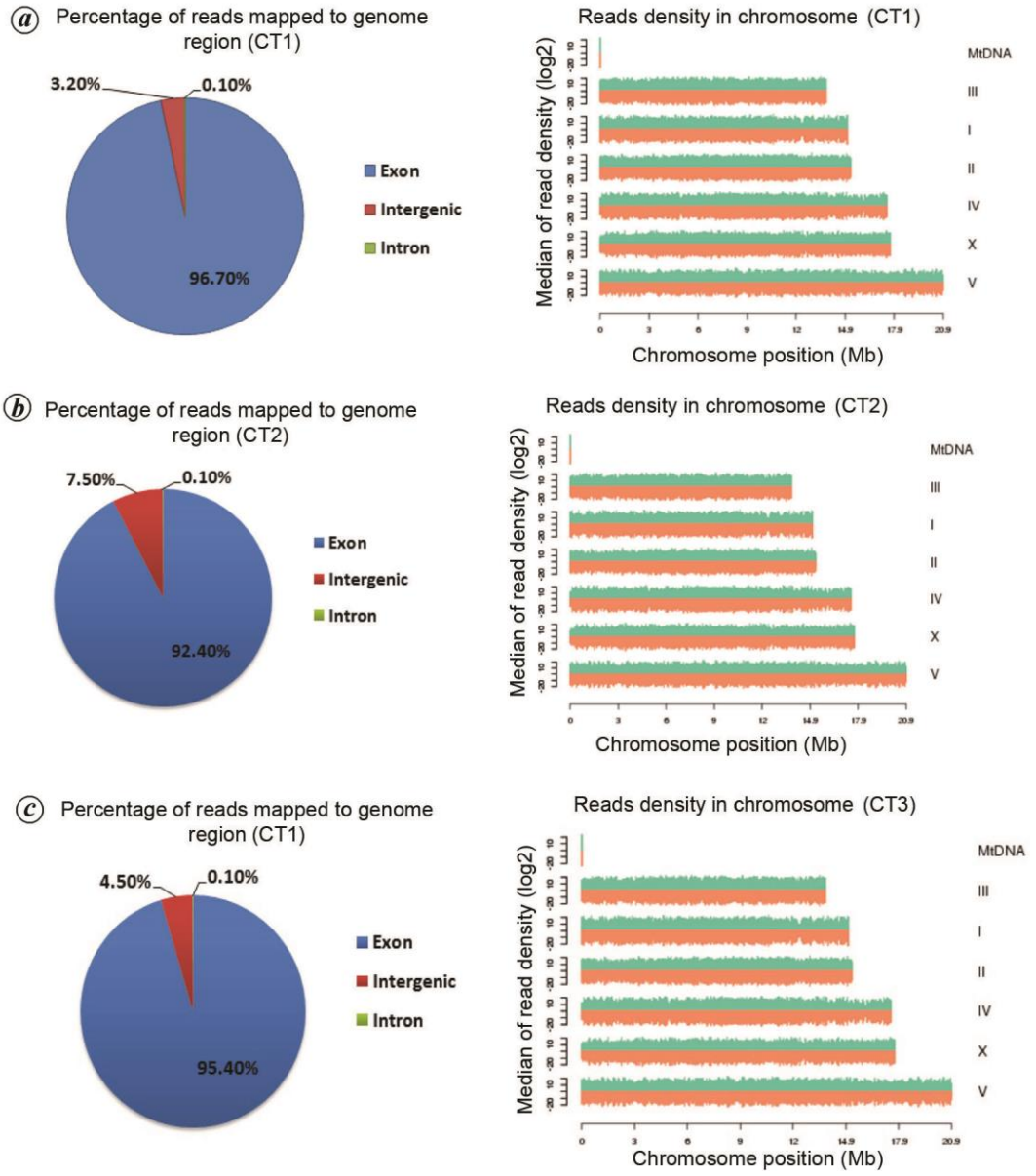

Figure 1. Statistics of mapped reads of control (CT) samples to *Caenorhabditis elegans* chromosome. ***a***–***c***, Replicates of CT samples. The lowest mapped reads (neglecting mtDNA) was in chromosome III followed by I, II, IV and X. The highest reads density was observed in chromosome V. Similar pattern was seen for all replicates.

the 'cellular component' category, the main subcategories for down-regulated GOs were collagen trimer, neuron part, extracellular region, neuron projection, cell projection, extracellular region part, synapse, somatodendritic compartment and axon. The major subcategories of the 'molecular function' category for down-regulated GO induced by Sal A included structural constituent of collagen and cuticulin-based cuticle, structural molecule activity, receptor binding and hormone activity. Figure 9 summarizes the most enriched down-regulated GO terms. The total down-regulated GO analysis is presented in Supplementary Material 4.

The most enriched GO categories that were classified as upregulated were 'biological process' and 'cellular component'. For 'biological process', the subcategories involved were cellular nitrogen compound, cellular metabolic process, nitrogen compound metabolic process, metabolic process, organic substance metabolic process, RNA processing and primary metabolic process. For 'cellular component', the subcategories involved were intracellular part, intracellular, intracellular organelle, organelle, cytoplasm, intracellular organelle part, cell part, intracellular membrane-bounded organelle, cell, membrane-bounded organelle, organelle part, macromolecular complex, cytoplasmic part, non-membrane-bounded organelle, intracellular non-membrane-bounded organelle, membrane-enclosed lumen, intracellular organelle lumen, organelle lumen, nuclear part, nuclear lumen, intracellular

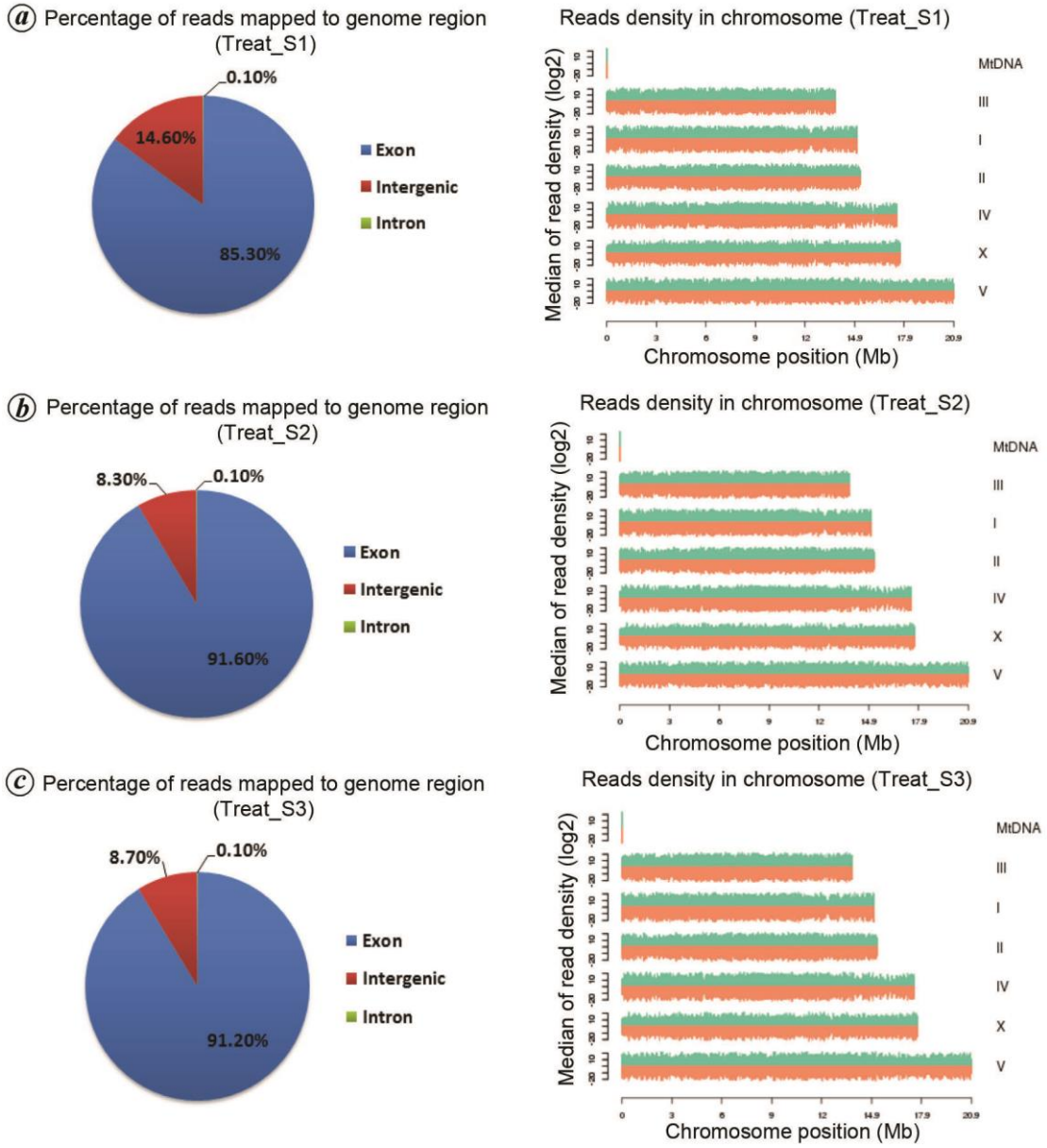

Figure 2. Statistics of mapped reads of treated samples to *C. elegans* chromosome. ***a***–***c***, Replicates of treated samples. The lowest mapped reads (neglecting mtDNA) was in chromosome III followed by I, II, IV and X. The highest reads density was observed in chromosome V. Similar pattern was seen for all replicates.

ribonucleoprotein complex, ribonucleoprotein complex and mitochondrion. Figure 10 summarizes the most enriched upregulated GO terms. The total upregulated GO analysis is presented in Supplementary Material 5.

KEGG pathway enrichment analysis

The assembled DEGs were also subsequently searched against the Kyoto Encyclopedia of Genes and Genomes (KEGG) database to determine those involved in metabolic pathways. Figure 11 shows the statistics of overall KEGG pathway enrichment. The four significant upregulated pathways ($P < 0.05$) induced by Sal A were those involving RNA polymerase (14 genes), pyrimidine metabolism (23 genes), ribosome (35 genes) and oxidative phosphorylation (29 genes). Figure 12 presents the overall statistics for enrichment of the upregulated pathway in KEGG. The significantly down-regulated pathways ($P < 0.05$) included those involving Wnt signalling (17 genes) and TGF-beta signalling (10 genes). Figure 13 summarizes the statistics of the down-regulated pathway enrichment in KEGG. Table 1 showed the hyperlink to the KEGG pathway. The total KEGG analysis is presented in Supplementary Material 6 for upregulated and in Supplementary Material 7 for down-regulated pathways.

Real-time PCR validation of transcriptomic results

Validation of DEGs that were identified through transcriptomic studies was performed by qPCR analysis on ten selected genes (five upregulated and five downregulated genes). The result is presented in Supplementary Material 8.

Discussion

The increase in life expectancy of the world population has indirectly caused the rise in AD patients. AD is the most common cause of dementia and the number of individuals affected by it is predicted to increase in the next two decades[26]. The search for effective treatments using drugs and natural products targeting multiple molecular pathways have been less successful. Disease-modifying symptom-reduction therapies have

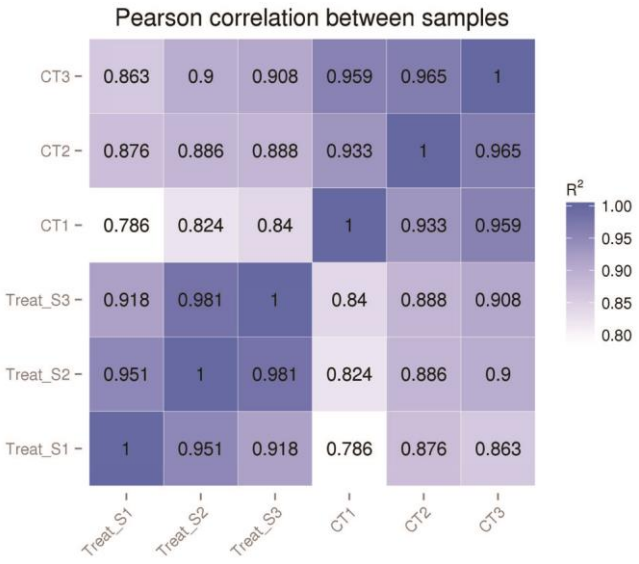

Figure 3. Pearson correlation graph of control versus treated samples.

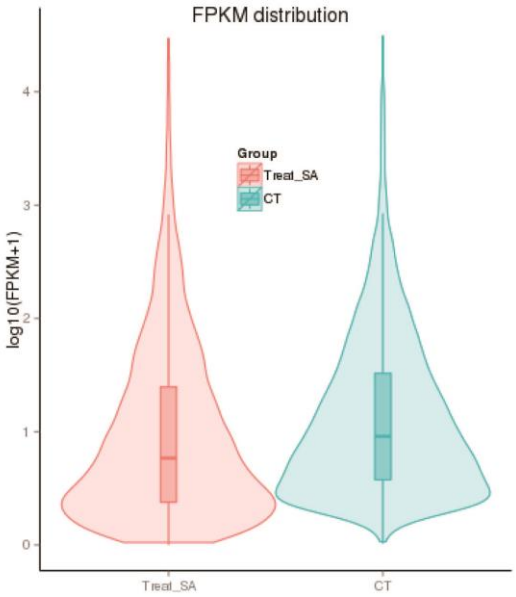

Figure 4. Distribution of Fragments Per Kilobase of transcript per Million mapped reads (FPKM) value of control versus treated samples.

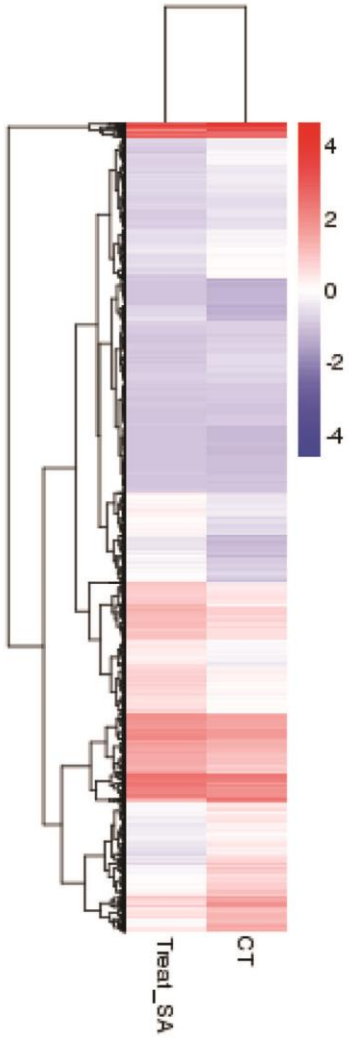

Figure 5. Cluster analysis of differentially expressed genes.

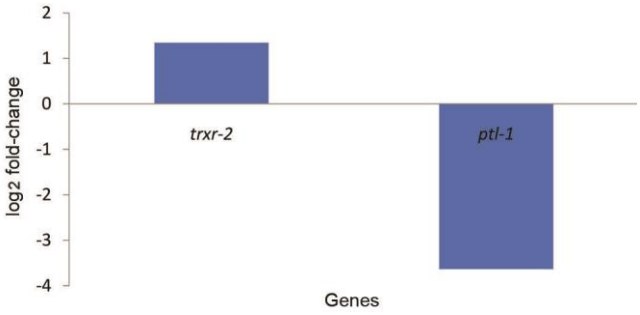

Figure 6. Genes related to Alzheimer's disease that were affected by Sal A.

encountered various shortcomings with adverse side effects. This drawback necessitates further screening for effective anti-AD drugs.

Danshen has been commonly used to treat cardiovascular diseases as well as cerebral ischaemia. The beneficial compounds from Danshen like diterpenoid quinones and hydroxycinnamic have shown to improve cognitive deficit in mice, protect neuronal cells, as well as prevent the formation of amyloid fibrils in AD. Sal A is among the main active compound in Danshen[27]. The structure of Sal A and curcumin are quite similar. A previous study showed that curcumin is an anti-Aβ aggregation agent[28]. Thus, we used Sal A to observe the global transcriptomic response of *C. elegans*.

Antioxidant response genes form an important part of the antioxidative defence system in combating stress due to accumulation of ROS[29–31]. In this study, three antioxidant response genes, namely *gst-4*, *gst-10* and *spr-1* were upregulated by Sal A. These genes most likely provide defence to oxidative stress caused by Aβ_{42}. The *sod-1* gene that was suggested to increase the lifespan of the *C. elegans* strain GMC101 was also upregulated.

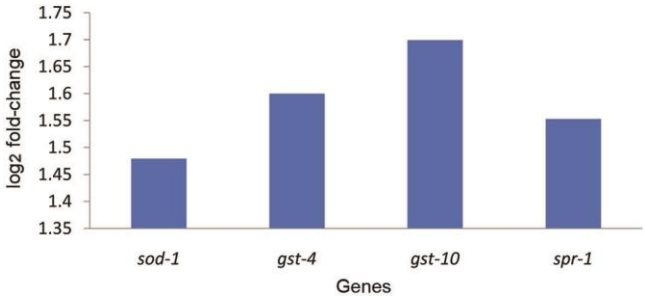

Figure 7. Antioxidant response genes affected by Sal A.

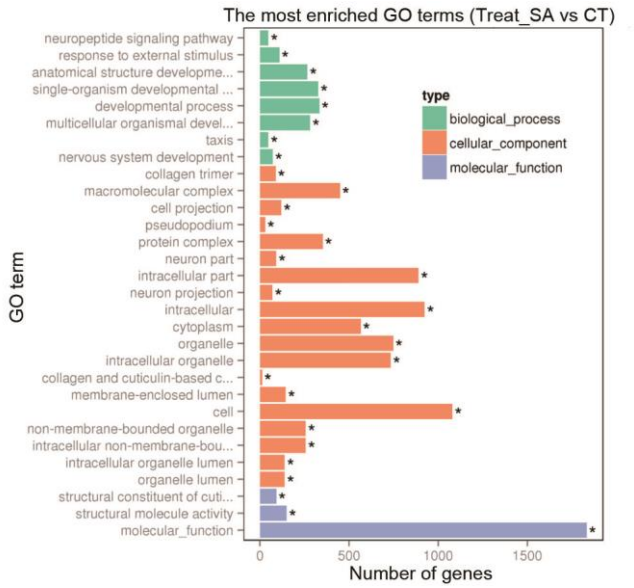

Figure 8. Enriched gene ontology (GO) classification.

The gene *gst-4*, which encodes for stress-responsive glutathione S-transferase (GST) was shown to provide protection to oxidative stress in *C. elegans*[30]. The overexpression of *gst-4* subsequently led to oxidative stress protection by paraquat, but did not increase the lifespan[30]. On the other hand, RNAi of *gst-4* was observed to decrease the lifespan of *daf-2* mutant[32].

The gene *gst-10* which encodes for glutathione S-transferase P 10 (EC:2.5.1.18) has the potential to detoxify 4-hydroxynon-2-enal (HNE), which is a lipid peroxidation product due to oxidative stress[29]. Overexpression of this gene appeared to extend the lifespan of

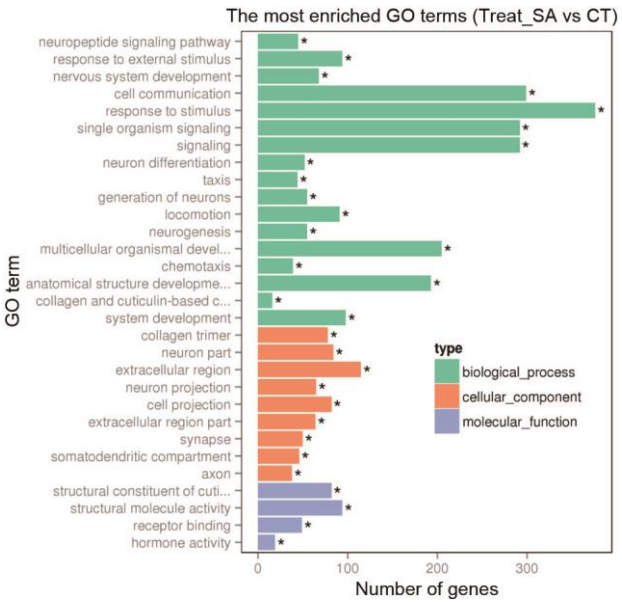

Figure 9. The most enriched down-regulated GO.

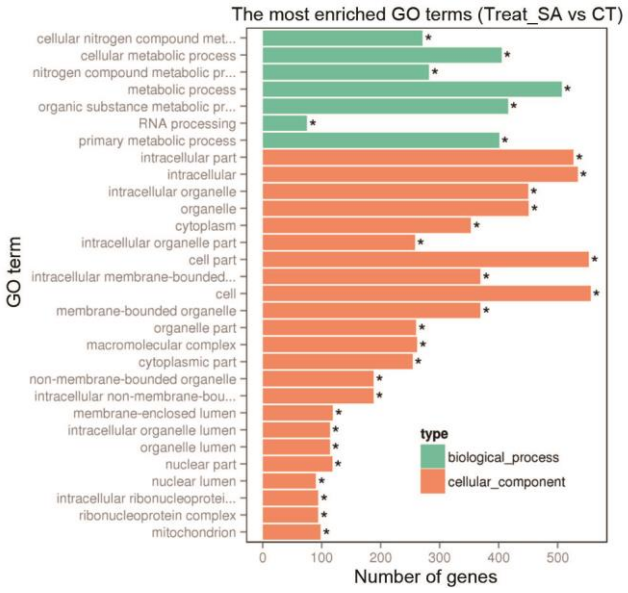

Figure 10. The most enriched up-regulated GO.

C. elegans. RNAi of *gst-10* increased sensitivity to HNE toxicity, and reduced lifespan in both wild-type and *daf-2* mutant populations[33].

In *C. elegans*, the gene *spr-1*, which codes for putative co-repressor protein SPR-1 was found to interact with SPR-4, which is the homologue of repressor element 1-silencing transcription factor (REST)[34]. Mutants of *spr-1* were shown to have reduced survival during oxidative stress[34]. This indicates that the upregulation of *spr-1* is important to reduce oxidative stress[31]. Thus, it is suggested that Sal A not only provides neuroprotection to *C. elegans*, but is also able to activate the antioxidant response genes in order to overcome oxidative stress caused by ROS.

The effects in manipulating the expression of *sod-1*, the major cytosolic Cu/Zn-SOD isoform, suggest that cytosolic $O^{2\bullet-}$ and the damage that it causes contribute to *C. elegans* ageing. Lifespan was shown to be slightly decreased with deletion of, or RNA-mediated interference (RNAi) of *sod-1* (refs 35–38), but overexpression of *sod-1* increased the lifespan[35]. However, it was reported that overexpression of *sod-1* did not reduce levels of oxidative damage. Furthermore, studies had shown that *sod-1* overexpression lines were hypersensitive and not resistant to oxidative stress[39]. This suggested that overexpression of SOD might not reduce ROS-induced damage in these strains.

There were reports indicating that increased expression of *gst-4* led to an increase of resistance to oxidative stress, but there was no impact on the lifespan[30]. Thus, this particular antioxidant response gene is responsible for reducing the oxidative stress induced by $A\beta_{42}$.

In this transcriptomic study, two genes that are shown to be involved in AD were affected by treatment with Sal A. The first gene is glutathione reductase 2, *trxr-2*, involved in Aβ peptide deposits. The second gene involved is *ptl-1*, is homolog to the MAP2/MAP4/tau family in the nematode. The *trxr-2* gene was observed to be upregulated, while *ptl-1* gene was down-regulated.

The *trxr-2* gene was shown to have a protective role for transgenic *C. elegans* CL2006 strain. The down-regulation of*trxr-2* resulted in enhanced paralysis and increase in TRXR-2 protein expression, did not attenuate the onset of paralysis[40]. Overexpression of TRXR-2 significantly reduced the total Aβ species. This is probably due to the interaction between TRXR-2 and proteins that are involved in amyloid degradation in mammals. The proteins responsible for the reduction of Aβ peptide and amyloid deposits could be angiotensin-converting enzyme, insulin-degrading enzyme or neprilysin. The orthologs of these enzymes are found in the nematodes[40]. In addition, the overexpression of TRXR-2 causes insulin signalling to diminish, eventually leading to Aβ autophagic-dependent degradation[40]. The molecular mechanisms of TRXR-2 in attenuating paralysis and reducing amyloid deposits in Aβ nematodes, can potentially contribute to the understanding of the same phenomena in human[40].

Another gene affected by Sal A treatment was down-regulation of *ptl-1* which encodes for protein with tau-like repeats. The PTL-1 protein was found to possess high sequence homology to the tau/MAP2/MAP4 family of MAPs over the repeat region[41,42]. With the effect of Sal A in reducing the expression of *ptl-1* gene, it may work

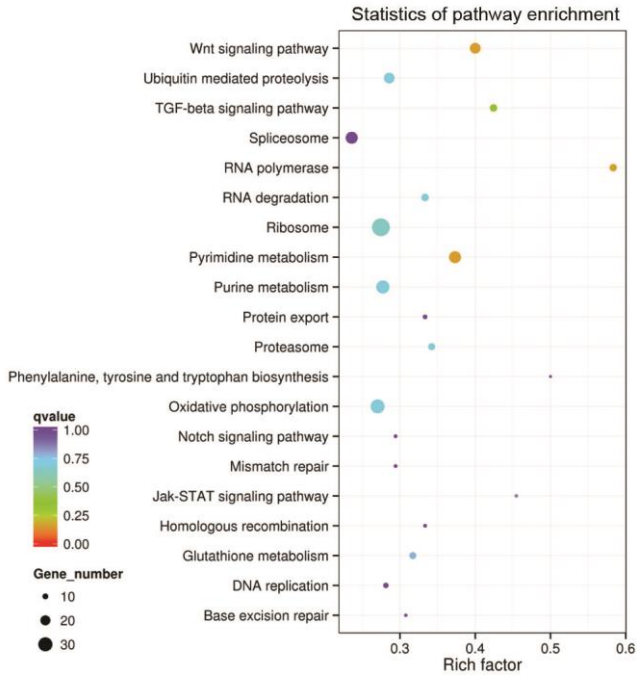

Figure 11. Statistics of overall KEGG pathway enrichment.

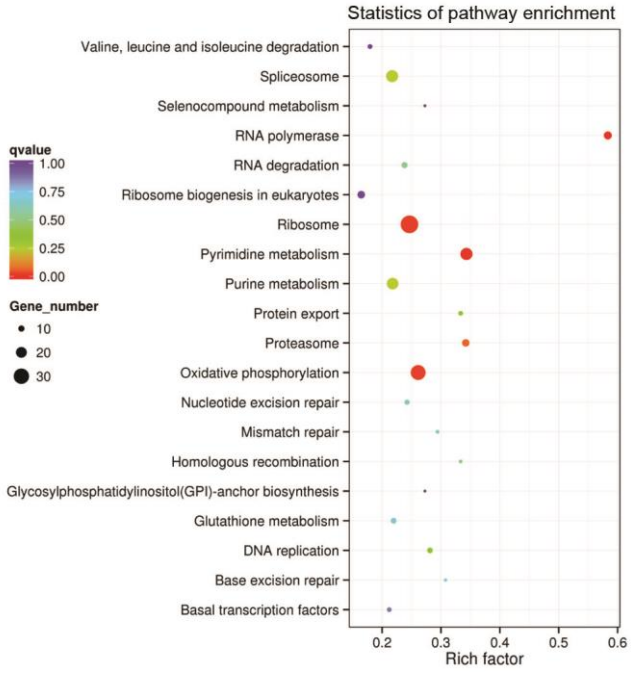

Figure 12. Statistics of down-regulated enriched KEGG pathway.

Table 1. Hyperlink of significant pathways to the KEGG pathway

Pathway	Regulation ($P < 0.05$)	Hyperlink
RNA polymerase	Up	http://www.genome.jp/kegg-bin/show_pathway?cel03020/cel:CELE_F14B4.3%09red/cel:CELE_Y37E3.3%09red/cel:CELE_C06A1.5%09red/cel:CELE_Y77E11A.6%09red/cel:CELE_F23B2.13%09red/cel:CELE_H27M09.2%09red/cel:CELE_C36B1.3%09red/cel:CELE_C15H11.8%09red/cel:CELE_W06E11.1%09red/cel:CELE_C48E7.2%09red/cel:CELE_W09C3.4%09red/cel:CELE_Y54E10BR.6%09red/cel:CELE_F26F4.11%09red/cel:CELE_W01G7.3%09red
Pyridine metabolism	Up	http://www.genome.jp/kegg-bin/show_pathway?cel00240/cel:CELE_Y77E11A.6%09red/cel:CELE_F12F6.7%09red/cel:CELE_C03C10.3%09red/cel:CELE_W09C3.4%09red/cel:CELE_B0001.4%09red/cel:CELE_W06E11.1%09red/cel:CELE_H27M09.2%09red/cel:CELE_T24C4.5%09red/cel:CELE_Y37E3.3%09red/cel:CELE_C15H11.8%09red/cel:CELE_C48E7.2%09red/cel:CELE_F08B4.5%09red/cel:CELE_C36B1.3%09red/cel:CELE_Y54E10BR.6%09red/cel:CELE_F26F4.11%09red/cel:CELE_W01G7.3%09red/cel:CELE_F14B4.3%09red/cel:CELE_ZK783.2%09red/cel:CELE_R53.2%09red/cel:CELE_C06A1.5%09red/cel:CELE_F23B2.13%09red/cel:CELE_R04F11.3%09red/cel:CELE_F49E8.4%09red
Ribosome	Up	http://www.genome.jp/kegg-bin/show_pathway?cel03010/cel:CELE_K02B2.5%09red/cel:CELE_C16A3.9%09red/cel:CELE_Y41D4B.5%09red/cel:CELE_T07A9.11%09red/cel:CELE_W01D2.1%09red/cel:CELE_T04A8.11%09red/cel:CELE_T22F3.4%09red/cel:CELE_T08B2.10%09red/cel:CELE_F28D1.7%09red/cel:CELE_T14B4.2%09red/cel:CELE_T23B12.3%09red/cel:CELE_F54E7.2%09red/cel:CELE_M01F1.6%09red/cel:CELE_Y37E3.8%09red/cel:CELE_R12E2.12%09red/cel:CELE_C04F12.4%09red/cel:CELE_K07A12.7%09red/cel:CELE_W04B5.4%09red/cel:CELE_C27A2.2%09red/cel:CELE_F45E12.5%09red/cel:CELE_ZK1010.1%09red/cel:CELE_F56D1.3%09red/cel:CELE_T01E8.6%09red/cel:CELE_K11H3.6%09red/cel:CELE_F52B5.6%09red/cel:CELE_F09G8.3%09red/cel:CELE_F33D4.5%09red/cel:CELE_Y119C1B.4%09red/cel:CELE_W09D10.3%09red/cel:CELE_K01C8.6%09red/cel:CELE_Y54E10A.7%09red/cel:CELE_T24B8.1%09red/cel:CELE_T25D3.2%09red/cel:CELE_B0303.15%09red/cel:CELE_F37C12.4%09red
Oxidative phosphorylation	up	http://www.genome.jp/kegg-bin/show_pathway?cel00190/cel:CELE_F45H10.2%09red/cel:CELE_Y54E10BL.5%09red/cel:CELE_T10E9.7%09red/cel:CELE_C16A3.5%09red/cel:CELE_F52E1.10%09red/cel:CELE_F54D8.2%09red/cel:CELE_Y56A3A.19%09red/cel:CELE_C18E9.4%09red/cel:CELE_F40G9.2%09red/cel:CELE_Y71H2AM.5%09red/cel:CELE_C17H12.14%09red/cel:CELE_F31D4.9%09red/cel:CELE_C53B7.4%09red/cel:CELE_F53F4.10%09red/cel:CELE_F32D1.2%09red/cel:CELE_T02H6.11%09red/cel:CELE_F46F11.5%09red/cel:CELE_F22D6.4%09red/cel:CELE_T27E9.2%09red/cel:CELE_Y51H1A.3%09red/cel:CELE_T26E3.7%09red/cel:CELE_D2030.4%09red/cel:CELE_F42G8.10%09red/cel:CELE_K07A12.3%09red/cel:CELE_T20H4.5%09red/cel:CELE_C33A12.1%09red/cel:CELE_JC8.5%09red/cel:CELE_F37C12.3%09red/cel:CELE_Y63D3A.7%09red
Wnt signalling	Down	http://www.genome.jp/kegg-bin/show_pathway?cel04310/cel:CELE_Y105C5B.13%09red/cel:CELE_C54D1.6%09red/cel:CELE_F47F2.1%09red/cel:CELE_Y71F9B.5%09red/cel:CELE_K08H2.1%09red/cel:CELE_Y47D7A.8%09red/cel:CELE_R10E11.1%09red/cel:CELE_Y47D7A.1%09red/cel:CELE_B0478.1%09red/cel:CELE_C52D10.8%09red/cel:CELE_C52D10.9%09red/cel:CELE_C52D10.6%09red/cel:CELE_C52D10.7%09red/cel:CELE_F49E10.5%09red/cel:CELE_F27E11.3%09red/cel:CELE_Y38F1A.5%09red/cel:CELE_Y37E11AR.2%09red
TGF-beta signalling	Down	http://www.genome.jp/kegg-bin/show_pathway?cel04350/cel:CELE_Y105C5B.13%09red/cel:CELE_F43C1.2%09red/cel:CELE_R10E11.1%09red/cel:CELE_Y47D7A.8%09red/cel:CELE_K08H2.1%09red/cel:CELE_Y47D7A.1%09red/cel:CELE_C52D10.6%09red/cel:CELE_C52D10.7%09red/cel:CELE_C52D10.8%09red/cel:CELE_C52D10.9%09red

well in humans by decreasing the mutated tau expression that causes AD.

Serpentine receptor, encoded by the *srt-13* gene, is also known as G-protein-linked receptor function as a receptor that acts as a mediator in response to various signal molecules like hormones and neurotransmitters[43]. The gene *fipr-11* which encodes for FIP (fungus-induced protein) encodes for possible antimicrobial peptides (AMPs)[44]. It is interesting to note that the antimicrobial activities of AMPs act as a defence system for *C. elegans*. AMPs work by disrupting the anionic cell walls and phospholipids membranes of the microorganisms[44].

The most down-regulated genes affected by Sal A treatment were *acr-23* and *daf-7*, which encoded for betaine receptor ARC-23 and dauer larva development regulatory growth factor DAF-7 respectively. In *C. elegans*, betaine receptor ACR-23 is known as ligand-gated cation channel. The protein is activated in the presence of betaine. It is involved in the mechanosensory neurons that regulate locomotion of the nematode[45]. A previous study also showed that *acr-23* was highly expressed in six mechanosensory neurons, body muscles and multiple interneurons. In addition, ACR-23 was highly expressed in larvae muscle, and less expressed in adults. Besides, the deletion of *acr-23* gene showed mild swimming defects, crawling sluggishly with some disrupted motion[45]. DAF-7 operated through a heteromeric TGF-β receptor that consisted of DAF-1 and DAF-4 that affect the activity

of the transcription factors DAF-8 and DAF-14 (ref. 46). DAF-7 is considered to affect the activity of neural circuits. Another possible function of DAF-7 is that it acts as a neuromodulator to some chemosensory neurons that work synergistically or antagonistically[47].

Other down-regulated protein annotated genes are Bardet–Biedl syndrome 1 protein homolog, homeobox protein CEH-23, chloride channel protein and *Caenorhabditis* frizzled homolog. The gene *bbs-1* in humans cause the Bardet–Biedl, an obesity syndrome[48]. In *C. elegans bbs-1* is a homolog to the human gene *bbs-1*, the repression of *bbs-1* transcription indicates that Sal A may disrupt the function of the gene in humans. As for the *cfz-2* gene, it is involved in axon development, cell migration and organization of the anterior ganglion[49], and as a receptor of Wnt proteins[50,51].

GO was performed to group DEGs into their biological functions[52]. In this study, GO analysis showed that Sal A upregulated genes were involved in cellular component process. On the other hand, down-regulated genes affected by Sal A treatment were mostly involved in biological processes and molecular functions.

In KEGG analysis, Sal A treatment was found to significantly upregulate genes that were involved in RNA polymerase, ribosome, pyrimidine metabolism and oxidative phosphorylation pathways. These pathways play important roles in gene regulation. In RNA polymerase pathway, genes that were associated with RNA polymerase I, II and III were upregulated. Pathways such as ribosome and oxidative phosphorylation are associated with energy supply and metabolism of materials. Hence, this might suggest that the production of antioxidant response proteins had increased to act as a defence system towards ROS produced by Aβ.

Wnt-signalling pathway and TGF-beta signalling pathway were found to be down-regulated in KEGG analysis. The Wnt signalling pathway is involved in the transferring of protein signals into a cell via cell-surface receptors.

Wnt signalling was responsible for regulating various cell processes throughout the embryonic development of *C. elegans*, especially in regulating cell fate specification, cell division and cell migration[53]. The transforming growth factor beta signalling pathway, was involved in the development of the postembryonic mesoderm which includes the antero-posterior pattern of the nematode[54]. Thus, it can be assumed that Sal A is involved in both the embryonic as well as postembryonic developmental stages of the nematode. Sal A may also act as a disease modifying drug for AD.

Conclusion

In this study, we found that *C. elegans* that expressed human Aβ_{42} gene showed positive response towards Sal A treatment. From transcriptomic analysis, the antioxidant genes in *C. elegans* was found to be upregulated. In addition, the genes related to the AD was also upregulated in response to the Sal A treatment towards AB42 toxicity. Thus, we infer that Sal A is a potential drug to combat AD.

Disclosure statement: The authors declare no conflict of interest.

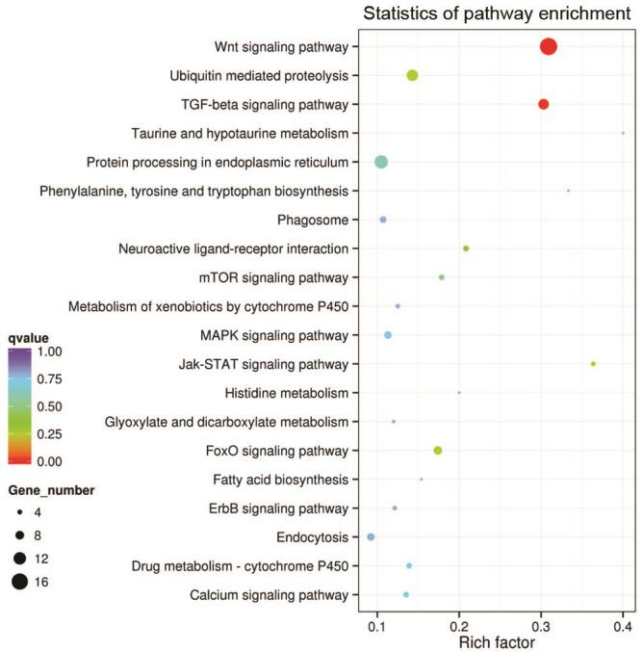

Figure 13. Statistics of upregulated enriched KEGG pathway.

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