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Original Article

The Analysis of Gene Expression on Fertility Decline in *Caenorhabditis elegans* after the Treatment with 5-Fluorouracil

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Abstract

Background: 5-Fluorouracil could lead to a decline in fertility in *Caenorhabditis elegans*. The aim of this study was to describe the mechanisms underlying such an altered fertility phenotype and to illustrate the specific genes and pathways that are involved in the related phenotypic changes in *C. elegans*.

Methods: We isolated total RNA from the samples and used a new method called Digital Gene Expression (DGE), which can rapidly identify genes with altered transcript levels. The random genes were confirmed by real-time RT-PCR.

Results: We analyzed the results of two methods to draw conclusions based on a comparison between *C. elegans* and other harmful parasites. Compared with controls, 1147 genes were up-regulated, and 1067 were down-regulated. Overall, 101 up-regulated genes had a log2 ratio higher than 8, whereas the log2 ratio of 141 down-regulated genes was higher than 8. After mapping to the reference database, 4 pathways were confirmed to be involved in this phenomenon, with statistically significant participation from 19 genes.

Conclusion: For the first time, the transcript sequence of 5-Fu-treated worms and controls was detected. We found that 4 possible pathways, i.e., ECM-receptor interaction pathway, TGF-beta signaling pathway, Focal adhesion and Hypertrophic cardiomyopathy, may be involved in the number decline in the embryos of *C. elegans*. Specifically, the ECM-receptor interaction pathway and Focal adhesion may be very important pathways that alter the reproduction of *C. elegans*.

Keywords: *Caenorhabditis elegans*, 5-Fluorouracil, Digital gene expression

Introduction

5-Fluorouracil (5-Fu) is a pyrimidine antagonist drug that is extensively used to treat carcinoma of various organs in humans (1). 5-Fu is also a prodrug and is converted into its active form through the pyrimidine biosynthesis pathway (2). The pathways involved in the function of 5-Fu in regulation at the molecular level are highly conserved between worms and human (3). 5-Fu has side effects such as cardiotoxicity, gastrointestinal disturbances and neurotoxicity (4, 5). *C. elegans* are a

free soil-living nematode with an adult body length of 1 mm and were first generally researched by Brenner, etc. in the 1970s. *C. elegans* are self-reproducing hermaphrodites, with each individual producing both sperm and ova, thus making it easy to produce descendants (6). They are easily cultured on nematode growth medium agar and can be fed with the *Escherichia coli* OP50 within the first 3 days of its life cycle. Therefore, *C. elegans* has been used as a tool to investigate the biology of a number of hosts and pathogens for which it was a simple and genetically tractable organism (7). In addition, the full DNA sequence of *C. elegans* has been identified (8, 9). Thus, *C. elegans* has become a wonderful model organism in toxicity, immune response, and infection studies (10-12).

Interestingly, Kim et al. confirmed that 5-Fu can induce germ cell death in *C. elegans* (13) and found that the dihydropridine dehydrogenase and thymidylate synthase expression levels were associated with 5-Fu function (14). D'Souza and Narayana demonstrated the toxicity of the reproductive system of 5-Fu (15). Toxic effects on the male reproductive system in rats after treatment with 5-Fu have also been confirmed (12). However, the mechanism of 5-Fu-induced fertility decline in *C. elegans* has not previously been specifically described. Kumar et al. found that lin-29 had a relationship with the 5-Fu repressed levels of mRNA encoding; however, it is only one component of this mechanism (16).

Our study aimed to illustrate the specific genes and pathways that are involved in the change of phenotype in *C. elegans* by differential gene expression. Uncovering the mechanism of fertility decline in *C. elegans* would help us further our understanding of the mechanisms of genes that regulate *C. elegans* reproduction.

Materials and Methods

Chemicals and Equipment

5-Fu was purchased from Sigma-Aldrich (American F6627). Trizol reagent was purchased from Invitrogen (USA 15596026). Diethyl pyrocarbonate (DEPC) was purchased from Amresco (E174). All other chemicals were of the highest quality.

A worm pick can be flattened by a hammer or pliers, and the worm pick was sterilized before use. The *C. elegans* and *Escherichia coli* OP50 were gifts from the Caenorhabditis Genetics Center (CGC) in the US.

C. elegans culture methods

C. elegans were cultured at 20°C in a glass petri dish (75 mm) on nematode growth medium (NGM). The *E. coli* strain OP50 was used as food. The methods of feeding *C. elegans* were provided by Brenner, with the OP50 seeded as a lawn on the nematode growth medium (NGM) (6). The wild-type strain background was Bristol N2.

Treatment of C. elegans

The 5-Fu was dissolved in double-distilled water in a concentration of 50 mM for the stock solution, and the working solution was 200 mM. The 5-Fu dilution was added to the NGM to obtain a final concentration of 200 mM. The petri dishes were prepared for use after 24 hours at 37°C. The NGM should be used within one week of preparation. The results were analyzed by the chi-square test using SPSS 13.0 software, with significant differences identified by statistical analysis when $P<0.05$.

Isolation of RNA in C. elegans

Approximately 500 adult worms that were treated by 5-Fu were collected from the 3 petri dishes and washed 3 times with 1 ml M9 buffer. The same was done with the controls. After collection, the solution with worms was placed in liquid nitrogen for 1 minute, followed by the addition of 1 ml of the Trizol reagent. The mixture was then placed into liquid nitrogen again 4 times. Next, the supernatant was transferred into an RNase-free tube. Total RNA was extracted in a one-step method following the instructions in a manual provided by Invitrogen. The total RNA was reverse transcribed into the cDNA using the prime script RT Master kit (Takara).

Preparation of libraries and sequencing

Sequencing libraries were prepared from 6 μg of total RNA, and Oligo(dT) magnetic beads were

used to purify the samples by adsorption. Then, double-strand cDNA was synthesized with the Oligo(dT) primer, which can be digested by endonuclease NIaIII, which recognizes and cuts off the CATG sites. The fragments with 3′ ends of cDNA were collected and purified with Oligo(dT). Then, the 5′ ends of the fragments were ligated with the Illumina adaptor1, which contained a restriction site for endonuclease MmeI. After digestion with Mme, the tags that were 17 bp downstream of the CATG site were produced. The 3′ ends of the tags were removed with magnetic beads, and Illumina adaptor 2 was ligated to the tags to construct tagged libraries with different adapters at both ends. Then, 15 cycles of linear PCR amplification were conducted, and 105 bp fragments were purified by 6% TBE PAGE gel, followed by fixing of the unlinked single chain to the Illumina Sequence Chip (flowcell). Each molecule became a single-molecule cluster template after the amplification. Sequencing by synthesis (SBS) was the method used after four nucleotides with four different colors were added. Each tunnel produced millions of raw reads, the length of which was 49 bp.

Data analysis

Low-quality sequences that were too long or too short or had unknown sequences were removed. The remaining tags, called "clean tags," contained CATG sites and were thus mapped to the reference sequences with which no more than one nucleotide was mismatched. A method established by Audic enabled us to obtain the results of the differentially expressed of genes (17). Then, we determined the FDR (False Discovery Rate) to control the range of the *P*-value. We focused on genes with an FDR≤0.001 and a $\log_2$ratio≥1 in our comparison (18). The transcripts of KEGG Ontologies were identified by searching the KEGG database, as were the results from differentially expressed genes and the Gene Ontology database; these terms were then mapped to these databases, and conclusions were made about the differences.

Quantitative PCR

The SYBR Green method was used for real-time RT-PCR using the SYBR Premix Ex Taq II kit (Takara). The primers and gene number for these genes are shown in Table 1.

Table1: The primers used in this study

gene	Forward(5′-3′)	Reverse(5′-3′)	GeneBank ID
daf-14	CAAGTGATGAGTGCCGAATGT	TCCAACTGTGTTTCGGATTTGT	NM_001268546
K11D12.6	TCTTCTTCTCCTTCCCTTCCTTT	AGTTATTCTCATTTCCTCCACATCC	NM_071949
col-165	GCCACCGAGCAACCATACA	GCGTTTCCGTCCTTTCCA	NM_076346
col-121	TATTGTCCATCCGATTGTGGAG	TTGCTGATTGGTAGTTGGCTGT	NM_069254
clec-85	CATACCTCCCTCCAGGCAAA	CTCGGCTCAAACCAATCCA	NM_067861
col-150	GAAGAAGTTTTGGATGGTGTGAAGA	CGCTTTTGACGGAAGACAGAG	NM_073246
col-131	CACATGCTCCATCAATCTCTCC	CCCGTGTTTTGTCCCATTTT	NM_070106
col-90	CTCTGCGTGATGAAGTTGAAGG	AGTTGGGGATTGAAGAACAAGAAG	NM_066328
col-175	TGAATCTCGTGCTGCTGCTT	CGTCTTTTCCATCCTCTCCATC	NM_077154
col-41	AACTCCAGGAAACGCTGGTC	TGGGGTTCTGTCGTCTTGTCT	NM_078121
col-38	CAGGAGGACGTGGAGATCAAG	AGATGATGGGTAACAGCCAGAGA	NM_063409
col-49	ATCGCAATTGTCGGATTGG	TCTTCCGTTCTCCGCCTTT	NM_058705
col-113	GGACAAATTGGAATGGTTGGA	GCTGAGAAGTTGATGGTTGTGG	NM_068722
col-124	CGGATATGCTGCCTTGACTTT	TTGCGAGTGTGATGGATGTAGTT	NM_069466
dpy-5	CTGCTATCGTTGCTTGCCTTT	AAGATTCCACGAACGTATTGCTC	NM_059194
emb-9	CAGCAATTCGTCCATACATTTCTC	CCACATACCCGACCATCCTT	NM_001027492
let-2	GAAACGAGAAATCCCATAACCAAG	TGAAGTCGCAGAACAGGAATG	NM_078262
mec-5	CGCAATCCAGACAGAAATCAA	TCTACCGCTCATTCCATCCTC	NM_171808
CELE_C24B9.3	CAATCTCCATCCGTTTCGTGT	AGTAGTGACCAGTGCGAAGGAAG	NM_001028500

The primers (Table 1) were designed by us using the primer 5.0 software. The gene numbers were from GeneBank, and the sequences were retrieved from NCBI (www.ncbi.nlm.nih.gov).

PCR was performed on a Roche LightCycler 480 (Roche Applied Sciences), with the steps of the reaction repeated for 40 cycles. The procedure included pre-incubation of the samples at 95°C

for 5 minutes, followed by 40 cycles of incubation at 95°C for 10 seconds and annealing at 61°C for 30 seconds. The extension step lasted for 10 seconds at 72°C. The results were normalized and analyzed using the Roche basic software (Roche Applied Science).

Results

The embryos were decreased in number and defective in development

The number of embryos decreased after the worms were cultured with one of three concentrations of 5-Fu: 5 μM, 10 μM and 20 μM.

Table2: The comparison of various concentration of 5-fu to control

Group	Embryos	Percentage (%)
20uM group	180	36.00*
10uM group	295	58.40*
5uM group	440	87.10*
Control group	505	100

Compared with Control group, * P <0.001.

The worms exposed to 20 μM 5-Fu had fewer embryos than did the normal worms. The worms cultured by the concentration of the 5 μM and 10 μM group had fewer embryos than the control group (P<0.001) but more embryos than the worms cultured in a concentration of 20 μM (P<0.001) (Table 2).

To understand the reasons for this phenomenon, we used the 20 μM sample with the DGE method to identify the differences in gene expression and the pathways in which they participate.

Digital gene expression library sequence and mapped sequences to the reference

Solexa DGE analysis was performed to obtain a general idea of the samples that were cultured with 5-Fu or control media. Two DGE libraries were sequenced after culture with 5-Fu and no other treatment.

We obtained approximately 4,207,526 total clean tags and 56,471 distinct clean tags in the control samples and approximately 4,167,565 total clean tags and 56,530 distinct clean tags in the 5-Fu treated samples. Overall, 71.16% and 71.78% of distinct tags were uniquely mapped to the reference (Table 3).

The gene detection would be saturated when the number of sequences reached 3 million. The distribution of total tags and distinct tags displayed similar patterns in 2 samples of the two DGE libraries (Fig. 1).

Table 3: The statistics of DGE in two samples

Summary		Control	5-fu
Raw Data	Total	4410363	4370074
	Distinct Tag	137741	137059
Clean Tag	Total number	4207526	4167565
	Distinct Tag number	56471	56530
All Tag Mapping to Gene	Total number	3142157	3129666
	Total % of clean tag	74.68%	75.10%
	Distinct Tag number	40183	40578
	Distinct Tag % of clean tag	71.16%	71.78%
Unambiguous Tag Mapping to Gene	Total number	2706754	2770479
	Total % of clean tag	64.33%	66.48%
	Distinct Tag number	38254	38753
	Distinct Tag % of clean tag	67.74%	68.55%
All Tag-mapped Genes	Number	12462	12397
	% of ref genes	55.74%	55.45%
Unambiguous Tag mapped Genes	Number	11638	11549
	% of ref genes	52.05%	51.65%
Mapping to Genome	Total number	897080	914567
	Total % of clean tag	21.32%	21.94%
	Distinct Tag number	10231	10861
	Distinct Tag % of clean tag	18.12%	19.21%

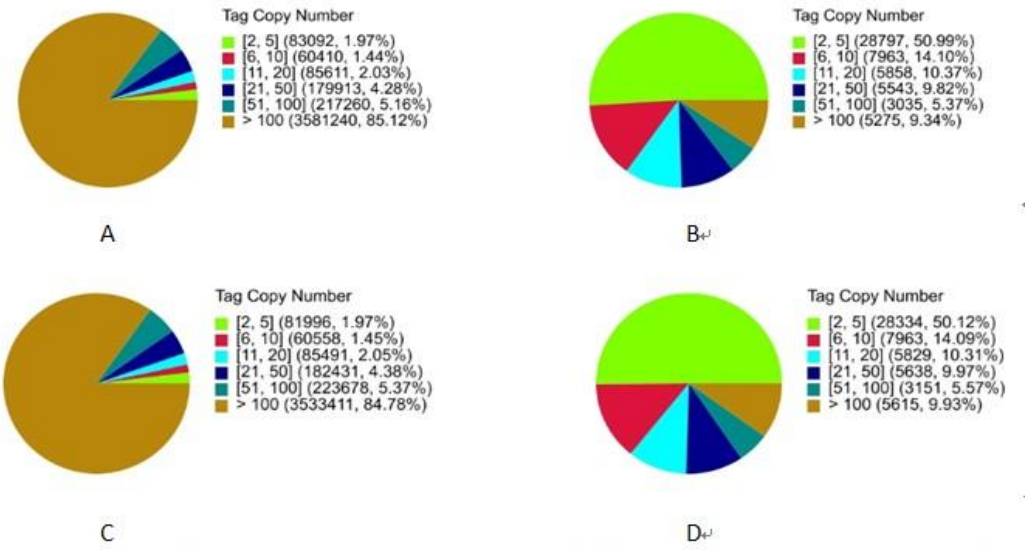

Fig.1: The distribution of control total clean tags(A)The distribution of control distinct clean tags(B)The distribution of 5-Fu total clean tags(C)The distribution of 5-Fu distinct clean tags(D)

Different gene expression in two libraries

To decipher the difference in gene expressions in the samples that were either cultured with 5-Fu or the control, the differentially expressed tags were analyzed by a comparison between the 5-Fu library and the control library. The standard of FDR≤0.001 and $\log_2$ratio≥1, as mentioned above, was to evaluate the significance of differential gene expression. The sample cultured with 5-Fu had 1,147 up-regulated genes and 1,067 down-regulated genes (Fig. 2).

From up-regulated genes, 101 had a $\log_2$ratio value greater than 8, whereas the absolute value of the $\log_2$ratio for 141 of the down-regulated genes was greater than 8. For a $\log_2$ratio≥1, we could consider the gene expression to be significantly different between the 5-Fu and control samples.

Identification of differentially expressed genes and signal pathway analysis

To obtain information regarding alterations after treatment with 5-Fu, we performed a pathway analysis of differentially expression genes based on the KEGG database (www.genome.jp/kegg/) (19). We selected several significant pathways that had distinct differential expression compared with the control. Among the 10,067 genes that were annotated in the database, 1,114 were significantly altered in expression.

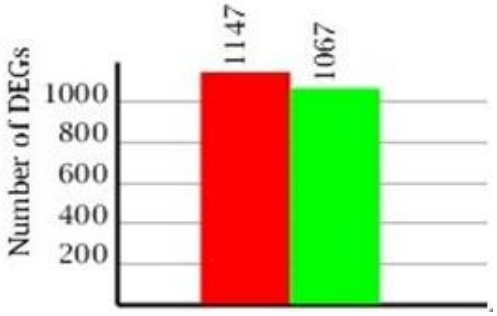

Fig.2: Distribution of different expressed genes between control and 5-Fu

These 1,114 unambiguous transcripts were present in 248 pathways. Lower Q values that were obtained after analyzing the pathways indicated greater confidence, and only pathways that had both a *P*-value<0.05 and a Q-value<0.05 were selected. The most creditable pathway involved was Protein digestion and absorption (ko04974), with a Q-value of 0.000023, followed by Amoebiasis (ko05146) and Focal adhesion (ko04510). We ultimately obtained 28 pathways according to the *P*-value<0.05 and Q-value<0.05 cutoffs, which was considered statistically significant. Four pathways were significantly enriched in genes that were associated with the changes of *C. elegans* cul-

tured with 5-Fu. The majority of the altered genes were clustered in two pathways: the TGF-beta signaling pathway (ko04350), with a Q-value of 0.003, and the ECM-receptor interaction (ko04512), which also had a correlation with the basement membrane integrity and had a Q value of 0.000067. Additionally, the above-mentioned genes had a minor role in two other pathways: Focal adhesion (ko04510), with a Q-value of 0.00004, and Hypertrophic cardiomyopathy (ko05410), with a Q-value of 0.00006.

Expression of genes in the TGF-beta signaling pathway and ECM-receptor interaction pathway

Based on the KEGG database and the analysis of the mapped genes, we found that several genes with altered expression were present in one or more pathways. The TGF-beta signaling pathway and ECM-receptor interaction pathway was representative of 5-Fu treatment-induced changes. Nineteen genes had differential expression compared with controls, based on the selection rule of a *P*-value<0.05 and FDR<0.05. These genes also had a relationship with the other two enriched pathways, i.e., Focal adhesion and Hypertrophic cardiomyopathy (Table 4).

The genes in these pathways had either up- or down-regulated expression, and interactions between these pathways resulted in heterogeneity in the expression of these genes (Fig. 3).

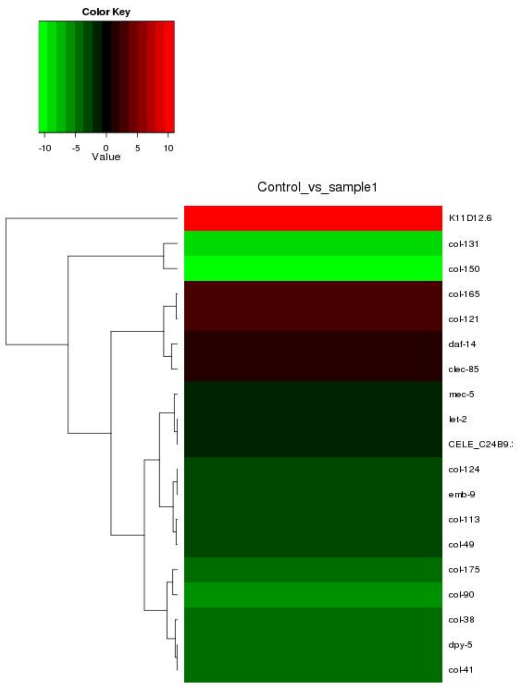

Fig. 3: the difference among the selected genes

Table 4: Upregulated and downregulated genes in selected pathways

Gene	log2 Ratio*	P-Value	FDR	Gene ID
daf-14	2.03	2.4*10-9	1.6*10-8	NM_001268546
K11D12.6	10.96	6.9*10-26	1.1*10-24	NM_071949
col-165	3.02	9.4*10-7	4.7*10-6	NM_076346
col-121	2.89	8.0*10-5	3.1*10-4	NM_069254
clec-85	1.29	1.6*10-28	2.8*10-27	NM_067861
col-150	-10.89	1.2*10-24	1.9*10-23	NM_073246
col-131	-9.15	6.7*10-8	3.8*10-7	NM_070106
col-90	-5.43	10.0*10-24	1.5*10-22	NM_066328
col-175	-4.85	3.5*10-44	9.4*10-43	NM_077154
col-41	-4.39	8.8*10-26	1.4*10-24	NM_078121
col-38	-4.12	7.0*10-180	7.7*10-178	NM_063409
col-49	-3.51	3.0*10-10	2.2*10-9	NM_058705
col-113	-3.36	4.4*10-11	3.4*10-10	NM_068722
col-124	-2.98	3.4*10-30	5.3*10-30	NM_069466
dpy-5	-4.36	6.5*10-56	7.8*10-55	NM_059194
emb-9	-3.01	1.6*10-137	1.4*10-135	NM_001027492
let-2	-1.61	7.9*10-60	2.9*10-58	NM_078262
mec-5	-1.23	2.1*10-5	8.6*10-5	NM_171808
CELE_C24B9.3	-1.62	1.2*10-4	4.4*10-4	NM_001028500

* The value of log2 Ratio greater than zero means upregulated and vice versa

The confirmation of different gene expression by real-time RT-PCR

To verify the reliability of this method, 19 genes that had been randomly selected from 4 pathways were confirmed. The results indicated that the expression of these 19 genes were consistent between the real-time RT-PCR and DGE (Table 5).

Table 5: The data from DGE and Quantitative PCR

Gene	DGE	Realtime-rtPCR	Pathways
daf-14	2.03	9.653	TGF-beta signaling
K11D12.6	10.96	5.685	ECM-R*, Focal adhesion
col-165	3.02	2.58	Focal adhesion
col-121	2.89	24.05	Focal adhesion
clec-85	1.29	1.565	ECM-R*, Focal adhesion
col-150	-10.89	9.41E-02	ECM-R*, Focal adhesion
col-131	-9.15	7.67E-03	ECM-R*, Focal adhesion
col-90	-5.43	3.54E-02	ECM-R*, Focal adhesion
col-175	-4.85	2.58E-03	ECM-R*, Focal adhesion
col-41	-4.39	4.45E-2	ECM-R*, Focal adhesion
col-38	-4.12	2.53E-4	ECM-R*, Focal adhesion
col-49	-3.51	4.53E-03	ECM-R*, Focal adhesion
col-113	-3.36	2.74E-03	ECM-R*, Focal adhesion
col-124	-2.98	1.59E-02	ECM-R*, Focal adhesion
dpy-5	-4.36	2.79E-03	ECM-R*, Focal adhesion
emb-9	-3.01	3.66E-01	ECM-R*, Focal adhesion
let-2	-1.61	7.82E-02	ECM-R*, Focal adhesion
mec-5	-1.23	9.03E-02	ECM-R*, Focal adhesion
CELE_C24B9.3	-1.62	5.85E-01	Hypertrophic cardiomyopathy

ECM-R* refers to ECM-receptor interaction pathway

Discussion

In the present study, we concluded that 5-Fu has induces reproductive toxicity in *C. elegans*, according to a comparative analysis of the 5-Fu and control groups using DGE. *C. elegans* is widely known to be a hermaphrodite. Interestingly, we also concluded that 5-Fu had an effect on male reproductive organs, which is inconsistent with the studies conducted by Inomata et al (20). The DGE provided a result of 10067 genes, of which 2214 were differentially expressed. GO classification and GO enrichment were included in the bioinformatics analyses. DGE revealed a large proportion of differentially expressed genes in 5-Fu-treated *C. elegans* compared with controls. This method has the advantages of being an easy procedure and providing quantitative gene expression data. Although we could describe the related genes from the transcript level, that approach has limitations, including an inability to align tags uniquely to the transcriptome of the samples or and that the database is not fully covered by previous research. The effects of 5-Fu were analyzed by the genes that were differentially expressed. Several conclusions could be drawn from our results. These genes were capable of participating primarily in 4 pathways: ECM-receptor interaction pathway, TGF-beta signaling pathway, Focal adhesion and Hypertrophic cardiomyopathy.

The ECM-receptor interaction pathway plays an important role in organizing tissues, defining shapes and contributing to the membranes (21). The extracellular matrix (ECM) is composed of glycoproteins, polysaccharides, amino acids, polysaccharides and other biological macromolecules

such as collagen and fibrin. It could also determine the shape of tissues because those constituents are embedded on the surface of the cells (22, 23). In some studies, gonad development was shown to be primarily determined by distal cells and extracellular matrix (24). The extracellular matrix sends signals to cells during cell adhesion with the help of the intergrin-specific receptor on the surface of the cell. It could transduce the signals to the cytoplasm and the nucleus, which is aimed to affect the gene expression and cell life activities (25). The focal adhesion pathway, which is involved in the regulation of cell activities or death, the reconstruction of the cell skeleton, and gene expression, senses the physiological and mechanical stress from the extracellular matrix and the surface of the cell through the integrins and caveolae. It can also embed the cell in the appropriate position, thereby maintaining the normal function of the cell (26). Focal adhesions are integrin-rich cell adhesion sites through which the ECM is physically linked to the actin cytoskeleton, thus allowing signals to transduce into the intracellular compartment (27, 28). Focal adhesion has the same effect in the cell regulation process as the ECM-receptor (29, 30). The Gonad morphogenesis of *C. elegans* is determined by the migration of distal tip cells and the ECM (31). In our study, the phenotype of *C. elegans* was that they could not ovulate as usual or even not at all. The DGE results showed that 32 of the genes involved in this phenomenon may have participated in the ECM-receptor interaction pathway and Focal adhesion. Fifteen genes were involved in ECM-receptor interaction, whereas 17 genes participated in Focal adhesion. *mec-5*, which encodes a unique collagen, has been identified in *C. elegans* and affected ECM (32). In our study, *mec-5* was down-regulated in both DGE analysis and real-time RT-PCR. This finding indicates that *mec-5* is inhibited by 5-Fu and that could work though the ECM-receptor interaction and Focal adhesion to induce the reproductive abnormalities of *C. elegans*. Some of the collagen family of genes, which has over 170 members, had been detected in our study, many of which have been previously studied (33-36). The genes *emb-9*, *let-2*, *col-165*, *col-121*, *clec-85*, *col-150*, *col-131*, *col-90*, *col-175*, *col-41*, *col-38*, *col-49*, *col-113*, *col-124* are all members of collagen families. We found that *col-165* and the *col-121* were up-regulated, whereas others exhibited a down-regulated effect. These two genes were involved in ECM-receptor interaction and Focal adhesion. Previous studies have shown that *emb-9* and *let-2* participate in the coding and formation of collagen proteins. They may be associated with the formation of the vulvas of *C. elegans*; however, the mechanism of this is not clear (37, 38). The effect of the 5-Fu might inhibit the development of germ cells and interfere with the regulation of the collagen protein family genes. These might inhibit the formation of the vulvas in *C. elegans,* ultimately leading to lowered fecundity. In our study, we found that many of the genes in this family played a major role in reproduction in *C. elegans*. This finding may indicate that this family had some correlation with the mechanism of 5-Fu. However, in our studies, we confirmed that the down-regulation of collagen families might contribute to the reproductive processes of *C. elegans*. However, another pathway is involved in mapping the results to the reference. The *daf-14* gene belongs to the TGF-beta signaling pathway, which many researchers consider to be involved in the differential expression of the genes. This pathway consists of a large number of structurally and functionally related polypeptide growth factors that are capable of regulating cellular processes, including differentiation, adhesion, and cell death (30-40). The SMAD proteins, which are located on the cell surface, activate target gene transcription in association with DNA-binding partners after they relocate into the nucleus. SMADs are divided into three subfamilies: receptor-activated Smads (R-Smad), including smad1, smad2, smad3, smad5, smad8; common mediator Smads (Co-Smad); and inhibitory Smads (I-Smad), including smad6, smad7 (41). In our study, *daf-14*, which regulates SMAD2 and SMAD3, was highly expressed in both the DGE analysis and RT-qPCR. The *daf-14* gene encodes a SMAD protein of atypical structure that lacks a DNA-binding domain and acts redundantly with *daf-8* (42). *daf-14* was required for non-dauer development at relatively high tempera-

tures. Our results show that this phenomenon may be associated with the regulation of *daf-14*. Hypertrophic cardiomyopathy has been seldom discussed in *C. elgans*, but we evaluated its involvement with 5-Fu. One component of its function may be to repress the expression of the related genes. Thus, our future studies will focus on this phenomenon.

In the present study, the transcriptome of *C. elegans* was characterized under 5-Fu using the digital gene expression method. Identifying differentially expressed genes under 5-Fu is useful for further research into the mechanism underlying the effects of reproductive toxins and toxicants and could represent candidate genes and pathways for the reproduction of harmful parasites. We speculate that this model animal, *C. elegans*, may have similar gene functions in reproduction mechanisms as other organisms, such as parasites and humans. This may open a new aspect of the control of harmful parasites and research initiatives for human reproductive defects. In summary, the conclusions from the DGE analysis of 5-Fu-treated *C. elegans* represents the basis of global reverse genetics, and further functional analysis of these genes would enable a deeper understanding of the mechanism of reproduction of vertebrates.

Ethical consideration

The research was conducted in accordance with the Declaration of Helsinki and with the Guide for Care and Use of Laboratory Animals as adopted and promulgated by the United National Institutes of Health. All experimental protocols were approved by the Review Committee for the Use of Human or Animal Subjects of Lanzhou University.

Ethical issues (including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been completely observed by the authors.

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