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Maternal linuron exposure alters testicular development in male offspring rats at the whole genome level

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Abstract

Linuron is a widely used herbicide; its toxicity on the male reproductive system has been recognized. The current study was designed to explore the molecular mechanism underlying linuron-induced reproductive toxicity. Pregnant rats received daily oral gavage of linuron at the dose of 120 mg/kg/d from gestation day (GD)12 to GD17. Tissues from male offspring rats were collected for pathological examination and microarray gene expression profiling. Changes in gene expression were further verified by quantitative real-time RT-PCR. Data showed that linuron-exposed offspring rats had a decreased sperm count (88% of controls) and disrupted acrosome formation. There were evident damages in seminiferous tubules and abnormal morphology in mesenchymal cells in samples from linuron-exposed animals. Microarray analysis indicated that the expressions of testosterone synthesis-associated genes, i.e., *Star*, *P450scc*, *3 β -Hsd*, *Abp*, *Cox7a2*, *Pcna*, *p450c17* and *17 β -Hsd* were significantly altered by linuron exposure, along with other genes involving in cell proliferation and apoptosis, such as *c-myc*, *S6K*, *Apaf1*, and *TSC1*. These data indicate that linuron upon entering male offspring body can directly or indirectly interact with the androgen production and function; linuron-induced alteration in genes encoding testosterone synthesis is likely a major factor in linuron-induced male reproductive toxicity.

Keywords

Linuron; Reproductive toxicity; Microarray; Pathway analysis; Testosterone

1. Introduction

Linuron is a low toxic herbicide commonly applied to suppress weeds in soybean, cotton, corn, wheat, sugar cane, and potato, among many other vegetables. Reports in literature show that linuron degrades rapidly after entering the animal body, and the ingestion of 125 mg/kg linuron for two years does not produce detectable distortion, carcinogenic and/or mutagenic effects, although minor residues can be found in blood, fat, liver, kidney, and spleen (Lambright et al., 2000; Santos et al., 2014; Wilson et al., 2009).

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The literature data on linuron-induced male reproductive toxicities are not entirely clear-cut. Many reports suggest that linuron likely acts as an anti-androgen agent. For example, Kang et al. (2004) observe that by oral gavage of 50 mg/kg and 100 mg/kg per day in rats, linuron causes the injury to the seminal vesicle and ventral prostate gland. Gray et al. (1999) report that a linuron dose at 100 mg/kg/d changes the differentiation of the androgen-dependent tissue in the pregnant rats, leading to testicular atrophy. Wilson et al. (2009) reveal that in utero exposure to linuron reduces testosterone production in the fetal rat testis.

However, Andrews and Gray (1990) report that rats orally exposed to linuron at 100 mg/kg/day do not cause any changes in both testosterone and luteinizing hormone (LH) levels. When the dose is increased at 200 mg/kg/day, the dose at which a severe neurotoxicity can be seen, linuron exposure increases the serum LH level by 25%. In addition, McIntyre et al. (2002a,b) show that by oral gavage of mother rats with 50 mg/kg/day during pregnancy, linuron does not cause significant pathological changes in testicular tissues of male offspring rats on the postnatal day 7 (PND7) and PND14, nor does it result in changes in testicular or serum levels of testosterone.

A recent study by this group has established that maternal exposure to linuron can cause the significant reproductive toxicity in male offspring rats (Ding et al., 2017). Following maternal exposure during pregnancy, male offspring rats display a shortened anogenital distance, a lack of fusion in urogenital folds, the damaged seminiferous tubules, and the injured Leydig cell ultrastructure. Further, our early study indicates a diminished serum testosterone concentration at PND2. These observations prompted us to investigate the mechanisms underlying linuron-induced tissue and cellular toxicities. Thus, the current study was designed to study the sperm production and quality in male offspring rats. We used the DNA microarray analysis and qPCR techniques to investigate the gene expression profile in order to identify the primary target genes associated with linuron-induced reproductive toxicity. Our results from both morphological and transcriptional studies provide strong evidence that linuron-induced male reproductive toxicities following maternal exposure is due primarily to its interference with the production and function of androgen hormone.

2. Materials and methods

2.1. Chemicals

Chemical reagents were purchased from the following sources: linuron (99.5%) was from Chemserv Inc. (West Chester, PA, USA); peanut oil from Shandong Luhua group (Yantai, China); Trizol reagent from Invitrogen (Carlsbad, CA, USA); ethidium bromide and Agarose from Sigma-Aldrich (St. Louis, MO, USA); RNAprep pure Tissue Kit from TIANGEN (Beijing, China), RevertAid™ First Strand cDNA Synthesis Kit from MBI (USA); Primer from Boruik (Changsha, China); and Taq DNA polymerase, PCR reaction buffer (10×), MgCl₂, and dNTP from Promega (Madison, WI, USA). All reagents were of analytical grade, HPLC grade, or the best available pharmaceutical grade.

2.2. Animals and treatment

Sprague-Dawley rats (6 week) of both sexes were purchased from the Laboratory Animal Center at the Third Military Medical University (Chongqing, PRC). Upon arrival, the animals (12 males and 24 females) were housed individually in a temperature (21 ± 1 °C)- and humidity ($55 \pm 5\%$)-controlled room under a 12-h light/dark cycle and allowed for acclimatization for 14 days prior to experimentation. At the time of experimentation, rats were 8 weeks old weighing $300 \text{ g} \pm 10 \text{ g}$ for males ($n = 12$) and $240 \pm 10 \text{ g}$ for females ($n = 24$). Rats had free access to food and tap water at libitum. The study was conducted in compliance with the Animal Care and Use Guidelines in China and approved by the Animal Care and Use Committee of Zunyi Medical College.

After acclimatization, each male rat was caged with two female rats for 24 h. Vaginal smears were performed on the following morning; the sperm-positive smear in female rats was considered as Gestational Day (GD) 0. The pregnant dams were housed individually and randomly assigned to the linuron-exposed group or the peanut-oil control group. The linuron suspension was prepared with peanut oil to the final concentration of 24 mg/mL. On GD12. The pregnant rats received the oral gavage at 2 mL/kg body weight, once daily, at the dose of 120 mg/kg of linuron as the exposed rats, or equivalent volume of peanut-oil as the control rats, for five consecutive days to GD17. Each exposure group had 10 dams. After birth, the male offspring rats were raised with the free access to food and tap water at libitum and were subjected to experimentation until sexual maturity at the postnatal day 30.

2.3. Pathological examination

Tissue samples of the testis, prostate, epididymis and genital tubercle were isolated from male offspring rats and prepared for pathological examination. Small tissue blocks (approximately 0.5 cm thick) were fixed in 10% formalin for 48 h; the samples were then dehydrated with ethanol from low to high concentration, treated with xylene, and embed in the paraffin according to the routinely used pathological sample preparation procedure. The tissues were then cut into 5–8 μm sections with a microtome and stained with hematoxylin-eosin. Each of tissue samples was made in triplicates and observed under a light microscope.

For electron microscopic examination, the testes were fixed in 3% glutaraldehyde solution for 6 h and treated with 1% osmium tetroxide for 45 min prior to electron microscopic analysis.

The rat cauda epididymis were weighed, placed in the dish preheated at 37 °C and containing M199 medium; the tissues were then cut and cultured at 37 °C for 5 min. An aliquot of 10- μL sperm suspension was added to 10 mL of the M199 dilution medium and thoroughly mixed. The 10- μL diluted sperm suspensions were then dropped into the blood cell counting plate and counted using an optical microscope.

2.4. Gene expression profiling by DNA microarray hybridization

Total RNA was isolated from the testicles of the postnatal day-30 rats for the gene expression microarray hybridization analysis, and for the bioinformatics analyses including gene ontology theory (GO) and pathway analyses using DAVID software. The GO analysis

included biological function, molecular function, and cellular component analyses. Microarray hybridization was run on a GeneChip[®] reg Scanner 3000 (P/N00-00212, Affymetrix). The original data were analyzed by a command console software 3.1 (Affymetrix) and the data qualified with the quality control were normalized using the gene spring software 11.0 (Agilent) and MAS 5.0 algorithm. The reproductive function-associated genes were obtained using the Affymetrix online Analysis Center.

2.5. Quantitative real-time reverse transcription-polymerase chain reaction (qPCR)

Testicle mRNA from the postnatal day-30 rats was isolated using the Trizol reagent. RNA was treated with DNase 1 and the RNA quality was examined using 1.2% agarose gel electrophoresis. Reverse transcription (RT) was performed using the Revert Aid[™] First Strand cDNA Synthesis Kit. The RT reactions quality was confirmed by the comparison of triplicate RT with no template control for each sample using the reference gene of glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*). The qPCR analysis was performed using TaqMan probe (5'-CTGCACCACCAACTGCTTAGC-3') and a reaction volume of 30 μ L. Table 1 shows the primer sequences of the target genes, i.e., steroidogenic acute regulatory protein (*StAR*), 3 β -hydroxyl steroid dehydrogenase (*3 β -Hsd*), proliferating cell nuclear antigen (*Pcna*), cytochrome P45017 α -hydroxylase (*p450c17*), 17 β -hydroxyl-steroid dehydrogenase (*17 β -Hsd*) and androgen receptor (*Ar*).

2.6. Statistical analysis

Statistical analysis of the real-time qPCR data was performed on the relative gene expression data. These data are shown graphically as percentages of the controls in the results. In addition, statistical analysis was performed on the individual sample data for each tissue normalized to *Gapdh* and then log-transformed to normalize the distribution. Significance in both analyses was determined by performing a *t*-test. Data was presented as means $\pm$ standard error (S.E.). The differences between two means were considered significant if *p* values were equal or less than 0.05.

3. Results

3.1. Reduced sperm numbers and altered acrosomes in male offspring rats following maternal exposure to linuron

Maternal exposure to linuron at 120 mg/kg/day by oral gavage from GD12 to GD17 had a significant impact on the sperm production in the offspring male rats. The numbers of sperm in the linuron-exposed group (Fig. 1A–C) under the light microscope were visibly reduced as compared to those in the control group (Fig. 1D–F). By routine sperm counts, the numbers of sperm in the linuron-treated group $(227 \pm 26) \times 10^6/\text{mL}$ was about 12% of those in the control group $(1868 \pm 86) \times 10^6/\text{mL}$, a reduction of 88% after maternal exposure (Fig. 1G) ($n = 6$, $p < 0.01$).

Moreover, a significant acrosome malformation could be observed in the linuron-exposed rats as compared with the control rats (Fig. 1A–C).

3.2. Pathologic changes in seminiferous tubules in male offspring after maternal exposure to linuron

Compared with the control group (Fig. 2B), parts of the seminiferous tubules in the linuron-exposed group were apparently destroyed (Fig. 2A). While spermatogonia could be seen along the incomplete basement membrane, there were few spermatids and sperm in the tubular lumen (Fig. 2A). The infiltration of a small amount of lymphocytes and plasma cells in the seminiferous tubules was also evident (Fig. 2A).

There were no obvious morphologic changes in spermatic cord vas deferens (Fig. 2C vs. D), prostate (Fig. 2E vs. F) and epididymis (Fig. 2G vs. H) between the linuron-exposed and control groups. The outcomes of these pathological examinations suggested that an early short-term maternal exposure to linuron caused the harmful effect on the male offspring's reproductive function, and the primary action site for linuron appeared to be on the seminiferous tubules.

Under the transmission electron microscope (Fig. 3A,B), the normal spermatogonia (mesenchymal cells) in the control offspring's testis showed abundant mitochondria, normal endoplasmic reticulum and intact nuclei (Fig. 3B). In the linuron-exposed spermatogonia, however, an abnormal morphology could be observed with swollen mitochondria and expanded endoplasmic reticulum as the arrowhead indicated (Fig. 3A). The data suggested that maternal exposure to linuron produced the damage to mesenchymal cells in male offspring's seminiferous tubules.

3.3. Effect of maternal linuron exposure on gene expression in male offspring

From the gene expression microarray profiling database, there were 168 differentially expressed genes in male offspring's testicles identified by using the criteria that p-value was less than 0.05 and the fold change (FC) was greater than 2 times. When using the signal log ratio 1 or -1 as threshold, we identified 89 up-regulated genes and 79 down-regulated genes in offspring testicle tissues following maternal linuron exposure (Fig. 4). The important differentially expressed genes with the implication in the reproductive function are listed in Table 2.

The gene ontology (GO) analysis was further used to profile the gene expression in three categories, i.e., molecular function, biological process and cellular component. In the molecular function category, the differentially expressed genes after maternal exposure were mainly in genes associated with the catalytic activity (26.2%), binding activity (24.7%), and transport activity (20%) (Fig. 5A). The affected genes in the category of the biological process mainly pertained the cellular process (19%), physiological process (12.4%), metabolism process (10%), biological regulation (8.6%), developmental process (7.6%), and regulatory process (6.5%) (Fig. 5B). Differentially expressed genes in the cell component category included the cell part (23%), cell (22.1%), organelle (15%), organelle part (14.8%), protein complex (6.4%), and macromolecular complex (6.1%) (Fig. 5C). These data suggest that maternal exposure to linuron may affect offspring's mesenchymal cell functions in cellular processing, metabolism and regulation, possibly by interfering enzymatic catalytic

activities as well as other molecular functions such as transport and signal transduction pathways.

Among the differentially expressed genes pertinent to the reproduction process, there were six genes identified; those down-regulated were steroidogenic acute regulatory protein (*Star*), cholesterol side-chain cleavage enzyme (*P450scc*), 3 β -hydroxyl steroid dehydrogenase (*3 β -Hsd*), androgen binding protein (*Abp*), 5 α -reductase, and one up-regulated gene was cytochrome c oxidase polypeptide 7a2 (*Cox7a2*).

3.4. Gene expression at the mRNA level

To study the gene expression at the translational level, a qPCR technique was used to quantify the mRNAs encoding the genes associated with testosterone synthesis including *Star*, *3 β -HSD*, proliferating cell nuclear antigen (*Pcna*), 17 β -hydroxyl-steroid dehydrogenase (*17 β -Hsd*), cytochrome P45017 α -hydroxylase (*p450c17*) and *AR*. In comparison to these genes expressed in the control group, the mRNA expression levels of *Star* (Fig. 6A), *3 β -Hsd* (Fig. 6B), *Pcna* (Fig. 6C), *17 β -Hsd* (Fig. 6D) and *P450c17* (Fig. 6E) in offspring testicle tissues were significantly decreased after maternal linuron exposure ($p < 0.05$). There was no statistically significant difference in the *Ar* mRNA expression between the linuron-exposed and the control groups (Fig. 6F).

4. Discussion

The current study, for the first time in literature, reveals that early exposure of the pregnant rats to 120 mg/kg/day of linuron from GD12 to GD17 results in a decreased sperm count, acrosome malformation, and pathological damage in seminiferous tubules of sexually matured offspring male rats. Further, DNA microarray and gene profiling analyses suggest that the linuron-induced male reproductive dysfunction seems likely to be associated with altered genes encoding testosterone synthesis, cell proliferation and apoptosis. Indeed, the mRNAs related to male testosterone synthesis, i.e., *Star*, *3 β -Hsd*, *Pcna*, *p450c17*, and *17 β -Hsd*, were significantly reduced in linuron-exposed animals as compared to controls.

The current data provided the strong evidence that maternal exposure to linuron could lead to the pathological damage of the testes and spermatid cord in the male offspring rats. Our finding is consistent with studies in literature that maternal linuron exposure can lead to smaller testes along with severe tissue lesion, sperm reduction and acrosomal structure developmental disorder among male offspring rats (Kang et al., 2004; Gray et al., 1999; Wilson et al., 2009).

Several mechanisms may explain the linuron-induced reproductive toxicity in male offspring. First, linuron may interact with the production and regulation of male sex hormone testosterone in fetus as well as during the maturation. It is known that the formation and differentiation of gonadal organs during embryonic development are regulated by a host of hormones through the hypothalamus-pituitary-gonad axis in mother and fetus (Kroupova et al., 2014), and the process is highly sensitive to variation of sex hormone levels in the fetal stage (Pusic et al., 2013). Oral exposure to linuron in pregnant mother rats may cause linuron to accumulate in maternal body. Upon passing across the blood-placenta

barrier and blood-testis barrier, linuron and its toxic metabolite(s) may interfere with the production and normal function of testosterone. In fact, our previous study using the similar exposure paradigm has shown that maternal exposure to linuron can cause a dose-dependent reduction of serum testosterone by about 60% (Ding et al., 2017). Santos et al. (2014) also report that short-term exposure to linuron in rats results in a reduced production of testosterone. Based on in-vitro data, Wilson et al. (2009) demonstrate that exposure to linuron leads to a significant reduction of testosterone synthesis. However, by using a low-dose exposure paradigm (50 mg/kg by oral gavage from GD12 to GD 17), McIntyre et al. (2002a,b) did not observe any changes in testosterone levels in testes and serum, nor did they observe any pathological changes in testis on postnatal days, although there was an obvious epididymis distortion on PND7 and PND14. These studies suggest that linuron-induced inhibition in testosterone production is apparently dose dependent. Noticeably also, literature reports suggest that linuron can be metabolized to form 3,4-dichloroaniline, a toxic metabolic product that is capable of competing with testosterone for binding with androgen receptor (AR) and functioning as an antagonist of androgen production (Bauer et al., 1998; Breugelmans et al., 2010). Thus, it seems plausible that linuron may have the dual effect on testosterone, by inhibiting its production to reduce the hormone level and by competitive binding with its receptor to disrupt its function.

Second, the molecular mechanism underlying linuron-induced inhibition of testosterone may be associated with a distorted expression of genes that are critical to testosterone production and regulation. The gene ontology (GO) analysis is a powerful tool to reveal the categories of genes affected by toxic exposure. Following maternal exposure, the genes associated with molecular functions such as cellular catalytic, binding and transport activities were significantly altered in the offspring male testes. For example, the expression of *c-myc* was significantly decreased, while the *Apaf-1* expression was increased, suggesting that maternal linuron exposure may cause the apoptosis disorder in the reproductive organ of the male offspring. Expressions of genes related to the biological processes such as the cell proliferation (TSC1 and S6K), differentiation, and growth (mTOR and PI3K) were also significantly altered. For example, the expression of genes encoding rapamycin (mTOR) and phosphatidylinositol 3-kinase (PI3K) signaling pathways, both of which are essential to cell growth and proliferation (Wullschlegel et al., 2006; Engelman, 2009), was significantly changed; this could lead to the altered development and maturity of male sex organs. Clearly, our GO analysis data provide the strong evidence that linuron-induced offspring male sexual dysfunction may be owing partly to its action on gene expression that are critical to cellular process, metabolism and regulation.

More specifically, our qPCR data verified that the differentially expressed genes related to testosterone synthesis, i.e., *Star*, *3 β -Hsd*, *Pcna*, *17 β -Hsd*, and *P450c17* were significantly reduced. Synthesis of androgen begins with cholesterol, which is delivered into the mitochondria by StAR protein and catalyzed by 3 β -HSD, 17 β -HSD and P450c17. Down-regulation of these active macromolecules can directly lead to a reduction of androgen production (Fitch et al., 1990; Katarzyna et al., 2011; Liu et al., 2015; Turner et al., 2003). PCNA (proliferating cell nuclear antigen), by binding to DNA polymerase δ in Leydig cells, participates in DNA replication and repair, chromatin remodeling and epigenetics, thus playing a decisive role in the early differentiation and development of gonad. Linuron

exposure apparently suppressed the mRNA level of *Pcna*. Thus, collectively our DNA microarray analysis and qPCR studies suggest that the linuron-induced male reproductive toxicity following maternal exposure is due to linuron's direct interaction with testosterone's function as well as its interference with the production of testosterone at transcription and translational levels; all this ultimately adversely influences the early differentiation and development of the male reproductive system.

Finally, linuron and its major metabolite 3,4-dichloroaniline may have a direct cytotoxicity on cellular structures of male sex organs. Based on the in vitro assay on the cytotoxicity and gap-junctional intercellular communication, Mazzoleni et al. (1994) report a time- and dose-related cytotoxic effect caused by linuron in the endothelial cell line F-BAE GM7373, an in vitro cell system known to be responsive to the biological effects of tumor promoters. In animal studies, 3,4-dichloroaniline has also been shown to cause the histopathological damage to liver and testis (Eissa et al., 2012). The toxicity is said to be related to the toxicant's ability to induce free radical generation and antioxidant depletion; the ensuing oxidative stress and lipid peroxidation in hepatocytes underlie cell degeneration and death. The question as to whether linuron and its metabolite have the direct cytotoxic effect on the cellular structure of male offspring's reproductive system remains unknown. Since both toxicants are highly lipophilic, it is likely that they could readily pass across the blood-placenta barrier and blood-testis barrier, and accumulate in the male reproductive organs, where they exert the direct cytotoxicity. This hypothesis deserves further investigation.

In summary, the current study demonstrates maternal exposure to linuron causes a significant damage to the male offspring reproductive system, manifested in reduced sperm counts, increased acrosome malformation, and damaged seminiferous structure. Our data further reveal that the mechanisms underlying linuron toxicity pertain to linuron's direct or indirect interaction with androgen production and function; altered expressions of genes encoding testosterone synthesis following linuron exposure explain partly linuron-induced male reproductive toxicity. Future studies are needed to understand the direct cytotoxicity of linuron at the cellular or subcellular level in male gonadal organs.

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Fig. 1. Decrease in the sperm number and malformation of acrosome following maternal exposure to linuron. Photographs show the typical morphology of the sperm from linuron-exposed animals (A, B and C) and control animals (D, E and F). The statistical analysis of sperm counts is presented in G. Data represent mean $\pm$ SD, $n = 6$. **: $p < 0.01$ as compared to the controls.

Fig. 2. Pathological examination of changes in testicular, spermatic cord, prostate and epididymis by HE staining. Panel A, C, E and G represent the typical photographs from a linuron-exposed animal and panel B, D, F and H represent those from a control animal.

Fig. 3. Transmission electron microscopic study of changes in seminiferous tubules. Tissues were collected from GD17 fetus following maternal linuron exposure. (A) Typical testicular tissue collected from a linuron-exposed fetus.(B) Typical testicular tissue from a control fetus.

Fig. 4.

The microarray Volcano plot. The plot was constructed by using fold-change and p values, which enables the visualization of the relationship between fold change (magnitude of change) and statistical significance (which takes both magnitude of change and variability into consideration). The vertical lines correspond to a 2.0-fold up or down, while the horizontal line represents $p = 0.05$. In the plot, the red points represent the differentially expressed genes with a statistical significance. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 5.

Gene ontology analysis of the differentially expressed genes in testicular samples from GD17 fetus. Differentially expressed genes are grouped into three categories: (A) molecular function, (B) biological process and (C) cellular component.

Fig. 6. qPCR analysis of mRNAs encoding StAR (A), 3β-HSD(B), PCNA (C), p450c17 (D), 17β-HSD (E) and AR (F). Tissues were collected from GD21 male fetus. Data are expressed as the relative change in expression compared to the control group where the mean expression is assumed to be 100%. Data represent mean + SD, n = 6. *: $p < 0.05$, **: $p < 0.01$ as compared to controls.

Table 1

Primer sequences of mRNAs selected for qPCR analysis.

Gene	Forward primer (5'–3')	Reverse primer (5'–3')
<i>SlAR</i>	CTGCTAGACCAGCCCATGGAC	TGATTTCCTTGACATTGGGTTCC
<i>3β-HSD</i>	CTGAATGTTACTGGCAAATTCTC	TGTAAAATGGACGCAGCAGGAA
<i>PCNA</i>	AAGAGGAAGCTGTGTCCATAGAG	CTTCATCTTCGATCTTGGGA
<i>p450c17</i>	GAGAAGCTAATCTGTCAGGAA	GCATCCACGATACCCTCAGT
<i>17β-HSD</i>	CAGAAGAGATTGAGAGGACCAG	CAGGAAATGACTTGGGAGCA
<i>AR</i>	GACATGCGTTTGGACAGTA	ACTTCTGTTTCCCTTCCGCA
<i>GAPDH</i>	TGGGTGTGAACCACGAGAA	GGCATGGACTGTGGTCATGA

Table 2

Genes differentially expressed in testis from linuron group and control group.

Genbank_ID	Definition	P value	Ratio
NM_031558.2	Rattus norvegicus steroidogenic acute regulatory protein (Star), mRNA	0.04323	0.489
NM_017286.2	Rattus norvegicus cytochrome P450, family 11, subfamily a, polypeptide 1 (Cyp11a1) mRNA	0.01383	0.401
NM_013821.3	Mus musculus hydroxy-delta-5-steroid dehydrogenase, mRNA	0.00271	0.485
NM_012650.1	Rattus norvegicus sex hormone binding globulin (Shbg), mRNA	0.01007	0.693
NM_017070.3	Rattus norvegicus steroid-5-alpha-reductase, alpha polypeptide 1, mRNA	0.02356	0.396
NM_022503.2	Rattus norvegicus cytochrome c oxidase subunit VIIa polypeptide 2 (Cox7a2), mRNA	1.36E-04	3.052
NM_021854.1	Rattus norvegicus tuberous sclerosis 1 (Tsc1), mRNA	0.01432	3.651
NM_012680.2	Rattus norvegicus tuberous sclerosis 2 (Tsc2), mRNA	5.94E-03	4.321
NM_021485.2	Mus musculus ribosomal protein S6 kinase, polypeptide 2 (Rps6kb2), mRNA	0.03115	0.325
NM_053974.2	Rattus norvegicus eukaryotic translation initiation factor 4E(Eif4e), mRNA	1.14E-03	0.498
NM_199501.1	Rattus norvegicus cyclin dependent kinase 2 (Cdk2), mRNA	0.00032	0.362
NM_080782.3	Rattus norvegicus cyclin-dependent kinase inhibitor 1A (Cdkn1a), mRNA	0.00589	2.156
NM_012603.2	Rattus norvegicus myelocytomatosis oncogene (Myc), mRNA	2.69E-04	0.469
NM_023979.1	Rattus norvegicus apoptotic peptidase activating factor 1 (Apaf1), mRNA	0.02694	4.256
NM_012922.2	Rattus norvegicus caspase 3 (Casp3), mRNA	0.03659	2.230
NM_001106874.1	Rattus norvegicus solute carrier family 25, member 41 (Slc25a41), mRNA	0.04532	0.31
NM_130894.3	Rattus norvegicus mitofusin 2 (Mfn2), mRNA	0.04892	0.496
NM_001134499.2	Rattus norvegicus regulatory associated protein of MTOR, complex 1(Rptor), mRNA	0.04792	0.458
NM_001034117.1	Rattus norvegicus beclin 1, autophagy related (Becn1), mRNA	0.04638	2.053