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FULL LENGTH ARTICLE

Bioinformatic identification of key genes and molecular pathways in the spermatogenic process of cryptorchidism

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Abstract This study aims to determine key genes and pathways that could play important roles in the spermatogenic process of patients with cryptorchidism. The gene expression profile data of GSE25518 was obtained from the Gene Expression Omnibus (GEO) database. Microarray data were analyzed using BRB-Array Tools to identify differentially expressed genes (DEGs) between high azoospermia risk (HAZR) patients and controls. In addition, other analytical methods were deployed, including hierarchical clustering analysis, class comparison between patients with HAZR and the normal control group, gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, and the construction of a protein–protein interaction (PPI) network. In total, 1015 upregulated genes and 1650 downregulated genes were identified. GO and KEGG analysis revealed enrichment in terms of changes in the endoplasmic reticulum cellular component and the endoplasmic reticulum protein synthetic process in the HAZR group. Furthermore, the arachidonic acid pathway and mTOR pathway were also identified as important pathways, while RICTOR and GPX8 were indentified as key genes involved in the spermatogenic process of patients with cryptorchidism. In present study, we found that changes in the synthesis of endoplasmic reticulum proteins, arachidonic acid and the mTOR pathway are important in the incidence and spermatogenic

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process of cryptorchidism. GPX8 and RICTOR were also identified as key genes associated with cryptorchidism. Collectively, these data may provide novel clues with which to explore the precise etiology and mechanism underlying cryptorchidism and cryptorchidism-induced human infertility.

Introduction

Cryptorchidism is one of the most common congenital malformations, and is defined as the absence of unilateral or bilateral testes from the scrotum in boys. The morbidity of cryptorchidism is approximately 3—4%, which continues to increase due to environmental endocrine chemical disruptors and environmental pollution.[1] Cryptorchidism is considered as part of the testicular dysgenesis syndrome (hypospadias, germ cell tumor, cryptorchidism, and subfertility), although the exact cause of cryptorchidism remains unknown.[2]

The etiology of cryptorchidism has been considered to be multifactorial, and includes numerous endocrine, environmental, genetic, anatomical and mechanical factors[3]. The therapeutic regimen for cryptorchidism includes hormonal treatment and orchidopexy. However, these treatment methods do not appear to be able to alter pre-existing pathological lesions.[4] Hence, the prognosis for these patients is not optimistic. A previous study reported that the incidence of azoospermia in patients with unilateral cryptorchidism was 13%, while its incidence increased to 89% in patients with untreated bilateral cryptorchidism. Consequently, children with cryptorchidism, particularly those with untreated bilateral cryptorchidism, are likely to face infertility issues throughout their life.

In recent years, a significant number of genetic studies have attempted to investigate cryptorchidism in humans. For example, Tannour-Louet et al revealed that the increased copy number of the *VAMP7* gene could upregulate the expression of estrogen-responsive genes, including *ATF3*, *CYR61* and *CTGF*, in the genitourinary tract, and thereby cause masculinization disorders in children.[5] Some studies that involved the analysis of blood samples identified the mutation of *CYP19A1*, *LIFR* and *GPRC6A* as potential reasons for cryptorchidism.[5—8] In another study, Ferlin et al reported that *NR5A1* mutation could become a novel genetic infertile phenotype in cryptorchidism patients.[9] Aside from genetic mutations, an animal study indicated that the inhibition of the Nrf2/HO-1 signaling pathway could improve cryptorchidism-induced infertility in a rat Leydig cell line.[10] In another study, the RXFP2 and Hsf1/Phlda1 signaling pathways were also identified as important pathways in the development of cryptorchidism in rats and mice.[11,12] Collectively, existing research in both human and animal material strongly indicates the fact that cryptorchidism is associated with genetic mutation and aberrant changes in a number of signaling pathways.

However, many of these previous genetic studies of cryptorchidism were limited to peripheral blood analysis, and did not involve the analysis of testicular tissues from patients with cryptorchidism. These limitations were imposed by ethics, particularly in China, a country associated with strong ethical values and cultural traditions. Merely few studies, one research was conducted by Hadziselimovic et al, attempted a detailed genetic analysis in this area by performing a whole-genome analysis that involved the high azoospermia risk (HAZR) group and control group; but these authors only screened differentially expressed genes (DEGs).[13] In the present study, the gene microarray data utilized by Hadziselimovic et al[13] were first analyzed by Biometric Research Branch Array Tools to identify DEGs. Next, a range of other analytical methods were incorporated, including hierarchical clustering analysis, class comparison between the HAZR group and control group, gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, and the construction of a protein—protein interaction (PPI) network.

The aim of the present study was to identify key genes and related signaling pathways in cryptorchidism and cryptorchidism-induced azoospermia. These findings may help to elucidate the etiology of cryptorchidism, and ultimately prevent azoospermia in cryptorchidism.

Materials and methods

Affymetrix microarray data

The gene expression profile data of GSE25518 (an ID code relating to specific expression data) based on the platform of GPL570 (Affymetrix Human Genome U133 Plus 2.0 Array) were obtained from the Gene Expression Omnibus (GEO) database, National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/geo/). This data was previously deposited by Hadziselimovic et al.[13] In addition, 23 testicular biopsies from 22 boys (19 testes from 18 boys with cryptorchidism, the HAZR group) and four contralateral descended testes from patients with testicular agenesis (the control group) were analyzed. The mean age of these patients at surgery was 3.4 years old (95% confidence interval [CI]: 0.6—6.1 years) and 3.9 years old (95% CI: 2.3—5.4 years) for the HAZR group and control group, respectively. All patients underwent extensive clinical examinations to exclude any clinical signs of developmental malformations or syndromes, and none of these patients had hypospadias. In addition, no clinical signs of Kallmann syndrome were identified. Furthermore, thyroid screening was normal, and no features of hypopituitarism were found in any of these patients.[14] Data was downloaded from the

GEO database in the form of a raw CEL, since this format can be conveniently analyzed.

Identification of DEGs

A total of 54,676 probes were obtained, and the expression profile data underwent log2 transformation before being imported into BRB-Array Tools (4_5_1_Stable, National Cancer Institute, Bethesda, MA, USA; http://linus.nci.nih.gov/BRB-ArrayTools.html). The threshold intensity was set at the minimum value if the spot intensity was below 10, and each array was normalized (centered) using quantile normalization. Genes were excluded from the analysis if <20% of the expression data had at least a 1.5-fold change in either direction from the gene's median, or if the proportion of missing or filtered-out data exceeded 50%.[15] A *t*-test was used to compare these two groups and identify DEGS, where $P < 0.01$. In addition, further prerequisites for inclusion was an FDR of <0.05, and an at least 2.0 fold-change in the data.[15]

Hierarchical clustering analysis

In order to collate genes with similar expression levels and investigate the expression values of DEGs in different samples, hierarchical clustering analysis was performed.[16] The expression values of DEGs in each group were selected according to the probe information obtained from the downloaded files. The hierarchical clustering map was prepared using BRB-Array Tools.

Comparison of DEGs between groups

The *t*-test was conducted in BRB-Array Tools to compare the relative expression of DEGs between the HAZR group and control group.[16] The prerequisite R/Bioconductor software package, which can provide an integrated solution for the data analysis obtained from gene expression experiments, was automatically downloaded from related websites using the BRB-Array Tools. $P < 0.01$ was used as the threshold value to test whether the expression of DEGs differed significantly between these two groups.

Gene ontology functional and pathway enrichment analysis

The database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov) is a gene functional enrichment analysis tool used to understand the biological meaning of genetic discoveries.[17] All DEGs identified in the present study underwent a GO and KEGG pathway enrichment analysis.[18] GO categories were divided into three systems: molecular function (MF), biological process (BP), and cellular component (CC).[19]

PPI network analysis

DEGs that were significantly upregulated or downregulated were uploaded to STRING[10.5] (http://www.stringdb.org) and analyzed online to determine the PPI network involved.[20]

Results

Screening for differentially expressed genes

A total of 9343 DEGs were identified. The *t*-test was used to identify DEGs between these two groups at $P < 0.01$. In addition, definitive DEG identification required an FDR < 0.05 and at least a 2.0-fold change. Following the univariate test, 2665 genes were identified, including 1015 upregulated genes (Table 1) and 1650 downregulated genes (Table 2). The hierarchical clustering of these DEGs is shown in Fig. 1. In order to express these results intuitively, the top 200 DEGs were visualized with Heatmap (Fig. 1). A Volcano plot that presents all DEGs is shown in Fig. 2.

Analysis of GO clustering

GO enrichment analysis indicated that the identified DEGs between the HAZR group and control group were significantly enriched in relation to the different GO terms. The enriched GO terms, which are expressed by BP, MF and CC, are shown in Table 3.

The GO functional annotation analysis of these DEGs revealed that (1) the BPs were mainly involved in kidney development, protein homo-oligomerization and intracellular signal transduction processes, (2) the MFs of the altered genes were mainly involved with axon guidance receptor activity, DNA replication origin binding and DNA replication origin binding, and (3) the CCs were mainly involved with the endoplasmic reticulum, endoplasmic reticulum membrane and the lumen of the endoplasmic reticulum.

Pathway enrichment analysis of DEGs

The KEGG pathway analysis results are presented in Table 4, which show the enrichment in the arachidonic acid pathway, the axon guidance pathway, the protein processing in the endoplasmic reticulum pathway, and the mTOR pathway. According to previous studies and KEGG results, it could be speculated that the arachidonic acid pathway and mTOR pathway are the most important pathways.[21–24] Charts arising from the KEGG pathway analysis are shown in Fig. 3.

PPI network construction

STRING[10.5] (http://www.stringdb.org) online analysis was used to construct the PPI network of DEGs, which were significantly upregulated or downregulated. The remaining DEGs in the PPI network after excluding disconnected nods are shown in Fig. 4. Each node gene in this network was subjected to statistical analysis. *CDH1*, *IRS1*, *RICTOR*, *GPX8* and *PTK2* were considered as "hub" genes. Combined with previous KEGG analysis results, it was determined that *RICTOR* and GPX8 also play key roles in the mTOR and

Table 1 The top 30 genes upregulated by a fold-change of >2, a false discovery rate of <0.05 and a *P*-value of <0.01.

ProbeSet	*P*-value	FDR	Fold-change	ProbeSet	*P*-value	FDR	Fold-change
228697_at	4.01×10^{-05}	0.00644	5.01	229074_at	1.36×10^{-04}	0.00723	2.78
204955_at	1.20×10^{-06}	0.00327	4.90	231411_at	3.69×10^{-04}	0.00967	2.78
238326_at	6.96×10^{-03}	0.0287	4.06	227044_at	1.07×10^{-04}	0.00711	2.74
208478_s_at	1.51×10^{-03}	0.0148	3.20	211833_s_at	1.77×10^{-03}	0.0155	2.74
232028_at	1.15×10^{-04}	0.00721	3.10	241671_x_at	5.82×10^{-04}	0.0107	2.72
210169_at	7.90×10^{-04}	0.012	3.08	205984_at	1.41×10^{-03}	0.0147	2.69
229947_at	8.43×10^{-03}	0.0319	2.98	229839_at	4.69×10^{-03}	0.0235	2.68
201131_s_at	5.91×10^{-03}	0.0264	2.97	241302_at	6.02×10^{-03}	0.0266	2.67
201430_s_at	9.14×10^{-05}	0.00678	2.96	203665_at	2.27×10^{-05}	0.00558	2.66
205433_at	4.74×10^{-03}	0.0236	2.93	238267_s_at	9.76×10^{-03}	0.0346	2.62
226533_at	7.48×10^{-05}	0.00647	2.90	223623_at	4.42×10^{-04}	0.0101	2.62
237444_at	5.54×10^{-04}	0.0105	2.87	228915_at	5.10×10^{-04}	0.0105	2.62
214844_s_at	2.39×10^{-05}	0.00563	2.87	218723_s_at	3.33×10^{-04}	0.00967	2.61
203797_at	1.53×10^{-05}	0.00517	2.83	218380_at	1.03×10^{-04}	0.00711	2.59
227059_at	1.21×10^{-03}	0.014	2.83	225954_s_at	3.35×10^{-03}	0.0201	2.59

Note: False discovery rate, FDR.

Table 2 The top 30 genes downregulated with a fold-change of <0.5, a false discovery rate of <0.05 and a *P*-value of <0.01.

ProbeSet	*P*-value	FDR	Fold-change	ProbeSet	*P*-value	FDR	Fold-change
242059_at	9.65×10^{-04}	0.013	0.19	216766_at	3.72×10^{-03}	0.211	0.24
214422_at	1.97×10^{-04}	0.00844	0.20	240485_at	2.12×10^{-03}	0.0167	0.24
230970_at	2.57×10^{-04}	0.00878	0.20	240141_at	4.97×10^{-04}	0.0105	0.25
1570571_at	2.51×10^{-03}	0.018	0.21	234032_at	3.17×10^{-03}	0.0197	0.25
215175_at	8.72×10^{-03}	0.0325	0.22	231644_at	2.73×10^{-03}	0.0186	0.25
1569041_at	3.11×10^{-03}	0.0196	0.22	243233_at	4.14×10^{-03}	0.0221	0.25
231956_at	3.65×10^{-04}	0.00967	0.22	243908_at	5.78×10^{-03}	0.0107	0.25
244267_at	7.02×10^{-05}	0.00647	0.23	238875_at	2.13×10^{-04}	0.00848	0.25
238970_at	6.45×10^{-03}	0.0276	0.23	217536_x_at	1.76×10^{-04}	0.00804	0.25
1560271_at	3.83×10^{-03}	0.0214	0.23	238593_at	8.08×10^{-05}	0.00653	0.25
240271_at	5.10×10^{-04}	0.0105	0.23	1556602_at	1.16×10^{-03}	0.0139	0.25
242440_at	1.76×10^{-03}	0.0155	0.24	242542_at	2.56×10^{-03}	0.0181	0.26
244464_at	6.73×10^{-04}	0.0113	0.24	1556849_at	6.10×10^{-06}	0.00517	0.26
215571_at	5.82×10^{-04}	0.0107	0.24	243756_at	5.40×10^{-06}	0.00517	0.26
1557270_at	9.56×10^{-04}	0.13	0.24	225239_at	2.72×10^{-03}	0.0186	0.26

Note: False discovery rate, FDR.

arachidonic acid pathways, respectively, as shown in Table 4 and Fig. 3.

Discussion

The gene expression profile data of GSE25518 was obtained from the GEO database, NCBI. Overall, 2665 genes were significant ($P < 0.01$) following the univariate test, which included 1015 upregulated genes and 1650 downregulated genes. The GO and KEGG analyses revealed enrichments in terms of changes to endoplasmic reticulum CCs and the endoplasmic reticulum protein synthetic process. The KEGG pathway analysis indicated that the arachidonic acid pathway and mTOR pathway were the most important pathways identified in the present study. Next, PPI analysis was performed, and it was revealed that *RICTOR* and *GPX8* represented as "hub" genes in the PPI network, which were significantly enriched in the mTOR pathway and arachidonic acid pathway. In summary, we believe that the *GPX8* and *RICTOR* genes may play a predominant role in the spermatogenic process in cryptorchidism.[25–27]

It is known that arachidonic acid metabolites are critical in sperm generation, and that polyunsaturated fatty acids may play important roles during sexual maturation and acrosomal reactions.[27,28] In the present study, bioinformatics analysis revealed that arachidonic acid metabolites are important in the human testis. This is in line with earlier animal studies, which revealed that unsaturated fatty acid supplements influence semen quality and testosterone concentrations in dogs.[29]

GPX8, also referred to as glutathione peroxidase 8, belongs to the glutathione peroxidase family, and is located on Chr. 5 q11.2.[30] In the present study, GPX8 was overexpressed and enriched in the arachidonic acid pathway by a factor of 5.7. The main biological role of glutathione peroxidase was to protect an organism from oxidative damage. Several previous studies have reported a

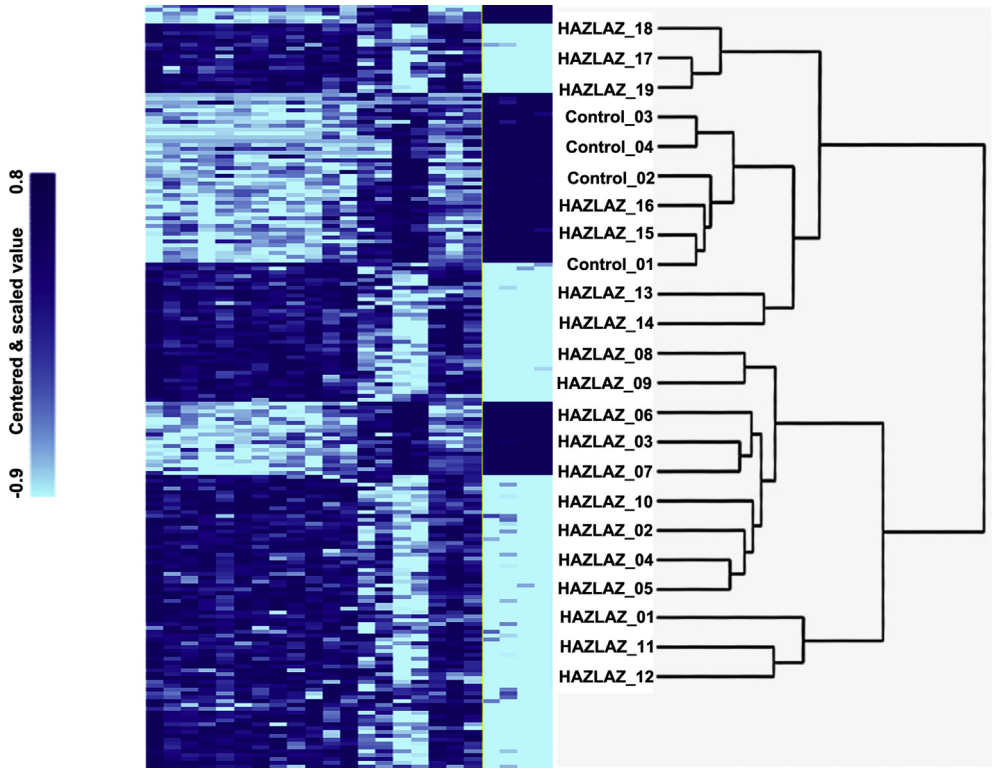

Fig. 1 The hierarchical clustering of DEGs and the heatmap of the top 200 DEGs. The dark blue shading on the left side of the figure represents the upregulated genes, while the light blue shading represents the downregulated genes. The hierarchical clustering trees are shown on the right.

significant increase in the reactive oxygen species (ROS) activity of human spermatozoa in certain forms of male infertility, and it is presently widely accepted that ROS contributes to sperm DNA damage and lipid peroxidation.[31—33]

It is interesting to note that 30%—80% of cases that involve male subfertility are considered to be due to the damaging effects of oxidative stress in sperm, and that the present analyses identified GPX8 as a key gene in the oxidative stress process. Furthermore, a recent study revealed that antioxidant supplementation in sub-fertile males may improve live birth outcomes and pregnancy rates.[34] It has also been reported that GPX8 plays an important role in protecting CCs, including nuclear DNA, against oxidative stress.[35] Consequently, we speculate that GPX8 plays a pivotal role in regulating arachidonic acid metabolites, protecting sperm from DNA damage, and repairing spermatogenic function in cases of cryptorchidism, thereby avoiding infertility and improving sperm quality.

Autophagy is a subject that has increasingly gained research attention from a medical perspective. Furthermore, macroautophagy is a term used to describe the processes involved in the elimination of infra-proteins, mitochondria and inflammasomes.[36] By coincidence, in the present study, the GO results revealed that huge number genes were related to intracellular signal transduction processes. Moreover, the KEGG pathway analysis revealed that the mTOR signaling pathway, an intracellular pathway involved in the regulation of cell cycle events, was critically related to cryptorchidism. Aberrant autophagic activity is known to contribute to a wide range of diseases, including diseases of the male reproductive system. It is also known that Sertoli cell function is heavily implicated in the normal spermatogenic process. A previous *in vitro* experiment using primary pre-pubertal Sertoli and adult Sertoli cell lines revealed that autophagy level could mediate the activation of caspase-1 and the secretion of IL-1β.[37] In other words, autophagy can exert a significant influence on Sertoli cells by regulating the production of inflammatory factors and the level of apoptosis. Another study revealed that the autophagy-related mTOR signaling pathway was required for the maintenance of spermatogenesis and the progression of germ cell development in Sertoli cells through regulating the pachytene spermatocyte stage.[38] In addition, the mTOR signaling pathway was identified as an important pathway in the present study. We found that RICTOR exerted the critical function and controlled the downstream expression of the mTOR gene (Fig. 3).

RICTOR is a regulatory binding partner of kinase mTOR, and forms part of the rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton.[38] RICTOR interacts with Cullin1-Rbx1 to form an E3 ubiquitin ligase complex that promotes the ubiquitination and degradation of SGK1.[39] In the present study, KEGG results revealed that RICTOR can regulate the downstream expression of mTOR, and control the level of autophagy. Autophagy has been reported to be activated during spermatogenesis. Furthermore, the levels of activated LC3 were previously

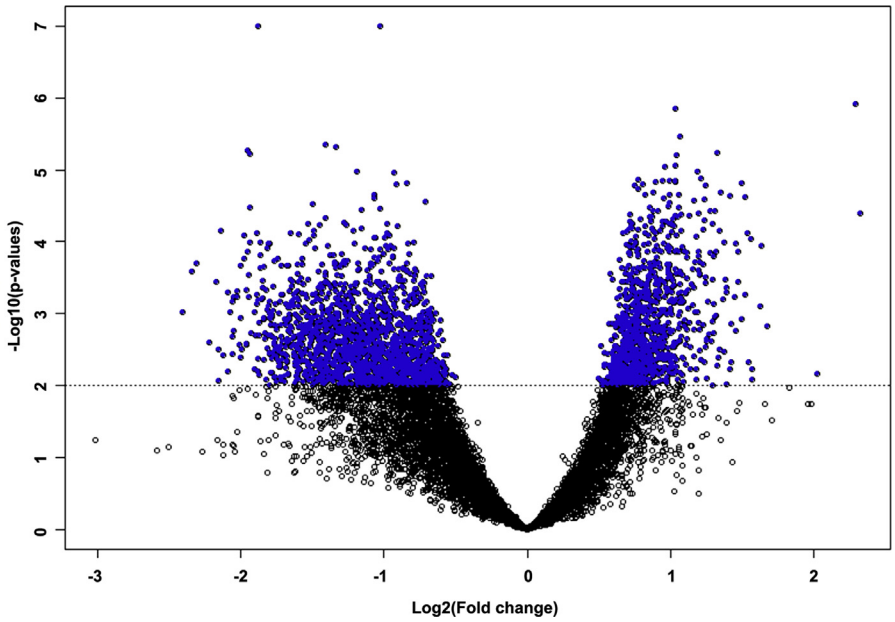

Fig. 2 Visualization of DEGs using a Volcano plot. Upregulated genes are presented as blue spots, while downregulated genes are presented as black spots.

Table 3 Function enrichment results of differentially expressed genes in different biological categories: molecular function, biological processes and cellular components.

GO-ID	Category	Term	Count	*P*-value
GO:0001822	BP	Kidney development	4	0.0294136
GO:0051260	BP	Protein homo-oligomerization	5	0.0477502
GO:0007420	BP	Brain development	5	0.0566024
GO:0035556	BP	Intracellular signal transduction	7	0.0904985
GO:0001525	BP	Angiogenesis	8	0.0017069
GO:0005783	CC	Endoplasmic reticulum	16	0.0012454
GO:0005789	CC	Endoplasmic reticulum membrane	15	0.0060193
GO:0005788	CC	Endoplasmic reticulum lumen	6	0.0166542
GO:0005743	CC	Mitochondrial inner membrane	8	0.0543134
GO:0070062	CC	Extracellular exosome	28	0.0948015
GO:0008046	MF	Axon guidance receptor activity	2	0.0541358
GO:0003688	MF	DNA replication origin binding	2	0.0837548
GO:0005003	MF	Ephrin receptor activity	2	0.0837548

Note: molecular function, MF; biological processes, BP; cellular components, CC.

Table 4 Results derived from the KEGG pathway analysis.

KEGG-ID	Term	Count	*P*-value	Gene Name
hsa00590	Arachidonic acid metabolism	5	0.024773	CBR1, CBR3, GPX7,GPX8, PLA2G1B
hsa01130	Biosynthesis of antibiotics	9	0.0363899	BPNT1, NSDHL, AK6, DBT, DLD, ICMT, GART, SHMT2, TKT
hsa04360	Axon guidance	6	0.077676	EEPHB2, EEPHB3, ARHGEEF12, SRGAP3, EEFNA5, UN5B
hsa04141	Protein processing in endoplasmic reticulum	7	0.082896	EED EEM2, RAD23B, SEEL1L, CKAP4, ERP29, PDIA4, UBE2D2
hsa00630	Glyoxylate and dicarboxylate metabolism	3	0.0838192	DLD, HYI, SHMT2
hsa04150	mTOR signaling pathway	4	0.0855002	RICTOR, RHEB, EIF4EBP1, EIF4E2

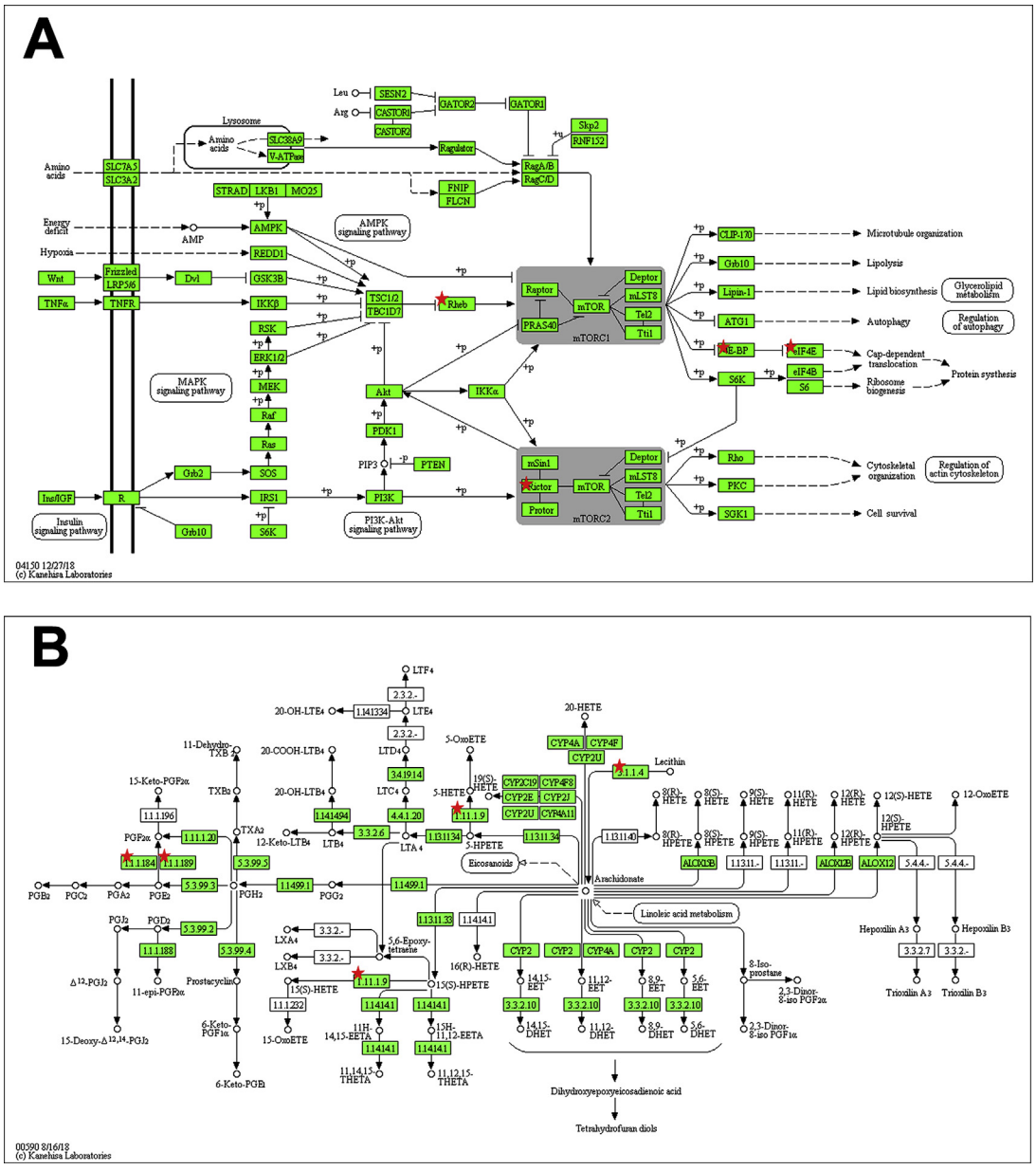

Fig. 3 **(A) The mTOR pathway.** The positions in which DEGs cause action are shown with red stars. **(B) The arachidonic acid pathway.** The positions in which DEGs cause action are shown with red stars.

associated with the viability of stallion sperm following a stressful intervention.[40,41] In mice, mTOR is necessary for sperm to progress through the pachytene spermatocyte stage, and can also regulate the distribution of gap junction alpha-1 protein in Sertoli cells.[42] Furthermore, autophagy can interact with ROS and apoptosis to regulate the spermatogenic process.[43] Collectively, this information suggests that RICTOR may represent a novel target to facilitate the elucidation of the mechanism underlying spermatogenesis, and the regulation of RICTOR gene expression. In addition, by controlling the mTOR pathway, it may be possible to regulate the relative levels of oxidative stress and apoptosis, protecting the testicular damage caused by the use of certain drugs in children with cryptorchidism.

The etiology of cryptorchidism and cryptorchidism-induced azoospermia is related to a multitude of different factors, which remains unclear. In the present study, GO enrichment analysis and KEGG analysis identified enrichment in terms of changes in endoplasmic reticulum-related genes and the synthesis of endoplasmic reticulum proteins. In adult mice with cryptorchidism, the absolute volumes of the endoplasmic reticulum have been shown to be significantly reduced.[44] We speculate that the alteration of endoplasmic reticulum proteins may be critical in the development of cryptorchidism. By reviewing related studies, several studies that reported on morphological changes and volume differences in the endoplasmic reticulum of

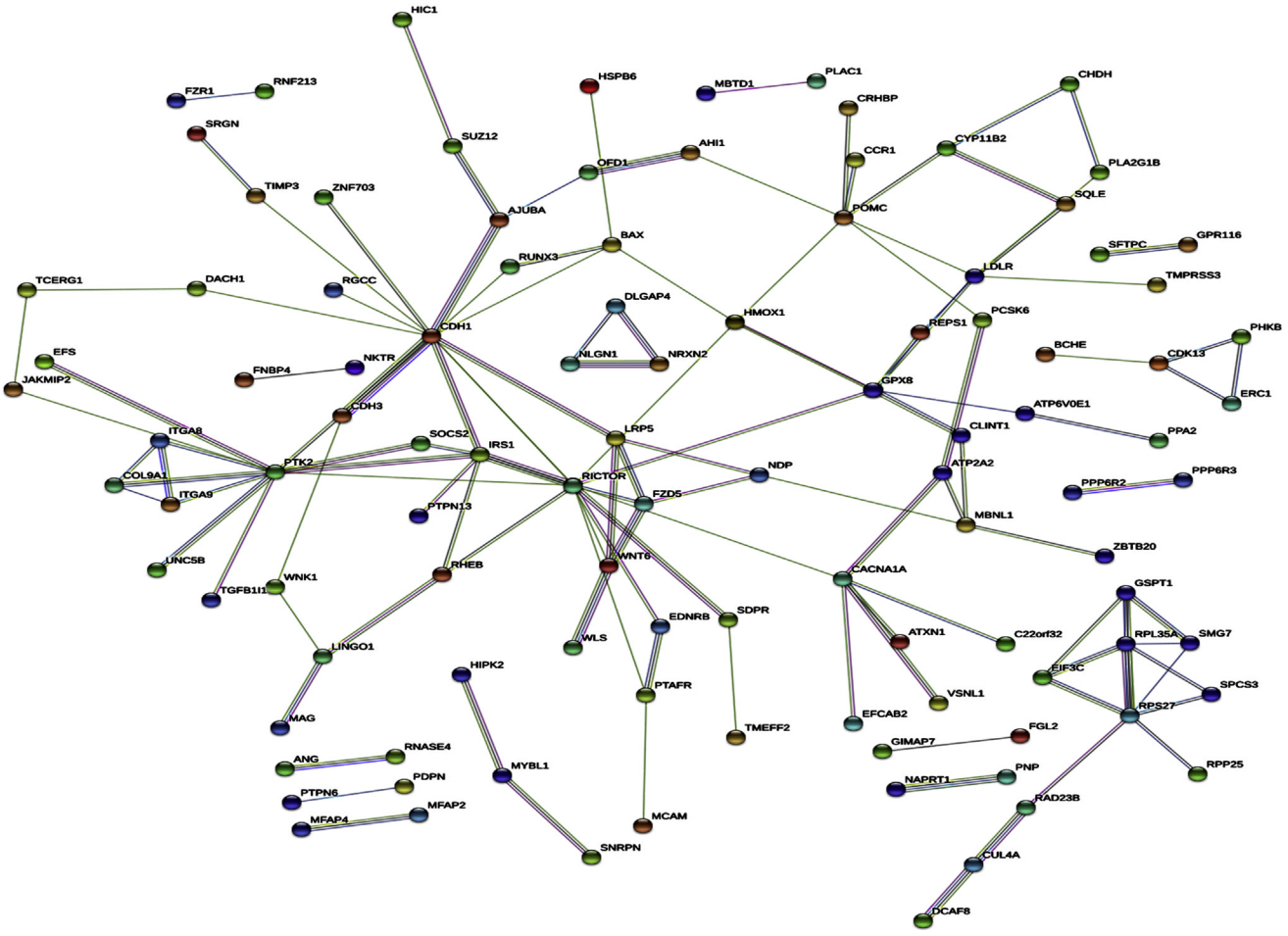

Fig. 4 The protein—protein interaction network of significantly upregulated DEGs and downregulated DEGs. Results derived from collated databases are annotated by , while those that were experimentally determined are annotated by . Predicted interactions are shown by (gene neighborhood), (gene fusions), and (gene co-occurrence). Text-mining interactions are represented by , co-expressed proteins are represented by and protein homology is represented by .

animal models with cryptorchidism were found.[44—47] However, these earlier studies did not investigate the specific levels of change in the synthesis of endoplasmic reticulum proteins in patients with cryptorchidism or dysgenesis. This was mostly related to the lack of suitable technology to study such changes at the level of the endoplasmic reticulum. However, the last decade has seen significant development in proteomics, and future studies should presently aim to use proteomic techniques to investigate changes in the synthesis of proteins in the endoplasmic reticulum in both animal models and human patients.

Previous studies of cryptorchidism used samples from either the tissues of animal models or from human peripheral blood. The present study was thereby more reliable than previous studies, because tissues from children with cryptorchidism were specifically analyzed. Next, we aimed to explore the application of antioxidant drugs and drugs that can regulate the mTOR pathway to ameliorate the spermatogenic function in a rat model of cryptorchidism. This should provide a foundation to prevent cryptorchidism-induced azoospermia in clinical scenarios.

Conclusions

In the present study, we identified that the arachidonic acid and mTOR pathways are important factors in the spermatogenic process, and that these pathways may play an important role in the occurrence of cryptorchidism. Furthermore, DEGs such as *GPX8* and *RITOR* were identified as key genes that may provide some new clues to explore the exact etiology and mechanism underlying cryptorchidism and cryptorchidism-induced infertility. However, the application and function of these pathways and genes should presently be studied in more specific detail, and on a larger scale, in both animal models and human patients.

Conflicts of interest

The authors have no conflict of interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2018.11.002.

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