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ORIGINAL ARTICLE

Lrrc34 Is Highly Expressed in SSCs and Is Necessary for SSC Expansion *In Vitro*

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Key words: spermatogonial stem cells; Lrrc34; high expression; apoptosis

Objective To discover critical genes contributing to the stemness and maintenance of spermatogonial stem cells (SSCs) and provide new insights into the function of the leucine-rich repeat (LRR) family member Lrrc34 (leucine-rich repeat-containing 34) in SSCs from mice.

Methods Bioinformatic methods, including differentially expressed gene (DEG), gene ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses, were used to uncover latent pluripotency-related genes. Reverse transcription-polymerase chain reaction (RT-PCR) and immunofluorescence analyses were utilized to verify the mRNA and protein expression levels, respectively. RNA interference of Lrrc34 using siRNA was performed to detect its transient impact on SSCs.

Results Eight DEGs between ID4-EGFP^{+} (G) and ID4-EGFP^{+}/TSPAN8^{High} (TH), eight DEGs between G and ID4-EGFP^{+}/TSPAN8^{Low} (TL) and eleven DEGs between TH and TL were discovered, and eleven protein-protein interaction (PPI) modules were found to be significant in the PPI network of DEGs. One of the DEGs, Lrrc34, was selected as a potential pluripotency-related gene due to its differential expression among ID4-EGFP^{+} spermatogonia subsets and its interaction with fibroblast growth factor 2 in the fifth module. Immunofluorescence experiments exhibited specific expression of Lrrc34 in a subpopulation of undifferentiated spermatogonia marked by LIN28A, and RT-PCR experiments confirmed the high expression of Lrrc34 in SSCs from P7 and adult

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mice. The transient knockdown of Lrrc34 in SSCs resulted in reduced colony sizes and significant changes in the transcriptome and apoptotic pathways.

Conclusion Lrrc34 is highly expressed in mouse SSCs and is required for SSC proliferation *in vitro* through effects on transcriptome and signaling transduction pathways.

SPERMATOGONIAL stem cells (SSCs), a subpopulation of undifferentiated spermatogonia (including A_{single}, A_{pair} and $A_{aligned}$ based on morphology), are the sole stem cell pool in the germline that maintains male fertility by ensuring a balance between self-renewal and differentiation *via* the continuous production of sperm capable of fertilization.[1, 2] The SSC pool in the undifferentiated spermatogonia forms in 2–3-day-postpartum mice and resides in the basement membrane of the seminiferous tubule throughout the life of mice.[3] The self-renewal of SSCs is an essential process in which their pluripotency is maintained by numerous extrinsic and intrinsic factors. For instance, the extrinsic factor glial cell line-derived neurotrophic factor (GDNF), which is mainly secreted by Sertoli cells, can bind to its receptor GFRA1/RET in SSCs and activate downstream signaling pathways to give rise to the transcription of GDNF-dependent intrinsic factors, such as ID4 and ETV5. The extrinsic factor fibroblast growth factor 2 (FGF2) is thought to enhance the function of GDNF.[4] The complex regulatory mechanism guarantees SSC maintenance and the source of mature spermatozoa.

Lrrc34 is predicted to belong to a subfamily of leucine-rich repeats (LRRs), which is called the ribonuclease inhibitor (RI)-like subfamily, and is predicted to have a protein structure that is identical to that of RI.[5, 6] The β-strands and α-helices of the RI-like subfamily provide a structural framework for protein-protein interactions with various biological functions, such as RNA protection from mammalian RIs.[7, 8] However, the function of Lrrc34 is not clearly understood. Previous studies have revealed that single nucleotide polymorphisms are closely associated with several diseases, such as coronary heart disease as well as systemic sclerosis-related interstitial lung disease.[9, 10] Lührig *et al*.[5] reported that Lrrc34 is highly expressed in pluripotent stem cells and germ cells in the testes but decreased after differentiation, which suggests that Lrrc34 is involved in the regulation of pluripotency. Nevertheless, no further studies have attempted to determine the role of Lrrc34 in SSCs. In the present study, we discovered that Lrrc34 is a differentially expressed gene (DEG) among subpopulations of undifferentiated mouse spermatogonia, identified its expression pattern in mouse SSCs and explored its function in the maintenance of SSCs *in vitro*.

MATERIALS AND METHODS

Materials

P7 and adult C57BL/6 male mice [SiBeiFu (Beijing) Biological Technology Company)]; type-IV collagenase (Gibco, 17104019); 0.25% (1×) trypsin Solution (HyClone, SH30042.01); CD90.2 MicroBeads, mouse (Miltenyi Biotec,130-049-101); RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, K1622); PowerUp™ SYBR® Green Power Master Mix (Thermo Fisher Scientific, A25742); Fluoroshield Mounting Medium with DAPI (Abcam, ab104139); anti-c-Kit (Santa Cruz, sc-19983); anti-hLIN-28A (RD Systems, AF3757); anti-LRRC34 antibody (Abcam, ab107820); Alexa Fluor® 647 AffiniPure Donkey Anti-Goat IgG (H+L) [Jackson ImmunoResearch (USA), 705-545-003]; Alexa Fluor® 594 AffiniPure Donkey Anti-Rabbit IgG (H+L) [Jackson ImmunoResearch (USA), 711-585-152]; Alexa Fluor® 488 AffiniPure Donkey Anti-Mouse IgG (H+L) [Jackson ImmunoResearch (USA), 715-545-150]; and RNAi Max (Invitrogen, 13778150) in GIBCO® Opti-MEM® (GlutaMAX™) Reduced Serum Medium (Invitrogen, 31985070).

Data downloading and DEG analysis

RNA-seq raw data (GSE83311) of spermatogonia subpopulations were downloaded from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo).

Deseq2[11] was used to analyze the expression levels of transcripts obtained from Cufflinks[12] and to predict the differentially expressed transcripts between distinct experimental conditions. Genes were considered significant if the false discovery rate (FDR) was less than 0.05.

GO and KEGG pathway enrichment analyses

Gene ontology (GO) is a bioinformatics tool that pro-

vides gene annotation and thus allows researchers to comprehend the biological processes in which genes participate.[13] Kyoto Encyclopedia of Genes and Genomes (KEGG) is a database resource that links genomic information with higher-order functional information by computerizing the current knowledge on cellular processes and standardizing gene annotations.[14] GO and KEGG were used to predict the biological processes in which the identified DEGs are involved. A standard hypergeometric distribution was used to measure the significant *P*-values with the R package named clusterProfiler.[15] Multiple hypothesis testing was performed using the Benjamini and Hochberg (BH) method.

Construction of the PPI network

The human protein-protein interaction (PPI) dataset was downloaded from the STRING database.[16] STRING (https://string-db.org/) is a database that stores information on functional protein interactions and covers 9.6 million proteins from 2031 species. A PPI network is defined as a graph, and the nodes and edges in this graph represent proteins and their interactions, respectively. We used Cytoscape[17] to construct the PPI network of the DEGs. The modules in the PPI network were calculated through Molecular Complex Detection (MCODE).

SOM clustering

Lrrc34-knockdown and negative control expression data were compiled and calibrated to establish self-organizing maps (SOMs) using R to align different expression patterns of mRNAs into a SOM.

Isolation of testis cells and enrichment of THY1$^+$ spermatogonia

THY1$^+$ and THY1$^-$ spermatogonia were isolated and enriched as described previously.[18] Testicular suspensions from P7 and adult C57BL/6 mice were prepared using a two-step enzymatic digestion procedure with 1 mg/ml type-IV collagenase and 0.25% trypsin. SSCs labeled with the THY1 antibody conjugated to magnetic microbeads were then selected from the suspensions by magnetic-activated cell sorting (MACS).

Immunofluorescent assay

Testes of adult C57BL/6 mice were fixed in 4% paraformaldehyde at 4°C overnight, dehydrated using graded ethanol and then embedded by paraffin. Tissue sections (5 μm thick) were produced, dewaxed and rehydrated. After antigen retrieval, the sections were blocked in 5% BSA (0.1% Triton X-100 in 5% BSA). Then the sections were incubated successively with primary antibody at 4°C overnight and secondary antibody at 37°C for 1 hours. DAPI was used to stain cell nuclei. After the sections were mounted with anti-quencher fluorescence decay, images were captured using a ZEISS 780 laser scanning confocal microscope.

Quantitative real-time PCR

Total RNA from THY1$^+$ and THY1$^-$ spermatogonia was extracted using the TRIzol reagent and then reversely transcribed to obtain cDNA using a RevertAid First-Strand cDNA synthesis kit according to the manufacturer's instructions. Quantitative real-time PCR was performed using PowerUpTM SYBR® Green Power Master Mix following the manufacturer's recommended protocol. Information on the primers is: Lrrc34, 5′-TTGCTGGTAACAATCGCTTAGAC-3′ (forward) and 5′-ACACCACTGATACATGGCTGG-3′ (reverse); GAPDH, 5′-AGGTCGGTGTGAACGGATTTG-3′ (forward) and 5′-TGTAGACCATGTAGTTGAGGTCA-3′ (reverse); ID4, 5′-CAGTGCGATATGAACGACTGC-3′ (forward) and 5′-GACTTTCTTGTTGGGCGGGAT-3′ (reverse); PLZF, 5′-TATCTCGAAGCATTCCAGCGAGGA-3′ (forward) and 5′-ACTCATGGCTGAGAGACCGAAAGA-3′ (reverse); GFRA1, 5′-CACTCCTGGATTTGCTGATGT-3′ (forward) and 5′-AGTGTGCGGTACTTGGTGC-3′ (reverse).

***In vitro* SSC culture**

SSCs were isolated from 6- to 8-day-old C57BL/6; 129S-Gt (Rosa) 26Sor/J mice (Jackson Laboratory, Bar Harbor, ME, USA), enriched using THY1 antibody-conjugated microbeads and MACS, and cultured with mitomycin C-inactivated mouse embryonic fibroblasts (MEFs) in serum-free MEMα supplemented with other components at 37°C with 5% CO_2 as previously described.[19]

RNA interference by siRNA

siRNAs were transfected into SSCs seeded in six-well plates in triplicate at a final concentration of 50 nmol/L using the transfection reagent RNAi Max and the diluent Opti-MEM according to the protocol provided by the reagent's manufacturer. Three siRNA oligonucleotides with different sequences that targeted Lrrc34 were pooled to achieve the best knockdown efficiency (labeled as Lrrc34-KD). The target sequences of lrrc34 for RNA interference were as follows: Lrrc34-1, CCGTAAATATTGCTGGTAA; Lrrc34-2, CCAACCTCGTACTGTC-

CAA; Lrrc34-3, GCACCGACTTATGGAGAAA. A negative control siRNA was used to exclude the potential non-specific binding of the siRNAs (labeled as NC-ctrl). Forty-eight hours later, the SSCs were transfected again with siRNA using the same method to silence Lrrc34 to the full extent. In addition, MEFs have no influence on the knockdown of Lrrc34 in SSCs because Lrrc34 is not expressed in MEFs.[5] Forty-eight hours later, representative bright-field images of Lrrc34-KD and NC-ctrl were captured by a DMI 4000B microscope (Leica, Germany). And then all images were reconstructed as projection images by ImageJ. Digital images were analyzed for diameters of SSC colonies by counting 5 random colonies per image.

Statistical analysis

Data were presented as mean ± SEMs and Student's *t*-test was performed by GraphPad Prism software. Significant *P* values are indicated with asterisks as follows: $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

RESULTS

Identification of DEGs among P6 ID4-EGFP+ spermatogonia

In a previous study, Mutoji *et al*. isolated ID4-EGFP^{+} spermatogonia (G) and subdivided them into ID4-EGFP^{+}/TSPAN8^{High} (TH) and ID4-EGFP^{+}/TSPAN8^{Low} (TL) populations. Three replicate samples of each population (**Figure 1A**) were used for independent RNA-seq analyses to demonstrate that TSPAN8 can distinguish SSC subtypes.[20] Thus, we conducted pairwise comparisons of the gene expression counts obtained for the sorted populations (G, TH and TL). We found eight DEGs between the G and TH populations, eight DEGs between the G and TL populations and 11 DEGs between the TH and TL populations. Genes with log-fold change (FC)>1 were considered upregulated candidates, whereas genes with logFC<-1 were considered downregulated candidates (**Figures 1B-1D, Supplemental Tables 1-3**). Although a small number of DEGs showed considerable similarity among the sorted cell populations referred to in a previous study,[20] a slight distinction was found among these cell populations.

PPI network of DEGs among P6 ID4-EGFP^{+} spermatogonia and module analysis

To determine the roles of the above-mentioned DEGs, we constructed the PPI network of the DEGs based on GO and KEGG enrichment analyses and human PPI information from the STRING database, and those DEGs

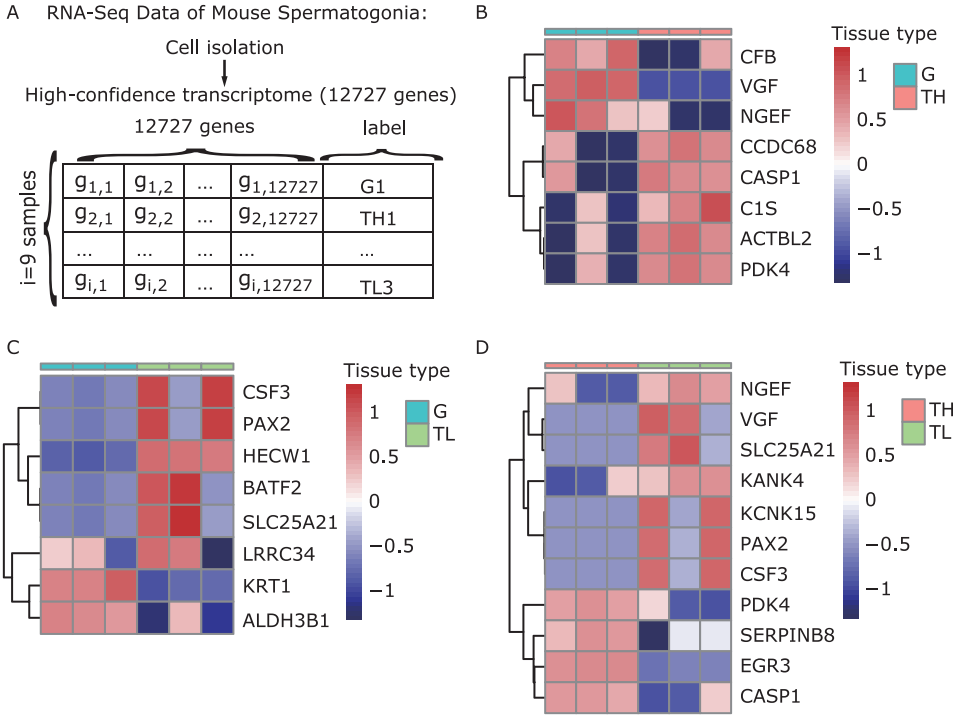

Figure 1. Differentially expressed genes (DEGs) among P6 ID4-EGFP+ spermatogonia [$P < 0.05$ and logFC > 1 or < -1]. A. RNA-seq data sources, B. DEGs between G and TH, C. DEGs between G and TL, D. DEGs between TH and TL.

with a higher degree of connectivity were considered candidate genes (**Figure 2A**). To identify important modules in the PPI network, we used the MCODE plug-in, and the 11 top modules were selected based on the following criterion: number of nodes > 3 (**Figure 2B**). These modules were mainly associated with the processes of gene transcription, mRNA translation, and protein processing and degradation, which could regulate cell growth, survival, proliferation and movement. For example, PPARA, CARM1, NCOR1 and CREB1 in the second module are all related to transcription regulation,[21-27] and TRIM69, FBXO22, SMURF2 and FBXL19 in the third module are relevant to the ubiquitination process *via* the E3 ubiquitin ligase, which includes substrate recognition.[28-31] Among these identified modules, the fifth module includes FGF2, FGF23, PIK3CB and LRRC34 and serves as the focus of this study due to the significant roles of FGF2 in SSC self-renewal and of the PI3K-AKT signaling pathway in SSC proliferation and division. Because FGF2, which is secreted mainly by Sertoli cells, is the secondary critical soluble factor for the self-renewal and expansion of SSCs *in vivo* and *in vitro*[2] and because the PI3K-AKT signaling pathway, which is activated by GDNF or Kit/SCF-R, controls the proliferation and division of SSCs,[32-34] the interaction of the pluripotency-related gene Lrrc34 with FGF2 and PI3K might suggest that LRRC34 is correlated with the self-renewal of SSCs and with the maintenance of their stemness.

LRRC34 is highly expressed in SSCs from P7 and adult C57BL/6 male mice

To better understand the role of the pluripotency-related gene Lrrc34 in DEGs between ID4-EGFP$^+$ spermatogonia subsets and in the module containing FGF2-interacting proteins, we detected its expression in SSCs from P7 and adult mice. As shown in the figures, an immunofluorescent assay revealed that LRRC34 was colocalized with the undifferentiated spermatogonia marker LIN28A in the P7 mouse testes (**Figure 3A**), but was only partially connected with LIN28A at the basement membrane of the seminiferous tubule in adult male mice (**Figure 3B**), which indicated that Lrrc34 was constantly highly expressed in SSCs. Subsequently, SSCs were enriched from P7 and adult male mice by MACS using an anti-THY1 antibody and were used to detect the expression of Lrrc34. RT-PCR results confirmed that LRRC34 was mainly detected in THY1$^+$-enriched SSCs from P7 and adult mice at levels similar to those found for ID4, PLZF, and GFRA1 (SSC markers) (**Figures 3C and 3D**). Thus, the results indicate that LRRC34 is highly expressed in SSCs from P7 and adult male mice.

The knockdown of Lrrc34 led to variations in the transcriptome signaling pathway in SSC colonies *in vitro*

To elucidate the functions of Lrrc34, we explored the variations in coding genes and their associated signaling pathways induced by the transient knockdown of Lrrc34 in *in vitro* SSC cultures through RNA-seq analyses. First, the treatment of SSCs with siRNA interference for 4 days resulted in a reduction in the SSC colony size (**Figures 4A, 4B**), and an RT-PCR analysis confirmed the knockdown of Lrrc34 (**Figure 4C**). The Lrrc34-KD and NC-ctrl expression data were then compiled and calibrated to establish SOMs. The changes in mRNAs after the knockdown of Lrrc34 were assessed by aligning the mRNAs onto an SOM (**Figure 4D**). According to the hexagonal units of NC-ctrl and Lrrc34-KD, clearly different expression patterns were obtained, which indicated the clustering of two different subsets of mRNAs regulated by NC-ctrl or Lrrc34-KD.

To further explore DEGs between the Lrrc34-KD and NC-ctrl groups, we compared the mRNA levels between the two groups using Ballgown. A total of 1120 mRNAs were differentially expressed in our study, and these included 305 upregulated and 815 downregulated genes in the Lrrc34-KD group compared with the NC-ctrl group (adjusted $P<0.05$, log FC > 1 or < -1) (**Figure 4E**, **Supplemental Table 4**). We then conducted GO and KEGG pathway analyses of these downregulated and upregulated mRNAs to determine the changes in the signaling pathways enriched with these genes. Glycosaminoglycan biosynthesis-heparan sulfate/heparin, carbon metabolism and other signaling pathways were enriched with the upregulated DEGs. The most significantly enriched pathway with the downregulated genes was the apoptotic signaling pathway, followed by I-kappaB kinase/NF-kappaB signaling and the MAPK cascade, which are closely associated with cell apoptosis and proliferation (**Figures 4F and 4G**). Therefore, the significantly altered apoptosis-related pathways might account for the observed decrease in the SSC colony size. These results imply that the loss of Lrrc34 leads to a decreased ability to colonize, likely through cell apoptosis, and support our

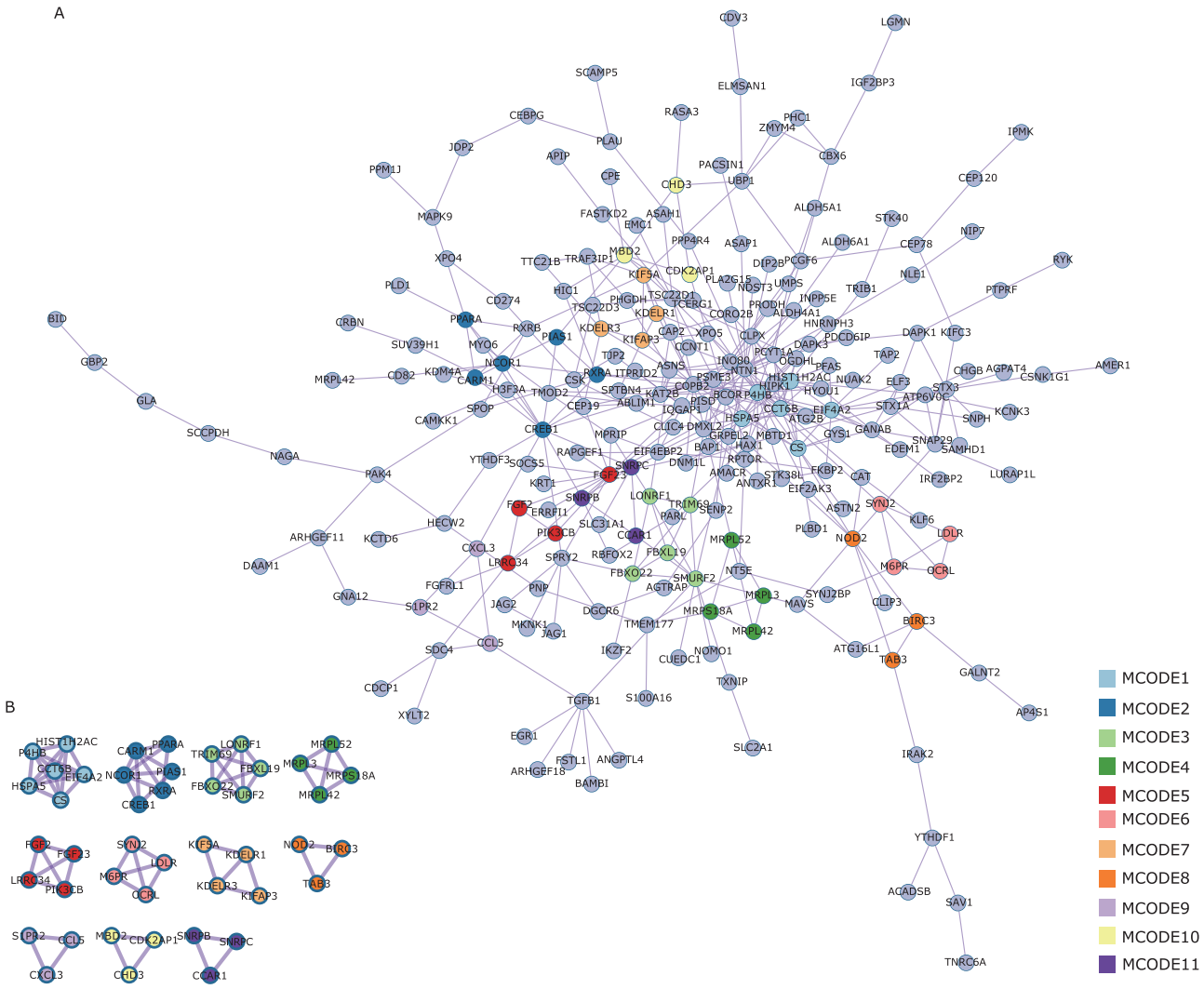

Figure 2. Protein-protein interaction network construction and module analysis.
A. Protein-protein interaction network of all above-mentioned DEGs, B. prominent interaction modules in the protein-protein interaction network.

contention that Lrrc34 plays an important role in SSC expansion.

DISCUSSION

Mutoji *et al*.[20] discovered a new cell surface marker, TSPAN8, for SSC enrichment by subdividing ID4-EGFP^{+} spermatogonia from P6 mice. This finding showed a reliable approach for the enrichment of SSCs because ID4-EGFP^{+}/TSPAN8^{High} spermatogonia formed 233 colonies/10^5 transplanted cells (stem cell concentration of 1:21) and exhibited a nearly 2-fold higher colonization capacity at 2 months after transplantation compared with the that of the ID4-EGFP^{+}/TSPAN8^{Low} subset. However, the ID4 population generated 516.6±98.1 colonies/10^5 transplanted cells in a previous study,[35] which indicated that changes in the stem cell concentration do not exhibit a simple additive relationship. Hence, we reused the RNA-seq data from ID4-EGFP^{+}, ID4-EGFP^{+}/TSPAN8^{High} and ID4-EGFP^{+}/TSPAN8^{Low} spermatogonia in a further analysis aiming to explore which factors affect the purity of SSCs obtained by marker selection.

Among a few DEGs, Lrrc34, which was differentially expressed between the G and TL populations, was found to be important due to its regulatory role in pluripotency and loss of expression in the W/W^{V} mouse testes, which is a germ cell-deficient testis model.[5] Furthermore, Lrrc34 was enriched in the significant module, and FGF2 was required for SSC expansion

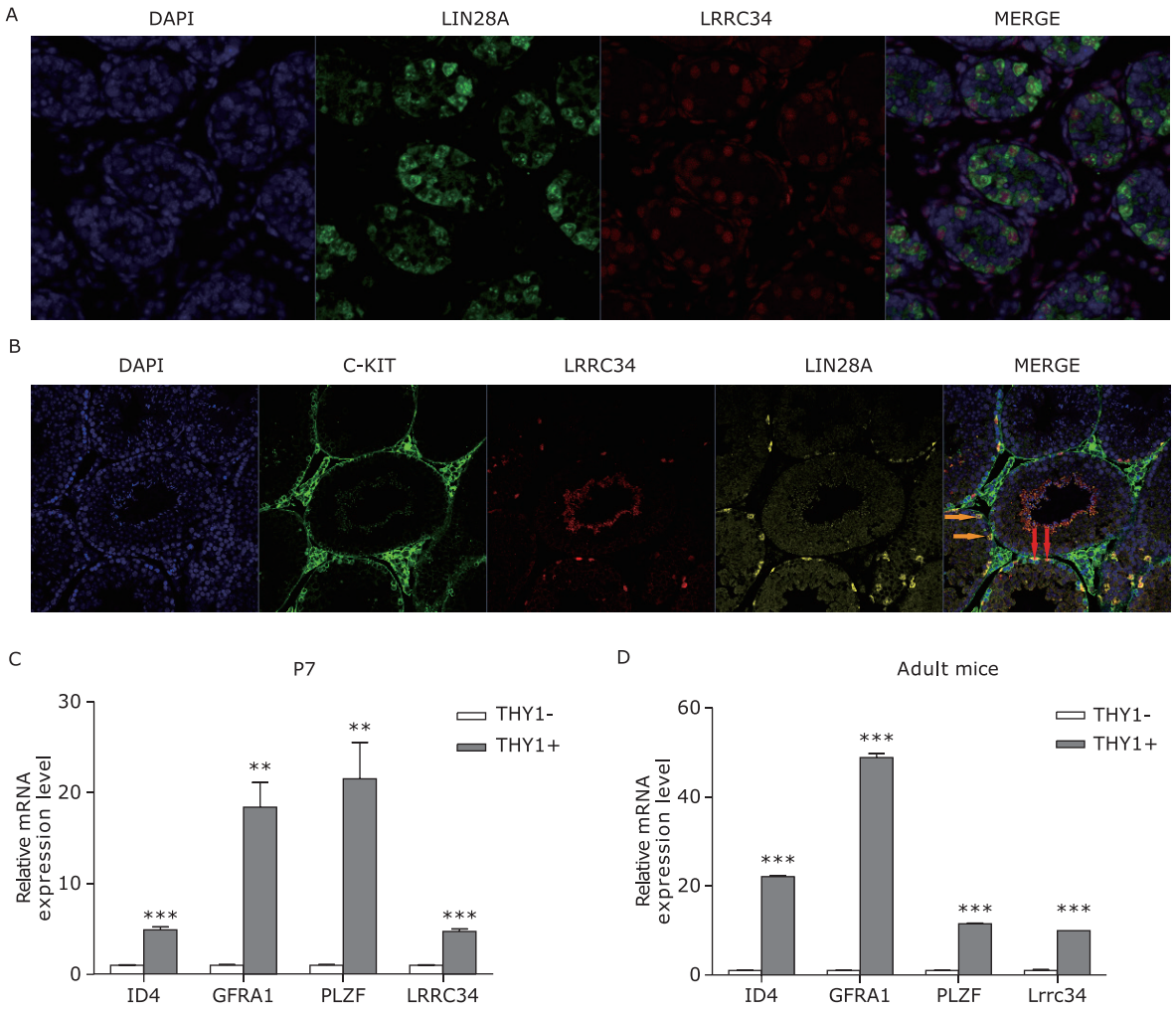

Figure 3. Expression pattern of LRRC34 in SSCs.

A and B. Protein localization of LRRC34 in the P7 (A) and adult (B) mouse testes. The red arrows indicate cells coexpressing LIN28A and LRRC34, whereas the yellow arrows indicate cells expressing LIN28A only.

C and D. Relative mRNA expression levels in THY1-isolated spermatogonia from P7 (ID4: 1.005±0.0692 *vs*. 4.912±0.3223, P=0.0003, t=11.85; GFRA1: 1.005±0.0727 *vs*. 18.42±2.712, P=0.0030, t=6.419; PLZF: 1.006±0.0788 *vs*. 21.54±4.002, P=0.0068, t=5.129; LRRC34: 1.004±0.0662 *vs*. 4.740±0.2806, P=0.0002, t=12.96) and adult (ID4: 1.003±0.0566 *vs*. 22.11±0.2694, P=0.0000, t=76.67; GFRA1: 1.000±0.0402 *vs*. 48.89 ±0.9009, P=0.0000, t=53.10; PLZF: 1.002±0.0400 *vs*. 11.53±0.1405, P=0.0000, t=72.07; LRRC34: 1.033±0.1746 *vs*. 9.970±0.7089, P=0.0003, t=12.24) mouse testes. The means ± SEMs are shown in the stacked bar graphs. The significance of the differences in gene expression levels between the TYH1$^-$ and THY$^+$ subpopulations were determined by t-test ($^{**}P$<0.01; $^{***}P$<0.001; n=3 biological replicates).

in vitro and *in vivo*. Thus, we concluded that Lrrc34 has an impact on the stem cell activity of SSCs. The Lrrc34 expression pattern highlighted this hypothesis. The protein expression of Lrrc34, particularly in undifferentiated spermatogonia, declined with age but was continuously distributed in the membrane basement where SSCs are located.[36] In addition, our results demonstrated that Lrrc34 is partially overlaps with LIN28A that is specifically expressed in undifferentiated spermatogonia,[37] including SSCs. These findings indicate that Lrrc34 likely exhibits specific expression in SSCs. Therefore, we isolated SSCs using a THY1$^+$ antibody and detected the Lrr34 mRNA levels in SSCs. As expected, Lrrc34 mRNA is highly expressed in SSCs labelled with ID4,[38] PLZF[39] and GFRA1[40] in both P7 and adult mice. Due to its specific expression in SSCs, Lrrc34 might be a candidate marker of SSCs similar to other markers that can be used to distinguish SSCs from other undifferentiated spermatogonia, such as markers for specific types of undifferentiated A-sper-

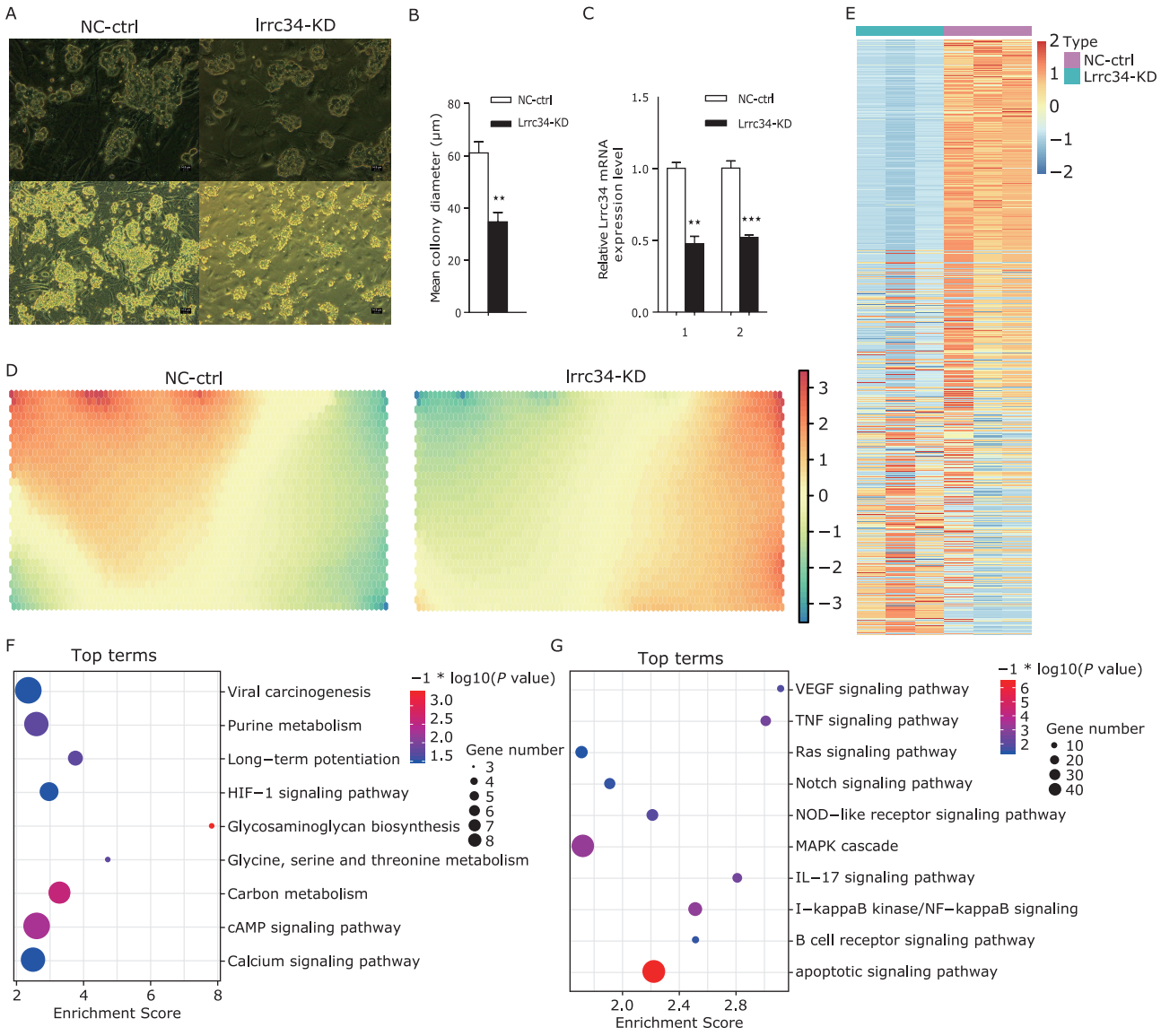

Figure 4. RNA-seq data analyses of the transcriptome characteristics after Lrrc34 knockdown.

A. The colony size of lrrc34-KD SSCs cultured *in vitro* was decreased compared with that of the negative control NC-ctrl SSCs. (bar = 14.9 μm)

B. The average diameter was quantified using ImageJ (61.09±4.242 *vs*. 34.70±3.624 μm, P=0.0015, t=4.731, n = 5 biological replicates). The data are presented as the means ± SEMs.

C. Lrrc34 was knocked down according to the relative mRNA level (1.002±0.0402 *vs*. 0.4768±0.0512, P=0.0013, t=8.064; 1.003±0.0505 *vs*. 0.5206±0.0166, P=0.0008, t=9.067, n = 3 biological replicates).

D. The overall transcriptome divergency between the lrrc34-KD and NC-ctrl SSCs was profiled by an SOM.

E. The DEGs between lrrc34-KD and NC-ctrl SSCs are presented in a heat map [P<0.05, logFC > 1 or < -1].

F and G. The signaling pathways enriched with upregulated (E) or downregulated (F) DEGs between lrrc34-KD and NC-ctrl SSCs are presented using a Bubble diagram.

matogonia (GFRA1, LIN28, NANOS2 and NGN3) and all undifferentiated A-spermatogonium markers (PLZF, SALL4 and CDH1).[41]

The knockdown of Lrrc34 in this study supported our hypothesis that Lrrc34 plays a specific role in SSC proliferation. A decrease in colony size was clearly observed after the transient knockdown of Lrrc34 in SSCs, and this phenotype showed that normal Lrrc34 function is vital for SSC expansion. Further analysis revealed that Lrrc34 knockdown led to marked changes in the

transcriptome, and notably enrichment of several apoptosis-related signaling pathways[42-45] instead of differentiation-related signaling pathways was observed, which suggested that the function of Lrrc34 is to support SSC expansion. It is well recognized that male fertility depends on the balance between the self-renewal and differentiation of SSCs; however, a precondition for maintenance of the SSC pool is cell survival.[46] Therefore, Lrrc34 is worth studying, and future research should elucidate the mechanism underlying the induction of SSC apoptosis due to the loss of Lrrc34.

In conclusion, our bioinformatic analysis identified Lrrc34 as a candidate gene that shows differential expression among ID4-EGFP$^+$ spermatogonia subsets. Based on the important role of FGF2 in SSC self-renewal and the expression of Lrrc34 in pluripotent stem cells, we speculated that Lrrc34 might act as a regulator of SSC expansion or maintenance. Verification experiments confirmed that Lrrc34 is particularly highly expressed in SSCs and is required for SSC expansion, which might contribute to the above-mentioned differences in the regenerative capacities of SSCs. The exact function and mechanism of Lrrc34 in SSCs need to be further investigated.

The Supporting Information is available free of charge on the CMSJ website at doi: 10.24920/003680.

Table S1. Differentially expressed genes between G and TH.

Table S2. Differentially expressed genes between G and TL.

Table S3. Differentially expressed genes between TH and TL.

Table S4. Differentially expressed genes between Lrrc34-KD and NC-ctrl.

Conflict of Interests Statement

The authors have no conflict of interests to disclose.

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