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RESEARCH ARTICLE

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Application of lncRNA–miRNA–mRNA ceRNA network analysis in the treatment of androgenic alopecia

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Abstract

Background: Long noncoding RNAs (lncRNAs) can be used as competitive endogenous RNAs (ceRNAs) to bind to microRNAs (miRNAs) to regulate gene expression. Previous studies have demonstrated that ceRNAs play an important role in the development of tumors. However, it is not clear whether the lncRNA–miRNA–mRNA ceRNA network plays a role in androgenic alopecia (AGA).

Methods: The hair follicles of three AGA patients and three healthy individuals were collected for high-throughput whole transcriptome sequencing to screen for differentially expressed lncRNAs. Differentially expressed lncRNA target genes were subjected to databases to predict miRNA–mRNA and lncRNA–miRNA relationship pairs, and a ceRNA network was constructed using Cytoscape software. Relative expression was verified by real-time quantitative reverse transcription–polymerase chain reaction (qRT-PCR).

Results: 84 lncRNAs were significantly differentially expressed between the hair follicles of AGA patients and those of healthy individuals; 30 were upregulated, and 54 were downregulated. The top 10 upregulated lncRNAs were ENST00000501520, ENST00000448179, ENST00000318291, ENST00000568280, ENST00000561121, ENST00000376609, ENST00000602414, ENST00000573866, ENST00000513358, and ENST00000564194. The top 10 downregulated lncRNAs were ENST00000566804, ENST00000561973, ENST00000587680, ENST00000569927, ENST00000340444, ENST00000424345, ENST00000589787, NR_024344, NR_073026, and NR_110001. The qRT-PCR validation results and receiver-operating characteristic curve analysis indicated that one upregulated lncRNA, LOXL1-AS1 (ENST00000564194), had the most significant clinical diagnostic potential. After further analysis, it was concluded that LOXL1-AS1 could be used as a sponge to target hsa-miR-5193, thereby regulating TP53 expression.

Conclusion: The ceRNA network-regulating AGA was constructed through high-throughput sequencing. Our study also identified a key lncRNA that is possibly related to the AGA pathological process.

Hanxiao Wei and Tian Yi contributed equally to this study.

KEYWORDS
androgenic alopecia, apoptosis, hsa-miR-5193, LOXL1-AS1, TP53

1 | INTRODUCTION

Androgenic alopecia (AGA) is the most common type of hair loss in men.[1] The pathogenesis of AGA is very complex and is associated with polygenic inheritance, androgen metabolism, and the abnormal expression of growth factors and their receptors.[2] In genetically susceptible individuals, undesirable androgen metabolism plays an important role in the pathogenesis of AGA. Testosterone produces dihydrotestosterone (DHT) under the action of increased 5a-reductase activity. The binding of DHT to the androgen receptor causes the gradual miniaturization of hair follicles, eventually leading to hair loss.[3] Scientifically proven treatments for AGA include oral finasteride and topical minoxidil, both of which have been approved by the US Food and Drug Administration. In addition, some new treatment methods have emerged, such as low-intensity laser irradiation,[4] autologous platelet-rich plasmas injections,[5] and microneedle therapy.[6] However, these methods have not been shown to be superior to the existing standard therapies for hair loss, and further clinical trials are needed to verify their efficacy.

For patients, hair loss usually reduces self-esteem, confidence, and quality of life. Therefore, early diagnosis and treatment are necessary. In recent years, with the development of high-throughput sequencing technology, biomedical studies have found that many new noncoding RNAs play important regulatory roles in diseases. However, there are few studies on the diagnosis and treatment of AGA. Exploring the regulation of AGA by long noncoding RNAs (lncRNAs) and the mechanism of action may lead to promising results for the treatment of AGA. LncRNAs are a class of RNA molecules that rarely encode proteins and have transcripts longer than 200 bp. LncRNAs play important regulatory roles in various biological processes, such as cell proliferation and apoptosis.[7] The expression of lncRNAs is significantly correlated with their neighboring protein-coding genes, and many lncRNAs act as regulatory factors for neighboring protein-coding genes.[8] In the process of hair follicle morphogenesis and hair follicle induction, key signaling molecules, such as Wnt/β-catenin, bone morphogenetic proteins, and Notch, are regulated by lncRNAs.[9]

LncRNAs regulate gene expression levels through different mechanisms, including epigenetic silencing, mRNA degradation, lncRNA–microRNA (miRNA) regulatory mechanisms, and lncRNA–mRNA regulatory mechanisms.[10] Various studies have demonstrated that lncRNAs or mRNAs can act as competitive endogenous RNAs (ceRNAs) to regulate gene expression levels through competitive binding with miRNAs via miRNA response elements. In this mode of regulation, lncRNAs negatively regulate target miRNA functions and positively regulate target gene expression by binding to complementary base sequences.[11] These lncRNA-related ceRNA networks are thought to play important regulatory roles in the occurrence and progression of tumors and other diseases.

This study compared differentially expressed lncRNAs in the hair follicles of individuals with AGA and healthy individuals through bioinformatics analysis, comprehensively analyzed the differential lncRNAs, predicted miRNA–mRNA and lncRNA–miRNA pairs using a database, and constructed a ceRNA network. The results of this study further elucidate the molecular mechanisms of AGA and provide a theoretical reference and research basis for the prevention and treatment of AGA.

2 | MATERIALS AND METHODS

2.1 | Clinical sample collection

The inclusion criterion was AGA diagnosed in accordance with the guidelines for the diagnosis and treatment of androgenetic alopecia in Chinese. Hair follicle samples were obtained from the posterior occipital hair follicles of middle-aged males (30–45 years old) during hair transplant surgery. All volunteers provided written informed consent, and the study was approved by the Institutional Review Committee of the Affiliated Hospital of Xuzhou Medical University.

2.2 | RNA extraction

TRIzol reagent (Invitrogen) was used to extract total RNA from hair follicle tissue according to the manufacturer's instructions. Then, the purity, concentration, and integrity of the RNA samples were determined by using a Qubit 2.0 (Invitrogen), Nanodrop 2000 (Thermo Fisher Scientific), and Agilent 2100 analyzer (Agilent), respectively. The purity (OD 260/280 ≥ 1.8; OD260/230 ≥ 1.0) and concentration (≥ 250 ng μL^{-1}) of the total RNA were adequate for library preparation.

2.3 | RNA library preparation and high-throughput sequencing

The qualified samples were used to construct sequencing libraries. The ribosomal RNA in the sample was removed, and cDNA was obtained by reverse transcription. Finally, the chain-specific library was constructed by PCR amplification, and RNA sequencing was carried out using an Illumina HiSeq 2500 sequencer. The library preparation and high-throughput sequencing of the lncRNAs were supported by NewCore Biotech.

2.4 | Real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was extracted from hair follicle tissue using TRIzol. The RNA quality and concentration were quantified using a Nanodrop 2000 system (Thermo Fisher Scientific), and cDNA was prepared using a ReverTra Ace real-time qPCR kit (Toyobo). All PCRs were carried out on an ABI 7500 System. The relative gene expression level was calculated using the $2-\Delta\Delta Ct$ method.

2.5 | Selection of differentially expressed lncRNAs and target gene analysis

LncRNAs in individuals with AGA and healthy individuals were compared, and differentially expressed lncRNAs between the two groups were identified using Cuffdiff software. The following thresholds were used for differential screening: fold change ≥1.5, p value ≤0.05, and fragments per kilobase of exon model per million mapped fragments (FPKM) value ≥0.1. Differentially expressed lncRNAs and their neighboring differentially expressed mRNAs were integrated to identify lncRNA target genes to further explore lncRNA function.

2.6 | Functional and pathway enrichment analyses

To annotate different biological functions and pathways significantly enriched in differentially expressed lncRNA genes, gene ontology (GO) functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed on all lncRNA target genes. GO functional analysis included molecular function (MF), biological process (BP), and cellular component (CC). These analyses allowed the pathways of the differentially expressed lncRNA target genes and their biological functions to be inferred. The p value was corrected by the Bonferroni method, and the threshold value for the corrected *p* Value was set at ≤0.05.

2.7 | Prediction of miRNA–mRNA and lncRNA–miRNA pairs

We used the miRanda and TargetScan databases to perform association analyses between lncRNAs and miRNAs using the sequences of the transcripts corresponding to the selected differential lncRNA target genes. We obtained the top five miRNAs and their sequences associated with each lncRNA, i.e., the interaction pair for each lncRNA and the obtained five miRNAs. Similarly, using the predicted miRNAs, association analyses between miRNAs and mRNAs were performed to obtain miRNAs associated with each selected mRNA, and miRNA–mRNA interaction pairs were obtained.

2.8 | Construction of the ceRNA regulatory network

Using the miRNA–mRNA and lncRNA–miRNA pairs, Cytoscape software was employed to visualize the lncRNA–miRNA–mRNA regulatory network. Linear correlations between genes were represented by the Pearson correlation coefficient (PCC). Genes with PCC > 0.95 and $p < 0.05$ were included in the AGA-related specific lncRNA–miRNA–mRNA regulatory network.

2.9 | Statistical analysis

All data are representative of three independent experiments. GraphPad Prism 8.0 and SPSS 18.0 software were used for all statistical analyses. Correlations between miRNAs and lncRNAs were analyzed by regression analysis with the Pearson's correlation coefficient. *p* Values <0.05 were considered statistically significant.

3 | RESULTS

3.1 | Differentially expressed lncRNAs

The samples were subjected to high-throughput whole transcriptome sequencing and analyzed using screening criteria (fold change ≥1.5 and *p* Value ≤0.05). Eighty-four lncRNAs were significantly differentially expressed (30 upregulated and 54 downregulated) between hair follicles from AGA patients and hair follicles from healthy individuals. The differentially expressed lncRNAs are shown in Table 1. In addition, we used volcano maps and heatmaps to visually display the distribution of all differential lncRNAs (Figure 1).

3.2 | GO and KEGG pathway analyses of differentially expressed lncRNA target genes

To interpret the differential analysis results, we performed functional clustering of important biological pathways using GO and KEGG pathway analyses. GO analysis of the upregulated lncRNA target genes indicated that the changes in BP were enriched in the regulatory process of apoptosis (Figure 2), the changes in CC were enriched in the guanylate exchange factor complex (Figure 3), and the changes in MF were mainly enriched in the activity of iron ion transmembrane transporters (Figure 4). KEGG pathway analysis (Figure 5) of the upregulated lncRNA target genes indicated that thyroid cancer and thyroid hormone synthesis were the main enriched pathways.

TABLE 1 The top 10 upregulated and downregulated differentially expressed lncRNAs.

lncRNA_id	gene_id	log_2(FC)	*p*_Value	Regulation
ENST00000501520	ENSG00000246465	2.26482	0.00575	up
ENST00000448179	ENSG00000230699	1.65388	0.00335	up
ENST00000318291	ENSG00000177406	1.58677	0.03045	up
ENST00000568280	ENSG00000260196	1.3784	0.0304	up
ENST00000561121	ENSG00000259478	1.35909	0.02915	up
ENST00000376609	ENSG00000204584	1.21002	0.032	up
ENST00000602414	ENSG00000269893	1.04319	0.0155	up
ENST00000573866	ENSG00000262202	1.03066	0.00495	up
ENST00000513358	ENSG00000250899	0.975583	0.03325	up
ENST00000564194	ENSG00000261801	0.949099	0.04465	up
ENST00000566804	ENSG00000261734	−2.38377	0.0243	down
ENST00000561973	ENSG00000260118	−2.37901	0.03205	down
ENST00000587680	ENSG00000186526	−2.2344	0.00195	down
ENST00000569927	ENSG00000260641	−2.07267	0.0028	down
ENST00000340444	ENSG00000188525	−2.00111	0.00005	down
ENST00000424345	ENSG00000238113	−1.97313	0.02065	down
ENST00000589787	ENSG00000186526	−1.83297	0.0001	down
NR_024344	MIR4697HG	−1.72444	0.0023	down
NR_073026	ZNF334	−1.67803	0.0264	down
NR_110001	LOC101926935	−1.66596	0.01455	down

3.3 | qRT-PCR validation

The expression levels of the top 10 upregulated lncRNAs in the AGA group were verified by qRT-PCR analysis of the hair follicles of the 30 AGA patients and 30 healthy volunteers. The expression differences of seven lncRNAs were statistically significant ($p < 0.05$) (Figure 6), which indicated that the sequencing results were reliable.

3.4 | Prediction of miRNA–mRNA and lncRNA–miRNA pairs

We used the miRanda and TargetScan databases to predict the relationships between differential lncRNAs and miRNAs and predicted 25 lncRNA–miRNA pairs and 12 miRNA–mRNA pairs (Table 2).

3.5 | Construction of the ceRNA regulatory network

A ceRNA network is connected by miRNAs. The upregulation of miRNAs is accompanied by the downregulation of mRNA and lncRNAs and vice versa. The prediction results were used to plot the lncRNA–miRNA–mRNA regulatory network (Figure 7). The ceRNA regulatory network contained a total of 18 nodes and 20 edges, including five lncRNA nodes, eight miRNA nodes, and five mRNA nodes.

3.6 | LOXL1-AS1 (ENST00000564194) has diagnostic potential for patients with AGA

The diagnostic value of LOXL1-AS1 for AGA was evaluated using receiver operating characteristic (ROC) curve analysis. Patients with AGA were considered true positive cases, and healthy controls were considered true negative cases. Among the seven statistically significant lncRNAs, the top three differentially expressed lncRNAs according to the qRT-PCR results were selected for ROC curve analysis. Among the lncRNAs, LOXL1-AS1 had the largest area under the curve (AUC), 0.7278, with a 95% confidence interval of 0.5975–0.8580 ($p = 0.0024$; Figure 8).

3.7 | LOXL1-AS1 competes with TP53 for binding to hsa-miR-5193

Based on the ceRNA regulatory network, LOXL1-AS1 may sponge hsa-miR-5193 by acting as a ceRNA, thereby participating in the regulation of TP53 expression. The genomic expression sites of LOXL1-AS1 were obtained using the Ensembl online database (Figure 9A). The putative base binding sites between the LOXL1-AS1 3'UTR and hsa-miR-5193 and between hsa-miR-5193 and the TP53 3'UTR were explored by

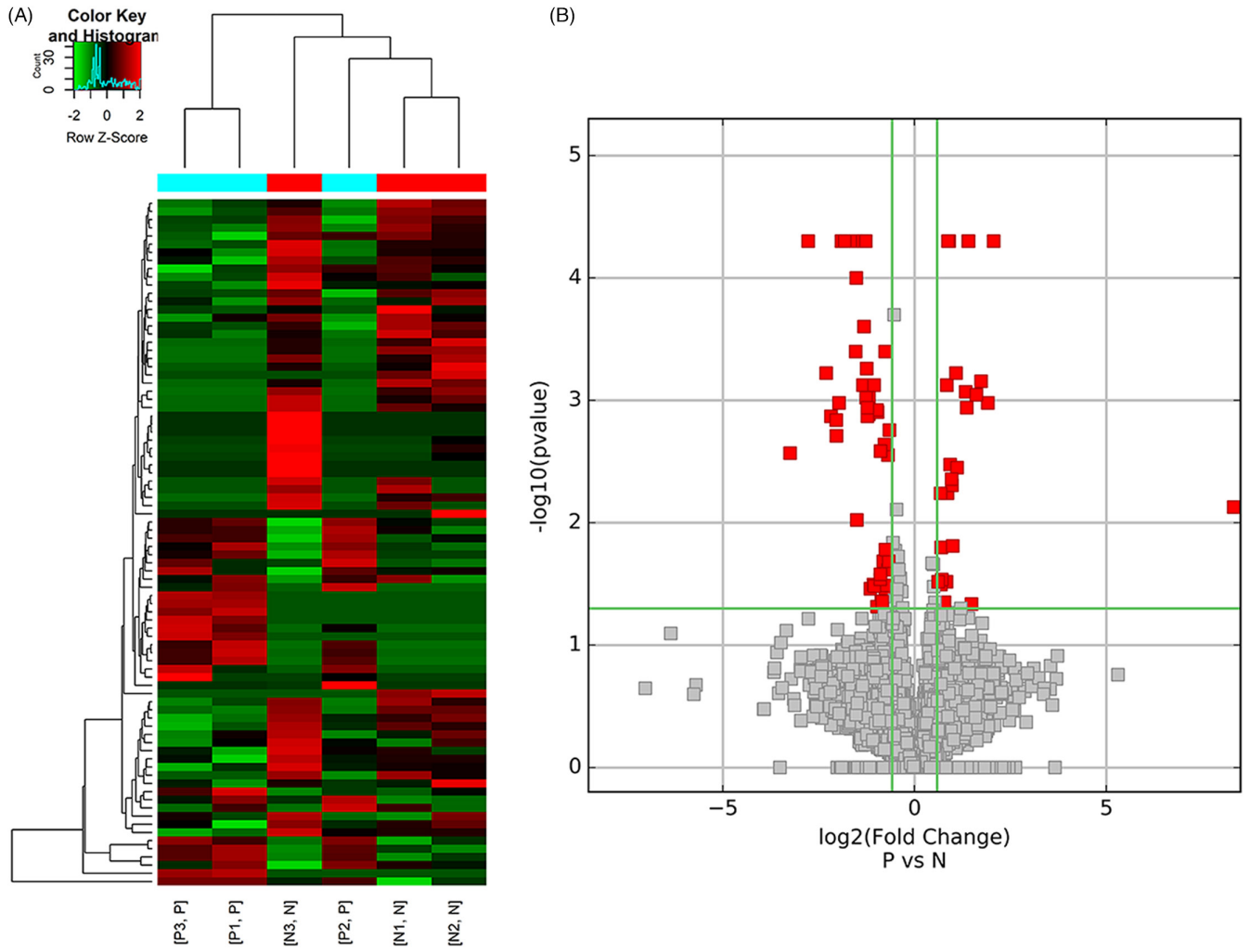


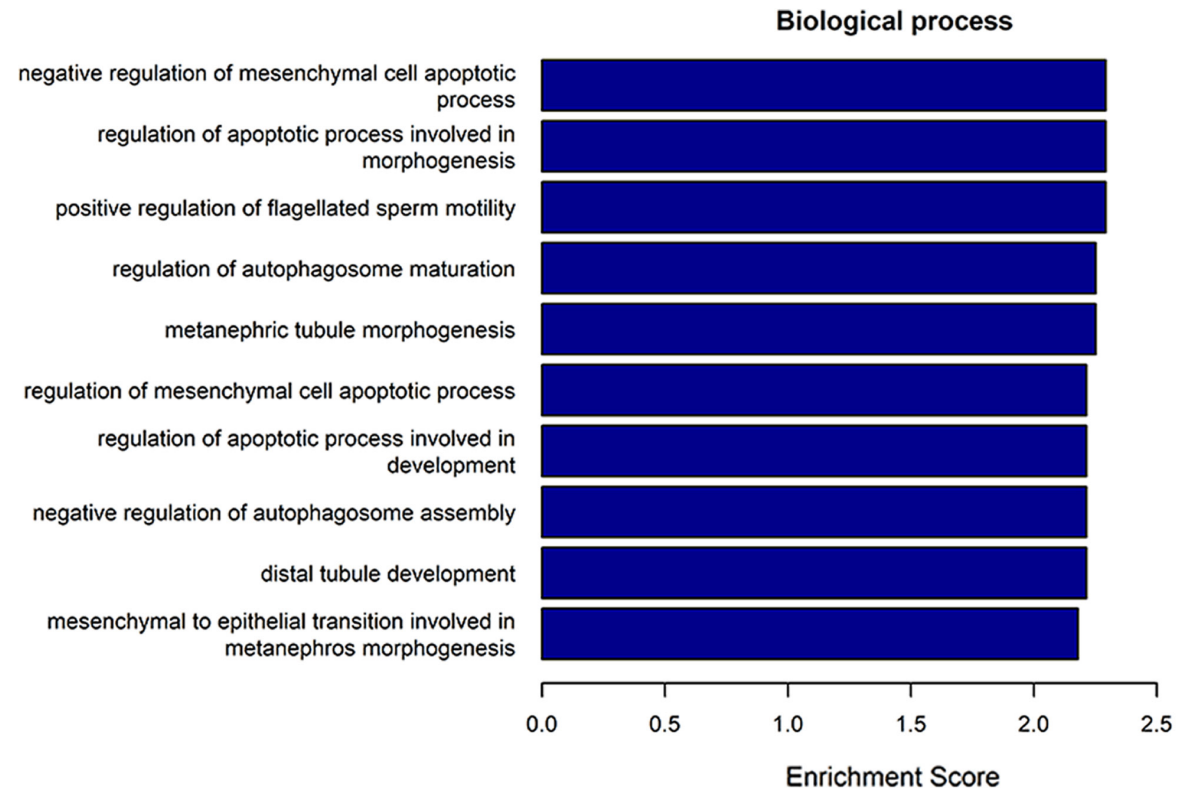
FIGURE 1 Differentially expressed lncRNAs between AGA patients and healthy controls. (A) Heat map of differentially expressed lncRNAs between AGA patients and healthy controls (NC). The blue bar at the top represents the patient sample, and the red bar represents the control sample. (B) In the volcano map, the red squares represent the significantly differentially expressed lncRNAs, the red dots in the left panel represent the significantly downregulated lncRNAs in the AGA group, and the red dots in the right panel represent the significantly upregulated lncRNAs in the AGA group.

miRanda and TargetScan database analysis (Figure 9B). We investigated the interaction between LOXL1-AS1 and hsa-miR-5193 and performed a correlation analysis by calculating the Pearson's correlation coefficient. LOXL1-AS1 expression was inversely correlated with that of hsa-miR-5193 in patients with AGA (Figure 9C).

4 | DISCUSSION

Recent studies have demonstrated that lncRNAs and miRNAs play important regulatory roles in a series of biological processes related to hair follicle regeneration, including proliferation, differentiation, transcription, and apoptosis.[11,12] Certain lncRNAs, such as lncRNA H19[13] and lncRNA-PCAT1,[14] can activate specific signaling pathways to maintain the characteristics of hair follicle regeneration induced by dermal papilla cells. The ceRNA mechanism links the functions of noncoding RNAs and protein-coding mRNAs, making these noncoding RNAs possible disease diagnostic and prognostic markers or therapeutic targets.[15] Recent studies have used whole-genome microarray technology to identify differential lncRNA expression profiles between AGA lesion areas and adjacent normal tissues,[16] suggesting that the occurrence of AGA may be associated with the differential expression of lncRNAs. In recent years, with the development of high-throughput sequencing technology, detection throughput has increased, while detection time and cost have decreased. Sequencing is different from closed-system microarrays. It is an open detection platform that can discover new sequences and new mutations and provide transcript information.[17]

Although some studies have sequenced hair follicles under different culture conditions, most data have been obtained from laboratory cell or tissue culture, which is not closely related to clinical practice, and these results are difficult to translate into clinical application. The sequencing data in this study are based on clinical surgical samples. The aim of this study was to explore the pathogenesis



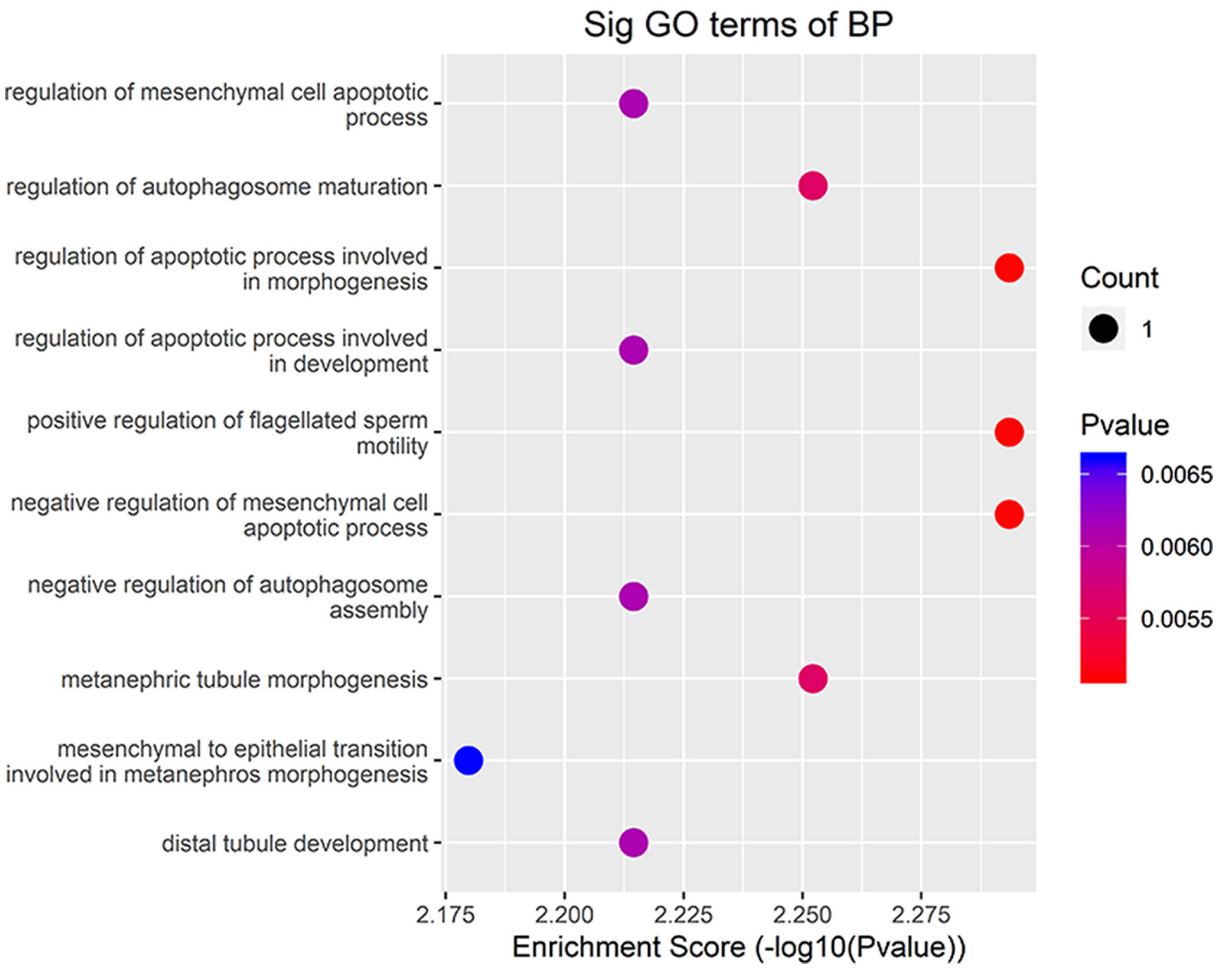
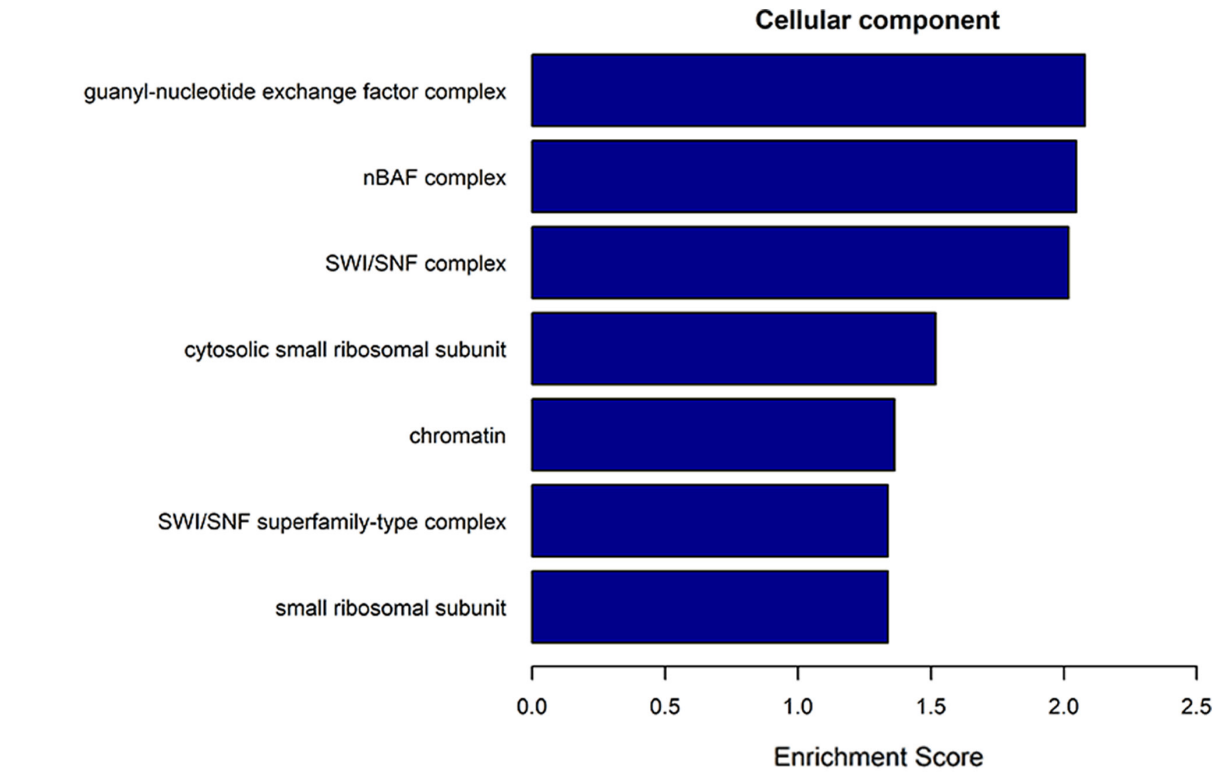


FIGURE 2 Significantly enriched gene ontology (GO) annotations based on upregulated lncRNAs. Top 10 biological process (BP) annotations in the enrichment analysis



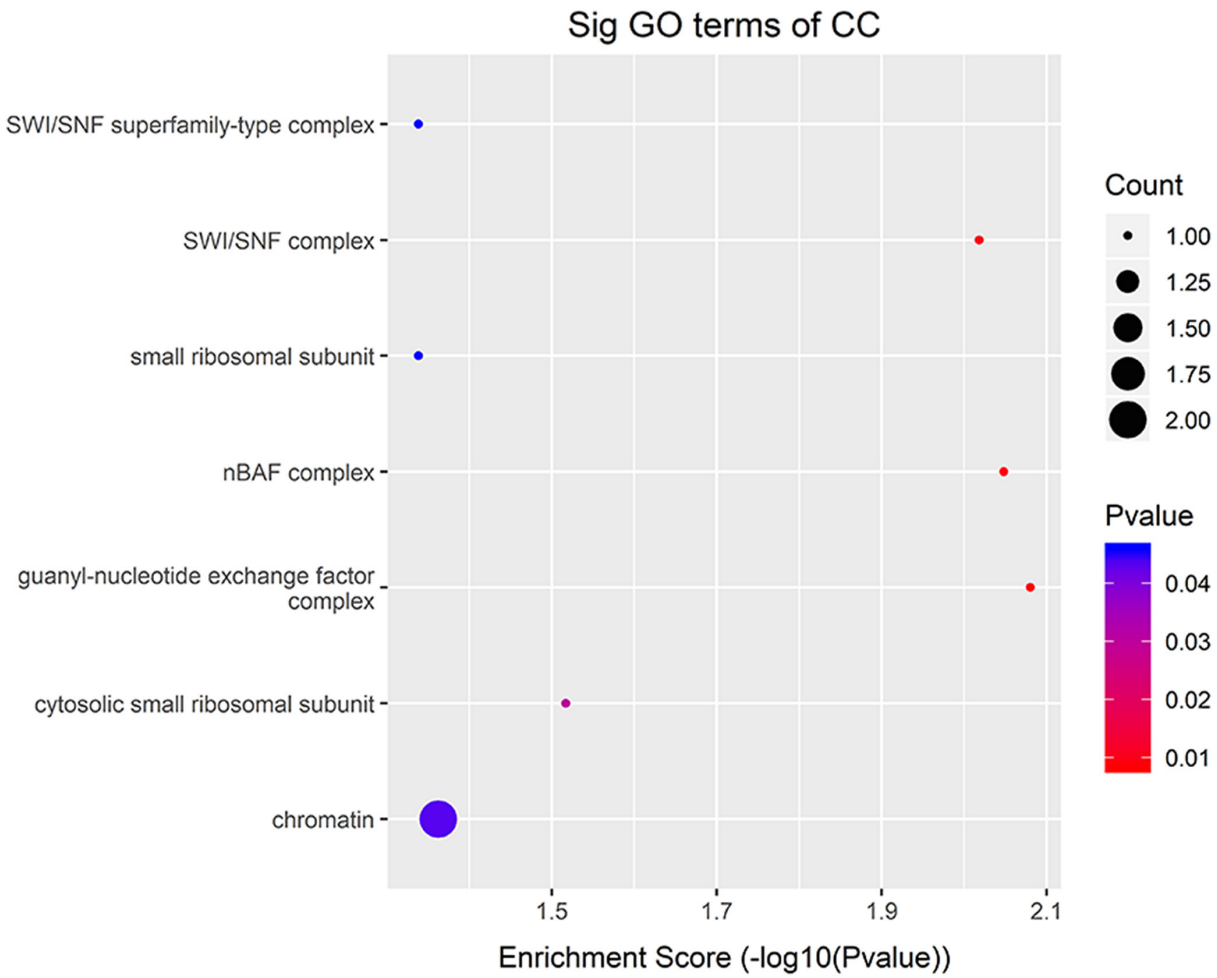


FIGURE 3 Significantly enriched gene ontology (GO) annotations based on upregulated lncRNAs. Top 7 cellular component (CC) annotations in the enrichment analysis

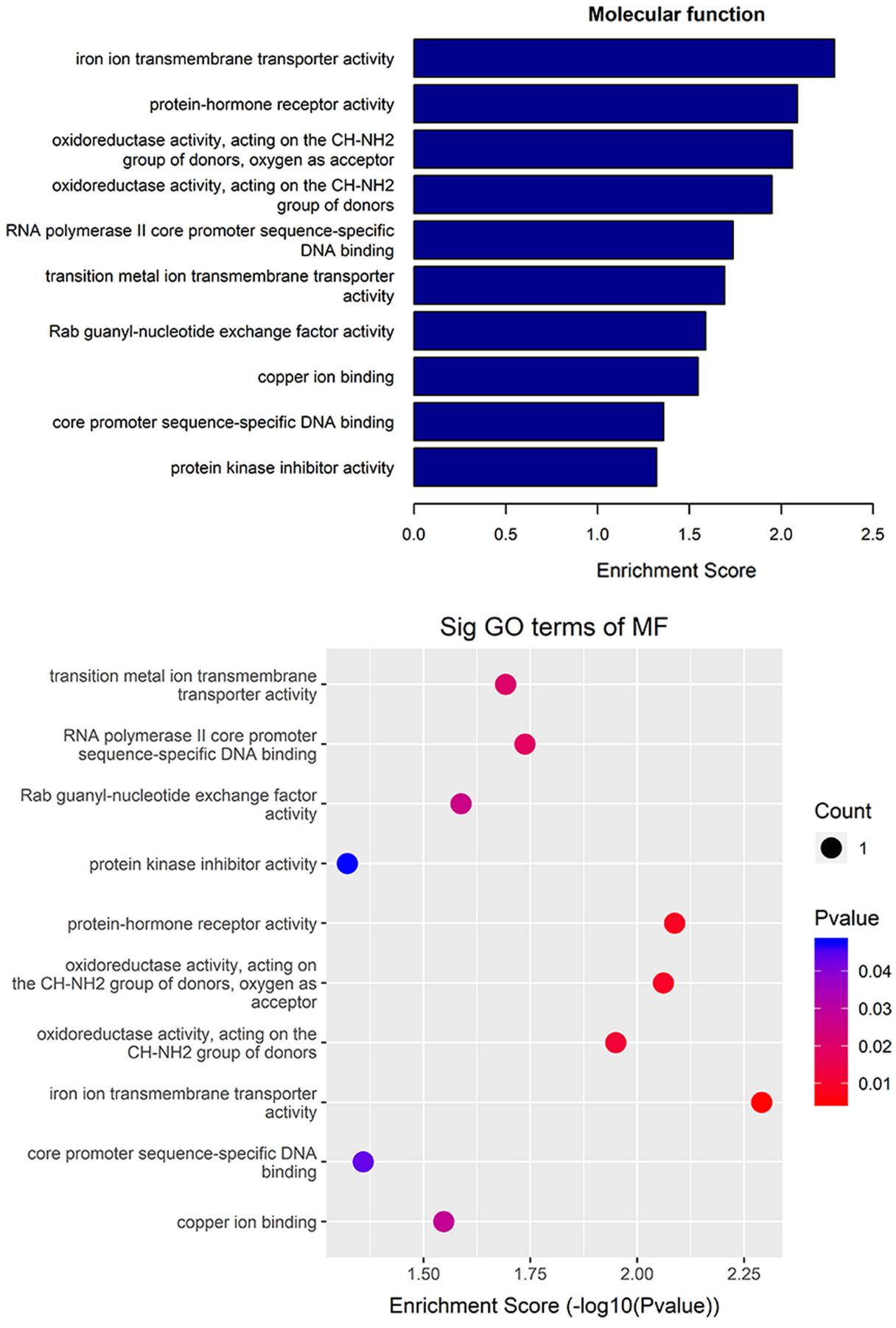


FIGURE 4 Significantly enriched gene ontology (GO) annotations based on upregulated lncRNAs. Top 10 molecular function (MF) annotations in the enrichment analysis

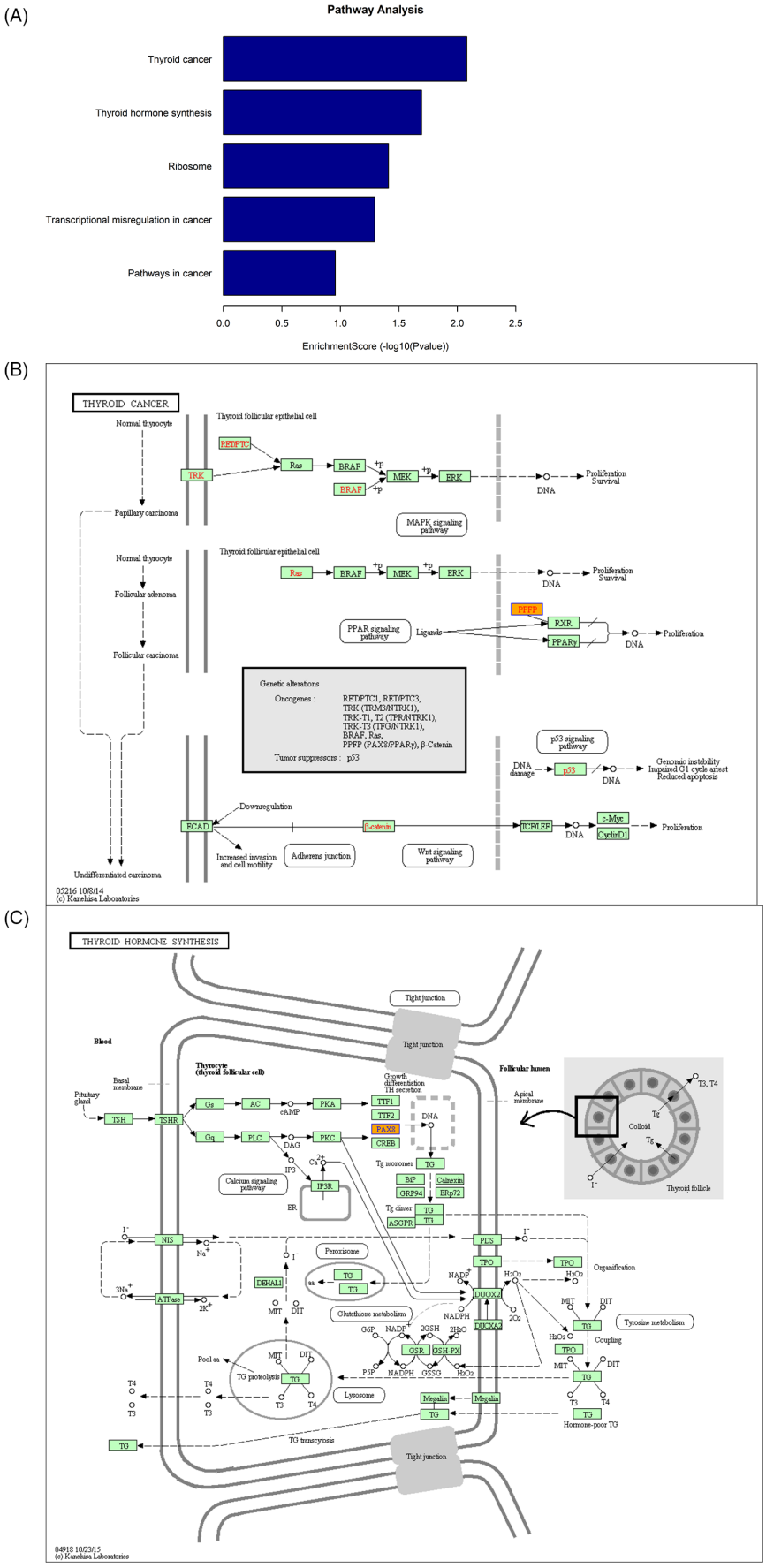


FIGURE 5 In the KEGG analysis, thyroid cancer, and the thyroid hormone synthesis pathways play a key role in the pathogenesis of AGA. (A) KEGG analysis revealed the top 5 signaling pathways with upregulated lncRNA enrichment. (B) Genes and response elements involved in the thyroid cancer pathway. (C) Genes and response elements are involved in the synthesis of thyroid hormones

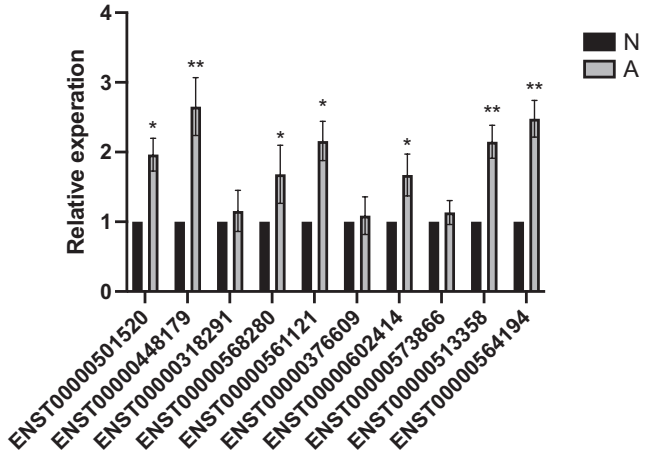


FIGURE 6 lncRNA sequencing results of samples acquired from 30 AGA patients and 30 normal volunteers were verified by qRT-PCR. In a comparison of the 2 groups, 7 lncRNAs in the AGA group were significantly upregulated (*$p < 0.05$, **$p < 0.01$)

of AGA to provide an experimental basis for the development of diagnostic markers and targeted therapies for AGA. In this study, we used high-throughput whole transcriptome sequencing to compare and analyze differentially expressed lncRNAs in the hair follicles of individuals with AGA and healthy individuals. We found that 84 lncRNAs were significantly differentially expressed, including 30 upregulated lncRNAs and 54 downregulated lncRNAs. The differentially expressed lncRNA target genes were subjected to GO and KEGG pathway enrichment analyses. GO enrichment analysis indicated that the differentially expressed lncRNAs were associated with the regulatory process of apoptosis, the guanylate exchange factor complex, and the activity of iron ion transmembrane transporters. KEGG pathway analysis indicated that differential lncRNAs were associated with thyroid cancer and thyroid hormone synthesis.

In terms of differential gene regulatory pathways, pathway enrichment analysis of the upregulated differentially expressed lncRNAs revealed that they were mainly involved in the development of thyroid cancer and the thyroid hormone synthesis pathway. Studies have demonstrated that thyroid hormone signaling is an important determinant of the mobilization of hair follicle stem cells from their niche in the hair bulge.[18] In clinical practice, insufficient and excessive levels of thyroid hormones (T3 and T4) both lead to hair loss in patients; T4 promotes hair keratinocyte proliferation, while T3 promotes cell apoptosis.[19] This finding indicates the importance of thyroid hormones for hair circulation. Therefore, we speculate that the upregulation of lncRNAs in the AGA group is related to signal transduction in thyroid hormone synthesis and might mediate hair loss.

We increased the number of specimens and performed qRT-PCR verification. We selected 10 upregulated lncRNAs based on their distribution of expression in each specimen. Seven lncRNAs that had the most obvious differences in expression are displayed in the PCR column chart. ROC analysis confirmed that ENST00000564194 (LOXL1-AS1) and ENST00000448179 could distinguish AGA patients from normal controls. Notably, LOXL1-AS1 appeared to have greater value as a diagnostic biomarker of AGA than the other lncRNAs. LOXL1-AS1 can target and compete with miR-5193, potentially leading to increased TP53 expression and promoting the apoptosis of hair follicle remodeling-related cells; this information further elucidates the pathogenesis of AGA and the identification of potential targets for treatment.

TABLE 2 lncRNA–miRNA relationship pairs and miRNA–mRNA relationship pairs.

LncRNA	miRNA
ENST00000318291	hsa-miR-2277-5p
ENST00000318291	hsa-miR-1539
ENST00000318291	hsa-miR-6887-3p
ENST00000318291	hsa-miR-4747-3p
ENST00000318291	hsa-miR-3937
ENST00000376609	hsa-miR-505-5p
ENST00000376609	hsa-miR-6805-5p
ENST00000376609	hsa-miR-6882-3p
ENST00000376609	hsa-miR-185-5p
ENST00000376609	hsa-miR-4450
ENST00000428504	hsa-miR-4433a-3p
ENST00000428504	hsa-miR-6804-3p
ENST00000428504	hsa-miR-3922-3p
ENST00000428504	hsa-miR-328-5p
ENST00000428504	hsa-miR-1273h-5p
ENST00000561121	hsa-miR-98-5p
ENST00000561121	hsa-miR-2909
ENST00000561121	hsa-let-7e-5p
ENST00000561121	hsa-let-7f-5p
ENST00000561121	hsa-miR-34c-5p
ENST00000564194	hsa-miR-30b-3p
ENST00000564194	hsa-miR-5193
ENST00000564194	hsa-miR-1273h-5p
ENST00000564194	hsa-miR-4421
ENST00000564194	hsa-miR-5699-3p
mRNA	**miRNA**
FAS	hsa-let-7f-5p
FAS	hsa-let-7e-5p
SMAD7	hsa-miR-4747-3p
TP53	hsa-miR-98-5p
FAS	hsa-miR-4433a-3p
TP53	hsa-let-7f-5p
TP53	hsa-let-7e-5p
FAS	hsa-miR-98-5p
SMAD7	hsa-miR-4433a-3p
WNT5A	hsa-miR-6882-3p
BAX	hsa-miR-6887-3p
TP53	hsa-miR-5193

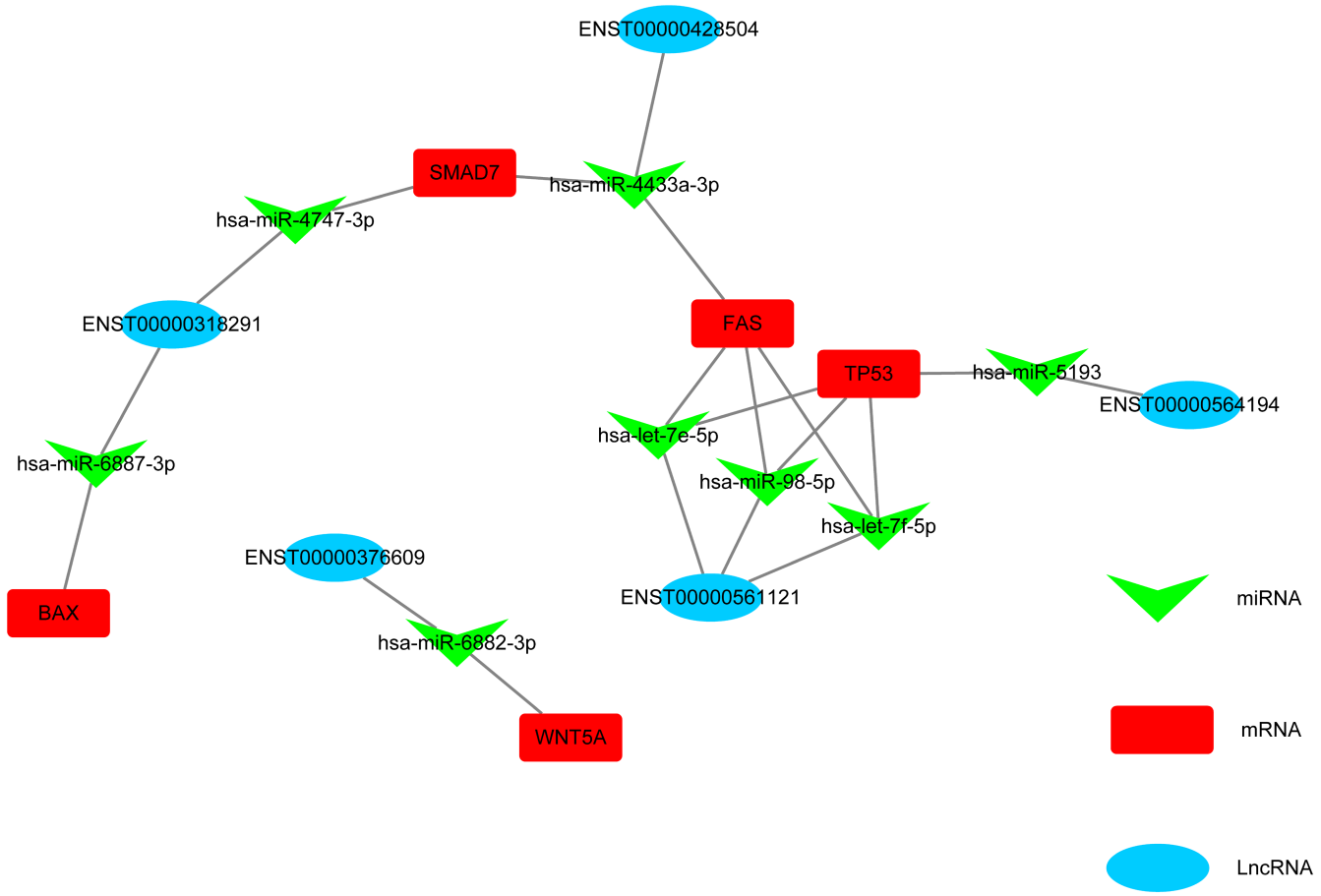
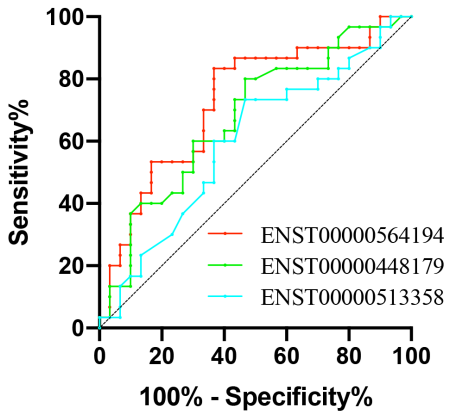


FIGURE 7 lncRNA–miRNA–mRNA regulatory network. Five lncRNAs (blue ellipses) may regulate target genes (red squares) through miRNAs (green arrows).

FIGURE 8 ROC analysis showing that the expression of LOXL1-AS1 (ENST00000564194) and ENST00000448179 had potential significance for the diagnosis of AGA, among which LOXL1-AS1 had better.



Variables	AUC	P value	95% CI	Sensitivity	Specificity
ENST00000564194	0.7278	0.0024	0.5975 to 0.8580	82.00	43.04
ENST00000448179	0.6767	0.0187	0.5401 to 0.8132	71.52	48.47
ENST00000513358	0.5983	0.1907	0.4533 to 0.7434	58.76	49.90

As a regulatory factor of posttranscriptional gene expression, miRNAs can bind to target genes, play important roles in the occurrence, development, and apoptosis of hair follicles, and regulate signaling pathways.[20] For example, miR-324-3p may promote the differentiation and migration of cultured keratinocytes through the regulation of mitogen-activated protein kinase (MAPK) and transforming growth factor (TGF)-β signaling.[21] In addition, the abnormally high expression of miR-133b in AGA patients indicates that miR-133b can be used as a biomarker to predict the occurrence of AGA and that miR-133b mediates the inactivation of the

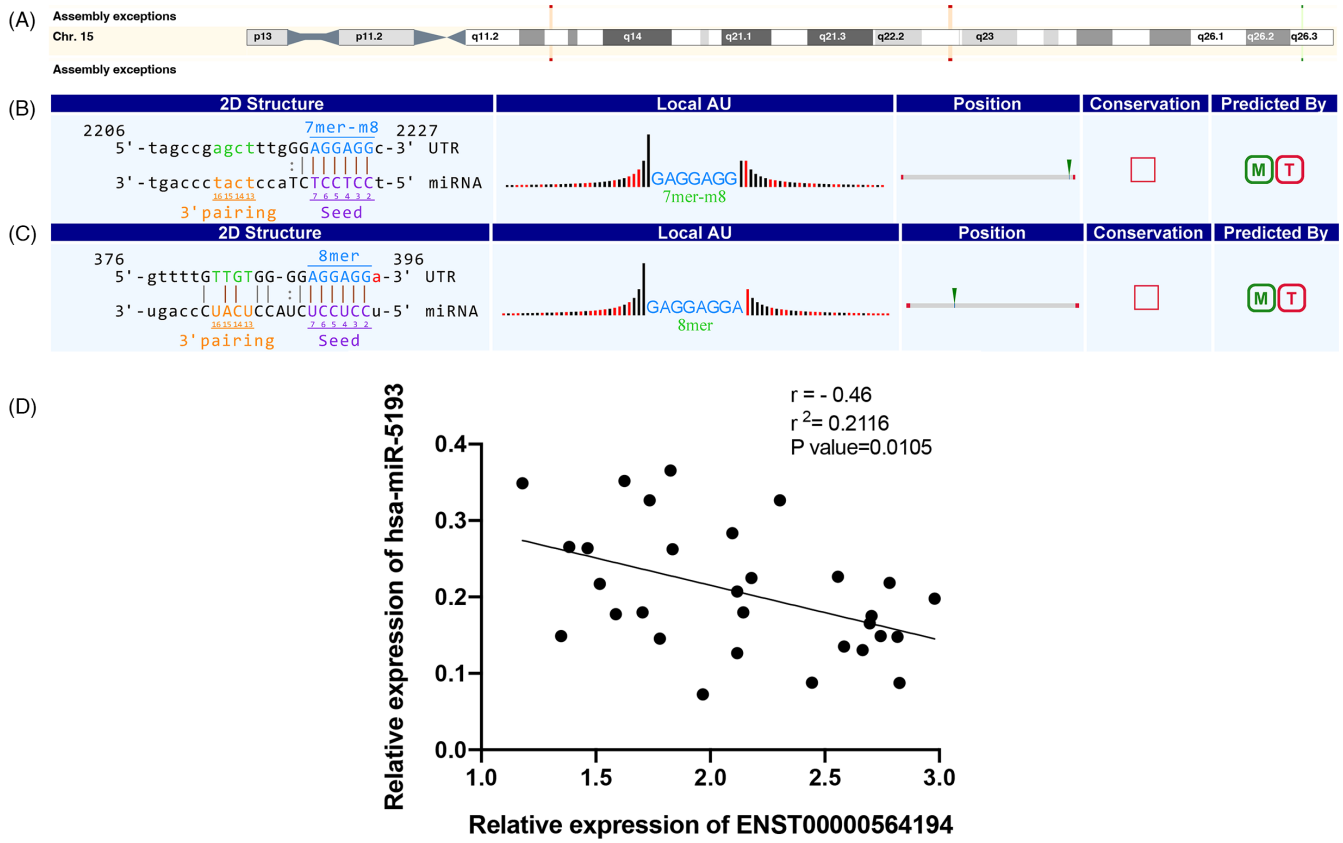


FIGURE 9 Predicted ceRNA relationship pairs discovered by sequencing. (A) The genetic locus of LOXL1-AS1 (ENST00000564194). (B) The binding sites of the LOXL1-AS1 3'UTR and hsa-miR-5193. (C) The binding site of the TP53 3'UTR and hsa-miR-5193. (D) In the AGA group, the expression of LOXL1-AS1 was negatively correlated with that of hsa-miR-5193 ($p = 0.0105$).

Wnt/β-catenin pathway and ultimately affects hair growth.[22] These studies confirmed the important role of miRNAs in AGA. miR-5193 is a newly discovered tumor-related miRNA. In vitro experiments have revealed that it can downregulate the expression of TRIM11 and upregulate the expression of P53, thereby inhibiting the proliferation and migration of ovarian cancer cells.[23] In addition, in prostate cancer tissues, miR-5193 acts on TRIM11 to mediate the development of prostate cancer, thereby increasing patient survival.[24] These studies have shown that miR-5193 regulates tumor progression, but there are few studies of its role in hair follicle growth-related cells.

Based on the sequencing results and biosynthesis analysis, we speculate that miR-5193 and TP53 have a targeting relationship and a reverse regulatory relationship. The tumor suppressor gene TP53 encodes P53.[25] The P53 protein induces cell cycle arrest or apoptosis through transcription-dependent and transcription-independent mechanisms. In addition, it is involved in the regulation of metabolism, antioxidant responses, and DNA repair.[26–28] In the process of P53-dependent cell apoptosis, some P53 proteins translocate to mitochondria to activate the caspase cascade pathway and mediate cell apoptosis.[29,30] Chemotherapy often stimulates hair follicles and leads to hair loss. However, in a P53-deficient mouse model, HF keratinocytes still actively proliferate after chemotherapy, and the animals do not exhibit hair loss after chemotherapy,[31] suggesting that P53 promotes cell apoptosis and is closely related to the regulation of hair loss.

In summary, the results of this study indicate that LOXL1-AS1 may bind to miR-5193 through a ceRNA mechanism, thereby regulating TP53 expression to mediate AGA. We used the miRanda and TargetScan databases to predict the potential binding sites between the LOXL1-AS1 3'UTR and hsa-miR-5193 and between hsa-miR-5193 and the TP53 3'UTR. Based on the results, we hypothesize that LOXL1-AS1 promotes cell apoptosis and the occurrence of AGA through positive regulation of TP53 expression. Thus, LOXL1-AS1/miR-5193/TP53 is expected to be a novel target for the diagnosis and treatment of AGA. However, future work should include functional studies and animal experiments to verify the above bioinformatics predictions and confirm the functional mechanism of LOXL1-AS1/miR-5193/TP53 in AGA.

AUTHOR CONTRIBUTIONS

All authors participated in the design, interpretation of the studies, and analysis of the data and review of the manuscript.

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CONFLICT OF INTEREST

The authors do not declare any financial interest. No ghostwriters were involved.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. York K, Meah N, Bhoyrul B, Sinclair R. A review of the treatment of male pattern hair loss. *Expert Opin Pharmacother*. 2020;21(5):603-612.
2. Piraccini BM, Blume-Peytavi U, Scarci F, et al. Efficacy and safety of topical finasteride spray solution for male androgenetic alopecia: a phase III, randomized, controlled clinical trial. *J Eur Acad Dermatol Venereol*. 2022;36(2):286-294.
3. Ceruti JM, Leirós GJ, Balañá ME. Androgens and androgen receptor action in skin and hair follicles. *Mol Cell Endocrinol*. 2018;465:122-133.
4. Guo Y, Qu Q, Chen J, Miao Y, Hu Z. Proposed mechanisms of low-level light therapy in the treatment of androgenetic alopecia. *Lasers Med Sci*. 2021;36(4):703-713.
5. Cecerska-Heryć E, Goszka M, Serwin N, et al. Applications of the regenerative capacity of platelets in modern medicine. *Cytokine Growth Factor Rev*. 2022;64:84-94.
6. Shi Y, Zhao J, Li H, et al. A drug-free, hair follicle cycling regulatable, separable, antibacterial microneedle patch for hair regeneration therapy. *Adv Healthc Mater*. 2022;11(19):e2200908.
7. Yang C, Shen C, Feng T, Li H. Noncoding RNA in NK cells. *J Leukoc Biol*. 2019;105(1):63-71.
8. Muskovic W, Slavich E, Maslen B, et al. High temporal resolution RNA-seq time course data reveals widespread synchronous activation between mammalian lncRNAs and neighboring protein-coding genes. *Genome Res*. 2022;32(8):1463-1473.
9. Yang M, Weng T, Zhang W, et al. The roles of non-coding RNA in the development and regeneration of hair follicles: current status and further perspectives. *Front Cell Dev Biol*. 2021;9:720879.
10. Dykes IM, Emanueli C. Transcriptional and post-transcriptional gene regulation by long non-coding RNA. *Genomics Proteomics Bioinformatics*. 2017;15(3):177-186.
11. Ma T, Li J, Li J, et al. Expression of miRNA-203 and its target gene in hair follicle cycle development of cashmere goat. *Cell Cycle*. 2021;20(2):204-210.
12. Zhu YB, Wang ZY, Yin RH, et al. A lncRNA-H19 transcript from secondary hair follicle of liaoning cashmere goat: identification, regulatory network and expression regulated potentially by its promoter methylation. *Gene*. 2018;641:78-85.
13. Zhu N, Lin E, Zhang H, et al. LncRNA H19 overexpression activates Wnt signaling to maintain the hair follicle regeneration potential of dermal papilla cells. *Front Genet*. 2020;11:694.
14. Lin BJ, Lin GY, Zhu JY, Yin GQ, Huang D, Yan YY. LncRNA-PCAT1 maintains characteristics of dermal papilla cells and promotes hair follicle regeneration by regulating miR-329/Wnt10b axis. *Exp Cell Res*. 2020;394(1):112031.
15. Tang W, Lu Q, Zhu J, et al. Identification of a prognostic signature composed of GPI, IL22RA1, CCT6A and SPOCK1 for lung adenocarcinoma based on Bioinformatic analysis of lncRNA-mediated ceRNA network and sample validation. *Front Oncol*. 2022;12:844691.
16. Bao L, Yu A, Luo Y, et al. Genomewide differential expression profiling of long non-coding RNAs in androgenetic alopecia in a Chinese male population. *J Eur Acad Dermatol Venereol*. 2017;31(8):1360-1371.
17. Kim IB, Lee T, Lee J, et al. Non-coding de novo mutations in chromatin interactions are implicated in autism spectrum disorder. *Mol Psychiatry*. 2022. https://www.nature.com/articles/s41380-022-01697-2
18. Contreras-Jurado C, Lorz C, García-Serrano L, Paramio JM, Aranda A. Thyroid hormone signaling controls hair follicle stem cell function. *Mol Biol Cell*. 2015;26(7):1263-1272.
19. van Beek N, Bodó E, Kromminga A, et al. Thyroid hormones directly alter human hair follicle functions: anagen prolongation and stimulation of both hair matrix keratinocyte proliferation and hair pigmentation. *J Clin Endocrinol Metab*. 2008;93(11):4381-4388.
20. He Y, Liu X, Qu Q, et al. Overexpression of miR-122 promotes apoptosis of dermal papilla cells by directly targeting IGF1R in androgenetic alopecia. *Cell Biol Int*. 2022;46(2):185-191.
21. Mohammadi P, Nilforoushzadeh MA, Youssef KK, et al. Defining microRNA signatures of hair follicular stem and progenitor cells in healthy and androgenic alopecia patients. *J Dermatol Sci*. 2021;101(1):49-57.
22. Deng W, Hu T, Han L, et al. miRNA microarray profiling in patients with androgenic alopecia and the effects of miR-133b on hair growth. *Exp Mol Pathol*. 2021;118:104589.
23. Song Z, Guo Q, Wang H, et al. miR-5193, regulated by FUT1, suppresses proliferation and migration of ovarian cancer cells by targeting TRIM11. *Pathol Res Pract*. 2020;216(11):153148.
24. Pan Y, Zhang R, Chen H, Chen W, Wu K, Lv J. Expression of tripartite motif-containing proteactiin 11 (TRIM11) is associated with the progression of human prostate cancer and is downregulated by MicroRNA-5193. *Med Sci Monit*. 2019;25:98-106.
25. Levine AJ. Spontaneous and inherited TP53 genetic alterations. *Oncogene*. 2021;40(41):5975-5983.
26. Hu W, Chen S, Thorne RF, et al. TP53, TP53 target genes (DRAM, TIGAR), and autophagy. *Adv Exp Med Biol*. 2019;1206:127-149.
27. Tang M, Xu H, Huang H, et al. Metabolism-based molecular subtyping endows effective ketogenic therapy in p53-mutant colon cancer. *Adv Sci (Weinh)*. 2022;9(29):e2201992.
28. Gonzalez-Rellan MJ, Fondevila MF, Fernandez U, et al. O-GlcNAcylated p53 in the liver modulates hepatic glucose production. *Nat Commun*. 2021;12(1):5068.
29. Shteinfer-Kuzmine A, Verma A, Arif T, Aizenberg O, Paul A, Shoshan-Barmaz V. Mitochondria and nucleus cross-talk: signaling in metabolism, apoptosis, and differentiation, and function in cancer. *IUBMB Life*. 2021;73(3):492-510.
30. Ferecatu I, Bergeaud M, Rodríguez-Enfedaque A, et al. Mitochondrial localization of the low level p53 protein in proliferative cells. *Biochem Biophys Res Commun*. 2009;387(4):772-777.
31. Botchkarev VA, Komarova EA, Siebenhaar F, et al. p53 is essential for chemotherapy-induced hair loss. *Cancer Res*. 2000;60(18):5002-5006.

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