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Bioinformatic analysis of a microRNA regulatory network in Huntington's disease

Zhi-Min Wang[1], Xiao-Yu Dong[1] and Shu-Yan Cong[1,*]

[1]*Department of Neurology, Shengjing Hospital of China Medical University, Shenyang, 110004, Liaoning Province, P. R. China*

*Correspondence: congsy@sj-hospital.org (Shu-Yan Cong)

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Huntington's disease is an autosomal dominant hereditary neurodegenerative disease characterized by progressive dystonia, chorea and cognitive or psychiatric disturbances. The leading cause is the Huntington gene mutation on the patient's chromosome 4 that produces a mutated protein. Recently, attention has focused on the relationship between microRNAs and Huntington's disease's pathogenesis. In Huntington's disease, microRNAs can interact with various transcription factors; dysregulated microRNAs may be associated with the Cytosine deoxynucleotide-Adenine ribonucleotides-Guanine ribonucleotide length and Huntington's disease's progression and severity. This study explores the role of microRNAs in the pathogenesis of Huntington's disease through bioinformatics analysis. By analyzing data from the Gene Expression Omnibus database, we identified a total of 9 differentially expressed microRNA. Subsequently, target genes and long non-coding RNAs were predicted, and a comprehensive regulatory network centered on microRNA was constructed. The microRNA integrated regulatory network, *Homo sapiens* (*hsa*)-miR-144-3p, interacted with the largest number of long non-coding RNAs, including X-inactive specific transcript and taurine upregulated gene 1. The miRNAs, hsa-miR-10b-5p and hsa-miR-196a-5p, regulated most of the target genes, including class I *homeobox* and brain-derived neurotrophic factor genes. Additionally, 59 Gene Ontology terms and eight enrichment pathways were identified by analyzing the target genes of hsa-miR-196a-5p and hsa-microRNA-10b-5p. In conclusion, hsa-miR-10b-5p and hsa-miR-196a-5p were significantly and differentially expressed in Huntington's disease, the long non-coding RNAs X-inactive specific transcript, taurine upregulated gene 1, and target genes such as *homeobox* or brain-derived neurotrophic factor may play critical roles in the pathogenesis of Huntington's disease.

Keywords

Huntington's disease; long non-coding RNAs; microRNA; target gene; regulatory network

1. Introduction

Huntington's disease (HD) is an autosomal dominant hereditary neurodegenerative disease with an incidence of 5-10 per 100,000 individuals in Western countries (Walker, 2007). It is characterized by progressive dystonia, chorea, cognitive or psychiatric disorders (Huang et al., 2016) and is caused by the dominant heterozygous amplification of the Cytosine deoxynucleotide-Adenine ribonucleotides-Guanine ribonucleotide (CAG) trinucleotide repeat sequence, which encodes multiple glutamine residues in the gene encoding the huntingtin (Htt) protein (Hu et al., 2010). Over the years, a large amount of literature has described the probable molecular mechanisms associated with neuronal dysfunction and degeneration in HD, including misfolding aggregation and the decreased clearance of mutant *Htt* (m*Htt*), autophagy, transcriptional dysregulation, mitochondrial dysfunction, and a damaged ubiquitin-proteasome system (McColgan and Tabrizi, 2018). Among various molecular changes in HD, understanding gene expression dysregulation may reveal possible pathogenesis and provide potential therapeutic HD strategies.

MicroRNAs (miRNAs) are a class of endogenous non-coding RNAs found in eukaryotes that have regulatory functions. They are about 20-25 nucleotides long and identify each target mRNA through complementary base pairing. In accordance with the degree of complementarity, differently guided silencing complexes target mRNA degradation or translation repression of the target mRNA (Rodriguez, 2004; Saraiva et al., 2017). MicroRNAs are expressed in all tissues and are thought to regulate around 30% of the entire human genome (Godnic et al., 2013). In the central nervous system, miRNAs appear to have the greatest diversity (Thomson et al., 2004). Emerging studies have shown that miRNAs may play a critical role in the pathogenesis and development of neurodegenerative disorders (Rinchetti et al., 2018). Indeed, several miRNAs can interact with several transcriptional cofactors involved in HD, including *RelA/NF-κB*, *p53* (Ghose et al., 2011), *Mitofusin2* (Bucha et al., 2015), Tata Binding Protein (*TBP*) (Sinha et al., 2010), *REST*, and *RE1* (Johnson et al., 2008). The level of miRNA dysregulation may correlate with the length of the CAG repeat in the m*Htt* allele and be involved in the progression or severity of HD (Langfelder et al., 2018). MiR-146a could target *TBP*, so dysregulation of TBP by miRNA-146a may contribute to HD pathogenesis (Sinha et al., 2010). Reynolds et al. (2018) found

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Table 1. Differentially expressed miRNAs list

Name	State	Sequence	$\log_2$FC	*P*-value	Chromosome location
hsa-miR-10b-5p	Up	uacccuguagaaccgaauuugug	3.94	1.28×10^{-20}	chr2: 176150303-176150412 [+]
hsa-miR-196a-5p	Up	uagguaguuucauguuguuggg	2.35	2.97×10^{-20}	chr17: 48632490-48632559 [-]
hsa-miR-615-3p	Up	uccgagccugggucucccucuu	1.59	2.33×10^{-16}	chr12: 54033950-54034045 [+]
hsa-miR-10b-3p	Up	acagauucgauucuaggggaau	1.45	2.13×10^{-12}	chr2: 176150303-176150412 [+]
hsa-miR-196b-5p	Up	uagguaguuuccuguuguuggg	1.31	2.33×10^{-8}	chr7: 27169480-27169563 [-]
hsa-miR-483-5p	Up	aagacgggaggaaagaagggag	1.16	1.6×10^{-3}	chr11: 2134134-2134209 [-]
hsa-miR-144-3p	Up	uacaguauagaugauguacu	1.08	9.16×10^{-6}	chr17: 28861533-28861618 [-]
hsa-miR-4449	Down	cgucccggggcugcgcgaggca	1.09	5.0×10^{-4}	chr4: 52712682-52712747 [-]
hsa-miR-4488	Down	agggggcgggcuccggcg	1.32	2.0×10^{-3}	chr11: 61508596-61508657 [+]

Abbreviations: miRNA, microRNA; FC, Fold Change.

the decreased levels of miRNA-34a-5p could increase p53 protein levels in brain tissue from R6/2 mice and promote progression in an HD model. These results suggested that dysregulated neuronal miRNAs might be related to the pathogenesis of HD. Moreover, modulating the expression of miRNAs could also exert therapeutic effects. It was suggested that miR-124 might slow down HD's progression through its essential role in neuronal differentiation and survival (Kim et al., 2015). Ban et al. (2017) suggested that miR-27a could reduce the HD cell's mHtt level by augmenting multidrug resistance protein-1 (MDR-1) function.

Long non-coding RNAs (lncRNAs) are a class of non-coding RNAs greater than 200 nt in length and similar in gene structure to mRNAs (Wang and Chang, 2011). LncRNAs have also been reported to participate in many important biological processes, including transcriptional and post-transcriptional regulation (Mercer et al., 2009). Several studies have shown dysfunctional or mutated lncRNAs are related to the pathogenesis of HD (Cheng et al., 2013). Chanda et al. (2018) reported that the *Neat1*, X-inactive specific transcript (*Xist)* and *Meg3* showed a significant increase in HD cell and animal models. Knock-down of *Meg3* and *Neat1* could down-regulate endogenous *Tp53* levels and reduce aggregates formed by m*Htt* in ell models of HD. Sunwoo et al. (2017) demonstrated the *NEAT1* was up-regulated in HD animal model, which may contribute to the neuroprotective mechanism against neuronal injury . Overexpression of *Abhd11os* could produce neuroprotection against an N-terminal fragment of m*Htt*, which indicated that the loss lncRNA *Abhd11os* probably contributes to striatal vulnerability in HD (Francelle et al., 2015). Therefore, investigating lncRNA's HD role is essential for understanding HD's pathogenesis and providing possible therapeutic strategies.

This study used data from Hoss et al. (2015) for bioinformatics analysis, who analyzed different miRNA expression profiles from the brains of 26 HD patients, two asymptomatic HD patients 36 normal controls. According to the identified differential expression of miRNAs, we predicted target genes and related lncRNAs and constructed a miRNA-centered integrated regulatory network. Pathway and functional enrichment analyses of target genes were analyzed using a database for annotation and visualization and comprehensive discovery software. Our purpose was to further explore the involvement of miRNAs in the pathogenesis of HD and to attempt to elucidate the comprehensive miRNA regulatory network in HD.

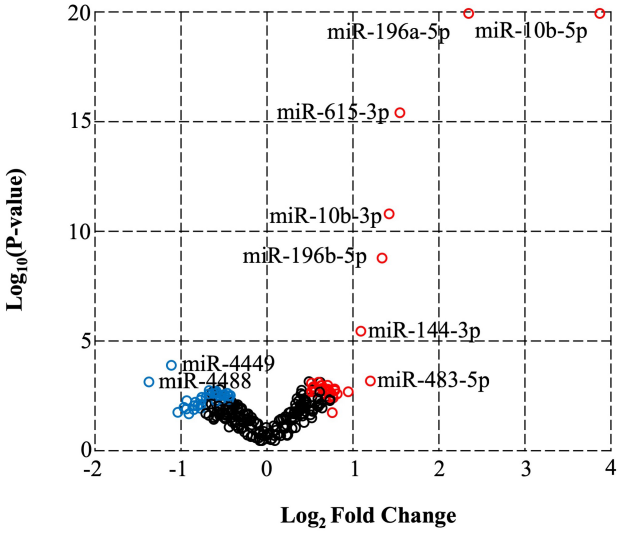

Fig. 1. Volcano diagrams of 9 significantly differentially expressed micro (mi) RNA after FDR-adjustment. Points labeled red were up-regulated and points labeled as blue were down-regulated in HD.

2. Materials and methods

2.1 Data source and pre-processing

The data in this study were extracted from Hoss et al. (2015). This investigation aimed to explore the possible mechanisms of dynamic regulation of miRNAs affecting HD gene expression and altering HD progression and severity. They conducted next-generation miRNA sequence analysis on the prefrontal cortex (Brodmann Area 9) of 26 HD patients, two asymptomatic HD patients, and 36 controls. Finally, 75 miRNAs that were differentially expressed in the HD brain (false discovery rate *P*-value < 0.05) were identified. We further screened nine miRNAs according to our criteria, where an adjusted $P < 0.05$ and $|\log_2$ fold-change (FC)$| > 1$ were used as thresholds. Based on the screening of miRNAs, a regulatory network was constructed to further elucidate the miRNAs' roles in HD's pathogenesis.

Target gene prediction. Five public algorithms predicted the target genes regulated by miRNAs, including TargetScan (http://targetscan.org) (Lewis et al., 2003), PicTar (http://pictar.bio.nyu.edu) (Krek et al., 2005), miRanda (http://microrna.sanger.ac.uk) (Enright et al., 2004), miRWalk (http://mirwalk.uni-h

d.de/), and miRecords (http://miRecords.umn.edu/miRecords) (Dweep et al., 2011; Xiao et al., 2009). When a miRNA gene pair was predicted by three of the algorithms mentioned above, it was recorded.

2.2 miRNA-lncRNA regulatory relationship construction

The StarBase v2.0 (http://starbase.sus.edu.cn/) was developed to decipher protein-RNA and miRNA-target interactions, such as protein-lncRNA, miRNA-lncRNA, miRNA-mRNA interactions, and networks from a 108 Cross-linking Immunoprecipitation combined with a high-throughput Sequencing dataset. StarBase also provided miR function web tools to analyze non-coding (nc) RNAs (miRNAs, lncRNAs) and target genes in miRNA-centered regulatory networks. In this study, the interaction networks of miRNA-lncRNAs related to the nine differentially expressed miRNAs were extracted from starBase v2.0 and reserved for further analysis.

2.3 Pathway and functional enrichment analysis of target genes

The Database for Annotation Visualization and Integrated Discovery (DAVID) (http://david.abcc.ncifcrf.gov) (Huang et al., 2009) online analysis tool constitutes a comprehensive bioinformatics database. This system can mine the biological functions of many genes and play a key role in further extracting biological, genetic information. The Gene Ontology Database (GO; www.Geneontology.org) describes the basic characteristics of genes and their products. Kyoto Encyclopedia of Genes and Genomes (KEGG; www.genome.jp/kegg) uses DAVID to perform pathway enrichment analysis on miRNAs targeting genes. $P < 0.05$ was considered statistically significant.

2.4 Construction of miRNA regulatory network and visualization analysis

Cytoscape is an open-source biological information analysis software that can build a visual network diagram of molecular interactions. The connection between nodes indicates interaction between each other and can integrate existing gene expression information into the network diagram. So it is easier to observe the correlation between protein and protein (Smoot et al., 2011). We reserved and obtained miRNA-lncRNA and miRNA-mRNA interaction networks and subsequently visualized these using Cytoscape. The GO plot package in R language was used to visualize the GO and KEGG results of target genes (Walter et al., 2016).

3. Results

3.1 Identification of differentially expressed miRNAs

An adjusted $P < 0.05$ and $|\log_2 FC| > 1$ were set as thresholds. A total of nine differentially expressed miRNAs were identified, including seven up-regulated and two down-regulated HD patients compared with healthy controls Table 1 and Fig. 1.

3.2 miRNA-mRNA and miRNA-lncRNA comprehensive regulatory network

According to predicted miRNA-mRNA and miRNA-lncRNA regulatory networks, a miRNA-centered, comprehensively regulated network was constructed (Fig. 2). The network showed the hsa-miR-144-3p regulated the largest number of lncRNAs, including *XIST* and taurine upregulated gene 1 (*TUG1*). The miRNAs, hsa-miR-196a-5p and hsa-miR-10b-5p, regulated most target genes, including class I *homeobox* (*HOX)* and brain-derived neurotrophic factor *(BDNF).*

3.3 Function enrichment analysis of hsa-miR-196a-5p target genes

Sixty-nine target genes of hsa-miR-196a-5p were identified by performing GO enrichment analysis. We found such genes were mostly enriched in 59 GO terms, such as anterior/posterior pattern specification ($P = 4.80 \times 10^{-7}$), sequence-specific DNA binding ($P = 2.97 \times 10^{-6}$), and transcription from the RNA polymerase II promoter ($P = 1.04 \times 10^{-4}$). The top 10 functions enriched for target genes are listed in Table 2; 18 GO terms ($P < 0.01$) are shown in Fig. 3. Pathway enrichment analysis for the target genes of hsa-miR-196a-5p was mainly enriched in the gonadotropin-releasing hormone (*GnRH*) signaling pathway ($P = 0.002$), neurotrophin signaling pathway ($P = 0.004$) and insulin signaling pathway ($P = 0.006$) (Table 3, Fig. 4).

3.4 Hsa-miR-10b-5p target genes GO enrichment analysis

Fifty-eight target genes of hsa-miR-10b-5p were identified, which were mainly enriched in nucleus ($P = 6.37 \times 10^{-4}$), nucleoplasm ($P = 1.55 \times 10^{-5}$), and transcription, DNA-templated (7.78×10^{-4}) (Table 4, Fig. 5).

3.5 Targeted lncRNAs of multiple differentially expressed miRNAs

By retrieving from starBase v2.0, five of nine differentially expressed miRNAs possessed a regulatory relationship with lncRNAs. We found hsa-miR-144-3p regulated the largest number of lncRNAs, including XIST and TUG1. By analyzing the regulatory network, we found some of the lncRNAs were targeted by multiple differentially expressed miRNAs, such as XIST, TUG1 and GS1-358P8.4. LncRNAs targeted by at least two miRNAs are shown in Table 5.

4. Discussion

HD is an autosomal dominant neurodegenerative disease caused by CAG repeated amplification of the *Htt*; however, HD's exact pathophysiology remains obscure. Although a large number of early investigations have focused on the effect of transcriptional dysregulation of gene expression on HD, recent studies have shown that post-transcriptional mechanisms are involved in the pathogenesis of HD (Juźwik et al., 2019). Specifically, neuronal miRNAs have been shown to participate in the post-transcriptional regulation of target genes (Miniarikova et al., 2018). The results from Hoss et al. (2015) are one of the latest and most important contributions to this field. They performed an unbiased analysis of next-generation miRNA sequence in the prefrontal cortex (Brodmann Area 9) of brains from 26 HD patients, two asymptomatic HD patients, and 36 controls. Seventy-five differentially expressed miRNAs were identified in the HD brain. Their results enabled us to focus on several miRNAs using more stringent screening criteria. In the present study, we chose an adjusted $P < 0.05$ and $|\log_2 FC| > 1$ as thresholds and identified nine differentially expressed miRNAs associated with HD. We consequently constructed a miRNA-centered comprehensive regulatory network. To our best knowledge, this was the first time that a regulatory network study was constructed containing lncRNAs in HD. Of these, hsa-miR-196a-5p and hsa-miR-10b-5p were highly and significantly regulated; they also had the largest number of target genes.

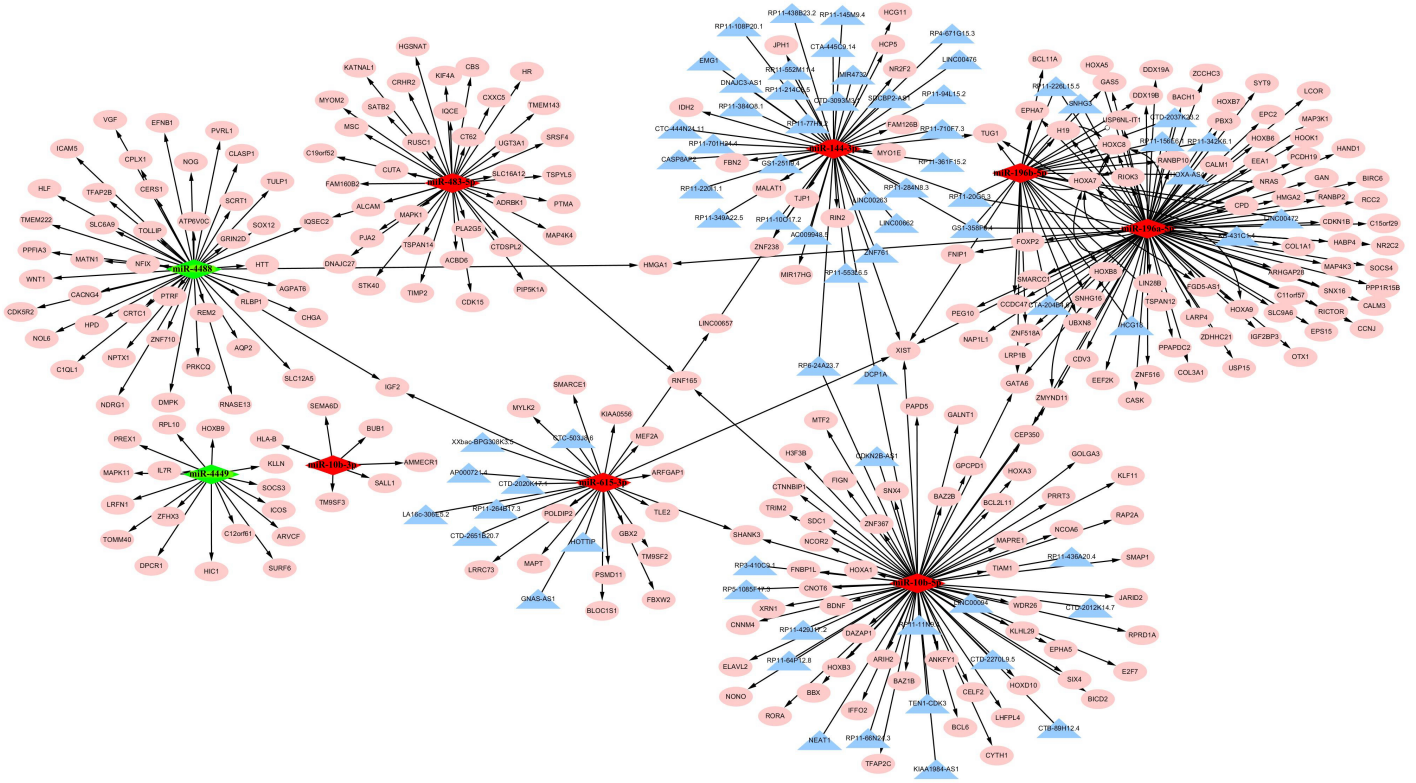

Fig. 2. A micro(mi)RNA-centered comprehensive regulatory network. The rhombus represents miRNAs (red represents up-regulated, green represents down-regulated); pink circles represent target genes; blue triangles represent long non-coding (lnc)RNAs.

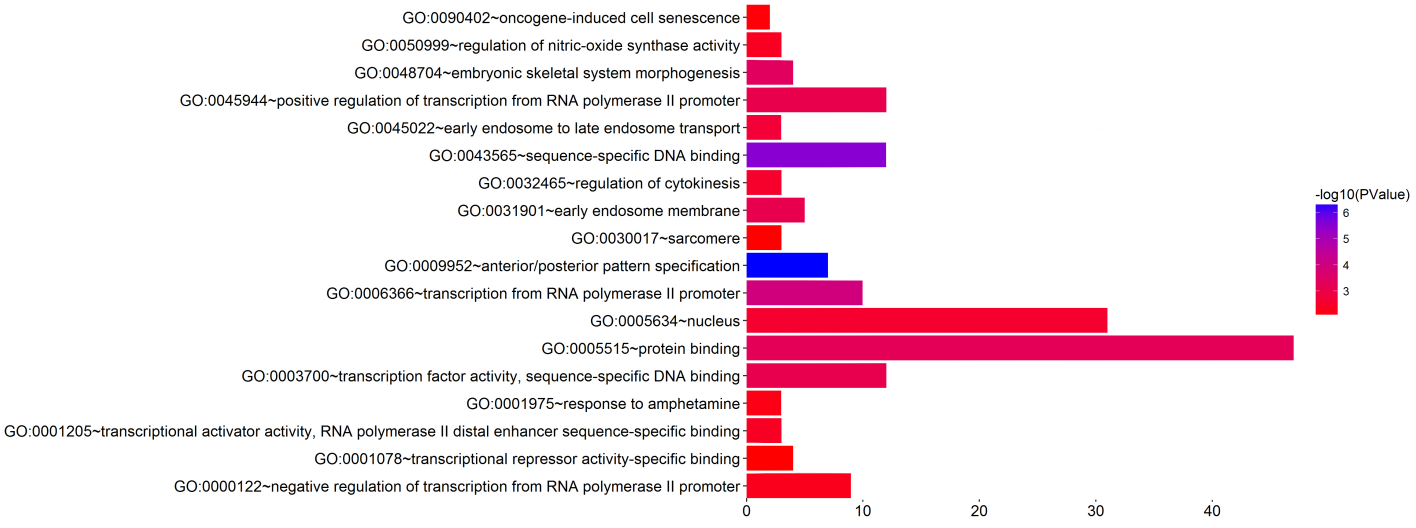

Fig. 3. To understand the biological processes, cell components, and molecular functions involved in target genes of (hsa)-miR-196a-5p. The GO gene enrichment analysis of target genes was obtained, as shown in (Fig. 3) (terms containing more genes tended to have a more significant *P*-value).

It has been reported the hsa-miR-196a participates in the pathogenesis and progression of HD (Cheng et al., 2013). Fu et al. (2015) believed hsa-miR-196a could alter the RIG-I-like receptor signaling pathway and change certain well-defined pathways expression in HD, such as cell adhesion and apoptosis. In this study, we found hsa-miR-196a-5p mainly interacted with *HOX* genes, which had been reportedly involved indirectly in the neuroprotective response in HD. Hoss et al. (2014) reported that increased *HOX* genes expression could enhance H3K27me3 or reduce Polycomb G group (PcG) repression; their findings suggested the possibility of increased miRNAs and *HOX* genes expression might be associated with enhanced H3K27me3 or reduced PcG repression, and that hsa-miR-196a-5p might be participated in the progression of HD by regulating *HOX* genes expression. Through

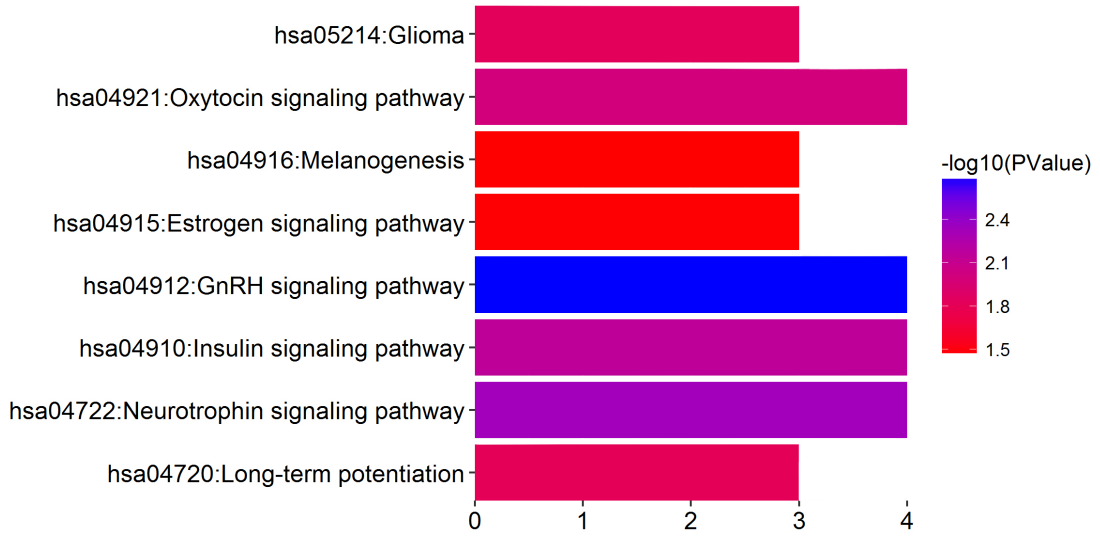

Fig. 4. Pathway enrichment analysis for the target genes of hsa-miR-196a-5p was shown in (Fig. 4), which is mainly enriched in the gonadotropin-releasing hormone GnRH signaling, Neurotrophin signaling and Insulin signaling pathways (terms containing more genes tended to have a more significant *P*-value).

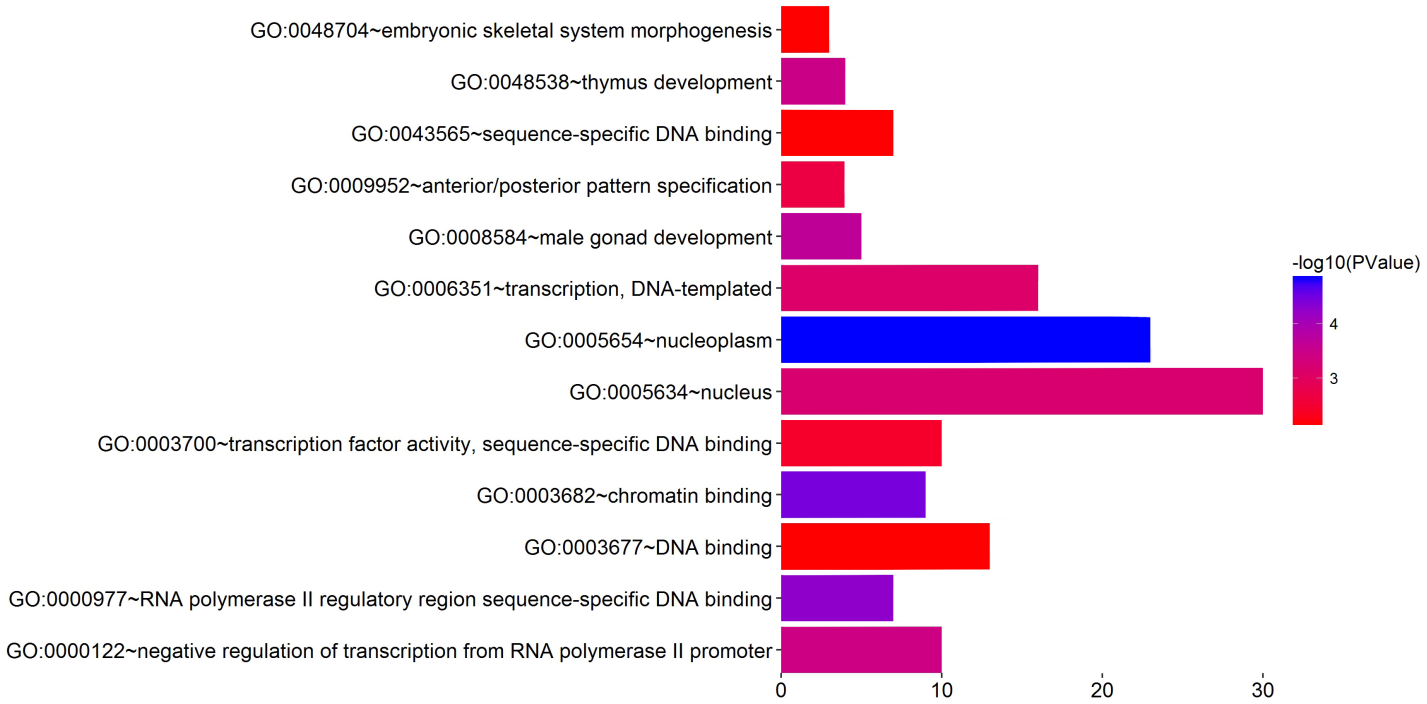

Fig. 5. The GO gene enrichment analysis of *Homo sapiens* (hsa)-miR-10b-5p target genes were obtained, as shown in (Fig. 5) (terms containing more genes tended to have a more significant *P*-value).

function enrichment analysis of hsa-miR-196a-5p target genes, we found that most genes were enriched in protein binding, the nucleus, the regulation of transcription from the RNA polymerase II promoter, and transcription factor activity. Such conclusions are consistent with previous research findings that suggested that the transcriptional dysregulation of gene expression might be one of the main pathogenic changes, which confirmed the role of hsa-miR-196a-5p in HD. Cheng et al. (2013) suggested that miR-196a suppresses m*Htt* directly in the brain and improves neuropathological progression. MiR-196a could also suppress the RAN binding protein 10 (*RANBP10*) expression by binding to its 3' UTR, which could exacerbate neuronal morphology and intracellular transport. Also, miR-196a might enhance neuronal morphology by suppressing *RANBP10* and increasing the ability of β-tubulin polymerization, suggesting its therapeutic effects in HD patients (Her et al., 2017). Furthermore, overexpression of miR-196a could surpass the expression of apoptosis-related genes and improve mitochon-

Table 2. Gene ontology enrichment analysis for the target genes of hsa-miR-196a-5p (top 10)

ID	Description	*P*-value	Total no. of genes	Genes
GO: 0009952	anterior/posterior pattern specification	4.80×10^{-7}	7	H*OXC8, HOXB7, HOXA5, OTX1, HOXA7, HOXB6, HOXA9*
GO: 0043565	sequence-specific DNA binding	2.97×10^{-6}	12	*BACH1, ZNF516, HOXC8, HOXB7, HOXA5, OTX1, HOXA7, HOXB6, HOXA9, PBX3, NR2C2, FOXP2*
GO: 0006366	transcription from RNA polymerase II promoter	1.04×10^{-4}	10	*BACH1, ZNF516, HAND1, HOXA5, GATA6, OTX1, HOXA7, HMGA2, PBX3, HMGA1*
GO: 0048704	embryonic skeletal system morphogenesis	3.98×10^{-4}	4	*HOXB7, HOXA5, HOXA7, HOXB6*
GO: 0005515	protein binding	5.34×10^{-4}	47	*BACH1, ZMYND11, COL3A1, NAP1L1, CASK, EEA1, IGF2BP3, LIN28B, NR2C2, HOOK1, PEG10, HAND1, GATA6, DDX19A, HOXA5, DDX19B, MAP3K1, HOXA9, RANBP2, C11ORF57, USP15, OTX1, CCDC47, BIRC6, SOCS4, GAN, RICTOR, HMGA2, HMGA1, FNIP1, FOXP2, MAP4K3, EPS15, EPHA7, CDKN1B, RCC2, CEP350, HOXB7, RIOK3, SMARCC1, HOXB6, HABP4, CALM3, COL1A1, LCOR, PPP1R15B, CALM1*
GO: 0031901	early endosome membrane	7.38×10^{-4}	5	*EPS15, SLC9A6, RCC2, SNX16, EEA1*
GO: 0003700	transcription factor activity, sequence-specific DNA binding	7.75×10^{-4}	12	*BACH1, ZNF516, HOXC8, HOXB7, HOXA5, GATA6, OTX1, HOXB6, PBX3, HMGA1, NR2C2, FOXP2*
GO: 0045944	positive regulation of transcription from RNA polymerase II promoter	8.35×10^{-4}	12	*BACH1, HAND1, HOXA5, GATA6, OTX1, SMARCC1, HOXA7, CASK, HMGA2, PBX3, HMGA1, NR2C2*
GO: 0045022	early endosome to late endosome transport	2.0×10^{-3}	3	*HOOK1, SNX16, EEA1*
GO: 0005634	nucleus	3.0×10^{-3}	31	*BACH1, ZMYND11, ZNF516, NAP1L1, IGF2BP3, NR2C2, PEG10, HAND1, GATA6, DDX19A, HOXA5, DDX19B, HOXA7, HOXA9, USP15, CCNJ, OTX1, HMGA2, HMGA1, FOXP2, CDKN1B, RCC2, HOXB7, HOXB6, CALM3, HABP4, CPD, PBX3, LCOR, CALM1, RANBP10*

Abbreviations: GO, Gene Ontology.

Table 3. Pathway enrichment analysis for the target genes of hsa-miR-196a-5p

ID	Description	*P*-value	Total no. of genes	Genes
hsa04912	GnRH signaling pathway	0.002	4	*NRAS, MAP3K1, CALM3, CALM1*
hsa04722	Neurotrophin signaling pathway	0.005	4	*NRAS, MAP3K1, CALM3, CALM1*
hsa04910	Insulin signaling pathway	0.007	4	*NRAS, CALM3, SOCS4, CALM1*
hsa04921	Oxytocin signaling pathway	0.01	4	*NRAS, EEF2K, CALM3, CALM1*
hsa05214	Glioma	0.01	3	*NRAS, CALM3, CALM1*
hsa04720	Long-term potentiation	0.02	3	*NRAS, CALM3, CALM1*
hsa04915	Estrogen signaling pathway	0.03	3	*NRAS, CALM3, CALM1*
hsa04916	Melanogenesis	0.03	3	*NRAS, CALM3, CALM1*

Abbreviations: GO, Gene Ontology.

drial morphology and activity by upregulating CREB Binding Protein (*CBP*) and peroxisome proliferator-activated receptorγcoactivator-1 (*PGC-1*α) expression (Kunkanjanawan et al., 2016).

Levels of plasma hsa-miR-10b-5p are significantly upregulated in HD patients compared with asymptomatic HD gene carriers and healthy controls (Hoss et al., 2017). Hoss et al. (2015) confirmed that hsa-miR-10b-5p showed the most prominent associations with age of onset, disease stage, and the extent of neuropathological impairment in HD patients. In many target genes, we found brain-derived neurotrophic factor (*BDNF)* was one of the target genes of hsa-miR-10b-5p, critical to striatal neurons (Zuccato et al., 2003). Reduced regulation of *BDNF* transcription in the cerebral cortex by the dysregulated expression of hsa-miR-10b-5p might be a leading candidate mechanism for striatal neuronal death in HD (Buckley and Johnson, 2011). Other genes, such as *BAZ2B*, are also thought to be related to the pathogenesis of HD (Maulik et al., 2018). Analysis of pathway enrichment for the target genes of hsa-miR-10b-5p showed that this miRNA targeted significantly enriched pathways in HD involved in the three pathways, including GnRH, neurotrophin, and insulin signaling pathways. Other involved miRNAs in the present study, including hsa-miR-10b-3p, hsa-miR-144-3p, hsa-miR-483-5p, hsa-miR-4488 had also been reported to be differentially expressed in HD samples; hsa-miR-10b-3p was considered to have a significant relationship with the CAG length of HD (Hoss et al., 2015).

LncRNA is a type of RNA with a length greater than 200 nucleotide units. Because there is no open reading frame (ORF), it is called non-coding RNA (Iyer et al., 2015). These show epigenetic, transcriptional, and post-transcriptional gene expression (Wapinski and Chang, 2011). Since the 1990s, several individual lncRNAs have been studied; however, the exact mechanisms underlying transcriptional regulation by lncRNAs are not precise. Recently, numerous studies have suggested that thousands of lncRNAs might interact with multiple inhibitory chromatin regulatory complexes, including PRC2, SMCX and RCOR1, and have shown that lncRNAs are involved in numerous neurodegenerative disorders, including HD (Khalil et al., 2009). Other lncRNAs have been shown to affect the function of transcription factors (TFs), either by directly inactivating TFs or promoting their export from the nucleus (Willingham, 2005).

Some of the lncRNAs have been confirmed involved in the pathogenesis of HD. Chung et al. (2011) found that the repeat expansion reduced *HTT-AS_v1* expression; the levels of *HTT-AS_v1* were reduced in the human HD frontal cortex. Also, *HTT-AS_v1* could negatively regulate *Htt* expression in a repeat length-dependent manner. It was known that the levels of *BDNF* were down-regulated in the brains of HD patients, possibly contributing to the clinical characteristics of HD. *BDNF-AS*, a lncRNA transcribed from the *BDNF* opposite strand, could inhibit *BDNF* transcription, suggesting a potential role of *BDNF-AS* dysregulation in HD pathogenesis (Riva et al., 2016). *Abhd11os* was enriched in the striatum, and its expression levels were markedly reduced in different mouse models of HD. Francelle et al. (2015) suggested over-expression of *Abhd11os* was neuroprotective against an N-terminal fragment of the m*Htt*, and the loss of *Abhd11os* likely might contribute to striatal vulnerability in HD. The human accelerated region 1 (HAR1) lncRNA was reported to be repressed by *REST* and expressed significantly lower HD patients (Johnson, 2010). Tcl1 upstream neuron-associated lincRNA (TUNA) expression in HD patients' striatum was associated with pathological disease severity, decreasing significantly as the disease grade increased (Lin et al., 2014).

According to our network, several lncRNAs were regulated by differentially expressed miRNAs, including *XIST* and *TUG1*. *XIST* is a lncRNA that is a necessary condition for female X chromosome silencing in placental mammals. *XIST* spreads from one of its X chromosome transcription sites to gradually cover it; it also interacts with the PRC2 complex, deposits suppressive histone markers, and effectively suppresses gene transcription. Previous studies concluded that the dysregulation of *XIST* in HD might directly cause changes in one or a few proximal target genes (Johnson, 2012). Chanda et al. (2018) also identified the *XIST* was a significant increase in HD cell and animal models. *TUG1* is highly expressed in mammalian brains. Initially found during genomic screening, the *TUG1* gene was up-regulated during taurine-treated retinal cell development. The expression of *TUG1* was down-regulated in HD patients (Johnson, 2012). Khalil et al. (2009) found in their research that *TUG1* was a direct downstream target and a regulator of p53; it was up-regulated in HD to counterbalance m*Htt* cytotoxicity pro-survival factor in neurons to activate p53 (Zhang et al., 2014).

Table 4. Gene ontology enrichment analysis for the target genes of hsa-miR-10b-5p (top 10)

ID	Description	*P*-value	Total no. of genes	Genes
GO: 0005654	nucleoplasm	1.55×10^{-5}	23	*ZMYND11, JARID2, E2F7, BBX, ZNF367, ELAVL2, RORA, DAZAP1, CTNNBIP1, NONO, HOXA3, WDR26, BAZ1B, CEP350, GATA6, MTF2, NCOA6, BCL6, H3F3B, TFAP2C, RPRD1A, NCOR2, GOLGA3*
GO: 0003682	chromatin binding	3.62×10^{-5}	9	*NONO, ZMYND11, BAZ1B, JARID2, GATA6, NCOA6, BCL6, NCOR2, HOXD10*
GO: 0000977	RNA polymerase II regulatory region sequence-specific DNA binding	5.71×10^{-5}	7	*ZMYND11, JARID2, GATA6, E2F7, BCL6, TFAP2C, RORA*
GO: 0008584	male gonad development	2.19×10^{-4}	5	*GATA6, H3F3B, TFAP2C, SIX4, BCL2L11*
GO: 0048538	thymus development	2.35×10^{-4}	4	*HOXA3, JARID2, SIX4, BCL2L11*
GO: 0000122	negative regulation of transcription from RNA polymerase II promoter	3.79×10^{-4}	10	*HOXB3, ZMYND11, JARID2, GATA6, MTF2, E2F7, KLF11, BCL6, TFAP2C, NCOR2*
GO: 0005634	nucleus	6.37×10^{-4}	30	*ZMYND11, E2F7, ZNF367, RORA, CNOT6, HOXD10, DAZAP1, NONO, HOXA1, ARIH2, HOXA3, FIGN, GATA6, RNF165, TIAM1, BCL6, BAZ2B, GOLGA3, JARID2, KLF11, SIX4, CTNNBIP1, HOXB3, MTF2, NCOA6, CELF2, H3F3B, TFAP2C, XRN1, NCOR2*
GO: 0006351	transcription, DNA-templated	7.78×10^{-4}	16	*ZMYND11, JARID2, E2F7, BBX, ZNF367, RORA, CNOT6, HOXD10, NONO, HOXB3, HOXA1, HOXA3, BAZ1B, BCL6, BAZ2B, NCOR2*
GO: 0009952	anterior/posterior pattern specification	2.0×10^{-3}	4	*HOXB3, CTNNBIP1, HOXA3, HOXD10*
GO: 0003700	transcription factor activity, sequence-specific DNA binding	3.0×10^{-3}	10	*HOXB3, HOXA3, GATA6, E2F7, KLF11, ZNF367, BCL6, TFAP2C, RORA, HOXD10*

Abbreviations: GO, Gene Ontology.

Table 5. LncRNAs targeted by multiple (≥ 2) differential miRNAs

LncRNAs	The number of miRNAs regulates the lncRNA
XIST	5
TUG1	3
GS1-358P8.4	3
RP11-20G6.3	3
LINC00657	2
DCP1A	2
RP6-24A23.7	2

Abbreviations: miRNA, microRNA, lncRNAs, long non-coding RNAs.

5. Conclusions

Although extensive studies have revealed the abnormal expression of miRNAs in HD, its pathological significance's exact molecular mechanisms remain unclear. Using the expression profile data of Hoss et al. (2014), a comprehensive regulatory network centered on miRNAs was constructed. We identified that hsa-miR-196a-5p and hsa-miR-10b-5p were significantly differentially expressed, which regulated most target genes. Targeted genes, including *HOX* and *BDNF*, and targeted lncRNAs, including *XIST* and *TUG1*, may play important roles in HD's development. Research in this area may help deepen our understanding of the role of miRNAs in HD's pathogenesis.

Author contributions

This manuscript was primarily written by ZW. Figures were produced by XD and ZW. SC contributed to editing the review and contributed to the review revision. All authors read and approved the final manuscript.

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Conflict of Interest

All authors have no conflicts of interest to declare.

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