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Association between long non-coding RNA and human rare diseases (Review)

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Abstract. Long non-coding RNAs (lncRNAs) are untranslated transcripts with longer than 200 nucleotides (nt), which possess many of the structural characteristics of mRNAs, including a poly A tail, 5'-capping, and a promoter structure, but no conserved open reading frame. Moreover, lncRNA expression patterns change during differentiation and exhibit a variety of splicing patterns. Many lncRNAs are expressed at specific times and in specific tissues during development. It has been proposed that lncRNAs are involved in the epigenetic regulation of coding genes, and thus exert a powerful effect on a number of physiological and pathological processes, including the pathogenesis of many human rare diseases.

Contents

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1. Introduction

At present, five classes of long non-coding RNAs (lncRNAs) are known: anti-sense lncRNAs, intronic non-coding RNAs, large intergenic non-coding RNAs (lincRNAs), promoter-associated lncRNAs, and untranslated region (UTR)-associated lncRNAs (1). These lncRNAs are involved in a variety of vital regulation processes, including X-chromosome inactivation, genomic imprinting, chromatin modification, transcriptional activation, transcriptional interference, and nuclear transport, suggesting a possible involvement in diseases (2). lncRNAs are aberrantly expressed in several complex disorders and appear to be directly linked to the incidence of some diseases (3). This review summarizes the potential association between lncRNAs and human rare diseases.

2. The association between long non-coding RNA and human rare diseases

In differentiation and development processes, the dysfunction of non-coding RNA often leads to disease. Abnormality of lncRNA might affect DNA methylation, histone modification, and chromatin remodeling in various ways. Moreover, as a precursor of microRNA, lncRNAs play an important role in the initiation and progression of disease. Recent studies (4,5,6,7) have found that C15orf2, H19, Ube3a-as, and DGCR5 are associated with rare diseases (Table I).

3. Prader-Willi syndrome

Prader-Willi syndrome (PWS) is a neurogenetic disorder that results from loss of the paternal contribution of a 1.5-Mb imprinted region on the proximal long arm of chromosome 15. PWS is characterized by neonatal muscular hypotonia and failure to thrive, hyperphagia and obesity starting in early childhood, as well as hypogonadism, short stature, small hands and feet, sleep apnea, behavioral problems and mild to moderate mental retardation (8). *C15orf2* is a testis-specific gene that maps between *NDN* and *SNURF-SNRPN* and is expressed by the two alleles. The novel genes Prader-Willi region non-protein-coding RNA 1 and 2 are located between *NDN* and *C15orf2*. *PWRN2* is expressed only in testis and is biallelic (4). *PWRN1* is biallelically expressed in testis and kidney. Investigation of *C15orf2* revealed that this gene is also expressed in the fetal brain but is monoallelic. Therefore,

Table I. Long non-coding RNAs and human rare diseases.

Name	Location	Length (bp)	Dysfunction type	Associated diseases
C15orf2	Chr15: 24920541-24928593	8053	Expression	Prader-Willi syndrome
H19	Chr11: 2016406-2019065	2559	Epigenetics	Beckwith-Wiedemann
Ube3a-as	Unknown	Unknown	Locus	Angelman syndrome
KCNQ1OT1	Chr11: 2661768-2721228	91671	Epigenetics	Beckwith-Wiedemann syndrome
DGCR5	Chr12: 18958027-18982141	3334	Expression and mutation	DiGeorge syndrome
NRON	Chr9q33.3: 129270966-129481601	2730	Expression	Down's syndrome
XIST	ChrX: 73040495-73072588	31093	Epigenetics	Klinefelter's syndrome
Ak042766	Unknown	1029	Expression	Restless legs syndrome
BX118339	Chr6: 21486545-21512123	25578	Mutation	West syndrome
ASFMR1	ChrX: 146990949-147003676	3026	Expression	Fragile X syndrome
CECR3	Chr22: 17737750-17747623	1915	Expression	Cat eye syndrome
CECR9	Chr22: 17809924-17810122	198	Expression	Cat eye syndrome
BPESC1	Chr3: 138823027-138844009	3518	Mutation	Blepharophimosis syndrome

PWRN1 and *C15orf2* may be involved in PWS. *ZNF127* and *ZNF127AS* are imprinted genes that may be associated with some of the clinical features of the polygenic Prader-Willi syndrome (9).

4. Angelman syndrome

Angelman syndrome is a complex genetic disorder primarily affecting the nervous system. Characteristic features of this condition include delayed development, intellectual disability, severe speech impairment, and problems with movement and balance (5). *Ube3a-as* is a lncRNA transcribed antisense to the maternally expressed *Ube3a* gene, a candidate gene for Angelman syndrome, suggesting that *Ube3a-as* may be responsible for the repression of paternal *Ube3a* expression (10).

5. Beckwith-Wiedemann syndrome

Beckwith-Wiedemann syndrome (BWS) is an imprinting disorder characterized by overgrowth, tumor predisposition, and congenital malformations. Approximately 85% of reported BWS cases are sporadic, while the remaining 15% are familial. BWS is caused by epigenetic or genomic alterations that disrupt genes in one or both of the two imprinted domains on chromosome 11p15.5 (11). Genetic alterations of *H19* and *LIT1* distinguish patients with BWS from those with cancer and birth defects. Microdeletions in the human *H19* DMR result in loss of *IGF2* imprinting and BWS (6). The *LIT1* CpG island acts as a negative regulator in cis for coordinate imprinting at the centromeric domain, suggesting a role for the *LIT1* locus in a BWS pathway, which results in the functional inactivation of *p57* (KIP2) (12). *In vitro* fertilization may increase the risk of BWS, and it may be linked to the abnormal imprinting of the *KCN1OT* gene, suggesting that *LIT1* (*KCNQ1OT1*) plays an important role in the development of BWS (13). In BWS, ~50% of patients show loss of DNA methylation accompanied by loss of histone H3 Lys9 dimethylation on maternal *KCNQ1OT*-DMR, known as imprinting disruption, which causes the decreased expression of *CDKN1C* (14). Imprinting disruption of the *CDKN1C/KCNQ1OT1* domain is involved in the development of BWS and cancer, and it changes the maternal epigenotype to the paternal type, resulting in a decrease of *CDKN1C* expression (15).

6. DiGeorge syndrome

The microdeletion 22q11.2 syndrome is one of the more common human deletion syndromes (1 in 4,000 live births) and encompasses several clinical entities. Approximately 35-90% of patients clinically diagnosed with DiGeorge syndrome, including cardiac abnormalities such as interrupted aortic arch, truncus arteriosus and tetralogy of Fallot, and 80-100% of those with velocardiofacial syndrome, including pharyngeal dysfunction, cardiac anomalies and dysmorphic facies, have this hemizygous deletion (16). The essential transcriptional repressor element 1-silencing transcription factor (REST) is crucial in the development of human disease by regulating a large cohort of neural genes (17). The DiGeorge syndrome-associated non-coding RNA, *DGCR5*, is repressed by REST through a proximal upstream binding site, and disrupted by the DiGeorge syndrome patient breakpoint (7).

7. Down's syndrome

Trisomy 21 or Down syndrome (DS), is the most frequent and recognizable cause of intellectual disabilities. DS is a major cause of mental retardation and congenital heart disease. Besides a characteristic set of facial and physical features, DS is associated with congenital anomalies of the gastrointestinal tract, an increased risk of leukemia, immune system defects and an Alzheimer-like dementia. Moreover, DS is a model for

the study of human aneuploidy (18). *NRON* (*Homo sapiens* non-protein coding RNA, repressor of *NFAT* non-coding RNA) is a lncRNA that mediates the cytoplasmic to nuclear shuttling of the *NFAT* transcription factor. In animal models, deregulation of the *DSCR1* and *DYRK1A* genes acts synergistically to prevent nuclear occupancy of *NFATc* transcription factors, leading to reduced *NFATc* activity and to a number of features of DS, suggesting a potential link between *NRON* activity and DS pathophysiology (19).

8. Klinefelter's syndrome

Klinefelter's syndrome, also known as 47,XXY or XXY syndrome, is a genetic disorder in which there is at least one extra X chromosome to a standard human male karyotype, with a total of 47 chromosomes rather than the 46 found in genetically normal humans. The presence of an isochromosome Xq in Klinefelter syndrome (KS) is an apparently rare condition (20). In all cases reported thus far, patients showed the classical phenotype. Severe *XIST* hypomethylation clearly distinguishes (SRY+) 46,XX-maleness from Klinefelter syndrome (21). Quantitative RT-PCR demonstrated that an active XIST RNA expression in blood lymphocytes of Klinefelter patients, comparable to that observed in the control females although >30,000-fold higher than the control males. The higher expression of *XIST* provides a clue to the diagnosis of Klinefelter syndrome (22).

9. Restless legs syndrome

Approximately 65% of restless legs syndrome (RLS) patients, particularly those with an early onset of symptoms, have at least one first-degree relative with the disease. The concordance rate between monozygotic twins has also been reported to be high. Most pedigrees suggest an autosomal dominant inheritance, although recessive models have been proposed as well. Sequence variants have also been proposed, in or around genes on 6p, 2p, or 15q (23). The most significant gene identified currently is *Meis1*. A recent study (24) suggested that the predisposition to RLS results from a reduced expression of *Meis1* mediated by intronic cis-regulatory elements. Of note, in the developing mouse brain, *Meis1* is co-expressed in the developing cerebellar granule cell layer along with a genomically-associated lncRNA *AK042766* (24).

10. Silver-Russell syndrome

Silver-Russell syndrome (SRS) is a genetically and clinically heterogeneous disease that is mainly characterized by pre- and postnatal growth restriction. The typical SRS phenotype includes a relative macrocephaly, a triangular shaped face, body asymmetry, clinodactyly of the fifth finger and other less constant features (25), as well as epigenetic mutations of the imprinted IGF2-H19 domain in SRS. The majority of the patients with methylation abnormalities showed hypomethylation at both the *H19* and *IGF2* genes. However, it was also identified that SRS patients with hypomethylation were restricted to the *H19* or *IGF2* gene. Epimutations were also identified in siblings of normal parents, most likely reflecting germ cell mosaicism in the fathers. In one family, epimutation was identified in an affected father and his likewise affected daughter (26).

11. West syndrome

West syndrome is a disabling, age-related epileptic encephalopathy that may be attributed to different aetiologies. This syndrome is characterized by a unique seizure-type of development (27). A *de novo* balanced t(2;6)(p15;p22.3) in a patient with West syndrome disrupts a lncRNA, and BX118339 spans the breakpoint of chromosome 6. It could be hypothesized that disruption of this non-coding transcript plays a role in the pathogenesis of the patient (28).

12. Fragile X syndrome

Fragile X syndrome, an X-linked dominant disorder with reduced penetrance, is associated with intellectual and emotional disabilities ranging from learning problems to mental retardation, and from mood instability to autism (29). Homo sapiens *FMR1* antisense RNA 1 (*FMR1-AS1*), transcript variant 1, is a non-coding RNA. *ASFMR1* is silenced in FXS patients and upregulated in pre-mutation carriers, suggesting that a common process is responsible for regulating the expression of these transcripts (30). A primate-specific non-coding RNA transcript (2.4 kb) that resides upstream likely shares a bidirectional promoter with FMR1. *FMR4* is a product of RNA polymerase II and has a similar half-life to FMR1. *FMR4* is also silenced in FXS patients because of a CGG expansion repeat in the 5' UTR of the *FMR1* gene but is upregulated in pre-mutation carriers (31).

13. Cat eye syndrome

Cat eye syndrome (CES), or Schmid-Fraccaro syndrome, is a rare condition caused by the short arm (p) and a small section of the long arm (q) of human chromosome 22 in trisomic or tetrasomic forms instead of the normal disomic form. CES is actually a genomic disorder (32), with a homo sapiens CES chromosome region, a candidate 3 (non-protein coding) (CECR3) for non-coding RNA. It was found to be a 1.1-Mb region of human chromosome 22q containing the dosage-sensitive gene(s) responsible for CES and the 450-kb homologous region on mouse chromosome 6 (33).

14. Blepharophimosis syndrome

The blepharophimosis syndrome (BPES) is a rare genetic disorder characterized by blepharophimosis, ptosis, epicanthus inversus and telecanthus (34). The BPES syndrome has been mapped to 3q23. *Homo sapiens* blepharophimosis, epicanthus inversus and ptosis, candidate 1 (non-protein coding) non-coding RNA (BPESC1), *BPESC1*, is disrupted by a balanced chromosomal translocation, t(3;4)(q23;p15.2), in a patient with BPES. It plays a significant role in the pathogenesis of BPES (35). BPES is driven by dysregulation of the *FOXL2* gene, and a number of extragenic mutations have been reported in patients. A particular deletion occurring 283 kb away from *FOXL2* disrupts a lncRNA, *PISRT1* (*Homo sapiens* polled intersex syndrome-regulated transcript 1), which was

shown by chromatin confirmation capture to be a physical loop with *FOXL2* (36).

15. Conclusions

A total of 195 functional lncRNAs have been identified (http://lncrnadb.com, accessed April 2, 2013). However, the number of currently identified functional lncRNAs is a small fraction of that estimated by bioinformatics. Given that lncRNAs are capable of regulating the expression of coding genes, aberrant lncRNA expression is likely to cause disorders associated with dysregulation of the specific proteins. Thus, studies of lncRNA regulation and its function may provide new insights into the disease etiology, while diseases associated with known protein pathways may reveal the function of lncRNA. Our study suggests that lncRNAs are involved in the progression of this type of disease, through the up- or down-regulation of specific mRNAs, methylation and regulation of specific gene polymorphisms.

Despite the present advances, the study of lncRNA is still in its infancy and the association between lncRNAs and the majority of other diseases (if any) remains to be determined. To explore lncRNAs as markers for clinical diagnosis, studies on lncRNA should compare the lncRNA expression profiles between healthy subjects and patients with the genetic disorders. Gene knockout, RNA interference, and transgenic strategies are likely to reveal more information regarding lncRNA regulation and function, leading to novel treatments for the diseases. Consequently, the study of lncRNA may be of great significance for understanding of the intricate and multi-level regulatory systems in the development, for prevention and treatment of human genetic diseases, and for identification of the principles in evolution.

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