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Long noncoding RNAs: Undeciphered cellular codes encrypting keys of colorectal cancer pathogenesis

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Abstract

Long noncoding RNAs are non-protein coding transcripts longer than 200 nucleotides in length. By the advance in genetic and bioinformatic technologies, the new genomic landscape including noncoding transcripts has been revealed. Despite their non-capacity to be translated into proteins, lncRNAs have a versatile functions through various mechanisms interacting with other cellular molecules including DNA, protein, and RNA. Recent research interest and endeavor have identified the functional role of lncRNAs in various diseases including cancer. Colorectal cancer (CRC) is not only one of the most frequent cancer but also one of the cancer types with remarkable achievements in lncRNA research. Of the numerous notable lncRNAs identified and characterized in CRC, we will focus on key lncRNAs with the high potential as CRC-specific biomarkers in this review.

Keywords

Long noncoding RNAs (lncRNA); Colorectal cancer (CRC); Colorectal cancer associated transcript (CCAT); Cancer-associated region long noncoding; RNA (CARLo); MYC-regulated long noncoding RNA (MYCLo); Colorectal Neoplasia Differentially Expressed (CRNDE)

1. Introduction

Colorectal cancer (CRC) is ranked third on the list of most diagnosed cancers [1]. Although mortality rate of CRC has declined for several decades, CRC is still the third leading cause of cancer death in both men and women in the United States [2]. The World Health Organization (WHO) estimates 774,000 deaths from CRC in 2015 [3]. The American Cancer Society expects that CRC will result in more than 50,200 deaths in 2017 [4]. The increased survival rate is mainly due to the dissemination and advance of screening tests and surgery. Of course, the adjuvant chemotherapy and radiation therapy options have also contributed to the better survival rate in part. Nevertheless, the efficacy of the therapeutic options is not satisfactory to prevent or dramatically lessen the number of CRC deaths.

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Cancer including CRC has been largely characterized and understood by intensive investigations for several decades. However, considering that cancer is caused not by a single cause but by complex causes, the observations focusing on only 1% protein-coding genes in the human genome are incapable of sufficient understanding to establish effective cancer treatment.

Noncoding RNAs account for 99% of total transcribed RNAs in the human genome [5]. In spite of their abundance in cells, their importance had been neglected due to the lack of protein-coding capacity. However, accumulating evidence has been elucidated the critical role of the noncoding RNAs highlighting those as pivotal molecules in cell functions and diseases [6]. The short noncoding RNAs including microRNAs have been intensively studied in cancer since the tumorigenic role of microRNA deletion was reported in chronic lymphocytic leukemia [7–10]. On the other hand, long noncoding RNAs (lncRNAs) have been less understood in cancer because it has been focused on more recently [11]. Compared to the short 20–22 nucleotides microRNAs, lncRNAs longer than 200 nucleotides can employ more various and complicated mechanisms in their functions, strongly suggesting variety of their biological roles. Indeed, lncRNAs are involved in numerous biological and cellular pathways by interacting with various macromolecules such as DNA, Chromatin, proteins, and various RNA species including mRNAs, microRNAs, and other lncRNAs [12–14]. Hence, the subcellular localization of lncRNAs is also a critical factor to determine their functions by providing them different opportunities to interact with different molecules [15]. For instance, lncRNAs localized in nucleus tend to be involved in transcriptional and epigenetic regulations by interacting with genomic DNA, chromatin, transcription factors, chromatin regulators, spliceosomes and other nuclear proteins [14]. Meanwhile, cytosolic lncRNAs are frequently implicated in post-transcriptional, translational, and posttranslational regulatory processes through interactions with various key factors in epigenetic and signaling pathways [13].

Along with technical evolution in genomics and bioinformatics, multiple lncRNA databases including GENCODE, lncRNAdb, NRED, RNAdb and T-UCRs in addition to the traditional Reference Sequence (RefSeq) and UCSC known genes datasets enable in-depth studies of lncRNAs by using high throughput technologies such as microarray and RNA sequencing (RNA-seq) [16]. As a result, it has been revealed that the broad-spectrum (~90%) of the human genome can be transcribed and that the noncoding transcripts account for more than 99% of the human transcriptome [17]. The lncRNAs can be broadly classified into 5 categories by their origins: (1) intergenic lncRNAs independent to any other protein-coding genes, (2) intronic lncRNAs generated from an intron of a host gene, (3) bidirectional lncRNAs transcribed from the host gene promoter in opposed direction, (4) sense lncRNAs (pseudogene) overlapped with coding exons of a host gene in same direction, (5) antisense lncRNAs overlapped with coding exons of a host gene in opposite direction [17].

Since lncRNA CCAT1 (CARLo-5) was identified in CRC, a number of lncRNAs dysregulated in CRC have been identified [16,18,19]. Furthermore, numerous lncRNAs have been characterized with their oncogenic or tumor suppressor functions in CRC [20,21]. In this review, we provide a brief overview of conspicuous lncRNAs as diagnostic markers and therapeutic targets in CRC.

2. Colorectal cancer associated transcript (CCAT)

The discovery of CCAT1 (CARLo-5) and CCAT2 suggesting the pivotal role of lncRNAs in CRC emphasized the necessity of comprehensive lncRNA profiling in CRC [18,19,22]. Consequently, recent high-throughput analysis of lncRNAs has determined the signatures of lncRNA dysregulation and specificity in CRC [23]. It revealed that ~2300 lncRNA among more than 33,000 lncRNAs tested are dysregulated at the genome-wide level, revealing additional CCAT family members (Fig. 1) [23]. Here, we discuss the CCAT family members identified by CRC-oriented research and their implications in CRC pathogenesis.

2.1. CCAT1 (CARLo-5)

In 2012, Nissan A et al. first discovered CCAT1 as a lncRNA biomarker of CRC due to a dramatically increased expression level in CRC [18]. The overexpressed lncRNA CCAT1 is detectable not only in primary CRC tissues but also in peripheral blood samples of CRC patients, suggesting its potential as a diagnostic marker in CRC. In 2014, CARLo-5 that was later revealed as a same lncRNA with CCAT1 was also identified as an oncogenic lncRNA overexpressed in primary CRC tissues. CARLo-5 (CCAT1) promotes cell proliferation, cell cycle progression and tumorigenesis by regulating various cell cycle regulators including CDKN1A (p21). It was also shown that normal colon tissues with high CRC risk frequently harbor higher expression of CARLo-5 (CCAT1), suggesting the putative role of CARLo-5 (CCAT1) in CRC initiation [19]. Furthermore, recent studies also show the potential roles of CCAT1 (CARLo-5) in the progress and prognosis of various cancer types in addition to CRC [24–30]. Additional long isoform of CCAT1, named CCAT1-L was also found to be overexpressed in CRC. CCAT1-L has been known a regulator of MYC transcription by interacting with CTCF [31]. In accordance with the lines of evidence, CCAT1 (CARLo-5) is one of the most putative diagnostic markers and therapeutic targets among the known CRC-associated lncRNAs to date.

2.2. CCAT2

The development and advance of a genome-wide association study (GWA study or GWAS) has revealed a huge number of single nucleotide polymorphisms (SNPs) associated with various diseases including cancer [32]. In general, the disease-associated SNPs are frequently found in/near disease-related genes and involved in the disease development by modulating the activity of the host gene at the transcription and post-transcription levels. The 8q24 gene desert has been highlighted due to the presence of a cohort of cancer-associated SNPs in spite of the lack of host genes that can intervene between the cancer-associated SNPs and cancer incidence [33]. For instance, homozygosity for the G allele of rs6983267 at 8q24 increases the risk of cancer in multiple cancer types including CRC [34–37].

Ling H et al. determined the presence of lncRNA CCAT2 at the mysterious cancer-associated 8q24 region, providing a critical clue of a coding gene-independent SNP function in cancer [22]. In accordance with the findings of the Calin laboratory, the lncRNA CCAT2 is expressed from the 8q24 genomic locus including rs6983267, leading to the altered activity and expression of CCAT2 by the genetic variants of rs6983267. CCAT2 is highly

expressed in microsatellite-stable CRC (MSS) displaying chromosome instability, suggesting the critical role of CCAT2 in cancer development [22]. Furthermore, several independent meta-analyses have determined CCAT2 as a prognostic marker in multiple cancer types including CRC [24,38–40]. Indeed, CCAT2 is involved in multiple critical CRC pathways including MYC gene regulation, WNT signaling, and cancer metabolism [22,41]. Overall, the discovery of CCAT2 raised new insight about how the cancer-associated SNPs influence cancer incidence through noncoding RNAs by a coding gene-independent manner.

2.3. New CCAT family members

The two CCAT family members such as CCAT1 (*CARLo-5*) and CCAT2 were found and investigated in CRC, supporting the critical functions of lncRNAs in CRC pathogenesis [18,19,22]. However, the endeavor of comprehensive analysis of lncRNAs in CRC was not done until the profile of lncRNAs in CRC-derived cell lines and patient tissues [23]. The recent high through-put analysis by using lncRNA microarray has profiled the coding and noncoding transcripts in multiple CRC cell lines and patient tissues, leading to the identification of a cohort of lncRNAs dysregulated in CRC at the genome-wide level (Fig. 1). Further validation in CRC-derived cell lines and tissues identified a squad of lncRNAs: 4 lncRNAs upregulated and 2 lncRNAs downregulated in CRC [23]. Those newly identified CCAT family members are CCAT3 (GenBank Accession # KT923687, two loci on chromosome 14, upregulated in CRC), CCAT4 (GenBank Accession # KT923688, chromosome 20, upregulated in CRC), CCAT5 (GenBank Accession # KT923689, a.k.a. MNX1-AS1, chromosome 7, upregulated in CRC), CCAT6 (GenBank Accession # JX046910, a.k.a. MYC_{Lo}-2 & ELFN-AS1, chromosome 7, upregulated in CRC), CCAT7 (GenBank Accession # KT923690, chromosome 20, downregulated in CRC), and CCAT8 (GenBank Accession # KT923691, four loci on chromosome 9, downregulated in CRC). Except for CCAT6, also referred to as MYC_{Lo}-2, the regulatory and functional mechanisms of the new CCAT members remain unknown despite their distinctive expression patterns in CRC [23].

3. Cancer-associated region long noncoding RNA (*CARLo*)

In addition to the discovery of CCAT2, the discovery of 7 lncRNAs (Cancer-Associate Region Long Noncoding RNA (*CARLo*) family) at the 8q24 region shows that the cancer-associated 8q24 region is the gene desert for coding genes but not for noncoding genes, suggesting the critical role of the lncRNAs in the SNP-mediated CRC pathogenesis [19]. Although the 8q24 cancer-associated genomic locus including rs6983267 produces the lncRNA CCAT2, the genomic region also harbors a strong promoter/enhancer activity evidenced by the enriched histone modification markers such as H3K4Me1 and H3K27Ac (ENCODE database) [42,43]. Indeed, the enhancer region including the rs6983267 could regulate the expression of *CARLo-5* (*CCAT1*) through a physical interaction with the core promoter of *CARLo-5* [19]. It indicates that various lncRNAs and machineries could be implicated in cancer development driven by the 8q24 cancer-associated region and variants. The other *CARLo* family members are significantly matched or overlapped with lncRNAs such as *CASC8* (*CARLo-1*), *CASC21* (*CARLo-2*), *PRNCR1* (*CARLo-3*), *PCAT2* (*CARLo-4*), *CASC19* (*CARLo-6*) and *CASC11* (*CARLo-7*) that were recently designated

by Human Genome Organization (HUGO). In fact, recent findings have reported the dysregulation and/or function of other CARLo family members in CRC. For instance, CARLo-6 (CASC19) is frequently overexpressed in CRC [24] and CARLo-3 (PRNCR1) promote cancer cell proliferation and cell cycle progression in CRC [44,45]. CARLo-7 (CASC11) is also known as an oncogenic lncRNA activating WNT/b-catenin-mediated cell proliferation in CRC [46]. To better understand the functional and regulatory mechanisms of CRC development through the lncRNAs at the 8q24 cancer-associated region, *CARLo* family members localized at the 8q24 region should be further investigated for their putative roles in various diseases and CRC that are associated with the 8q24 SNP variants.

4. MYC-regulated long noncoding RNA (MYCLo)

The proto-oncogene MYC known as a transcription factor is frequently activated, overexpressed and/or amplified in various types of cancer including CRC [47]. Although MYC drives cancer through the activity of transcription enhancer, many of critical downstream genes are actually repressed by MYC activation [48–50]. The finding of MYC-regulated lncRNAs named MYCLo family revealed that MYC regulates not only coding genes but also lncRNAs at the genome-wide level [23,51]. It was also shown how MYC-regulated lncRNAs serve as key regulators contributing the diversity of MYC-driven cancer pathway. For instance, MYC-induced lncRNAs such as MYCLo-1, MYCLo-2 (CCAT6) and MYCLo-3 suppress the transcription of critical MYC-repressed downstream genes including *CDKN1A* (p21) and *CDKN2B* (p15). In particular, MYCLo-2 (CCAT6) has a function in cancer transformation and development [23]. In addition, MYC-repressed lncRNAs such as MYCLo-4, MYCLo-5 and MYCLo-6 have a tumor suppressor role by activating cell cycle regulators such as GADD45A that is also a known MYC-repressed downstream gene [51].

For a long time of MYC research, numerous MYC-regulated genes and their roles in MYC-driven cancer have been investigated [50]. And it has been revealed that the numerous downstream genes contribute to the oncogenic role of MYC through various pathways. Interestingly, however, most of the critical downstream genes have been less commonly found in multiple types of MYC-driven cancers in spite of the consistent and conserved function of the oncogene MYC in the cancers. It suggests the presence of other unknown downstream factors of MYC. The MYC-mediated modulation of MYCLo expression is consistently found in multiple types of solid tumors including breast, liver, lung and prostate cancers [23]. Furthermore, the profiling of MYC-regulated lncRNAs done by the Vogt laboratory also found the MYCLo family members in blood cells [52]. Overall, it indicates that MYC-regulated lncRNAs could be the conserved key factors of the MYC-driven cancer pathways across cancer types.

5. Colorectal Neoplasia Differentially Expressed (CRNDE)

In 2011, Graham LD et al. found a novel transcript overexpressed in colorectal adenomas and adenocarcinomas, suggesting its putative role in the early stages of CRC development [53]. They also found that the transcript referred to as Colorectal Neoplasia Differentially Expressed (CRNDE) is a lncRNA transcribed into multiple transcript variants [53]. It was also reported that the lncRNA CRNDE is the upstream regulator of various cell metabolic

factors and downstream effector of insulin/IGF signaling [54]. However, a coding transcript (84 amino acids) overlying the non-coding transcripts was later found in the *CRNDE* gene locus and the peptide level of *CRNDE* is also highly detected in human tissues [55]. Nevertheless, various functional mechanisms of *CRNDE* as a lncRNA have been reported in PI3K/AKT, Ras/MAPK, EGFR, TLR3-NF-KB, and Wnt/b-catenin signaling pathways to date [56–60]. In addition to the functional mechanisms of *CRNDE* in CRC initiation, the research endeavor to discriminate the functionality of non-coding transcripts and coding transcripts of *CRNDE* in CRC is also required.

6. LncRNAs in WNT/b-catenin pathway

The highly conserved Wnt/b-catenin signaling pathway is a key player of CRC development, displaying abnormal activation in more than 90% of CRC cases [61]. The Wnt/b-catenin pathway is involved in numerous cellular functions such as proliferation, differentiation, invasion, migration, stem cell renewal, apoptosis, and genetic instability in CRC [62]. Recently multiple lncRNAs have been studied with their functions in Wnt/b-catenin pathway of CRC [21].

CASC11 (CARLo-7) activates Wnt/b-catenin pathway by interacting with other protein. The RNA-binding protein hnRNP-K is stabilized by interacting with CASC11 (CARLo-7), leading to the activation of b-catenin [46]. Similarly, the b-catenin-interacting lncRNA RBM5-AS1 also activates Wnt/b-catenin pathway by enhancing the transcription activity of the b-catenin/TCF7L2 complex [63]. LncRNA-CCAL enhances Wnt/b-catenin pathway through a different mode. LncRNA-CCAL destabilizes and deactivates AP2a (Activator Protein 2a) which is a negative regulator of Wnt/b-catenin pathway [64]. Distinctively, lncRNA *CRNDE* enhances Wnt/b-catenin pathway by regulating the expression or activity of microRNAs targeting key factors of the pathway [60]. Whereas other lncRNAs negatively regulate Wnt/b-catenin pathway. LncRNA-BC032913 upregulates TIMP3 causing Wnt/b-catenin inhibition [65]. LncRNA TINCR also represses Wnt/b-catenin pathway by preventing EpCAM hydrolysis and subsequent EpICD release [66]. In addition, lncRNA-CTD903 was reported to repress WNT/b-catenin pathway although the regulatory mechanism has been unknown [67].

Interestingly, recent findings show the role of lncRNA in Wnt/b-catenin signal pathways through microRNA bullets embedded in the lncRNA. The lncRNA MIR100HG is actually the primary micro-RNA of the miR-100/let-7a-2/miR-125b-1 cluster. The Coffey and Fan laboratories found that the increased expression of MIR100HG and subsequent processing of the microRNAs are implicated in cetuximab resistance through the activation of Wnt/b-catenin pathway [68]. To determine whether MIR100HG harbors a cellular function as a lncRNA in CRC, the microRNA-independent role of MIR100HG is required to be investigated. Likewise, H19 in which miR-675-5p is encoded is also an example conducting various functions as both lncRNA and primary microRNA, activating Wnt/b-catenin signaling in CRC [69]. However, the role of the embedded miR-675-5p in H19-modulated Wnt signaling has been less understood in CRC.

7. Perspective

In spite of the recent accomplishments of lncRNAs in cancer, the clinic application of lncRNAs is still at the conceptual level. One of the most critical concerns in the clinic application of lncRNAs is that lncRNAs have been less conserved during evolution across species [70]. Actually, only ~5% of lncRNAs are conserved in mammals [71]. Furthermore, very few conserved lncRNAs are found in cancer [70,72]. So the current translational medicine strategy inevitably adopting animal models tends to be unfavorable to the mostly unconserved genome products. Nevertheless, accumulated evidence indicates that the less conserved lncRNAs disseminated through the whole genome have functional roles at the cellular and molecular levels, suggesting lncRNAs as causes of diseases including cancer [12,13]. Moreover, lncRNAs are providing clues of many mysteries that were not explained with coding genes [23,51]. Therefore the less or non-conserved lncRNAs will enable to explain the discrepancy between human and animal models in biomedical research of various diseases including cancer. In particular, CRC has an advantage to translate the basic lncRNA findings to the clinic side because the organoid culture system of the intestine including the colon has been remarkably developed [73]. It will largely or partly allow the replacement of animal models during the process toward clinical trials. The efforts to translate the lncRNA discoveries into clinics should be continued for better understanding of human cancer and diseases that have been differentially evolved with those of other species through the adaptation of human-specific machineries. In addition, other lncRNAs associated with additional CRC-causing pathways and factors such as Kras, p53, DCC and APC should be identified and accessed in CRC.

The recent research endeavors have identified a number of lncRNAs in CRC. Although the current research focusing on the identification of differentially expressed lncRNAs in CRC provides the groundwork to emphasize the importance of lncRNAs in CRC, our understanding of lncRNAs is in the early stage. For instance, sequence variants of lncRNAs and their roles in cancer has been less investigated. A huge number of genetic mutations occur in non-coding regions of the genome [74–76]. In addition, RNA sequences can be altered and modified by various epigenetic mechanisms such as RNA editing, splicing and methylation [77–79]. These genetic and epigenetic mechanisms could modulate lncRNA activities by sequence, splicing and structural alterations. Many oncogenic activities are generated by mutations in non-oncogenic proteins. Likewise, the mutation and alteration in RNA sequences should be scrutinized to elucidate the lncRNA contributions in cancer development. Another interesting aspect of lncRNAs is their talent of small polypeptides production. lncRNAs are known as noncoding RNAs unable to produce peptides. However, recent studies revealed that some RNAs known as lncRNAs can be translated into small polypeptides [80,81]. As we introduced here, CRNDE also has an ability to produce a peptide [55]. Furthermore, very recently, additional lncRNA-derived small peptide has been studied in CRC [82]. The report shows that lncRNA HOXB-AS3 produces a peptide regulating the growth of colon cancer. As evidence of the paradox ‘coding peptides from noncoding genes’ has been accumulated, the discrimination and characterization of the coding lncRNAs are required to be explored by using various genomic, genetic and bioinformatic technologies including Ribosome profiling (Ribo-seq) [83].

8. Discussion

The remarkable attention to noncoding RNAs has emerged after indifference for a long period of time. Among various types of noncoding RNAs, lncRNAs have been highlighted even later than other short noncoding RNAs such as microRNAs. Nevertheless, the interest on lncRNAs has tremendously raised in cancer research in the world. As a result, many attractive lncRNA candidates in cancer have been introduced. Especially, the lncRNA research in CRC has had an impressive progress during the past five years. As we reviewed, a number of critical lncRNAs possessing strong potential as biomarkers in CRC have been unearthed in the short period of time. Besides, more lncRNAs that are not introduced in this review are also reported to contribute to CRC development [16,20,21].

Current trend of cancer treatment engages the concept of precision medicine significantly driven by systems biology. Along with the evolution of genetic and genomic technologies, the generation and development of bioinformatics and systems biology have made it possible to manage the huge quantity and complexity of the accumulated data generated by traditional basic research and large-scale high throughput analyses. Thus, the cutting edge precision medicine has raised based on extensive findings from the bench side in the long history of basic research. However, our attention on noncoding genes accounting for 99% of genomic products has appeared very recently. Although many noncoding RNAs have been characterized for a couple of decades and it has added the knowledge in the systems biology resource, it should be insufficient considering the vast spread of noncoding RNA areas in the human genome. In turn, the current fundamental resource for precision medicine is covering only 1% of human genome products and the extensive investigations of noncoding RNAs, particularly the less studied lncRNAs, should be continued.

Due to the discrepancy between the rules of lncRNAs and coding genes, it is difficult to define and characterize lncRNAs with previously established formulas for coding genes. Unlike the 1% coding genes on which cancer research has been centered, lncRNAs do not follow the current gene mapping standards such as open reading frames and splicing machineries. However, the catalogue of whole noncoding RNAs including lncRNAs at the genome-wide level is under development and advancement through various technologies and strategies including *in silico* analysis to predict lncRNAs, Chromatin immunoprecipitation-sequencing (ChIP-seq) for transcription and promoter markers, total RNA sequencing for whole transcriptome analysis, Ribosome profiling (Ribo-seq) to determine coding/noncoding RNAs and so on. In addition, the characterization of functional and regulatory mechanisms of lncRNAs is also a critical aspect to accumulate the data to complement the deficiency of current precision medicine. Given the diverse roles, various functional machineries, versatile structures, and enormous mutation spectrum of lncRNAs, the lncRNA research necessarily requires comprehensive participation and cooperation of a wide-range of biomedical fields including biochemistry, cell biology, molecular biology, genetics, genomics, systems biology, bioengineering, biomathematics, bioinformatics, integrative biology and biophysics and so on. To accelerate the clinical application of lncRNAs in cancer treatment, the development and improvement of lncRNA inhibitors and delivery systems are also the essential part of the future lncRNA research.

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Abbreviations

CRC	colorectal cancer
WHO	World Health Organization
lncRNA	long noncoding RNA
CCAT	colorectal cancer associated transcript
GWAS	genome-wide association study
SNP	single nucleotide polymorphism
MSS	microsatellite-stable

CARLo	cancer-associated region long noncoding RNA
H3K4me1	monomethylation of lysine 4 on histone H3
H3K27Ac	acetylation of lysine 27 on histone H3
MYCLo	MYC-regulated long noncoding RNA
CRNDE	colorectal neoplasia differentially expressed
ChIP-seq	chromatin immunoprecipitation-sequencing
Ribo-seq	Ribosome profiling



Fig. 1. LncRNA dysregulation signature at the genome-wide level (analyzed based on Human lncRNA microarray data from Kim et al., 2015) [23].