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Long non-coding RNAs in colorectal cancer: implications for pathogenesis and clinical application

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Long non-coding RNAs (lncRNAs) are a class of newly identified non-coding RNA molecules that are emerging as key regulators of tumor initiation and development. Colorectal cancer (CRC) remains a major health problem worldwide, and there remains a need to further refine the current screening approaches as well as provide tailored diagnostic and therapeutic approaches. Multiple dysregulated lncRNAs participate in tumorigenesis through a variety of molecular mechanisms, and various regulatory factors frequently contribute to the aberrant expression of lncRNAs in CRC, thereby allowing malignant transformation. Additionally, the association of dysregulated lncRNAs with specific developmental stages and clinical outcomes indicates their potential as strong diagnostic and prognostic predictors as well as therapeutic targets. Here we provide a brief overview of the known functions of CRC-associated lncRNAs, describe some potential molecular mechanisms that underlie changes in lncRNA expression in CRC, and attempt to uncover their clinical and therapeutic potential.
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High-throughput genome-scale studies have demonstrated that more than 93% of the DNA sequences in the human genome are actively transcribed.[1] However, only approximately 5–10% of the sequences are stably transcribed into mRNA or non-coding RNA (ncRNA). Genome tiling arrays have revealed that the amount of non-coding sequence is at least four times larger than the amount of coding sequence, which indicates that only 1% of the human genome is composed of protein-coding genes and the remaining 4–9% is transcribed into ncRNAs.[2] Therefore, ncRNAs constitute a very large proportion of the total RNA molecules.

The function and clinical significance of short regulatory ncRNAs, such as microRNAs (miRNAs) and small interfering RNAs (siRNAs), were elucidated first,[3] and the regulatory roles of miRNAs have been broadly recognized in almost all physiological and pathological processes in the body, including carcinogenesis.[4] For example, we previously reported that MIR95 promotes cell proliferation and targets sorting nexin 1 in human colorectal carcinoma;[5] moreover, in colorectal cancer (CRC) patients, the plasma levels of MIR29a and MIR92a are significantly upregulated and the plasma levels of MIR601 and MIR760 are significantly downregulated; thus the levels of these miRNAs have good diagnostic value for CRC screening.[6,7]

According to their transcript size, ncRNAs are grouped into two major classes: (i) small ncRNAs with transcripts <200 nucleotides (nt; eg, aforementioned siRNAs and miRNAs, Piwi-interacting RNAs, and some retrotransposon-derived RNAs) and (ii) long non-coding RNAs (lncRNAs), this class includes five broad categories: sense, antisense, bidirectional, intronic, and intergenic, based on the proximity between neighboring transcripts.[8] For a time, lncRNAs was commonly defined as a protein-coding transcripts that is >200 nt.[9] However, this definition is arbitrary and limits in distinguishing lncRNAs from small regulatory ncRNAs, without taking its distinct structure or function characteristics into account. For example, lncRNAs bear many signatures of mRNAs, including 5′ capping, frequently transcribed by RNA polymerase II,

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poly-adenylation and splicing, but have little or no open reading frame (ORF).[10] And moreover, a significant proportion of lncRNAs may possess coding and non-coding activities.[11] Combining with these characters, Mercer *et al*[2] proposed a definition that describes lncRNAs as 'RNA molecules that may function as either primary or spliced transcripts and do not fit into known classes of small RNAs or into classes of structural RNAs'. This undated definition overcomes the restriction of ORF and length that was arbitrarily set. However, so far there still a lack of standard nomenclature that makes comprehensive comments on the general features of lncRNAs, which to some extent demonstrate how little we currently know about this newly discovered class of ncRNA transcripts.

According to the LNCipedia database (www.lncipedia.org), 32 183 human lncRNAs have been annotated.[12] Emerging studies have revealed that particular lncRNAs are involved in diverse physiological and pathological processes, such as cell growth, apoptosis, stem cell pluripotency, and development, by acting as transcriptional, post-transcriptional, or epigenetic regulators. These studies have revealed the functional potential of these molecules;[9,10,13,14] however, the majority of lncRNAs have not yet been functionally characterized.Notably, observations of a few known lncRNAs have suggested that their dysregulation is linked to tumor pathogenesis, and these molecules perform essential regulatory functions by acting on cellular proliferation, apoptosis, or metastasis by participating in a variety of key signaling pathways.[11,15–17]

Here, we summarize the recent research concerning alterations in and roles of lncRNAs in cancer pathogenesis, with a specific focus on CRC. Additionally, we discuss the significant and almost wholly untapped potential of lncRNAs as biomarkers for the early diagnosis of CRC, as indicators of CRC prognosis prediction, or even as targets in CRC treatment strategies.

LncRNAs in cancer

Cancer is the result of a wide array of cellular transformation processes that occur due to oncogene activation and tumor-suppressor gene defects and lead to uncontrolled cell proliferation and escape from apoptosis. Using a range of techniques, such as microarray,[18] RNA sequencing,[19] and real-time PCR,[20] many studies have confirmed that lncRNA expression levels differ between normal and tumor tissues and vary among tumor types.[21–23] Although some of these abnormal expression patterns may be secondary effects from the cancer transformation, several factors suggest that lncRNAs could influence tumorigenesis. These factors include the tremendous complexity and diversity of the lncRNA landscape, the finding that lncRNAs are often located at crucial sites (eg, regions of single-nucleotide polymorphisms (SNPs), amplifications, or at common breakpoints), the presence of sequence motifs and other elements that result in specific structures, their regulation, and their functional relationships with other nucleic acids and proteins.[11,16] In fact, previous studies have shown that particular deregulated lncRNAs are important regulators of tumor formation and progression.[17,24] For example, the antisense intergenic RNA HOX Antisense Intergenic RNA (HOTAIR) is highly expressed in multiple types of primary somatic tumors, such as hepatocellular carcinoma,[25,26] pancreatic cancer,[27] and gastric cancer,[28] as well as some metastatic tumors, including metastatic breast tumors[29] and melanoma.[30] Moreover, high HOTAIR level is associated with poor patient survival rate[20,27] and tumor recurrence.[25] It was discovered that HOTAIR represses transcription across 40 kb of the HOXD locus by acting as a scaffold of histone modification complexes; it binds with polycomb chromatin remodeling complexes in trans, which lead to alterations of cells' epigenetic state and subsequent gene expression.[31] Moreover, HOTAIR can also interact with the LSD1/CoREST/REST complex, which coordinates targeting of LSD1 to chromatin for coupled histone K4 demethylation.[32] Given its important role in the epigenetic regulation of gene expression by mediating the modulation of chromatin structures, it is not surprising that HOTAIR performs vital functions in increasing cancer invasiveness and metastasis[25,26,29] and shows immense clinical relevance. Particularly, lncRNA expression levels may be extremely useful for monitoring tumors, and we consider lncRNAs to be novel cancer diagnostic tools and a source of future therapies.[33]

The involvement of lncRNAs in CRC

CRC remains a major health problem and represents the third most common cause of cancer-related death worldwide.[34] Most cases of CRC usually progress from benign polyps to malignant adenocarcinomas and distant metastases; therefore, screening and early diagnosis, as well as early treatment, help to extend the long-term survival of patients.[35] Given that the initiation, progression, and metastasis of CRC involve multiple genetic and epigenetic alterations, which permit the adaptations characteristic of malignant tumors, previous detection and treatment technologies based on genomic and proteomic analyses have got encouraging achievements to ease the disease burden.[36–39] However, only a limited number of CRC-associated gene and protein candidates have been clinically validated and demonstrated clinical utility. Additionally, the sensitivity and specificity of the current biomarkers used for the detection of precancerous lesions and early stages of tumor formation is weak, and their

ability to predict the chemotherapeutic response is poor.[36] Consequently, there is still a need for further research regarding the complex regulatory networks in CRC to refine the existing programs and to provide tailored diagnostic and therapeutic approaches.

Research conducted during the past 30 years has uncovered several critical important genes and pathways involved in the pathogenesis of many kinds of tumors including CRC, such as the WNT, RAS-MAPK, PI3K, TGF-β, P53, and DNA mismatch-repair pathways. More recently, with the aid of high-throughput techniques, a variety of systematic cancer genomics projects, such as The Cancer Genome Atlas Project (TCGA) (http://cancergenome.nih.gov/), are being used to investigate different molecular pathways and the genomic, transcriptomic, proteomic, and epigenomic alterations in each specific cancers, including CRC.[40–42] This disease-specific focus has identified novel oncogenic drivers, and the genes contributing to functional change,[43] importantly, revealed that different molecular features contribute to individual differences that occurred in clinicopathological characteristics, disease behavior, prognosis, and response to treatments, which thus helped to establish definitions of molecular subtypes and identified new biomarkers on the basis of omic alterations.[44–46]

It is now well known that some CRC cases are linked to some factors, such as environment,[47] inflammation,[48,49] immunity,[50] and epigenetic alterations[51,52] rather than heritable genetic changes. An interesting thing is that these factors can influence each other, for instance, epigenetic aberrations induced by environmental factors contribute to cancer processes;[53] interaction of drug and molecular characteristics can influence lncRNAs and clinical outcome;[46,54,55] and epigenetic factors such as lncRNAs can also coordinate cellular responses to environment in turn.[56]

The significance of lncRNAs in human CRC was realized in 2001 when Tanaka *et al*[57] determined that a loss of imprinting of long QT intronic transcript 1 (LIT1/KCNQ1OT1) was frequently observed in CRC patients, suggesting a link between lncRNAs and CRC. Following this research, several studies focused on the aberrant expression of lncRNAs during colorectal carcinogenesis, and an accumulating number of studies indicated that specific lncRNAs had potential biological and clinical relevance in CRC (Table 1). According to these data, understanding the pathophysiological roles of lncRNAs in CRC undoubtedly represents an important aspect of current and future research, as these molecules may be the hallmark features of CRC. Furthermore, the detection and identification of potentially functional lncRNAs in CRC is an emerging avenue of lncRNA research, which will be necessary before the application of lncRNAs in cancer diagnosis and therapy (Figures 1 and 2).

Role of lncRNAs in Tumorigenesis and Progression

LncRNAs may be involved in carcinogenesis and the progression of tumors through a variety of mechanisms. Generally, some lncRNAs function as oncogenes or tumor suppressors by participating in pivotal signaling pathways, while others have roles in malignant transformation by interacting with other regulatory molecules, such as DNA, RNA, and proteins.[17]

The expression level of the lncRNA colon cancer-associated transcript 2 (CCAT2) in microsatellite-stable (MSS) CRC patients was higher than that in microsatellite instable-high (MSI-H) CRC patients, and cancer cells transduced with CCAT2-containing retrovirus exhibited numerical and structural chromosomal changes, illustrating that CCAT2 can contribute to the MSS phenotype by inducing chromosomal instability.[58] Additionally, CCAT2 itself has a role in CRC pathogenesis through its direct involvement in the regulatory network. CCAT2 participates in loop formation between the genomic locus rs6983267 and the MYC promoter and works with the enhancer element to activate the transcription of the MYC oncogene. Moreover, CCAT2 enhances WNT activity by binding to TCF7L2, a pivotal transcription factor in the WNT signaling pathway, and facilitates MYC function, thereby enhancing cancer cell invasion and metastasis.[58] Intriguingly, CCAT2 levels can then be reduced by WNT signaling under TCF7L2 knockdown conditions, suggesting that a feedback loop may exist between CCAT2 and WNT signaling.[58] An attractive therapeutic strategy might involve attenuating CCAT2 activity and blocking the function of the downstream oncogenes in the WNT signaling pathway.

Data obtained from functional manipulations of lncRNAs have confirmed that lncRNAs have roles in tumor development by participating in a 'competitive endogenous RNA' network and by acting as endogenous miRNA 'sponges' or decoys.[59–62] Previous investigations revealed that an effector lncRNA downstream of TP53—LOC285194, also referred to as tumor-suppressor candidate 7—is significantly deregulated in CRC[63] and can suppress tumor cell growth both *in vitro* and *in vivo*.[64,65] Exon 4 of LOC285194, which is responsible for tumor cell growth inhibition, contains an active region that harbors two MIR211 binding sites. Moreover, *in vivo* studies confirmed that LOC285194 can bind to and downregulate MIR211.[64] Thus LOC285194 may modulate the cancer phenotype through its function as an endogenous miRNA sponge. Additionally, a reciprocal repression feedback loop exists between LOC285194 and MIR211, suggesting that miRNAs can also modulate lncRNAs.[64] In this scenario, lncRNAs and miRNAs may interact as components of a new genomic regulatory network, thereby greatly expanding the functional genetic information in the human genome.

Table 1 LncRNAs that are linked to colorectal cancer

LncRNA	*Size (bp)*	*Cytoband*	*Expression level*	*Potential function and mechanism*	*Indication*	*References*
CCAT1	2407	8q24.21	Increased	NA	NA	84,85,90
CCAT2	340	8q24.21	Increased	Mediates MYC and WNT signaling, promotes tumor growth, metastasis, and chromosomal instability	NA	58
CRNDE	1070	16q12.2	Increased	Promotes growth and suppresses apoptosis	Diagnostic biomarker	82,99
E2F4 antisense	~5000	16q21-22	Increased	Induced by WNT/beta-catenin signaling, which leads to decreased levels of E2F4	NA	100
HOTAIR	2158	12q13.13	Increased	Promotes cell invasion	Metastasis, prognosis	20
HULC	500	6p24.3	Increased in LMN	NA	Diagnostic biomarker	89
MALAT1	8708	11q13.1	Increased	Promotes proliferation, invasion, and metastasis	NA	67
H19	2322	11p15.5	Increased or LOI	The absence of the H19 locus increases the number of polyps in the APC murine model, H19-derived MIR675 regulates RB	NA	101–104
PCAT1	1.9	8q24.21	Increased	NA	Prognosis	88
uc.73a	201	2q22.3	Increased	Promotes proliferation and suppresses apoptosis	Prognosis	62,87
uc.388	590	12q13.13	Increased	NA	Distal location	87
UCA1/CUDR	2314	19p13.12	Increased	NA	NA	94,95
XIST	19 296	Xq13.2	Increased in MSI sCRC	NA	NA	105
BA318C17.1	673	20p12.1	Decreased	NA	NA	66
lncRNA-LET/ NPTN-IT1	2606	15q24.1	Decreased	Hypoxia-induced histone deacetylase 3 represses lncRNA-LET by reducing the histone acetylation-mediated modulation of the lncRNA-LET promoter region, which leads to cancer cell invasion	NA	68
LOC285194/ TUSC7	2105	3q13.31	Decreased	A TP53-regulated tumor suppressor, inhibits growth through the repression of MIR211	Prognosis	63,64
MEG3	1595	14q32.2	Decreased	Mediates TP53 signaling, inhibits cell proliferation in the absence of TP53	NA	106,107
PTENP1	3932	9p21	Decreased	A decoy of the PTEN-targeting microRNAs, inhibits cell growth	NA	60
KCNQ1OT1/ LIT1	59461	11p15.5	LOI	NA	NA	57,108

Abbreviations: LMN, liver metastatic nodules; LOI, loss of imprinting; MSI, microsatellite instable; NA, not available; sCRC, sporadic colorectal cancers.

Regulation of lncRNA Expression

The expression of lncRNAs can be modulated by multiple regulatory factors, which naturally raises the question of how particular lncRNAs become deregulated in tumors. Although the underlying mechanism is generally unknown, it has been proposed that the expression of certain lncRNAs in CRC can be initiated or modulated by genetic (eg, SNPs in BA318C17.1,[66] sequence mutation of MALAT-1[67]), epigenetic (eg, MIR211-dependent regulation of LOC285194, histone deacetylation-induced hypoxia-mediated downregulation of lncRNA-LET/NPTN-IT1[68]), and transcriptional (eg, TCF7L2-dependent regulation of CCAT2,[58] TP53-dependent regulation of PVT1[69]) regulatory factors and may allow for the exact execution of malignant transformation to some extent.[58,67,68] Intriguingly, in addition to being affected by MIR211,[64] LOC285194 was also found to be regulated by genetic deletion (copy number variation) and TP53,[64,65] suggesting that the expression of specific lncRNAs may be regulated by several regulatory mechanisms.

Most variations in regulatory sequences lead to relatively subtle phenotypic changes.[70] SNPs are the most abundant DNA variations in the human genome and contribute to human phenotypic

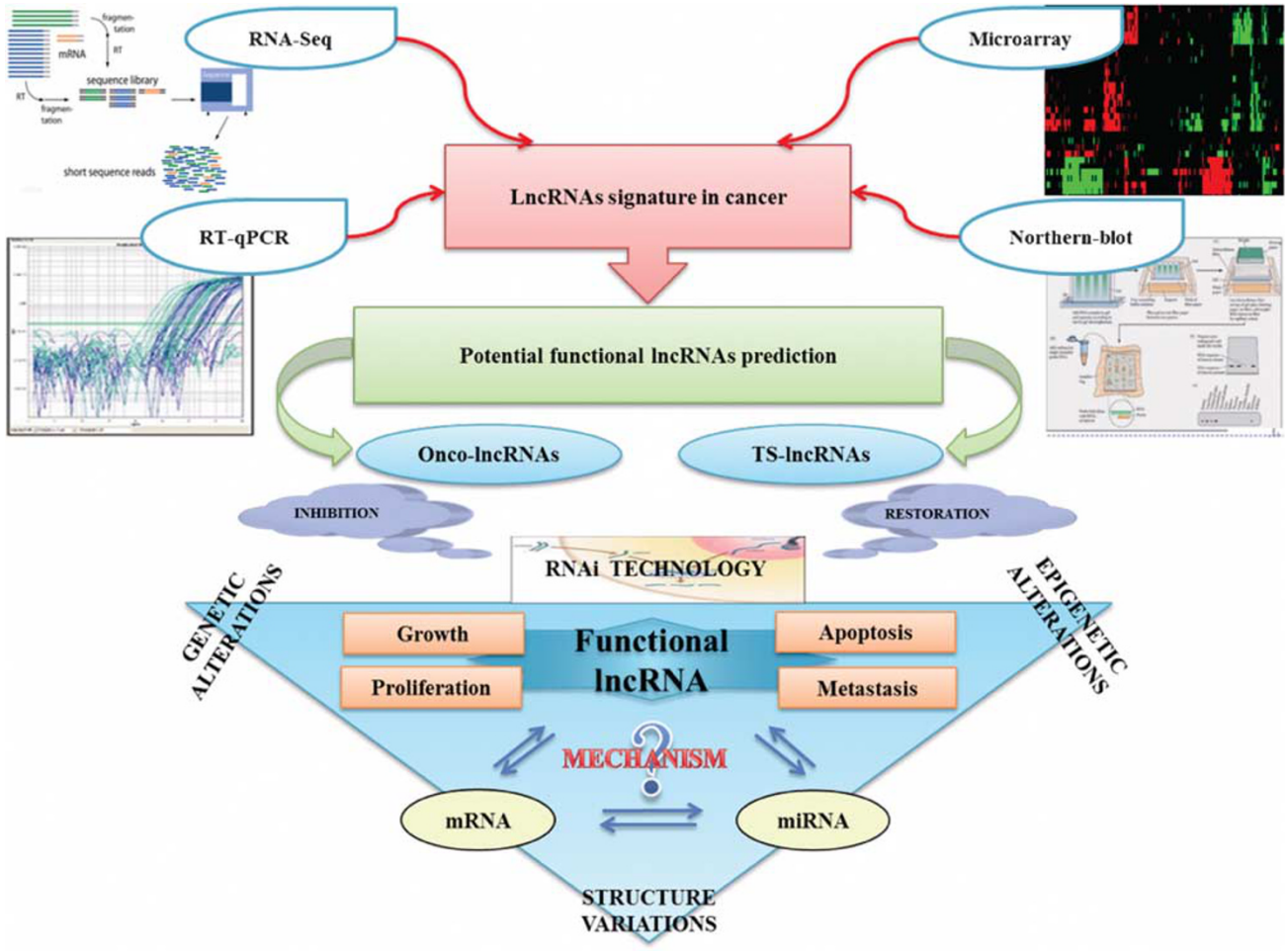

Figure 1 Strategy for the detection and identification of functional lncRNAs.

differences. The rs6983267 SNP region, located upstream of the MYC oncogene on chromosome 8q24, has been found to be consistently associated with an increased risk of CRC.[71] The G allele of rs6983267, in addition to being related to a significantly increased risk of CRC,[72] results in more CCAT2 transcripts than the T allele, which further affects the regulation of MYC by CCAT2; thus different rs6983267 alleles can affect CCAT2 expression and function.[58] Studies examining other cancer types have shown that SNPs in the key regulatory regions of an lncRNA can alter its structural motifs[73] and may consequently affect its expression and function in malignancies,[74] additionally contributing to cancer risk.[75,76] SNPs in the cancer-associated lncRNAs could potentially have pivotal roles in colorectal tumorigenesis and progression. Nevertheless, the means by which these alleles affect the lncRNAs and ultimately participate in colon carcinogenesis remains to be investigated.

LncRNAs in CRC may also harbor structural variations. Calin and colleagues recently identified a class of lncRNAs termed non-coding transcribed ultraconserved regions (T-UCRs); in humans, this class of lncRNAs shares 100% sequence conservation with both mouse and rat. In CRC patients, these loci frequently contain DNA sequence variations, including somatic variations, germline variations, and SNPs, compared with the general population; four T-UCRs were shown to harbor sequence variations in 35 CRC patients, whereas none of these mutations existed in a cohort of 175 cancer-free control patients.[77] Nevertheless, because no studies verified that these T-UCRs were deregulated or involved in CRC predisposition, it is difficult to determine whether the sequence mutations in these T-UCRs have specific impacts on colon carcinogenesis. Therefore, an examination of mutated lncRNAs in CRC will be important in future investigations.

Clinical applications of lncRNAs in CRC

Given the versatile biological and pathological roles played by lncRNAs, it is not surprising that these molecules are associated with cancer progression. Therefore, their expression levels may be an

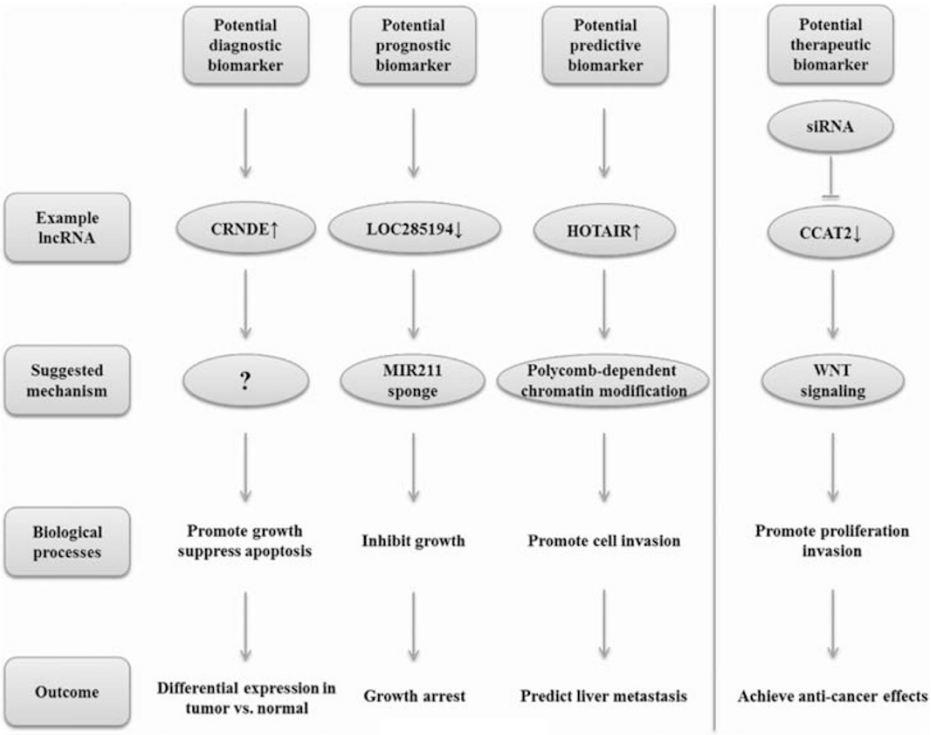

Figure 2 Potential uses of lncRNAs as diagnostic biomarkers, prognostic predictors, indicators of metastasis, and possible therapeutic targets in colorectal cancer.

indicator of the intrinsic characteristics of the tumor. As molecules that enable epigenetic regulation, aberrant expression of lncRNAs occurs before the phenotypic changes observed during the progression to carcinoma. Additionally, due to their tissue specificity, lncRNAs could potentially be more sensitive for diagnosis and more specific prognostic predictors than the current DNA, protein-coding RNA, or protein biomarkers (Figure 2).

Tumor Diagnosis

Tumor-derived DNA and RNA can be released and circulated in the peripheral circulation of cancer patients, allowing for non-invasive gene expression profiling by body fluid analysis. Indeed, there is a wealth of information indicating a correlation between tumor-associated changes in genomic, epigenetic, or transcriptional patterns and alterations in the levels of cell-free circulating nucleic acids (cfCNAs).[78] Our recent studies and others have suggested that cancer-specific DNA, mRNA, and miRNAs are stable and detectable in the peripheral blood of cancer patients, suggesting that cfCNAs may be promising biomarkers for cancer diagnosis and monitoring.[6,7,79–81] Notably, similar to other nucleic acids, lncRNAs can also be present in peripheral blood components, such as serum, plasma, and peripheral blood mononuclear cells.[82–84] The peripheral lncRNA profiles may potentially contribute to the characterization of novel biomarkers for CRC patients. Indeed, some lncRNAs appear to have strong diagnostic potential as blood biomarkers for colorectal neoplasia. For example, the expression of colorectal neoplasia differentially expressed-h in the plasma had a sensitivity of 87% and a specificity of 93% for detecting CRC.[82] It is likely that circulating lncRNAs may represent new, relatively non-invasive molecular markers of tumor activity. However, no lncRNA-based diagnostics have been developed for use in CRC as of yet.

Because most cases of CRC usually undergo sequential progression, and polypectomy can prevent colorectal adenomas from developing into malignant lesions, biomarkers for the clinical screening of premalignant conditions could result in early detection and early treatment of CRC, leading to the prevention of advanced disease. Recently, based on its unique expression characteristics in CRC, the lncRNA CCAT1 has emerged as a potential biomarker for screening precancerous lesions. CCAT1 is markedly overexpressed in CRC[84] and is also upregulated in precancerous tissues, including benign inflammatory colonic tissues and adenomatous polyps.[85] CCAT1 can be detected in CRC tissue and in the blood and stool samples of CRC patients.[86] Thus a CCAT1-based blood/stool assay may be explored for the screening and early detection of CRC.

Tumor Prognosis

Several studies have documented a link between the dysregulation of lncRNAs and the pathogenesis/prognosis of CRC (Table 1); thus lncRNA signatures may be utilized as a valuable tool for the prediction of disease outcomes in conjunction with the prevailing mRNA and miRNA expression signatures. The expression level of *uc.73a* is lower in CRC tissues than in the corresponding noncancerous tissues;[87] moreover, patients with low *uc.73a* expression have a relatively poor overall survival. Similarly, the expression level of PCAT-1 in 108 CRC patients can successfully distinguish between short-term and long-term survivors.[88] Our group also found that the downregulation of LOC285194 is correlated with several clinicopathological factors, such as tumor size and metastasis, in patients suffering from CRC. Indeed, in a multivariate analysis with other clinicopathological risk factors, LOC285194 served as a significant and independent predictor of disease-specific survival.[63] These lncRNAs may be exploited as novel prognostic tools to aid in CRC patient assessment and management in oncology clinics.

Particularly, some lncRNAs in CRC tissue samples exhibit differential expression patterns during specific tumor stages or in specific locations. One such lncRNA, HOTAIR, was highly expressed in cancerous tissues compared with the corresponding noncancerous tissues in stage IV CRC patients, and the levels of HOTAIR were correlated with liver metastasis.[20] HULC is an lncRNA that was identified in human HCC tissues; intriguingly, HULC expression was found to be high in CRC liver metastatic nodules but not in primary CRC tissues or the corresponding normal tissues.[89] These results suggest that individual lncRNAs or lncRNA panels could potentially be utilized as tailored biomarkers for monitoring CRC metastasis. With the aid of the appropriate pharmaceutical vehicles, such as lncRNA-specific peptide nucleic acid (PNA)-based molecular beacons,[90] it may be possible to differentiate malignant lesions from benign tissues and to identify metastatic lesions during surgery using real-time *in vivo* imaging in the near future.

Tumor Treatment

The expression levels of lncRNAs may aid in tumor diagnosis and prognosis; additionally, lncRNAs may be interesting therapeutic targets for CRC. The finding that certain pathologically dysregulated lncRNAs have crucial roles in the modulation of tumor behavior by acting as oncogenes (onco-lncRNAs) or tumor suppressors (TS-lncRNAs) suggests the potential for the development of lncRNA-oriented therapies (Figure 2).

SiRNAs can easily be designed to inhibit the function of specific lncRNAs, and the regulation of specific lncRNAs that are tightly linked to key carcinogenesis processes may have promising anticancer effects. In the case of CRC, the administration of specific siRNAs targeting the oncogenic HOTAIR, CCAT2, and *uc.73a* transcripts was shown to decrease the invasion or proliferation of colon cancer cells.[20,58,62] Although the results presented in these studies are only preliminary *in vitro* data, these reports suggest that RNAi-based therapy strategies are amenable to further development, particularly for cellular therapies.

In contrast to blocking onco-lncRNAs, restoring the expression of TS-lncRNAs in cancer patients could also achieve anticancer effects based on their intrinsic tumor-suppressive functions. By implanting colon cancer cells into nude mice after transfection with LOC285194 expression vectors, Liu *et al*[64] successfully demonstrated the tumor-suppressive effects of LOC285194 *in vivo*; both the tumor growth rate and the final tumor weight were significantly decreased when LOC285194 was overexpressed.

Given that lncRNAs can have structural interactions with other molecules, therapeutic effects can also be achieved by disrupting their structures or functional motifs or by modulating their transcriptional activity. For example, one study reported that targeting the interactions between Xist and chromatin remodeling complexes resulted in the activation of PRC2-regulated genes.[91] However, the practical feasibility of these novel strategies has not been studied in detail for CRC.

The acquisition of drug resistance is one of the main obstacles encountered in cancer chemotherapy, and some publications have suggested that lncRNAs may be critical regulators of drug sensitivity or resistance.[92,93] Cancer upregulated drug resistant (CUDR) is an lncRNA that exhibits elevated expression in multiple cancers, including CRC.[94,95] Tsang *et al*[94] provided evidence that increasing CUDR gene levels *in vitro* in human squamous carcinoma cells resulted in increased resistance to doxorubicin and etoposide and decreased levels of drug-induced apoptosis via the downregulation of caspase-3. The question of whether similar mechanisms operate in CRC should be examined further, but this observation suggests that lncRNA manipulation-based gene therapy may be beneficial in overcoming drug resistance and in designing personalized therapeutics for cancer patients.

Additionally, to reduce the treatment-related side effects during transgene-mediated treatment, the use of certain tumor-specific lncRNAs as therapeutic agents in CRC is currently being explored in preclinical models. For example, H19 is highly expressed in cancer tissues and is only marginally or not at all expressed in the corresponding normal tissues. This characteristic was utilized to refine the regional therapeutic methods, and a chain plasmid vector driven by specific H19 regulatory sequences was developed. This plasmid harbors diphtheria

toxin-A and was administered intra-arterially to animals with colon cancer liver metastases. The plasmid was selectively distributed to the tumor region and successfully delayed tumor growth.[96]

Conclusions and future perspectives

There is no doubt that the dysregulation of lncRNAs affects various cancer-related signaling pathways and has a significant role in tumor development. Currently, a variety of large-scale genomic studies, such as TCGA, are being used to investigate the abnormal expression profiles of lncRNAs in tumor;[97] however, the molecular nature of most lncRNAs remains poorly characterized. Due to their complicated structural characteristics, tissue-specific expression, and specific temporal and spatial expression patterns, the detailed functions and mechanisms of one lncRNA may be significantly different in different tumors.[24] Therefore, to attain a thorough understanding of the role of lncRNAs, besides to elucidate the expression patterns of lncRNAs in CRC, further structural, functional, and mechanistic characterizations should also be performed.

Nevertheless, while fulfilling critical roles as transcriptional, post-transcriptional, or epigenetic regulators, lncRNAs themselves are extensively modulated by multiple inherited genetic and epigenetic alterations in addition to structural variations and transcriptional regulators; all of these factors contribute to their dysregulation in malignancies and are consequently implicated in the etiology and pathophysiology of cancer. For this reason, it is of great interest to investigate the association between these lncRNA regulatory mechanisms and their subsequent effects on lncRNA structure, expression, and function in CRC and other cancers.

Moving forward, the systematic identification and annotation of tissue-specific lncRNA signatures and their expression patterns in tumors hold great promise for the development of accurate, non-invasive biomarkers that can be utilized in early CRC detection and prognosis prediction. Although several studies have already uncovered some candidate lncRNAs and demonstrated their association with clinicopathological characteristics, there are considerable limitations to the translation of lncRNAs into clinical practice. First, one of the prerequisites of an ideal cancer marker is stability, and the stability of circulating lncRNAs remains largely unknown. Second, although numerous studies have demonstrated the presence of lncRNAs in the circulation, the levels of lncRNA transcripts and their post-transcriptional modifications are unstable and variable or are difficult to detect during different disease stages. Third, there is no simple standard assay or universal endogenous control/control set for the quantification of circulating lncRNAs. Finally, it is unknown whether these lncRNAs are secreted from the cells of tissues or whether they are present due to hematocyte contamination. It is difficult to determine the origins of circulating lncRNAs that have been isolated and quantified. Future approaches should focus on overcoming these obstacles. Even if these problems are successfully solved, the lncRNA biomarkers remain needs for further analytic and clinical validation, and demonstration of clinical utility before it should be considered for use in general practice to become generally useful.[98]

Finally, investigations of the molecular mechanism(s) by which lncRNAs act and *in vivo* animal studies exploring lncRNA intervention demonstrate the potential for the use of lncRNAs in novel anticancer therapies. Like other important ncRNAs, such as miRNAs, lncRNAs are also natural nucleic acids that regulate many gene networks involved in cancer cell transformation; thus the rationale for using lncRNAs in CRC therapies is clear. However, several common challenges in RNA therapeutics, such as the lack of reliable delivery methods, limited effective vector types, lack of optimal dosage regimes, and side effects, have made lncRNA-based therapy difficult to achieve. Moreover, due to the large size and extensive secondary structures of lncRNAs, it is difficult to interfere with their expression using conventional RNAi technologies, such as siRNA and antisense oligonucleotides, and it is also difficult to use them directly as therapeutic agents Because these technical bottlenecks cannot be ignored, novel effective and stable strategies for genome editing—as well as effective gene therapy delivery systems—warrant further investigation. Nevertheless, although at present it may be too optimistic or premature to affirm the potential of lncRNA-targeted treatment, the momentum in the lncRNA-based research field will undoubtedly stimulate research in the field of ideal therapeutics for CRC patients in the near future.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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