Screening of lymph nodes metastasis associated lncRNAs in colorectal cancer patients

Jun Han, Long-Fei Rong, Chuan-Bin Shi, Xiao-Gang Dong, Jie Wang, Bao-Lin Wang, Hao Wen, Zhen-Yu He

Abstract

AIM: To screen lymph nodes metastasis associated long noncoding RNAs (lncRNAs) in colorectal cancer through microarray analysis.

METHODS: Metastatic lymph node (MLN), normal lymph node (NLN) and tumor tissues of 3 colorectal cancer (CRC) patients were collected during the operation and validated by pathological examinations. RNAs were extracted from MLN, NLN, and cancer tissues separately. RNA quantity and quality were measured with a NanoDrop ND-1000 spectrophotometer and RNA integrity was assessed by standard denaturing agarose electrophoresis. Agilent Feature Extraction Software (Version 11.0.1.1) was used to analyze acquired array images. Four differently expressed lncRNAs were confirmed by quantitative real-time polymerase chain reaction (qRT-PCR) in 26 subsets of MLN, NLN, and tumor tissues.

RESULTS: Of 33045 lncRNAs, 1133 were differentially expressed in MLN compared with NLN, of which 260 were up-regulated and 873 down-regulated (>2 fold-change). Five hundred and forty-five lncRNAs were differentially expressed in MLN compared with tumor tissues, of which 460 were up-regulated and 85 down-regulated (>2 fold-change). Compared with NLN and cancer tissues, 14 lncRNAs were specifically up-regulated and 5 specifically down-regulated in MLN. AK307796, ENST00000425785, and AK021444 were confirmed to be specifically up-regulated in MLN and ENST00000465846 specifically down-regulated in MLN by qRT-PCR in 26 CRC patients.

CONCLUSION: The specifically expressed lncRNAs in MLN may exert a partial or key role in the progress of lymph nodes metastasis of CRC.

Key words: Long noncoding RNAs; Colorectal cancer; Lymph nodes metastasis; Quantitative real-time polymerase chain reaction; MicroRNA

Core tip: Long noncoding RNAs (lncRNAs) have been reported to be aberrantly expressed in a variety of human cancers. However, no data are available regarding their functions in the lymph nodes metastasis of colorectal cancer (CRC). Our study is the first study to focus on lymph nodes metastasis associated lncRNAs in CRC by microarray. Obvious changes of lncRNAs expression profiles were observed in metastatic lymph node, normal lymph node, and tumor tissues of CRC. These changes of lncRNAs may serve as new diagnostic biomarkers and therapeutic targets for lymph node metastasis of CRC.
Han J et al. LncRNAs and colorectal cancer

INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignant tumors worldwide. According to the result of IARC (International Agency for Research on Cancer), approximately 1.23 million CRC patients were diagnosed yearly worldwide and 0.6 million CRC patients died in 2008[1]. As we all know, the prime cause of death among CRC patients is metastasis such as blood metastasis and lymph node metastasis. Among various metastatic pathways, lymph node metastasis is the most frequent pathway, which plays an important role in affecting the prognosis of CRC patients. Clinically, approximately 50% of patients with lymph node metastasis of CRC will experience disease recurrence[2-4]. Lymph node metastasis is also one of the critical clinical markers to judge the tumor stage and to make specific therapeutic schedule in CRC patients. Thus, how to detect the lymph node metastasis at early stage and how to further study molecular mechanisms are essential for diagnosis and therapy of CRC.

Long noncoding RNAs (lncRNAs), longer than 200 nucleotides (nt) in length, are a kind of RNAs that do not encode proteins. LncRNAs, transcribed by RNA polymerase II (RNA pol II), were ever thought to be “transcriptional noise” without biomedical functions. However, lncRNAs have been validated to have comprehensive functions in biological processes such as inactivating X chromosome, regulating DNA metabolism, and activating transcription by recently published studies[5-7]. Increasing evidence indicated that lncRNAs play important roles in many human diseases, including various types of cancer[8]. LncRNAs were reported to be abnormally expressed in various cancers and were associated with tumor cell proliferation, growth, apoptosis, invasion, and metastasis[9,10]. When it comes to CRC, several lncRNAs have been reported to be oncogenic factors by inhibiting apoptosis and promoting cell proliferation and so on[11,12]; while several other lncRNAs have been reported to be tumor suppressive factors by inhibiting cell growth[13,14]. However, the roles lncRNAs play in the progress of lymph node metastasis of CRC remain unknown.

In this study, we profiled the lncRNA expression in metastatic lymph node (MLN), normal lymph node (NLN), and tumor tissues from 3 CRC patients by using Human LncRNA Array. Differentially expressed lncRNAs were identified by comparing 3 different tissues with each other. Meanwhile, we selected specifically expressed lncRNAs in MLN by comparing MLN with NLN and tumor tissues out of these specifically expressed lncRNAs in MLN, and 4 were evaluated by qRT-PCR in 26 additional subsets of MLN, NLN and tumor tissues. Our findings indicated that lncRNAs may play a significant role in the process of lymph node metastasis of CRC.

MATERIALS AND METHODS

Patient samples

Twenty-six CRC patients who were surgically treated at Second Affiliated Hospital of Nanjing Medical University from June 2011 to June 2012 were included in our study. All patients recruited in this study received neither chemotherapy nor radiotherapy before the surgery. Written informed consent was obtained from all patients and permission for this study was obtained from the ethics committee of Second Affiliated Hospital of Nanjing Medical University. A set of lymph node and tumor tissues were collected during the operation and stained with HE by two experienced pathologists. Lymph nodes were divided into MLN and NLN according to the HE staining results. All samples were frozen in liquid nitrogen and stored at -80 °C until further analysis. Out of 87 samples, 9 (3 MLN, 3 NLN and, 3 tumor tissues) from 3 patients were used for microarray analysis of lncRNAs and the others were used for an extra evaluation.

RNA extraction

Total RNAs were extracted from 26 snap frozen subsets of MLN, NLN, and tumor tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, United States) according to the manufacturer’s protocol. RNA quantity and quality were measured with a NanoDrop ND-1000 spectrometer and RNA integrity was assessed by standard denaturing agarose gel electrophoresis.

RNA labeling, array hybridization and data analysis

Sample labeling and array hybridization were performed according to the Agilent one-color microarray-based gene expression analysis protocol (Agilent Technology) with minor modifications. Briefly, mRNA was purified from total RNA after removal of rRNA (mRNA-ONLY™ eukaryotic mRNA isolation kit, Epicentre). Then, each sample was amplified and transcribed into fluorescent cRNA along the entire length of the transcripts without 3’ bias utilizing a random priming method. The labeled cRNAs were purified by RNeasy Mini Kit (Qiagen). The concentration and specific activity of the labeled cRNAs (pmol Cy3/μg cRNA) were measured with a NanoDrop ND-1000 spectrometer. Each labeled cRNA (1 μg) was fragmented by adding 5 μL 10 × Blocking Agent and 1 μL of 25 × Fragmentation Buffer, then the mixture was heated at 60 °C for 30 min, and finally 25 μL 2 × GE Hybridization Buffer was added to dilute the labeled cRNA. Fifty microliters of hybridization solution was dispensed into the gasket slide and assembled to the lncRNA expression microarray slide. The slides were incubated for 17 h at 65 °C in an Agilent hybridization oven. The hybridized arrays were washed, fixed and scanned using an

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Agilent DNA microarray scanner (part number G2505C). Agilent feature extraction software (version 11.0.1.1) was used to analyze the array images. Quantile normalization and subsequent data processing were performed using the GeneSpring GX v11.5.1 software package (Agilent Technologies). A normalized value is a relative number that comes from the ratio of the raw data value of the listed probe to that of the control. After quantile normalization of the raw data, lncRNAs and mRNAs with 9 samples that have flags in present or marginal were chosen for further data analysis. Differentially expressed lncRNAs and mRNAs with statistical significance were identified through Volcano Plot filtering and fold change filtering. Hierarchical clustering was performed using the Agilent GeneSpring GX software (Version 11.5.1). GO analysis and pathway analysis were performed using the standard enrichment computation method. The microarray work was performed by KangChen Bio-tech Shanghai, China.

**Statistical analysis**

Quantitative real-time polymerase chain reaction (qRT-PCR) was used to verify differential expression of 4 lncRNAs that were detected to be specifically expressed in MLN by the lncRNA expression microarray. The first strand cDNA was synthesized using SuperScript™ III Reverse Transcriptase (Invitrogen), RNase Inhibitor (Epicentre), and 1.25 mmol/L dNTPs Mix. Each qRT-PCR reaction (in 10 μL) contained 2 × Super Array PCR master mix 5 μL, 10 μmol/L PCR forward primer 0.5 μL, 10 μmol/L PCR reverse primer 0.5 μL, diluted first strand cDNA synthesis reaction 2 μL, and ddH2O 2 μL. The cycling conditions consisted of an initial, single cycle of 10 min at 95 ℃, followed by 40 cycles of 10 s at 95 ℃, 60 s at 60 ℃, and 15 s at 95 ℃. For each sample, we performed qRT-PCR for target genes and a housekeeping gene. A standard curve was constructed using serial 10-fold dilutions (from 1 to 10^9) of the PCR products to quantify the results. According to the standard curve, the gene concentration of each sample is generated directly using Rotor-Gene Real-Time Analysis Software 6.0. For each sample, the relative amount of the target gene is determined by calculating the ratio between the concentration of the target gene and that of the housekeeping gene. For each target gene, we took the relative amount of the control sample as 1, and then the relative amount of the other samples as n fold of the control sample. The lncRNA expression differences between two groups were analyzed using Student’s t test. P < 0.05 was considered statistically significant.

**RESULTS**

**Patients and array information**

Clinical and pathological information of 3 patients were used for microarray analysis. Briefly, more than 15 lymph nodes were harvested and at least one was confirmed to be metastatic by HE staining in each patient (Figure 1).

Arraystar human lncRNA microarray V2.0 is designed for the global profiling of human lncRNAs and protein-coding transcripts. A total of 33045 lncRNAs and 30215 coding transcripts can be detected by this second-generation lncRNAs microarray. The lncRNAs are carefully collected from the most authoritative databases such as RefSeq_NR, UCSC_Known Genes, Ensembl, H-invDB, UCR, lincRNA, and the related literature. Each transcript is represented by a specific exon or splice junction probe which can identify individual transcript accurately. Positive probes for housekeeping genes and negative probes are also printed onto the array for hybridization quality control.

**LncRNA expression profile in MLN, NLN and tumor tissues**

After quantile normalization of the raw data, lncRNAs with 9 samples that have flags in present or marginal were chosen for differentially expressed lncRNA screening. Out of 33045 lncRNAs, 20705 were in present or marginal in all 9 samples.

**Quality assessment of lncRNA data after filtering**

The box-plot is a convenient way to quickly visualize the distributions of a dataset. It is commonly used for comparing the distributions of the intensities from all samples. After normalization, the distributions of expression values for the samples are nearly the same, indicating...
that it is suitable for further analysis. The scatter-plot is a visualization method used for assessing the lncRNA expression variation (or reproducibility) between the two compared samples or two compared sample groups.

**Screening of differentially expressed lncRNAs**

Initially, we performed a volcano plot filtering between two groups to identify differentially expressed lncRNAs with statistical significance (fold change $\geq 2.0\text{, }P \leq 0.05$). From the data of microarray, variations of lncRNA and mRNA expression were shown in Tables 1 and 2. Briefly, compared with the NLN group, 873 probe sets representing 471 lncRNAs were down-regulated, while 2038 probe sets representing 1396 lncRNAs were up-regulated in the NLN group. Then, we performed a fold change filtering between every two samples in each patient to identify differentially expressed lncRNAs (fold change $\geq 2.0$). The

Table 1 Summary of data from long noncoding RNA microarray for three pairs of metastatic lymph nodes, normal lymph nodes and tumor tissue of colorectal cancer

| Sample ID | InRNA | Fold change $\leq 4$ | Fold change $> 4$ | Total Changes lncRNA |
|-----------|-------|----------------------|-------------------|----------------------|
| R1        | MLN vs NLN | Up-regulation | 704 | 161 | 865 | 2087 |
|           | Down-regulation | 1034 | 188 | 1222 |
|           | MLN vs tumor | Up-regulation | 2864 | 1720 | 4584 | 9052 |
|           | Down-regulation | 2421 | 2047 | 4468 |
| R2        | MLN vs NLN | Up-regulation | 1223 | 554 | 1777 | 3923 |
|           | Down-regulation | 1934 | 412 | 2346 |
|           | MLN vs tumor | Up-regulation | 488 | 105 | 593 | 1109 |
|           | Down-regulation | 457 | 59 | 516 |
| R3        | MLN vs NLN | Up-regulation | 1557 | 399 | 1956 | 3707 |
|           | Down-regulation | 1441 | 310 | 1751 |
|           | MLM vs tumor | Up-regulation | 915 | 114 | 1029 | 1572 |
|           | Down-regulation | 482 | 61 | 543 |

MLN: Metastatic lymph nodes; NLN: Normal lymph nodes; Tumor: Tumor tissue of colorectal cancer. lncRNA: Long noncoding RNA.

Table 2 Summary of data from message RNA microarray for three pairs of metastatic lymph nodes, normal lymph nodes and tumor tissue of colorectal cancer

| Sample ID | Message RNA | Fold change $\leq 4$ | Fold change $> 4$ | Total Changes lncRNA |
|-----------|-------------|----------------------|-------------------|----------------------|
| R1        | Positive vs negative | Up-regulation | 1421 | 310 | 1731 | 2818 |
|           | Down-regulation | 910 | 177 | 1087 |
|           | Positive vs tumor | Up-regulation | 4028 | 2762 | 6790 | 10525 |
|           | Down-regulation | 2112 | 1623 | 3735 |
| R2        | Positive vs negative | Up-regulation | 1354 | 544 | 1898 | 4354 |
|           | Down-regulation | 1960 | 496 | 2456 |
| R3        | Positive vs negative | Up-regulation | 1706 | 612 | 2318 | 4317 |
|           | Down-regulation | 1562 | 437 | 1999 |

MLN: Metastatic lymph nodes; NLN: Normal lymph nodes; Tumor: Tumor tissue of colorectal cancer.
number of up-regulated and down-regulated lncRNAs varied in different patients compared with that of different samples. Interestingly, we found that the number of differently expressed lncRNAs between NLN and tumor tissues was much larger than that of differently expressed lncRNAs both between MLN and NLN and between MLN and tumor tissue. M: Metastatic lymph nodes; N: Normal lymph node; T: Tumor tissue of colorectal cancer.

Next, we collected the specifically expressed lncRNAs in MLN by comparing MLN with NLN and tumor tissues, respectively. Only 9 probe sets representing 5 lncRNAs were specifically down-regulated and 19 probe sets representing 14 lncRNAs specifically up-regulated in the MLN group compared with the NLN group and tumor tissue group (fold change ≥ 2.0, P ≤ 0.05). Of these, AK021444 (absolute fold change MLN/NLN = 7.63431269) was the most significantly up-regulated one, while BC042589 (MLN/NLN = 4.323467267) was the most significantly down-regulated one in the MLN group (Table 3). In each patient, there were 548, 193, and 134 specifically down-regulated probe sets (representing 424, 144, 113 lncRNAs), and 862, 135, and 693 specifically up-regulated ones (representing 593, 111, 522 lncRNAs) in MLN, NLN and tumor tissues, respectively.

Additionally, we determined whether lncRNAs changed gradually from tumor tissue to MLN and then from MLN to NLN. We summarized the gradually increased and decreased lncRNAs from tumor tissue to MLN then to NLN. These lncRNAs may also play an important role in facilitating the CRC tumor cell transfer from the primary tumor to lymph nodes.

As reported, lncRNAs exerted important roles in gene expression, especially their nearby coding genes. The possible relationships of lncRNAs with their nearby coding genes included natural antisense [18-20], exon sense-overlapping [21], intron sense-overlapping, intronic anti-
sense\textsuperscript{[22,23]}, bidirectional\textsuperscript{[24-26]}, and intergenic\textsuperscript{[27]}. We analyzed the relationship between lncRNAs and their nearby coding genes and each lncRNA was annotated by their associated genes and proteins.

**Heat map and hierarchical clustering**
Hierarchical clustering is one of the most widely used clustering methods for analyzing lncRNA expression data. Cluster analysis arranges samples into groups based on their expression levels, which allows us to hypothesize the relationships among samples. Here, hierarchical clustering was performed based on “differentially expressed lncRNAs”. The result of hierarchical clustering showed distinguishable lncRNA expression profiles among samples which were divided into two main subgroups, signifying two different tissues.

**LncRNA classification and subgroup analysis**
Some specific classes of lncRNAs, such as enhancer lncRNAs, rinn lncRNAs, HOX cluster, lincRNAs near coding genes, and enhancer lncRNAs near coding genes, have been reported to be involved in the development of many diseases, especially cancers\textsuperscript{[28-31]}. Thus, we analyzed the expression levels of these lncRNAs in 9 samples. LncRNAs with enhancer-like function (LncRNA-a) are identified using GENCODE annotation of the human genes\textsuperscript{[28]}. Depletion of these lncRNAs led to decreased expression of the neighboring protein-coding genes, including the master regulator of hematopoiesis, SCL (also called TAL1), Snai1, and Snai2. LncRNAs were demonstrated to be necessary for the activation of gene expression\textsuperscript{[29]}. Nine hundred and one enhancer lncRNAs were identified to be expressed in samples by microarray. Rinn et al\textsuperscript{[31]} characterized the transcriptional landscape of the four human Hox loci and identified a total of 407 discrete transcribed regions in the four human Hox loci. Transcription of these lncRNAs may demarcate chromosomal domains of gene silencing at a distance, which contains profiling data of all probes in the four human Hox loci.

**Overview of mRNA profiles**
17159 mRNAs out of 30215 coding transcripts could be detected in 9 samples. Generally, hundreds of mRNAs were statistically aberrantly expressed among the 3 different kinds of tissues. GO and pathway analyses showed that the different expressed mRNAs might be involved in T/B cell receptor signaling pathway and primary immunodeficiency, which indicated that lymph node metastasis of CRC was closely associated with deficiency of immune cells.

**Verifying the microarray results using qRT-PCR**
To verify the result of lncRNA array, we selected 4 specifically expressed lncRNAs in MLN to confirm their expression levels by qRT-PCR in 26 CRC patients with lymph node metastasis (Table 4). The data were in agreement with the results of the microarray analysis. Out
Figure 3  Comparison between microarray data and quantitative real-time polymerase chain reaction results. AK021444, ENST00000425785, AK307796 and ENST00000465846 were determined to be differentially expressed by microarray in 3 colorectal cancer patients, and this result was validated by quantitative polymerase chain reaction result (qPCR). AK021444, ENST00000425785, and AK307796, 3 specifically up-regulated lncRNAs in metastatic lymph nodes (MLN), were confirmed to be highly expressed in MLN, while ENST00000465846 was confirmed to be down-regulated in MLN. The validation results for the four long noncoding RNAs (lncRNAs) indicated that the microarray data correlated well with the qPCR results. M: Metastatic lymph nodes; N: Normal lymph nodes; T: Tumor tissue of colorectal cancer.

DISCUSSION

Recent advances in genome analysis, including massively parallel sequencing and microarray, have shown that a much greater portion of the human genome is pervasively transcribed into RNA than previously recognized. Moreover, much of the evidence emerging in recent years has highlighted the biological and pathological importance of RNAs that lack protein-coding potential; these are collectively referred to as noncoding RNAs (ncRNAs)\cite{9,33-35}. MiRNAs are a kind of well-defined ncRNAs that play critical roles in many diseases including cancer. In contrast, functions of a majority of lncRNAs remain unknown, but recent studies have begun to shed light on the critical roles played by lncRNAs in a variety of cellular processes such as tumorigenesis and malignancy transformation in various types of cancer\cite{9,33-35,8145}. However, the potential use of lncRNA expression profiling to identify lymph node metastasis of CRC has not been systematically explored. To uncover the potential role lncRNAs play in lymph node metastasis of CRC, we investigated the lncRNA expression signatures in MLN, NLN, and tumor tissues of CRC.

Arraystar Human LncRNA Microarray V2.0 is one of the most commonly used commercial microarrays in human cancer profiling\cite{8145,33,36}. LncRNAs were collected from databases that were very comprehensive and authoritative. It allows the analysis of mRNA and lncRNA expression at the same time and is easier to follow than transcript sequencing analysis.

In this study, to be comprehensive, we selected 3 CRC patients including 1 right colon cancer patient, 1 left colon cancer patient, and 1 rectal cancer patient. The results indicated that rectal cancer and colon cancer had a relatively greater diversity in lncRNA expression. For instance, 9051 lncRNAs altered between MLN and tumor tissues in the rectal patient while only 1109 and 1572 lncRNAs altered in the two colon cancer patients, respectively. The lncRNA expression features may be greatly different between colon cancer and rectal cancer, but more information was needed to confirm this disparity. In each patient, to reduce the histological difference, we compared the MLN with NLN and tumor tissues, respectively. The numbers of differentially expressed lncRNAs were 389 between MLN and tumor tissue, 829 between MLN and NLN, and 1867 between NLN and tumor tissue. Our results indicated that tumor tissue and lymph nodes are two quite different kinds of tissues. Thus, it was reasonable to compare MLN both with NLN and tumor tissue to identify the specifically altered lncRNAs in MLN.

Although hundreds of lncRNAs were deregulated when comparing the MLN group with the NLN group and tumor tissue group, respectively, only 14 lncRNAs were specifically up-regulated in MLN (both compared with NLN and tumor tissue): uc004aej.2, ENST00000393311, ENST00000447552, AK025180, AW449673, ENST00000450572, ENST00000483126, ENST00000425785, CR599788, AK097728, uc002fs.1, BC036914, AK021444, and AK307796; and only 5 were specifically down-regulated in MLN: BC042589, ENST00000429729, uc002txz.3, ENST00000465846, ENST00000418346. These lncRNAs were specifically expressed in MLN, indicating that their potential important role in facilitating the occurrence of lymph node metastasis of CRC. Unfortunately, after careful retrieval, no additional information of direct functions of these lncRNAs was available except the papers that first described their
discovered. Further research is urgently needed to clarify the functions and the underlying mechanism of these lncRNAs in the process of lymph node metastasis of CRC.

Generally, tumor cells transfer from the primary tumor to adjacent lymph nodes and finally reach distant lymph nodes. To determine whether certain lncRNAs gradually change during lymph node metastasis, we identified those lncRNAs that gradually up-regulated and down-regulated from tumor tissue to MLN then from MLN to NLN. Four and 66 lncRNAs were identified to be gradually up-regulated and down-regulated from tumor tissue to MLN and NLN, respectively. Unfortunately, as identified above, functions of these lncRNAs were also unclear. We supposed that these gradually changed lncRNAs may also play partial roles in the progress of lymph node metastasis. But more evidence should be collected to confirm this hypothesis.

To validate the consistency of the gene chip, we selected 4 specifically expressed lncRNAs (AK021444, ENST00000425785, AK307796, and ENST00000465846) to evaluate their expression in 26 CRC patients with lymph node metastasis. The results of qRT-PCR were consistent well with the chip results. AK021444 is a 1611 bp lncRNA collected from ncRNA Expression Database (http://jsm-research.imb.uq.edu.au/nred/cgi-bin/ncrnadb.pl). It is located on chromosome 13 and associated with gene (nearby gene) POSTN (periostin). All of 4 isoforms of peristin protein could be detected to be associated with AK021444 in our samples. POSTN was reported to be up-regulated in various cancers including non-small cell lung cancer, pancreatic cancer, breast cancer, and CRC. Furthermore, Bao et al. reported that POSTN in those hepatic metastases was 2.6-fold of that in the matched colon primary tumors. POSTN was also reported to be overexpressed in serum of CRC patients and the overexpression of POSTN was correlated with distant metastasis of CRC. In addition, POSTN was reported to be correlated with lymph node metastasis of many other malignant neoplasms. Furthermore, Bao et al. reported that POSTN in those hepatic metastases was 2.6-fold of that in the matched colon primary tumors. POSTN was also reported to be overexpressed in serum of CRC patients and the overexpression of POSTN was correlated with distant metastasis of CRC.

As far as we know, this is the first study to describe the expression profiles of human lncRNAs in MLN, NLN, and tumor tissues of CRC by microarray assay. In this study, we have identified hundreds of differentially expressed lncRNAs among these 3 different tissues. Thirteen lncRNAs and 5 lncRNAs were identified to be specifically up-regulated and down-regulated in MLN, respectively. AK021444, ENST00000425785, AK307796, and ENST00000465846 were validated to be specifically expressed in MLN of CRC. Our findings suggest that lncRNAs may play important roles in lymph node metastasis of CRC. However, the mechanism by which ENST00000425785 interacts with DPYD/DPD and the biological functions of ENST00000425785 need further study.
tasis of CRC. The clinical implications of our findings include the potential use of lncRNAs as molecular diagnosis markers and therapeutic targets. However, this is just a pilot study, and further studies are needed to expand the sample size for clinical research, and determine whether these lncRNAs can serve as new diagnostic biomarkers and therapeutic targets for lymph node metastasis of CRC.

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