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ORIGINAL ARTICLE

# Evaluation of the Effect of Circulating lncRNAs in Colorectal Cancers: As a Potential Biomarker

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**OBJECTIVE**

This study aimed to investigate the possibilities of using the *ARHGAP5-AS1, LOC152578, SNHG16, ZNRF3-IT1, CCAT1, CRNDE,* and *XLOC_000303* circulating lncRNAs as a non-invasive biomarker in colorectal cancers (CRCs).

**METHODS**

In this study, we enrolled plasma samples of 65 CRC patients (50 stages III/IV and 15 stages I/II) and plasma samples of 31 individuals in the control group of similar ages. Thereafter, we performed plasma separation and total RNA extraction; then, RNAs were reversely transcribed to complementary DNA. And then, we analyzed using a quantitative real-time polymerase chain reaction technique for lncRNA expression analysis.

**RESULTS**

Our results showed that the expression levels of *ZNRF3IT1* (p=0.011), CCAT1 (p=0.007), *CRNDE* (p=0.002), and *XLOC_000303* (p=0.001) were significantly upregulated in the CRC when it was compared to with the control group. *ZNRF3IT1, CCAT1, CRNDE,* and *XLOC_000303* lncRNAs were observed to have similar discriminating power. The calculated area under the curve of receiver operating characteristic was 0.66, 0.67, 0.70, and 0.70, respectively.

**CONCLUSION**

Our results revealed a high discriminatory power of *ZNRF3-IT1, CCAT1, CRNDE,* and *XLOC_000303* lncRNA in distinguishing CRC patients from healthy individuals. We also found that increased *XLOC_000303* expression is a protective factor against metastasis formation. This study demonstrated that *ZNRF3-IT1, CCAT1, CRNDE,* and *XLOC_000303* circulating lncRNAs may be used as a potential non-invasive biomarker in CRC. We think that the increased expression of *XLOC_000303* may be a protective factor against metastasis formation.

**Keywords:** Circulating lncRNA; colorectal cancer; plasma; real-time polymerase chain reaction.


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## Introduction

Colorectal cancer (CRC) is the 3rd most common type of cancer in the world and is also the 2nd leading cause of cancer-related deaths.[1-3] The etiology of the disease includes some factors such as age, gender, race and ethnicity, polyp history, and lifestyle. About 60-65% of CRC cases are sporadic, 25% of CRC have a family history, and 5% of CRC have a genetic predisposition.[4] The diagnosis and treatment of CRC has a great development in the past two decades. However, the fatality rate is still high, especially in advanced stage cases with distant organ metastasis.[5,6]

Understanding the molecular basis of colorectal carcinogenesis is important for both the prognosis and treatment of CRC. It is suggested that individualization of the treatment according to the pathological and molecular characteristics of the tumors and the better evaluation of the disease stage may have effective results in the progress of the disease. Many studies have been carried out in recent years to elucidate the molecular mechanisms of genetic factors that are effective in the development of CRC. Data, from these studies, have shown that circulating ncRNAs such as miRNA and lncRNA play a role in the progression of tumorigenesis, invasion, and metastasis in carcinomas.[7,8]

Long non-coding RNAs represent a heterogeneous group of RNAs ≥200 bp in size. LncRNAs effect to the regulation of numerous processes such as the cell cycle, apoptosis, histone modifications, chromosome imprinting, and cell differentiation.[5] They can cause to metastatic transformation at the transcriptional, post-transcriptional, and epigenetic levels. Moreover, they may exhibit oncogene or tumor suppressor-like behavior.[9,10] LncRNA participates in the physiological and pathological processes of the cell by regulating the expression of protein-coding genes. Therefore, the change in the expression level of lncRNA can lead to the emergence and development of various malignant diseases.[10,11]

The present study evaluated the possibilities of using circulating candidate lncRNAs as a non-invasive biomarker in CRC and also discussed, which is the power of these circulating lncRNAs to discriminate between patient and control groups.

## Materials and Methods

### LncRNA Selecting

For selecting potential CRC-associated lncRNA, it was used to obtain from the LNCipedia version 5.2 and NONCODE v5, LncRNADisease v2.0 database that has specific data in cancer development. In addition, candidate lncRNAs were determined by scanning related studies in the literature. The filtration was carried out by entering keywords such as circulating lncRNA, CRC, lncRNA, colon, rectum, plasma, serum, and tissue while searching these databases. As a result, these circulating lncRNAs were determined as *ARHGAP5-AS1, LOC152578, SNHG16, ZNRF3-IT1, CCAT1, CRNDE,* and *XLOC_000303*.

### Study Population

In this study, 96 participants were enrolled and divided into two groups. The first group included plasma samples of 65 patients who had been diagnosed with colon cancer or rectum cancer in the Department of Medical Oncology between February 2007 and April 2021. The second group included 31 age- and sex-matched healthy individuals who did not have a history of malignant disease in the study as a control group. In addition, the case group was evaluated in two subgroups as early-stage (15 patients) and advanced stage (50 patients).

### Plasma Preparation and Total RNA Extraction

Peripheral blood samples were collected from each participant in the study and were placed in the EDTA-anticoagulant tube. Blood samples were centrifuged within 2 h after collection at 3000 rpm for 15 min at +4°C, followed by 3000 rpm for 10 min at +4°C, and plasma was separated. The supernatant plasma was recovered and stored at -80°C until analysis. We extracted total RNA from 800 µl plasma by Trizol reagent according to the protocol of the manufacturer's instructions (A.B.T. Blood/Tissue RNA Purification Kit for Leukemia, Atlas Biotechnology Laboratory Materials Industry and Trade Ltd. Şti, Ankara, Turkey) and stored at -80°C until analysis. The purity and concentration of RNA were assessed using the Qubit device (Qubit 3 Fluorometer, Invitrogen, by Thermo Fisher Scientific, Malaysia).

### cDNA Synthesis

Total RNA was reversely transcribed and cDNAs were synthesized using a reverse transcription kit (RevertAid H Minus First Strand cDNA Synthesis Kit; Thermo Fisher Scientific Baltics UAB, Vilnius, Lithuania) according to the manufacturer's instructions. RNA was added to reverse transcription master mix (4 µl) to a final volume of 20 µL. Then, incubation period (including 5 min at 65°C), cDNA was synthesized with the following program of 5 min at 25°C, 60 min at 42°C,

**Table 1** Primer sequences for amplification of the studied lncRNAs

| Target gene | Forward sequence (5'→3') | Reverse sequence (5'→3') |
|---|---|---|
| *CCAT1* | CCTGGGCCACAAATCAACAA | TTGAGAAGGGGTGAAGGGAC |
| *CRNDE* | TTTCCGGAGTAGAGCCCTTG | CTCCTCCTTCCAATAGCCAGT |
| *LOC152578* | AGCTCCTCACTTCTTGGCTT | AGAGCCGGTATTGCAGTTCA |
| *XLOC_000303* | ACCTTGCAACACTCTCTGGA | TCCAACCTCTTCAGCTCCAG |
| *ARHGAP5-AS1* | TCAGCTTCCCTCTTTCTCCG | GTTCCTTTGCAGTACGGTGG |
| *SNHG16* | TGCGTTCTTTGGGCTTCATC | CAATCCTTGCAGTCCCATCG |
| *ZNRF3-IT1* | GATGTGGAGAGAGGAAGGGG | TCATCTCTAGCCCTGACCCT |
| *BETA-ACTIN* | GCCAACTTGTCCTTACCCAGA | AGGAACAGAGACCTGACCCC |

and 5 min at 70°C. cDNA Synthesis reactions were completed in a Thermocycler (GeneAmp PCR System 9700, Applied Biosystems, Singapore).

### Quantitative Real-Time Polymerase Chain Reaction (PCR) for lncRNA Expression Analysis

Quantitative RT-PCR (qRT-PCR) was performed using CFX96 real-time PCR systems (BIO-RAD, C1000 Touch Thermal Cycler, Bio-Rad Laboratories, Inc, California, USA). PCR amplification mix contained 10 µl SYBR Green Master Mix (Thermo Scientific™ Maxima SYBR Green/ROX qPCR Master Mix (×2), Thermo Fisher Scientific Inc, California, USA), 2 µl of cDNA product, 2 µl of primer, and 6 µl of RNase-free water. Thus, a final volume was completed to 20 µL. *β-actin* was used as a reference gene. The real-time PCR amplification mix was incubated at 95°C for 10 min for Taq activation, followed by 95°C for 15 s for denaturation and 60°C for 40 s annealing and extension during 40 cycles. The changes in the plasma expression levels of candidate lncRNAs were investigated using a qRT-PCR technique by lncRNA-specific oligonucleotide primers (Table 1). The specificity of the primer was confirmed by melting curve analysis. All PCR reactions were completed in duplicate and the mean Ct (threshold cycle) data for patients and control group were realized using cycle threshold settings. A comparative CT method ($2^{-\Delta\Delta Ct}$) was used to calculate candidate lncRNAs expression in plasma of CRC patients samples normalized to *β-actin* expression and relative to healthy controls.

### Statistical Analysis

All data were statistically analyzed and graphically represented using the Statistical Package for the Social Sciences (SPSS) 21.0 software (SPSS Inc., Chicago, IL). The conformity of quantitative variables to normal distribution was evaluated with the Shapiro-Wilk test. The comparison of the two groups was made with the t-test for normally distributed variables, and with the Mann-Whitney U-test for non-distributed variables. The relationship between qualitative variables was evaluated with Chi-square analysis, and the relationship between quantitative variables was evaluated with Spearman correlation analysis. The discriminating power of lncRNA expressions, which are suggested to be used in distinguishing the patient and control groups, the areas under the curve (AUC), sensitivity, and specificity values were evaluated by receiver operating characteristic (ROC) analysis. Survival analysis evaluations and life functions graphs of the diagnosis period were obtained by the Kaplan-Meier method. Cox regression analysis was used to assess the metastasis risk of candidate circulating lncRNAs. $P<0.05$ was considered significant. This work received ethics committee approval (Ethics committee no: 2019-349). A signed and written informed consent form was obtained from the individuals in the patient and control groups.

## Results

### Association between Plasma Expression Levels of lncRNAs and Clinicopathological Characteristics

We have analyzed the relationship between expression levels of lncRNAs and clinicopathological features. Our results showed an association between *CRNDE* lncRNA expression level and tumor differentiation ($p=0.023$) and primary tumor location ($p=0.009$). In addition, an association was observed between tumor size and expression level of *CCAT1* ($p=0.021$), *CRNDE* ($p=0.010$), and *SNHG16* ($p=0.023$). Moreover, we have shown that there is a strong association between the expression level of *XLOC_000303* lncRNA ($p=0.016$) and metastasis. We have evaluated the association of circulating lncRNAs with neo/adjuvant treatment. However, there was no significant difference between candidate circulating lncRNAs and

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**Table 2** Clinical characteristics of patients with colorectal cancer and of control group. It was evaluated by Chi-square analysis

| Variable | Clinical parameter | Patient group | | Control group | | p |
|---|---|---|---|---|---|---|
| | | n | % | n | % | |
| Gender | Male | 40 | 61.5 | 16 | 51.6 | 0.483 |
| | Female | 25 | 38.5 | 15 | 48.4 | |
| Age (Years) | ≤50 | 9 | 13.8 | 4 | 12.9 | 0.343 |
| | >50 | 56 | 86.2 | 27 | 87.1 | |
| TNM Stage | I | 4 | 6.2 | | | |
| | II | 11 | 16.9 | | | |
| | III | 6 | 9.2 | | | |
| | IV | 44 | 67.7 | | | |
| Tumor Grade | Poor differentiation | 1 | 1.5 | | | |
| | Middle differentiation | 57 | 87.7 | | | |
| | Well differentiation | 7 | 10.8 | | | |
| Histolojical Type | Adenocarcinoma | 61 | 93.8 | | | |
| | Stone ring component adenocarcinoma | 2 | 3.1 | | | |
| | Adenocarcinoma with a mucinous component | 2 | 3.1 | | | |
| LVI | Absence | 28 | 43.1 | | | |
| | Presence | 34 | 5.3 | | | |
| | Unknown | 3 | 4.6 | | | |
| PNI | Absence | 15 | 23.1 | | | |
| | Presence | 47 | 72.3 | | | |
| | Unknown | 3 | 4.6 | | | |
| Localization of the primary tumor | Right colon | 17 | 2.2 | | | |
| | Left colon | 48 | 73.8 | | | |
| Response to treatment | Full Pesponse | 1 | 1.5 | | | |
| | Partial response | 57 | 87.7 | | | |
| | Stable disease | 5 | 7.7 | | | |
| | Progress | 2 | 3.1 | | | |
| Neo adjuvant therapy | No | 27 | 41.54 | | | |
| | Yes | 38 | 5.46 | | | |
| Survive | Alive | 60 | 92.3 | | | |
| | Death | 5 | 7.7 | | | |

P<0.05 was considered significant. TNM : Tumor, node, and metastasis; LVI: Lymphovascular invasion; PNI: Perineural ınvasion

neo/adjuvant treatment. Clinicopathological features of CRC patients are summarized in Table 2.

**Evaluation of Plasma lncRNAs Expression Levels in CRC Patients, Compared to the Control Group**

In our results, *ZNRF3-IT1, CCAT1, CRNDE,* and *XLOC_000303* circulating lncRNAs (Fig. 1a, b) were found to be significantly different in the patient group, but no significant difference was found in other lncRNAs (*ARHGAP5-AS1, LOC152578,* and *SNHG16*) (Table 3).

**Evaluation of Expression Level in Candidate Circulating lncRNAs between Stages in CRC Patients**

The findings showed that only the *XLOC_000303* lncRNA was significant between the stages (p=0.001). In addition, according to Cox regression analysis, it was determined that the *XLOC_000303* lncRNA was a protective factor against the metastasis formation (p=0.045, HR=0.90, 95%CI=0.812-0.997) (Table 4). No significant difference was found in terms of stages in the other genes examined.

**Prognostic Value of Expression Level of Candidate lncRNAs (ROC and AUC Analyzes)**

To use candidate circulating lncRNAs as a biomarker, the AUC of ROC of the test was calculated. The patient group and control group were compared. The AUC of ROC of *ZNRF3-IT1* (95% confidence interval [CI]: 0.531-0.790; p=0.011; AUC: 0.660), of *CCAT1* (95% CI: 0.534-0.810; p=0.007, AUC: 0.672), of *CRNDE* (95% CI: 0.586-0.814; p=0.002, AUC: 0.700), and of

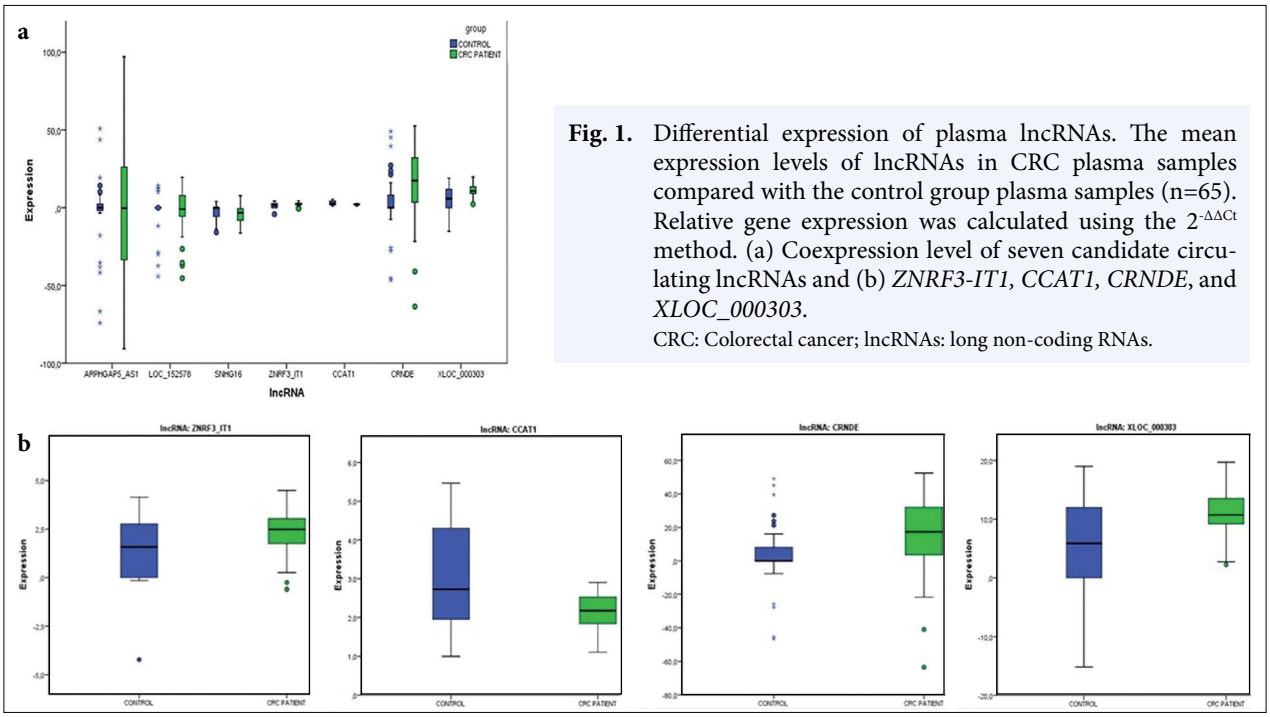


**Fig. 1.** Differential expression of plasma lncRNAs. The mean expression levels of lncRNAs in CRC plasma samples compared with the control group plasma samples (n=65). Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method. (a) Coexpression level of seven candidate circulating lncRNAs and (b) *ZNRF3-IT1, CCAT1, CRNDE*, and *XLOC_000303*.
CRC: Colorectal cancer; lncRNAs: long non-coding RNAs.

**Table 3** Comparison of patient and control group expression levels is shown as median and percentile (Q1-Q3)

| Circulating lncRNA | Groups | | | | | | p |
|---|---|---|---|---|---|---|---|
| | Control | | | CRC patients | | | |
| | Median | Percentile 25 0 (Q1) | Percentile 75 (Q3) | Median | Percentile 25 (Q1) | Percentile 75 (Q3) | |
| *ARHGAP5-AS1* | 0.000 | -2.172 | 3.442 | -0.334 | -33.535 | 26.185 | 0.909 |
| *LOC152578* | 0.000 | 0.000 | 0.002 | -0.859 | -5.552 | 7.784 | 0.984 |
| *SNHG16* | 0.000 | -5.728 | 0.000 | -3.311 | -8.095 | -0.583 | 0.226 |
| *ZNRF3-IT1* | 1.579 | 0.000 | 2.840 | 2.488 | 1.757 | 3.044 | 0.011* |
| *CCAT1* | 2.727 | 1.927 | 4.303 | 2.175 | 1.843 | 2.529 | 0.007** |
| *CRNDE* | 0.000 | 0.000 | 9.260 | 17.388 | 3.554 | 32.108 | 0.002** |
| *XLOC_000303* | 5.890 | 0.000 | 12.481 | 10.725 | 9.169 | 13.531 | 0.001** |

*p<0.05İ **p<0.01. CRC: Colorectal cancer

**Table 4** Comparison of the risk of candidate genes between stages according to the metastasis status of the patient group with CRC, is analyzed by Cox regression test

| Circulating lncRNA | Mean | SE | HR | HR 95% CI | p |
|---|---|---|---|---|---|
| *ARHGAP5-AS1* | -7.409 | 0.004 | 0.998 | 0.990-1.005 | 0.494 |
| *LOC152578* | -1.266 | 0.012 | 1.011 | 0.988-1.035 | 0.352 |
| *SNHG16* | -4.293 | 0.034 | 1.046 | 0.978-1.119 | 0.189 |
| *ZNRF3*-IT1 | 2.313 | 0.147 | 0.948 | 0.711-1.263 | 0.716 |
| *CCAT1* | 2.167 | 0.346 | 1.181 | 0.599-2.326 | 0.631 |
| *CRNDE* | 15.125 | 0.006 | 1.001 | 0.989-1.014 | 0.851 |
| *XLOC_000303* | 10.990 | 0.052 | 0.900 | 0.812-0.997 | 0.045* |

*p: p<0.05. CRC: Colorectal cancer; SE: Standart error; HR: Hazard ratio; CI: Confidence interval

Turk J Oncol 2022;37(3):305–13
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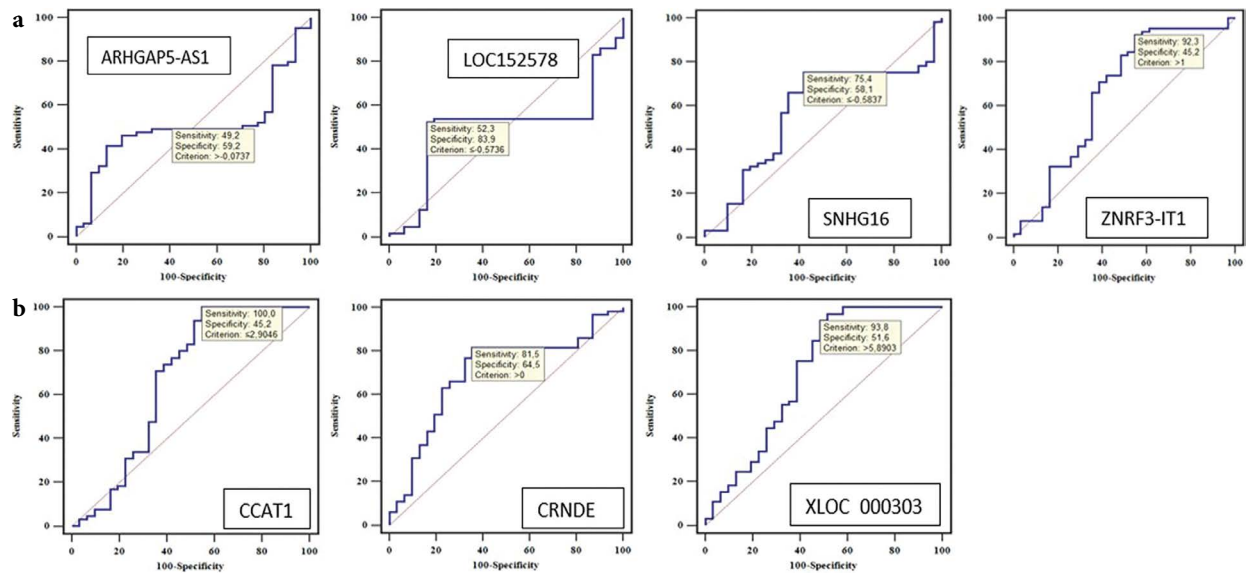


**Fig. 2.** Receiver operating characteristic (ROC) curves for evaluating the diagnostic power of lncRNAs. (a) *ARHGAP5-AS1, LOC152578 SNHG16,* and *ZNRF3-IT1*, and (b) *CCAT1, CRNDE,* and *XLOC_000303*. ROC curve and the area under the curve were calculated to determine the sensitivity and specificity CRC patients as a clinical biomarker.
CRC: Colorectal cancer.

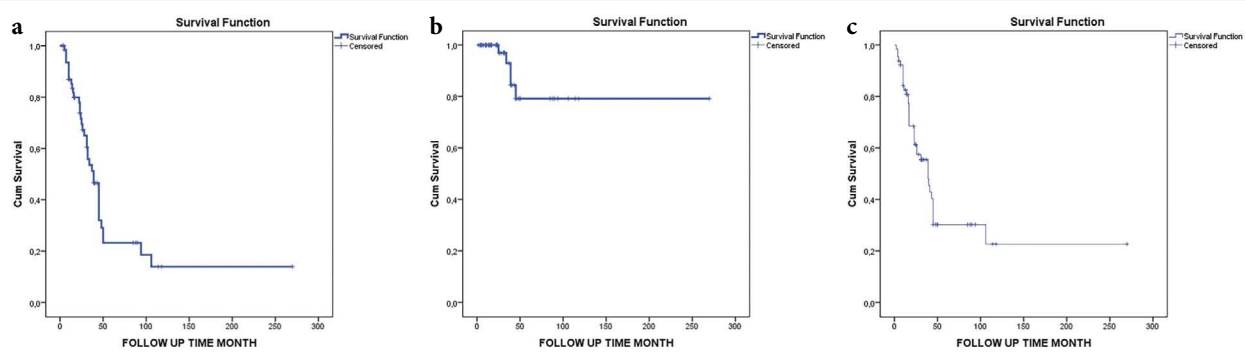


**Fig. 3.** (a) Analysis of survival time in diagnosis of metastasis, (b) analysis of overall survival, and (c) analysis of progression-free survival are shown. It was made by Kaplan-Meier analysis. The median time could not be calculated.

*XLOC_000303* (95% CI: 0.579-0.833; p=0.001, AUC: 0.706) was detected (Fig. 2).

### Survive Analyzes of Candidate lncRNAs

The median value for the overall survival analysis of the cases could not be calculated. Therefore, overall survival analysis could not be evaluated. The mean survival time after diagnosis of metastasis was 69.95±14.528 months (95% CI: 41.480-98.429) and a median of 39.00±3.968 months (95% CI: 31.223-46.777) (Fig. 3). The mean progression-free survival time was 86.864±17.564 months (95% CI: 52.439-121.289), and the median was 39.00±5.544 months (95% CI: 28.134-49.866) (Fig. 3).

## Discussion

CRC is one of the most common types of cancer. The findings obtained as a result of studies aiming to explain the molecular mechanisms of genetic factors affecting the development of CRC have shown that ncRNAs can be used as potential biomarkers for CRC patients. In this context, the potential to use circulating lncRNAs as non-invasive biomarkers in CRC has increased. In this study, we aimed to examine the potential of circulating lncRNAs to be used as non-invasive prognostic biomarkers.

In this study, seven lncRNAs (*ARHGAP5-AS1, LOC152578, SNHG16, ZNRF3-IT1, CCAT1, CRNDE,*

and *XLOC_000303*) were selected to investigate their expression profiles in the plasma of CRC patients. Expression analysis results showed that the case group had significantly higher plasma expression levels of *ZNRF3-IT1* (p=0.011), of CCAT1 (p=0.007), of *CRNDE* (p=0.002), and *XLOC_000303* (p=0.001). By further analysis, it was revealed that there is a high discriminatory power of these genes (*ZNRF3-IT1* [AUC=0.66; p=0.012], *CCAT1* [AUC=0.67; p=0.007], *CRNDE* [AUC=0.70; p=0.002], and *XLOC_000303* [AUC=0.706; p=0.001]) in plasma. Moreover, we found that there may be potential for use as clinical biomarkers in plasma.

*ZNRF3-IT1* is lncRNA that belongs to a class of sense intronic ncRNA. It has been reported to cause cellular proliferation in various cancer types such as CRC, breast cancer, and hepatocellular carcinoma.[12] Studies on *ZNRF3*, one of target genes of *ZNRF3-IT1*, have shown that it is one of the negative regulators of the WNT/β-catenin signaling pathway.[13] In another study, it was shown that higher expression of *ZNRF3* contributes to the good prognosis of colorectal carcinoma by suppressing cancer cell growth and inducing apoptosis in CRC patients.[14] In addition, in RNA-seq data, it has been reported that the expression profile of this gene is down-regulated in various cancer types and decreases approximately 5.6 fold.[15]

In our study, we were determined that the *ZNRF3-IT1* lncRNA was upregulated an average of 0.22-fold in the CRC patient group when compared to the control group. We found that the expression profile of this circulating lncRNA showed a statistically significant difference in the patient group, while it was compared with the controls (p=0.011), but no difference was found according to the stages. This suggests that *ZNRF3-IT1* may alter the tumor suppressor function of target gene *ZNRF3* of it. Our findings showed that lncRNA *ZNRF3-IT1* has a strong distinguishing feature between the patient and control group, and also it can be used as a biomarker.

*CCAT1* has been identified as an oncogene in CRC. It was found that CCAT1 expression was highly upregulated in the tumor sample, regional node, distant liver metastasis, and plasma samples in CRC.[16] Zhang et al.,[17] found a significant increase in *CCAT1* expression in tumor tissue of bladder cancer patients and this expression increase was positively correlated with tumor stage, grade, and size.[17] In the plasma sample of CRC patients, *lnc-ATB, lnc-CCAT1*, and *lnc-OCC-1* were found to be significantly upregulated, but only *lnc-ATB* and *CCAT1* were found statistically significant. There was no significant difference between clinical stages. The AUC of *lnc-ATB* (AUC=0.78; p<0.001) and *lnc-CCAT1* was 0.64 (95% CI: 0.811-0.94; p=0.024). According to these results, it was concluded that the discrimination power of *lnc-ATB* and *CCAT1* was high.[1] Siddique et al.[18] found that *MALAT1, CCAT1,* and *PANDAR* lncRNAs were significantly upregulated (1.86, 4.54, and 4.68-fold, respectively) in CRC plasma samples, and were differed statistically. They emphasized that their findings supported that *MALAT1, CCAT1*, and *PANDAR lncRNA* expression may be a potential biomarker in CRC prognosis.[18]

In this study, we determined *CCAT1 lncRNA* was upregulated by an average of 0.006-fold in the CRC patient group, and there was a significant difference between the patient and the control (p=0.007), but no significant difference was found between the stages. A strong correlation was found between the expression level of *CCAT1* and tumor size (p=0.021). Our results support the informations in direction that *CCAT1* increases proliferation and invasion in CRC. It has been observed that there is a strong discrimination feature between the patient group and control group. These data suggest that *CCAT1 lncRNA* can be used as a biomarker in CRC patients.

*CRNDE lncRNA* is localized next to the *IRX5* gene.[19] CRC has been identified as an oncogene that affects the PI3K/AKT signaling pathway in some cancers such as glioma,[20] gastric cancer,[21] and cervical cancer.[22] It has been shown to promote proliferation, migration, and invasion, interact with miRNAs, and affect the regulation and expression of the target gene.[22] It has been reported that upregulation of its expression is strongly correlated with tumor size, regional lymph node metastasis, distant metastasis, poor prognosis, and advanced tumor progression.[23]

In this study, it was found that *CRNDE* was upregulated by an average of 0.008-fold in the CRC patient group, with a statistically significant difference in the patient group (p=0.002), but there was no significant difference between the stages. A strong association was found between the expression level of *CRNDE*, tumor size (p=0.010), tumor differentiation (p=0.023), and primary tumor location (p=0.009). Our findings are concordant with the literature and support the information that *CRNDE* is effective in processes such as proliferation, migration, invasion, and suppression of apoptosis. However, when the relationship between *CRNDE* and neoadjuvant therapy was examined in our study, no statistically significant difference was found. This finding contradicts the knowledge that *CRNDE* has a role in oxaliplatin-

Turk J Oncol 2022;37(3):305–13
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based chemotherapy resistance.[24] We believe that more studies are needed to evaluate the chemoresistant effect. Moreover, it was observed that *CRNDE* had a strong discriminating feature between the patient group and control group. Our results support the hypothesis that *CRNDE lncRNA* could be used as a biomarker in CRC patients.

*XLOC_000303* is a newly identified intergenic lncRNA. It has been suggested that it is highly expressed in the plasma of CRC patients and can be used as a new and rapid diagnostic biomarker.[2] It has also been suggested that *XLOC_000303* is upregulated in the plasma of cervical cancer patients and can be used as a potential biomarker in the development of tumorigenesis.[25] In the present study, *XLOC_000303* was found to be upregulated by an average of 0.25-fold in the CRC patient group. It was found that there was a statistically significant difference between the patient group and control group (p=0.001), and there was also a statistically significant difference between the stages (p=0.001). It was observed that there was a strong association between *XLOC_000303* and metastasis (p=0.016). Moreover, *XLOC_000303* (p=0.045, HR=0.90) was a protective factor against metastasis formation (1.111 fold). *XLOC_000303* is different between stages and is a protective factor in the formation of metastases. It suggests that this circulating lncRNA has an active role in the development of tumorigenesis and disease progression in CRC patients. We observed that *XLOC_000303* circulating lncRNA has a strong distinguishing feature between the patient group and the control group. We think that it may be used as a biomarker in CRC patients.

### Limitations of the Study

Our study has some limitations. First, the study population was relatively small. Working with a larger population in the future may improve the diagnostic and prognostic accuracy of the lncRNAs. Second, median values could not be calculated, because the follow-up period was short and the number of deaths were not sufficient in the overall survival analysis of the patients. In addition to the expression profile, additional studies on polymorphic and mutant variants may contribute to explain the molecular mechanism.

## Conclusion

We have determined four *lncRNAs, ZNRF3IT-1, CCAT1, CRNDE,* and *XLOC_000303* differentially expressed in plasma of the CRC patient group. In addition, differing of the expression profile of *XLOC_000303 lncRNA* between stages indicates that it can be used as a protective factor in metastasis formation in the transition from early-stage to the advanced stage. According to these results, we suggest that *ZNRF3-IT1, CRNDE, CCAT1,* and *XLOC_000303* may contribute to CRC tumorigenesis by promoting CRC cell proliferation and may be a potential clinical target for therapy. We think that our results will contribute to the literature. To the best of our knowledge, this is the first study to show the association of *ZNRF3-IT1* circulating lncRNA with CRC. Moreover, *XLOC_000303* is the first study to show that circulating lncRNA is a protective factor against metastasis formation in CRC.

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**Peer-review:** Externally peer-reviewed.

**Conflict of Interest:** All authors declared no conflict of interest.

**Ethics Committee Approval:** The study was approved by the Eskisehir Osmangazi University Non-interventional Clinical Research Ethics Committee (No: 2019-349, Date: 10/12/2019).

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