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Serum circITCH and lncRNA HOTAIR expression signatures: Novel diagnostic and metastatic biomarkers for colorectal cancer

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Abstract

Background Colorectal cancer (CRC) remains one of the leading causes of death worldwide because of its late detection which results from being asymptomatic in the early stages and poor adherence to the invasive screening procedure such as colonoscopy. The existing biomarkers used in the blood for early detection of this disease include carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9), but they have poor sensitivity. Non-coding RNAs have proven to be key epigenetic factors for CRC progression and metastasis, including circular RNA ITCH (circITCH) and long non-coding RNA HOTAIR (lncRNA-HOTAIR).

Methods In a case-control study on 200 subjects (120 CRC patients and 80 healthy controls) whose specimens were histopathologically confirmed, the expressions of circITCH and lncRNA-HOTAIR were analyzed using qRT-PCR along with CEA and CA19-9. There were conducted clinicopathological correlations, ROC curves and multiple logistic regression analysis.

Results Serum levels of circITCH were significantly lower in CRC patients than in healthy control group (0.54 ± 0.24 vs. 1.03 ± 0.31, $P<0.001$), whereas the level of lncRNA-HOTAIR was significantly higher (3.21 ± 1.38 vs. 1.06 ± 0.42, $P<0.001$). The individual AUCs of the biomarkers HOTAIR and circITCH were 0.941 (95% CI: 0.909–0.974) and 0.889 (95% CI: 0.840–0.938), respectively. The combined panel (circITCH + lncRNA-HOTAIR) showed superior diagnostic efficacy with the highest AUC of 0.969 (95% CI: 0.947–0.990; sensitivity= 93.3%; specificity= 91.3%), surpassing significantly the traditional protein biomarkers.

Conclusion Circulating levels of circITCH + lncRNA-HOTAIR display complementary expression profiles and, when combined serve as effective, minimally invasive diagnostic biomarkers for CRC.

Keywords colorectal cancer | circITCH | lncRNA-HOTAIR | liquid biopsy | diagnostic biomarkers

Introduction

Colorectal cancer (CRC) is one of the most significant problems of global health, and is one of the biggest causes of cancer related morbidity and mortality worldwide. GLOBOCAN 2022 reports global new cases of CRC at over 1.9 million and global deaths at around 904,000 cases (Bray *et al.,* 2024). Despite recent developments in screening and treatment, prognosis is very strongly dependent on the stage at diagnosis; therefore, the development and identification of effective, minimally invasive markers for early diagnosis and disease monitoring are extremely important.

Conventional serum markers including carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) is not very sensitive or specific, especially in early-stage CRC. Therefore, non-coding RNAs (ncRNAs), such as, circular RNAs (circRNAs) and long non-coding RNAs (lncRNAs) with their regulatory functions in tumor proliferation, apoptosis, invasion, metastasis and response to treatment, have attracted more attention (Syakir and Zuhan, 2024; He *et al.,* 2021).

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CircRNAs are covalently closed RNA molecules that are formed by backsplicing and are highly resistant to exonuclease degradation (Sun and Yang, 2023). They are promising circulating biomarkers because of their stability in tissues and body fluids, and their involvement in cancer related signaling pathways (Goodall and Wickramasinghe, 2021). Of these, circular RNA itchy E3 ubiquitin protein ligase (circITCH) has been mainly reported as a tumor-suppressive circRNA. circITCH is a part of the ITCH gene; it can regulate cancer-related pathways by interacting with miRNAs and downstream targets (Chen, 2020b). Cir-ITCH has been reported to be significantly downregulated in CRC and to inhibit the development of tumors by blocking Wnt/β-catenin signaling pathway (Liu *et al.,* 2022). While circITCH primarily functions as a tumor-suppressive circRNA, lncRNA HOTAIR exerts potent oncogenic effects in cancer progression. LncRNAs are transcripts of greater than 200nt in length that control or modulate the expression of genes by transcriptional, post-transcriptional and epigenetic mechanisms. The role of lncRNAs in CRC development, invasion and resistance to therapy treatment has been established and blood-based detection of these molecules represents another promising minimally invasive marker for CRC.

The biological basis for the parallel assessment of circITCH and HOTAIR is not just related to their inverse expression patterns in tumor suppression and oncogenesis but is likely to involve convergence on shared miRNA regulated signaling networks. circITCH has been found to bind many oncogenic miRNAs such as miR-7, miR-20a and miR-214 and act as a competing endogenous RNA (ceRNA). This activity can upregulate the expression of its parental ITCH gene and in turn, inhibit Wnt/β-catenin signaling in CRC cells. Interestingly, HOTAIR can also be part of miRNA mediated regulatory networks. In CRC, HOTAIR has been reported to directly interact with miR-214 and regulate the miR-214/ST6GAL1 axis, which affects the sialylation of c-Met and subsequent JAK2/STAT3 signaling (Liu *et al.,* 2019). Therefore, miR-214 may be an area of overlap between circITCH- and HOTAIR-regulated networks. However, direct competition between circITCH and HOTAIR to compete for miR-214 has not been shown in CRC and remains a mechanistic hypothesis. Combined evaluation can still convey complementary aspects of CRC biology, such as the loss of CRC suppressing circRNA activity and the activation of oncogenic lncRNA associated pathways. Based on this, the aim of the present study was to measure the serum expression of circITCH and lncRNA HOTAIR in CRC patients, analyze the association between circITCH/lncRNA HOTAIR and clinicopathological features and disease stage, and examine the potential of the serum expression of circITCH/lncRNA HOTAIR as a minimally invasive liquid biopsy tool for CRC diagnosis (Li RD *et al.,* 2021).

HOX transcript antisense intergenic RNA (HOTAIR) is a well characterized oncogenic long non-coding RNA (lncRNA) that plays a role in epigenetic regulation and cancer progression. CRC has been shown to be associated with higher levels of HOTAIR expression, which has been linked to increased proliferation, invasion, metastasis and poor prognosis in CRC (Li *et al.,* 2020; Kristensen *et al.,* 2022; Mao and Lu, 2024). Furthermore, the level of HOTAIR has been observed to be increased in CRC patients compared to healthy people, making it a potential biomarker for CRC (Latifi-Pakdehi *et al.,* 2024; Huang *et al.,* 2015).

circITCH is known to have tumor suppressive properties, whereas HOTAIR is linked to oncogenic properties;therefore, it is hypothesized that they may provide complementary information on CRC development and progression when evaluated together. Thus, the present study set out to explore the expression levels of circITCH and lncRNA HOTAIR in the serum of CRC patients, analyze their correlations with clinicopathological parameters and cancer stages, and determine their individual and combined utility as minimally invasive diagnostic biomarkers in CRC. However, the combination of diagnostic potential and significance of the circITCH/HOTAIR axis in CRC has not been sufficiently explored. Therefore, the purpose of this research are to determine the expression of circITCH and HOTAIR in CRC tissue samples, establish its correlation with clinicopathological parameters, and evaluate their combined prognostic power.

Methods

Study design and participants

A case control study was conducted at Baghdad Teaching Hospital, Medical City, Baghdad, Iraq between March 2026 and August 2026. All 200 participants had confirmed colorectal cancer (CRC) by histopathology (120) and 80 were apparently healthy controls. The control group was frequency matched based on age and sex to the CRC group (Huang *et al.,* 2015).

CRC patients were selected from Gastroenterology and Oncology units of Baghdad Teaching Hospital. Diagnosis was made by clinical evaluation, colonoscopy, radiological tests and histopathology of colorectal biopsy or resection specimens. Blood samples were collected prior to the start of chemotherapy, radiotherapy, immunotherapy or other systemic anticancer drugs. The sample size was chosen to ensure good representation of the different CRC stages and to ensure that there was a large enough control group to compare circulating RNA expression and receiver operating characteristic (ROC) curve analysis (Huang *et al.,* 2019; Latifi-Pakdehi *et al.,* 2024).

Inclusion and exclusion criteria

Eligible patients were those aged ≥18 years, with newly diagnosed and histopathologically-proven colorectal adenocarcinoma. Patients who were not treated with chemotherapy, radiotherapy, targeted therapy, immunotherapy or surgery prior to blood sample collection were included.

Patients were excluded if they had a history of recurrent CRC, hereditary CRC syndromes, inflammatory bowel disease, CRC adenomas, other known precancerous CRC lesions, another current or previous active malignancy, acute infectious or inflammatory disease, severe liver or kidney disease or autoimmune disorders. The control group comprised 80 apparently healthy individuals who were free from a history of malignancy, inflammatory bowel disease, major gastrointestinal disease, and other important chronic inflammatory conditions.

Sample size calculation

The sample size was pre-calculated using the postulated diagnostic value of circulating non-coding RNAs in CRC. The pooled area under the receiver operating characteristic curve (AUC) for circRNAs as CRC diagnostic markers in a previous systematic review and meta-analysis was approximately 0.80–0.81. Hence, to estimate sample size, an expected AUC of 0.81 was used.

This was done by comparing the expected AUC to the null hypothesis value of 0.50 (no discriminatory ability). Type 1 error (α) of 0.05, power (β = 0.1) of 90% and an approximate 0.67 ratio of controls to cases were taken into account. With these assumptions, the necessary sample size was approximately 32 participants, 19 cases of CRC and 13 controls.

To further assess biomarker expression across CRC stages, lymph-node involvement, distant metastasis, and their relationships with conventional tumor markers, as well as to perform multivariable logistic regression, a significantly larger cohort was recruited. This ensured appropriate representation of clinically relevant subgroups and yielded more precise estimates of the ROC curve. The study population comprised 200 participants: 120 patients with pathologically proven CRC and 80 healthy controls.

Clinicopathological assessment and staging

Data for demographic and clinicopathological data were derived from medical records and comprised age, sex, tumor location, histological type, histological grade, lymph-node involvement, distant metastasis and disease stage. The American Joint Committee on Cancer (AJCC) TNM classification was used to stage CRC. Of the proposed cohort, 20 (16.7%) had stage I, 35 (29.2%) had stage II, 35 (29.2%) had stage III and 30 (25.0%) had stage IV disease. For selected analyses, stage I and II were merged and defined as the early-stage group (n=55; 45.8%) followed by stage III (n=35; 29.2%) for node-positive non-metastatic disease and stage IV (n=30; 25.0%) for metastatic CRC.

Blood collection and serum preparation

Peripheral venous blood was obtained from each participant (5 mL) using sterile disposable syringes. Blood was placed in serum separator tubes and left to clot at room temperature for 15–20 min. and then centrifuged at 4,000 rpm for 10 min. The separated serum was immediately placed in individual sterile RNase-free microcentrifuge tubes and subdivided into aliquots to reduce the risk of multiple freeze thaw cycles. The serum was frozen at −80 °C until biochemical and molecular analyses were performed.

Measurement of serum CEA and CA19-9

Carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) levels were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits. CEA and CA19-9 were measured using the Human CEA ELISA Kit (Elabscience Biotechnology Inc., Wuhan, China) and the Human CA19-9 ELISA Kit (Elabscience Biotechnology Inc., Wuhan, China), respectively, following the manufacturer's protocol.

The optical density was determined by microplate reading at 450 nm, and calibrated by a standard calibration curve. The results of CEA were reported in ng/mL, while the CA19-9 concentrations were reported as U/mL. The conventional tumor markers were added to the panel for comparison with the markers of circITCH, lncRNA HOTAIR and to examine the relationship between the conventional tumor markers and non-coding RNA expression.

Total RNA extraction

First, 200μL of serum was used to isolate total RNA, including cRNAs and lncRNAs, using the miRNeasy Serum/Plasma Advanced Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Briefly, the serum samples were lysed with the provided lysis buffer, proteins were precipitated, and isopropanol was added. This resulted in an RNA-containing lysate, which was transferred to an RNeasy UCP MinElute column, where the RNA was specifically bound to its membrane. Sequential washing was performed and purified RNA was eluted with 20μl of RNase-free water. The NanoDrop One spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to assess both the concentration and purity of RNA. The RNA was immediately stored at −80°C for reverse transcription.

Reverse transcription and cDNA synthesis

The extracted RNA was reverse transcribed to complementary DNA (cDNA) following the manufacturer's instructions of the QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany). sufficient RNA was available was used for cDNA synthesis in each sample (approximately 500 ng of total RNA). The overall volume of reaction mixture was 20 μL. The genomic DNA elimination was carried out following the kit protocol and reverse transcript was performed at 42°C for 15 min and enzyme inactivation at 95°C for 3 min. The synthesized cDNA was kept at −20°C until quantitative real-time PCR analysis.

Quantitative real-time PCR

CircITCH and lncRNA HOTAIR were quantified relative to the expression level of their corresponding reference gene by quantitative real-time polymerase chain reaction (qRT-PCR) with the QuantiNova SYBR Green PCR Kit (Qiagen, Hilden, Germany). A Rotor-Gene Q real-time PCR cycler (Qiagen, Hilden, Germany) was used for amplification reactions. All reactions were carried out in a final reaction volume of 20μL, with 10μL of 2× SYBR Green PCR Master Mix, 1μL of each forward and reverse primer, 2μL of cDNA template, and final volume was made up with nuclease-free water.

The amplification protocol included a single activation cycle at 95°C for 2 min and 40 cycles of denaturation at 95°C for 5 s, in combination, at 60°C for 10 s. To determine the specificity of the amplification product a melting-curve analysis was conducted. All reactions were carried out in duplicate and no-template controls were used to detect potential contamination in each PCR reaction.

Primer sequences and validation of circITCH circular structure

Specific primers were used for the quantitative real-time PCR (qRT-PCR) detection of circITCH and lncRNA HOTAIR. GAPDH was used as the endogenous reference gene for nor-

malization. All primers used in this study are listed in **Table 1**.

The primer sequences for the detection of circITCH were derived from Huang *et al.* (2015) who identified a colorectal cancer (CRC)-specific circITCH. The forward primer sequence was 5′-GCAGAGGCCAACACTGGAA-3′ and the reverse primer sequence was 5′-TCCTTGAAGCTGACTACGCTGAG-3′. The circular transcript was preferentially amplified using divergent primers that surround the characteristic back-splice junction of circITCH, and amplified much less of the linear ITCH mRNA.

To further confirm the circular form of the detected circITCH transcript, RNase R digestion was performed prior to reverse transcription. RNase R was used to treat total RNA and remove rRNA. The treated aliquot was treated with RNase R following the manufacturer's protocol, while the untreated aliquot was used as a control. The circular nature of the transcript was further confirmed by the significant resistance of circITCH amplification to RNase R treatment; RNase R is more likely to digest linear RNA compared to circular RNA.

Amplification specificity was also investigated using melting curve analysis of SYBR Green based quantitative real-time PCR. Only one distinct melting peak detected was deemed to be an amplification product, with no significant nonspecific amplification or primer dimer. For the purpose of eliminating the possibility of cross-amplification of linear ITCH mRNA, the qRT-PCR product obtained with the divergent circITCH primers was purified and then sequenced by the Sanger method. The resulting sequence was then compared to the expected circITCH sequence, and the occurrence of the expected head-to-tail back-splice junction was confirmed as the amplified sequence being the circular circITCH transcript as explain in (**Table 1**).

The forward and reverse primer sequences for lncRNA HOTAIR were 5′-CAGTGGGGAACTCTGACTCG-3′ and 5′-GTGCCTGGTGCTCTCTTACC-3′ (Wu *et al.,* 2014). The forward and reverse primers for GAPDH were 5′-GTCAACGGATTTGGTCTGTATT-3′ and 5′-AGTCTTCTGGGTGGCAGTGAT-3′ respectively (Wu *et al.,* 2014).

Normalization and relative gene expression

Because normalization of circulating RNA in serum represents an important analytical challenge, the suitability of GAPDH as an endogenous reference was evaluated in the present cohort before relative-expression analysis. The raw Ct values of GAPDH were compared between CRC patients and healthy controls and across CRC stages to assess whether its expression varied systematically with disease status. GAPDH was retained as the endogenous reference only after demonstrating no statistically significant difference in its Ct values between the study groups and acceptable inter-sample stability.

For each participant, the ΔCt value was calculated as:

$$\Delta Ct = Ct\ (target\ RNA) - Ct(GAPDH)$$

The relative expression levels of circITCH and lncRNA HOTAIR were subsequently determined using the comparative $2^{-\Delta\Delta Ct}$ method, with the healthy control group serving as the calibrator:

$$\Delta\Delta Ct = \Delta Ct(sample) - mean\ \Delta Ct(control\ group)$$
$$Relative\ expression = 2^{-\Delta\Delta Ct}.$$

This normalization strategy was consistently applied to all samples. Nevertheless, because GAPDH is more commonly validated as a reference gene in cellular and tissue specimens than in cell-free serum RNA, the absence of an exogenous RNA spike-in control represents an analytical limitation of this study.

Data analysis

IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 10.4 (GraphPad Software, Boston, MA, USA) software were used for statistical analysis. Data for continuous variables were reported as mean ± standard deviation (SD) or median and interquartile range (IQR) depending on data distribution and data for categorical variables as frequencies and percentages. The Shapiro–Wilk test was used to determine normality. Student's t-test or Mann–Whitney U test was used to compare the differences in each patient group and control group, while one-way ANOVA or Kruskal–Wallis test was used to compare the differences between each stage of CRC. The chi-square or Fisher's exact test was used to compare categorical variables. Pearson's or Spearman's correlation analysis (interpreted as weak: $r<0.4$, moderate: $r=0.4–0.69$, strong: $r\geq0.7$) was used to assess the correlation between circITCH, HOTAIR, CEA, CA19-9 and clinicopathological parameters. ROC curve analysis was used to evaluate the diagnostic performance of each and combined biomarkers using AUC, 95% CI, sensitivity, specificity, and optimum Cut-off values. The combined circITCH + HOTAIR model was developed using binary logistic regression and independent predictors of metastatic CRC were

Table 1 Primer sequences used for qRT-PCR analysis of circITCH, lncRNA-HOTAIR, and GAPDH

Target gene	Primer	Amplicon size (bp)	Annealing temp. (°C)
circITCH	Fwd: ACTCAGAACACCCCACAGGA Rev: TCGTCAGTACCACCACCAGT	134	60
lncRNA HOTAIR	Fwd: CAGTGGGGAACTCTGACTCG Rev: GTGCCTGGTGCTCTCTTACC	118	60
GAPDH (Reference)	Fwd: CTGGGCTACACTGAGCACC Rev: AAGTGGTCGTTGAGGGCAATG	101	60
U6 (Reference)	Fwd: CTCGCTTCGGCAGCACA Rev: AACGCTTCACGAATTTGCGT	89	60

identified using binary logistic regression. A P value of <0.05 was deemed to be statistically significant for a two-sided test. Relative expression was determined using the 2−ΔΔCt method. Genes were considered upregulated when the relative fold change was >1.0 (log2 FC>0) with P<0.05, and downregulated when the relative fold change was <1.0 (log2 FC<0) with P<0.05. The median value of gene expression was used as the cut-off point for stratifying patients into high- and low-expression groups.

Ethical considerations

This study protocol was approved by Institutional Review Board/Ethics Committee of Baghdad Teaching Hospital, Medical City Directorate, Baghdad, Iraq. The inspection was carried out in accordance with the ethical principles of the Declaration of Helsinki and institutional guidelines.

Results

Characteristics of the study population

The study included 200 subjects, 120 of whom were patients with histologically confirmed CRC and 80 apparently normal controls. Among the CRC patients, 20 (16.7%) were classified as stage I, 35 (29.2%) as stage II, 35 (29.2%) as stage III, and 30 (25.0%) as stage IV. Thus, 55 (45.8%) patients had an early stage (I-II), 35 (29.2%) had a stage III disease and 30 (25.0%) had a stage IV (metastatic).

There was no significant difference between CRC patients and healthy controls with regard to the mean age (59.8 ± 10.7 years vs. 58.1 ± 9.9 years, respectively, P=0.259) (**Table 2**). There was no significant difference in sex distribution (P=0.727); the male group had a higher proportion (60.0%) than the female group (40.0%) of the CRC group, and the male group (57.5%) had a higher proportion than the female group (42.5%) in the control group.

The levels of both serum CEA and CA199 were significantly higher in patients with CRC than in healthy controls. Mean serum CEA increased from 2.81 ± 1.29 ng/mL in controls to 7.36 ± 4.21 ng/mL in CRC patients (P<0.001), whereas CA19-9 increased from 17.6 ± 7.3 U/mL to 34.8 ± 19.6 U/mL (P<0.001).

Serum expression of circITCH and lncRNA HOTAIR in CRC patients

Serum expression of circITCH and lncRNA HOTAIR was significantly changed in CRC patients compared with healthy controls as verified by quantitative real-time PCR analysis (**Table 3**). The fold expression of circITCH was significantly reduced in CRC patients compared with the healthy controls (0.54 ± 0.24 vs 1.03 ± 0.31, P=0.00091). In contrast, the expression of serum HOTAIR was significantly increased in patients with CRC. The mean relative expression of HOTAIR was 3.21 ± 1.38fold higher in the CRC group than in the healthy control group (1.06 ± 0.42fold; P=0.00044). These results showed the contrary expression of the two non-coding RNAs, with HOTAIR upregulated and circITCH downregulated in CRC.

Expression of circITCH and HOTAIR according to CRC stage

As the CRC stage increased, a progressive change in the levels of both biomarkers was noted (**Table 4**). Serum circITCH expression decreased progressively from 0.76 ± 0.22 in stage I to 0.63 ± 0.20 in stage II, 0.48 ± 0.18 in stage III, and 0.32 ± 0.15 in stage IV. The overall difference among the CRC stages was statistically significant (P=0.0001). In contrast, HOTAIR expression gradually increased as the disease developed. The mean relative HOTAIR expression increased from 2.01 ± 0.76 in stage I to 2.59 ± 0.91 in stage II, 3.42 ± 1.06 in stage III, and 4.43 ± 1.29 in stage IV (P=0.0006). The concen-

Table 2 Demographic and biochemical characteristics of CRC patients and healthy controls

Variable	Healthy controls (n=80)	CRC patients (n=120)	P-value
Age (years), mean ± SD	58.1 ± 9.9	59.8 ± 10.7	0.259
Male, n (%)	46 (57.5%)	72 (60.0%)	0.727
Female, n (%)	34 (42.5%)	48 (40.0%)	
CEA (ng/mL), mean ± SD	2.81 ± 1.29	7.36 ± 4.21	<0.001
CA19-9 (U/mL), mean ± SD	17.6 ± 7.3	34.8 ± 19.6	<0.001

Table 3 Relative serum expression of circITCH and HOTAIR in CRC patients and healthy controls

Biomarker	Healthy controls (n=80)	CRC patients (n=120)	P-value
circITCH relative expression	1.03 ± 0.31	0.54 ± 0.24	0.00091
HOTAIR relative expression	1.06 ± 0.42	3.21 ± 1.38	0.00044

Table 4 Expression of molecular and conventional biomarkers according to CRC stage

Biomarker	Stage I (n=20)	Stage II (n=35)	Stage III (n=35)	Stage IV (n=30)	P-value
circITCH	0.76 ± 0.22	0.63 ± 0.20	0.48 ± 0.18	0.32 ± 0.15	0.0001
HOTAIR	2.01 ± 0.76	2.59 ± 0.91	3.42 ± 1.06	4.43 ± 1.29	0.0006
CEA (ng/mL)	4.21 ± 2.03	5.62 ± 2.84	7.48 ± 3.61	10.93 ± 4.92	0.0003
CA19-9 (U/mL)	23.8 ± 10.6	28.7 ± 13.4	36.2 ± 16.8	47.5 ± 22.1	0.0007

trations of CEA and CA19-9 also increased with stage; the highest levels were seen in the stage IV group.

Relationship between circITCH and HOTAIR expression

The results of correlation analysis showed a significant negative correlation between the expression of circITCH and HOTAIR in the serum of CRC patients (**Table 5**). Negative correlation between the expression of circITCH and that of HOTAIR was observed (r=−0.521, P=0.0002). There were also significant negative correlations between circITCH expression and serum CEA (r=−0.358, P=0.0004) and CA19-9 (r=−0.291, P=0.0009). Conversely, HOTAIR expression was positively correlated with CEA (r=0.467, P=0.0001) and CA19-9 (r=0.382, P=0.0009). There was no significant correlation between patient age and expression of circITCH or HOTAIR.

Association of circITCH and HOTAIR with clinicopathological characteristics

The correlation between serum circITCH and HOTAIR expression levels and selected clinicopathological parameters of CRC patients is shown in **Table 6**. No apparent sex differences were observed for either biomarker. The mean relative expression of circITCH was 0.55 ± 0.25 and 0.53 ± 0.23 in male and female patients, respectively (P=0.612). Likewise, there was no difference in HOTAIR expression between male and female patients (3.17 ± 1.36 vs. 3.27 ± 1.42, respectively; P=0.684).

In contrast, both biomarkers were significantly associated with lymphnode involvement. Patients with positive node involvement had significantly lower circITCH expression than those with negative node involvement (0.44 ± 0.20 vs. 0.68 ± 0.22, respectively; P<0.001). However, HOTAIR expression was significantly higher in patient with lymph-node positivity than in those with lymph-node negativity (3.75 ± 1.39 vs. 2.45 ± 0.94; P=0.0005).

This was a similar expression pattern as that seen with distant metastasis. The serum circITCH expression was significantly lower in patients with distant metastasis than in patients without distant metastasis (0.32 ± 0.15 vs. 0.61 ± 0.22; P < 0.001). In contrast, compared with patients without metastasis, those who developed distant metastasis showed significantly higher levels of HOTAIR expression (4.43 ± 1.29 vs. 2.80 ± 1.10; P = 0.0003).

Overall, these results show that both circRNAs have opposite relationships with poor clinicopathological parameters. Lower circITCH expression was related to both lymph-node involvement and distant metastasis, while higher HOTAIR expression was related to both these two parameters; there was no significant relationship between these two parameters and patient sex.

Diagnostic performance of circITCH and HOTAIR for CRC

ROC curve analysis was performed to determine the ability of circITCH and HOTAIR to discriminate CRC patients from healthy controls. This is because the diagnostic performance of serum circITCH was good, with an AUC of 0.889 (95% CI 0.840–0.938). CircITCH had the highest sensitivity and specificity at a relative-expression cutoff of ≤0.73, which were 83.3% and 82.5%, respectively (**Table 7**).

The diagnostic performance of HOTAIR was higher with an AUC of 0.941 (95% CI: 0.909–0.974). With a cut-off value >1.72-fold expression, the sensitivity of HOTAIR was 90.0% and the specificity was 87.5%. For comparison, CEA produced an AUC of 0.831 (95% CI: 0.774–0.888), with sensitivity of 76.7% and specificity of 77.5%, whereas CA19-9 yielded an AUC of 0.748 (95% CI: 0.680–0.816), with sensitivity of 70.0% and specificity of 68.8%. The combined circITCH + HOTAIR model achieved the best diagnostic performance with an AUC of 0.969 (95% CI: 0.947–0.990), a sensitivity of 93.3%, and a specificity of 91.3%.

Table 5 Correlations between circITCH, HOTAIR, and conventional serum tumor markers in CRC patients

Variables	Correlation coefficient (r)	*P*-value
circITCH vs HOTAIR	−0.521	0.0002
circITCH vs CEA	−0.358	0.0004
circITCH vs CA19-9	−0.291	0.0009
HOTAIR vs CEA	0.467	0.0001
HOTAIR vs CA19-9	0.382	0.0009

Table 6 Association of serum circITCH and HOTAIR expression with selected clinicopathological characteristics

Characteristic	n	circITCH, mean ± SD	*P*-value	HOTAIR, mean ± SD	*P*-value
Sex					
Male	72	0.55 ± 0.25	0.612	3.17 ± 1.36	0.684
Female	48	0.53 ± 0.23		3.27 ± 1.42	
Lymph-node involvement					
Negative	50	0.68 ± 0.22	<0.001	2.45 ± 0.94	0.0005
Positive	70	0.44 ± 0.20		3.75 ± 1.39	
Distant metastasis					
Absent	90	0.61 ± 0.22	<0.001	2.80 ± 1.10	0.0003
Present	30	0.32 ± 0.15		4.43 ± 1.29	

Table 7 ROC analysis of circITCH, HOTAIR, CEA, and CA19-9 for discrimination of CRC patients from healthy controls

Biomarker	Cutoff	AUC	95% CI	Sensitivity (%)	Specificity (%)	*P*-value
CEA	>4.10 ng/mL	0.831	0.774–0.888	76.7	77.5	0.0002
CA19-9	>22.4 U/mL	0.748	0.680–0.816	70.0	68.8	0.0007
circITCH	≤0.73	0.889	0.840–0.938	83.3	82.5	0.0005
HOTAIR	>1.72	0.941	0.909–0.974	90.0	87.5	0.0004
circITCH + HOTAIR	-	0.969	0.947–0.990	93.3	91.3	0.0001

ROC curves demonstrate the diagnostic performance of (a) CEA, (b) CA19-9, (c) circITCH, (d) lncRNA HOTAIR, and (e) the combined circITCH + HOTAIR model (**Figure 1**). The corresponding AUC values were 0.831, 0.748, 0.889, 0.941, and 0.969, respectively (all P<0.001). The combined circITCH + HOTAIR model showed the highest diagnostic performance for distinguishing CRC patients from healthy controls.

Diagnostic performance for metastatic CRC

AnROC curve analysis was used to assess the discriminative capacities of circITCH, HOTAIR, CEA, and CA19-9 to distinguish patients with metastatic CRC (stage IV) from those with non-metastatic CRC (stages I-III) (**Table 8**). The discriminatory performance of serum circITCH was good with AUC of 0.814 (95% CI: 0.731–0.897). When the cut-off set to ≤0.40, the sensitivity of circITCH was 80.0% and specificity was 73.3%. The discriminatory performance of HOTAIR was better, with an AUC of 0.856 (95% CI: 0.784–0.928); a sensitivity of 83.3% and a specificity of 76.7% at a cutoff value of >3.65. Among the conventional serum tumor markers, CEA yielded an AUC of 0.771 (95% CI: 0.679–0.863), with a sensitivity of 73.3% and specificity of 68.9%, whereas CA19-9 showed an AUC of 0.704 (95% CI: 0.598–0.810), with a sensitivity of 66.7% and specificity of 64.4%.

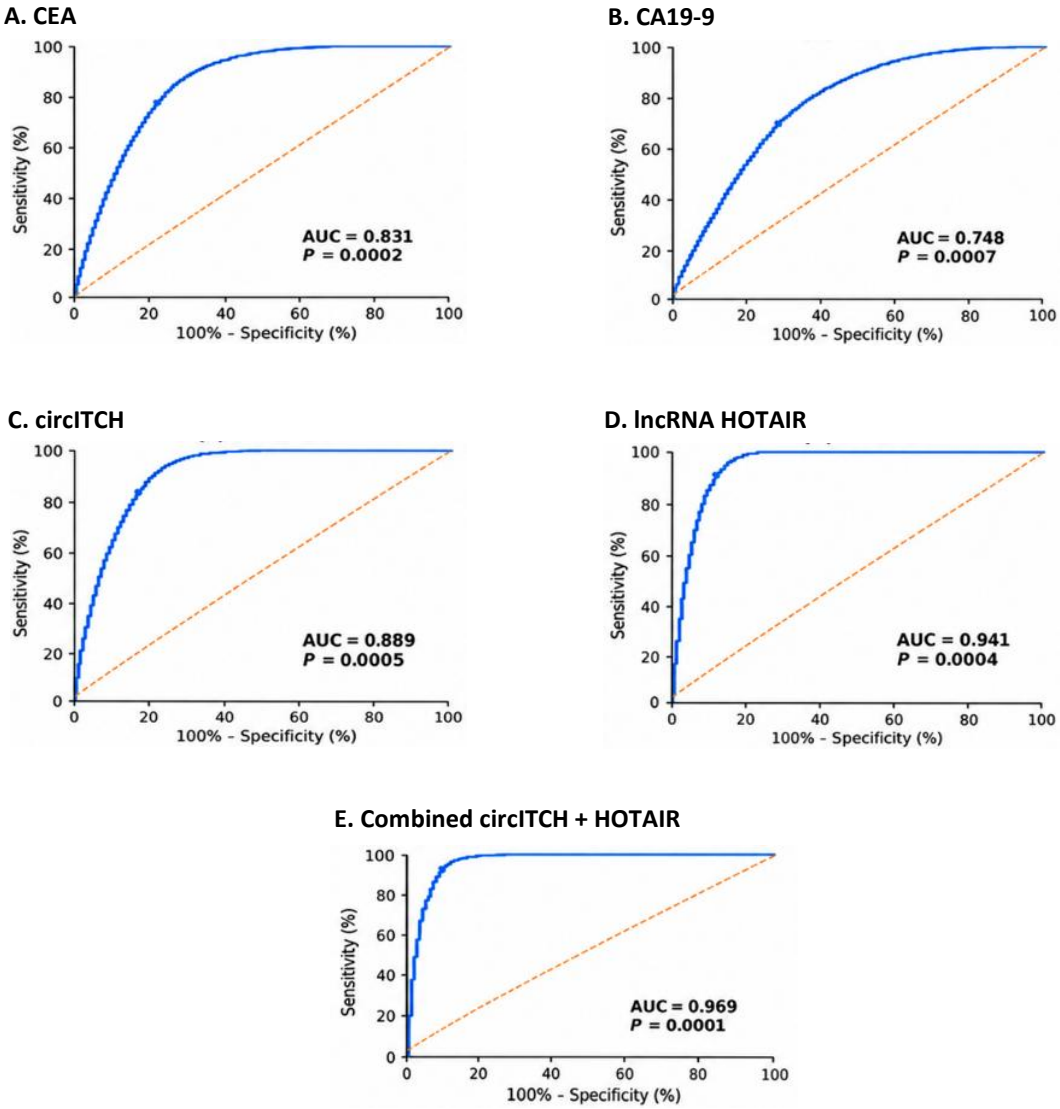


Figure 1 Receiver operating characteristic (ROC) curve analysis of serum biomarkers for discriminating between patients with colorectal cancer (CRC) and healthy controls. ROC curves demonstrate the diagnostic performance of (**A**) CEA, (**B**) CA19-9, (**C**) circITCH, (**D**) lncRNA HOTAIR, and (**E**) the combined circITCH + HOTAIR model.

Table 8 ROC analysis for discrimination of metastatic from non-metastatic CRC

Biomarker	Cutoff	AUC	95% CI	Sensitivity (%)	Specificity (%)	*P*-value
CEA	>8.20 ng/mL	0.771	0.679–0.863	73.3	68.9	0.0005
CA19-9	>37.5 U/mL	0.704	0.598–0.810	66.7	64.4	0.0009
circITCH	≤0.40	0.814	0.731–0.897	80.0	73.3	0.0004
HOTAIR	>3.65	0.856	0.784–0.928	83.3	76.7	0.0001
circITCH + HOTAIR	-	0.901	0.842–0.960	86.7	82.2	0.0008

The model that performed best in terms of discriminatory ability was the combined circITCH + HOTAIR model (AUC: 0.901; 95% CI: 0.842–0.960; sensitivity 86.7%, specificity 82.2%). Hence, the combined non-coding RNA model had significantly better discriminatory ability for metastatic CRC than to CEA (AUC=0.771),CA19-9 (AUC=0.704) and improved discriminatory ability than either circITCH or HOTAIR alone.

ROC curves show the diagnostic performance of (a) CEA, (b) CA19-9, (c) circITCH, (d) lncRNA HOTAIR, and (e) the combined circITCH + HOTAIR model (**Figure 2**). The corresponding AUC values were 0.771, 0.704, 0.814, 0.856, and 0.901, respectively. The combined circITCH + HOTAIR model showed the highest ability to distinguish metastatic stage IV CRC from non-metastatic stages I–III. All associations were statistically significant, with P<0.001 except for CA19-9 (P=0.001).

Multivariable logistic regression analysis of metastatic CRC

Multiple binary logistic regressions were performed to determine which biomarkers were independently associated with metastatic CRC (**Table 9**). The relationship between the expression of circITCH and HOTAIR and distant metastasis was preserved after simultaneous adjustment of the analyzed biomarkers.

The odds of the presence of metastatic disease were higher with high HOTAIR expression (OR=2.31, 95% CI: 1.42–3.76, P=0.001). There was an inverse association (OR=0.21, 95% CI: 0.06–0.71, P=0.012) between higher circITCH expres-

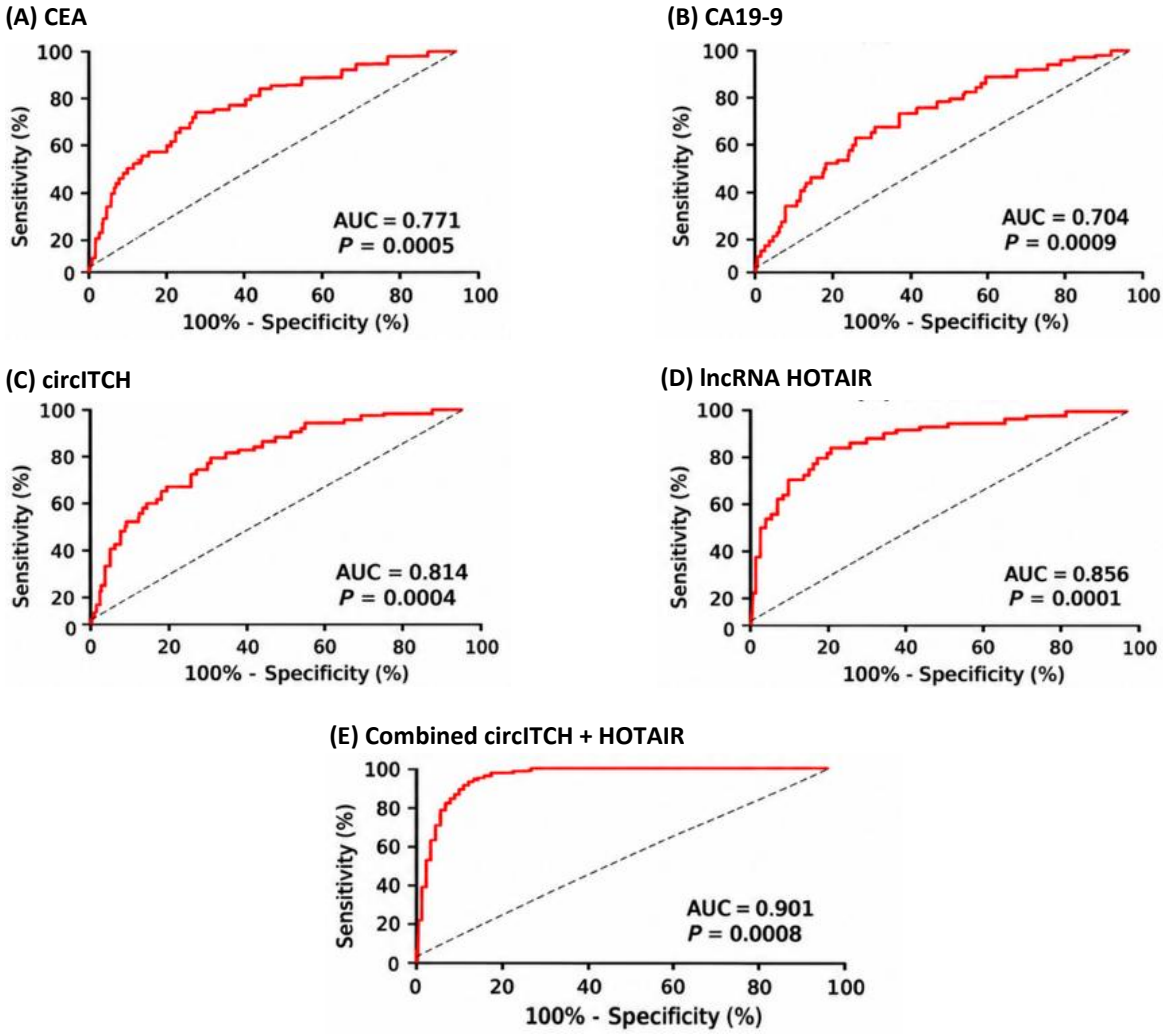


Figure 2 Receiver operating characteristic (ROC) curve analysis for discrimination between metastatic and non-metastatic colorectal cancer (CRC). ROC curves show the diagnostic performance of (**A**) CEA, (**B**) CA19-9, (**C**) circITCH, (**D**) lncRNA HOTAIR, and (**E**) the combined circITCH + HOTAIR model.

Table 9 Multivariable logistic regression analysis of factors associated with metastatic CRC

Variable	β	OR	95% CI for OR	*P*-value
circITCH	−1.56	0.21	0.06–0.71	0.012
HOTAIR	0.84	2.31	1.42–3.76	0.001
CEA	0.12	1.13	1.02–1.25	0.021
CA19-9	0.02	1.02	0.99–1.05	0.184

sion and metastatic CRC. After adjustment, CEA remained significantly associated with metastatic disease (OR=1.13, 95% CI: 1.02–1.25, P=0.021) whereas CA19-9 lost its significance (P=0.184).

In total, the results showed a differential expression of circITCH and HOTAIR in CRC patients, with a decrease in circITCH and an increase in HOTAIR expression in the serum of these patients. The two changes grew increasingly severe as the disease progressed and were highly correlated with involvement of the lymph nodes and distant metastases. The individual diagnostic performance of HOTAIR was superior to the performance of circITCH, CEA, and CA19-9, and the combined model of circITCH + HOTAIR had the highest discriminatory performance for CRC. The combined model also had good discriminatory power between metastatic and non-metastatic disease.

Discussion

The present study aimed to examine the expression patterns of circulating circITCH and lncRNA HOTAIR and their clinical roles in colorectal cancer (CRC). The main findings showed that serum circITCH was significantly downregulated in CRC patients, whereas serum HOTAIR is significantly upregulated compared with healthy controls. The latter became more distinct as the tumor progressed in stage and were significantly correlated with lymph-node involvement and distant metastasis. Furthermore, the two biomarkers showed clinically relevant diagnostic power, with HOTAIR showing the greatest discrimination among CRC patients and healthy controls and their combination showed significantly more ability to discriminate CRC patients and healthy controls compared to the two conventional tumor markers, CEA and CA19-9. These observations underscore the hypothesis of using a joint assessment of tumor-suppressive and oncogenic ncRNAs as complementary molecular clues to the development and progression of CRC.

No significant difference was found in the sex or age distribution of the study groups, indicating comparability between the CRC patients and healthy controls. This is crucial because this could lead to problems when comparing molecular biomarkers across different age cohorts. However, serum CEA and CA19-9 concentrations were found to be significantly elevated in CRC patients, and both markers were significantly elevated as the disease progressed from earlier end stages (stage IV). The results of these measurements are similar to those of the conventional tumor markers that correlate with tumor burden and disease progression. CEA has a limited sensitivity for the diagnosis of CRC, but is useful to assess the response to treatment, to monitor for recurrence and as a postoperative test. When used individually, CA19-9 is also not very sensitive or specific for the disease. The diagnostic value of each marker, CEA and CA19-9, was found to be low, and the simultaneous use of markers was shown to enhance the diagnostic efficiency in a recent meta-analysis of 95 studies (Wang *et al.,* 2019; Chen *et al.,* 2020a) involving over 22,000 patients.

One of the most salient findings in the current study was the significant decrease in the serum expression of circITCH in patients with CRC compared to healthy controls (1.03 ± 0.31 vs. 0.54 ± 0.24). This discovery is biologically plausible and matches the previously described tumor-suppressive activity of circITCH. Huang *et al.* (2015) revealed that the expression of cir-ITCH is significantly reduced in CRC tissues compared to their corresponding non-tumorous tissues, and restoration of the expression of cir-ITCH suppressed CRC-related signaling via the Wnt/β-catenin pathway. It is hypothesized that circITCH could act as a competitive endogenous RNA that sequesters certain miRNAs and promotes the expression of its parental ITCH gene. ITCH is an E3 ubiquitin ligase that can facilitate the degradation or suppression of components of oncogenic signaling. This inhibitory regulatory network may therefore be impaired in colorectal cancer cells with reduced expression of circITCH, allowing activation of pathways that promote the proliferation and malignant transformation of the colorectal epithelium (Galamb *et al.,* 2019; Chen *et al.,* 2023; Xie *et al.,* 2020).

The gradual decrease in circITCH from stage I to stage IV indicates that the loss of this tumor-suppressive RNA may not only be linked to the initial process of CRC transformation, but also to itsprogression. The expression in the present data was found to be reduced from 0.76 ± 0.22 in stage I to 0.32 ± 0.15 in stage IV. This stage-specific profile is consistent with the literature, which has correlated the dysregulated expression of circRNAs in CRC with tumor invasion, metastasis, tumor stage, CRC therapeutic resistance, and CRC prognosis (Lin *et al.,* 2024). In a systematic review of 55 studies on CRC, some circRNAs were consistently reduced in CRC, which may be considered as tumor suppressors, and the loss of these circRNAs is suggested to promote CRC progression by affecting downstream signaling pathways (Flynn and Chang, 2014). Circular RNA are also very stable to exonuclease degradation, further validating their use as circulating biomarkers (Chen, 2020b).

Unlike circITCH, serum HOTAIR expression was significantly elevated in CRC patients with mean relative expression elevated from 1.06 ± 0.42 in controls to 3.21 ± 1.38 in CRC. This is consistent with the known oncogenic activity of HOTAIR. HOTAIR plays a role in epigenetic regulation and various complexes containing chromatin-modifying enzymes

can bind to it and change the expression of genes that regulate cell proliferation, survival, invasion, and metastatic behavior. Huang *et al.* showed that HOTAIR is highly expressed in CRC cells and biopsies and that it enhances the growth of cancer cells partly through its ability to recruit enhancer of zeste homolog 2 (EZH2) (Huang *et al.,* 2019). The interaction of HOTAIR and EZH2 may help to epigenetically remodel target genes and create a pro-oncogenic context.

In the present study, we discussed the moderate inverse correlation observed between the tumor suppressor circITCH and the oncogenic markers (HOTAIR, CEA, and CA19-9), reflecting their opposing roles in CRC pathogenesis. We found that HOTAIR expression increased gradually with advancing CRC stage (2.01 ± 0.76 in stage I to 4.43 ± 1.29 in stage IV), which further supports the correlation of HOTAIR with CRC aggressiveness. Increased HOTAIR expression in colon cancer has been reported to be significantly associated with deeper invasion, lymph-node metastasis, distant organ metastasis, vascular invasion, advanced stage, and poor prognosis in colon cancer survival (Zhou *et al.,* 2026). They also found that HOTAIR was involved in cancer cell migration and invasion and correlated with epithelial–mesenchymal transition (EMT) in their experiments. Suppression of HOTAIR led to the upregulation of E-cadherin and downregulation of vimentin and MMP9 expression, thus suggesting a role for HOTAIR in the acquisition of a more invasive cellular phenotype (McShane *et al.,* 2005). The mechanisms of these pathways are biologically plausible to explain the increasing expression of HOTAIR seen at increasing stages of disease in the current study.

The correlation of HOTAIR with advanced disease in this study is also in agreement with the summarized prognostic evidence. In a CRC-specific meta-analysis of 629 patients, high expression of HOTAIR was found to correlate with poor overall and recurrence-free survival (pooled hazard ratio 2.46; Chen *et al.,* 2020c). Therefore, the observed elevation in HOTAIR levels in advanced and metastatic forms could be a reflection of its role beyond simply as a circulating marker, but rather as a part of a molecular network that plays a role in malignant progression (Mao and Lu, 2024).

A negative correlation was also noted between circITCH and HOTAIR ($r=-0.521$, $P<0.001$). When circITCH was expressed at a low level, HOTAIR was expressed at a high level. This connection is conceptually similar to their opposing biological functions, with circITCH having mainly tumor-suppressive roles, while HOTAIR promotes oncogenic and metastatic pathways. Together, as CRC develops, the gain of oncogenic signaling and epigenetic reprogramming might occur concurrently with the loss of tumor-suppressing regulatory RNAs and gain in oncogenic lncRNAs. The current correlation should not be interpreted as direct regulation of HOTAIR by circITCH, or vice versa, as there was no experimental investigation of the molecular interaction. Instead, the inverse relationship could be the result of two opposing and parallel molecular effects of CRC progression. Further mechanistic studies are required to elucidate if these RNAs share downstream miRNAs, transcriptional regulators, epigenetic pathways, and/or signaling networks.

This is further corroborated by positive correlations with conventional tumor markers. There were negative correlations between circITCH and CEA, circITCH and CA19-9, and positive correlations between HOTAIR and CEA, as well as between HOTAIR and CA19-9. The negative correlation of circITCH with tumor burden markers, CEA and CA19-9, is consistent with the loss of a tumor suppressive signal with disease progression. On the other hand, HOTAIR elevation along with CEA and CA19-9 helps their correlation with increased burden of malignancy. However, the moderate level of these correlations suggests that circITCH and HOTAIR are not just rehashes of the information that can be obtained from traditional tumor markers. Instead, they may represent several different molecular features of tumor biology, and this could account for the high diagnostic power of the combination of ncRNAs.

This is supported by the clinicopathological features. CircITCH was significantly lower and HOTAIR was significantly higher in patients with lymph node involvement than in those without lymph nodes involvement. The levels of circITCH were lowest in patients with distant metastases, whereas the levels of HOTAIR were highest. This correlation between decreased circITCH and metastatic phenotype may be attributed in part to loss of circITCH's inhibitory effects on oncogenic signaling pathways, such as Wnt/β-catenin signaling, which plays a key role in CRC proliferation, invasion, stemness, and progression. In contrast, the correlation between elevated HOTAIR and metastatic disease is consistent with pro-EMT, cell migration and invasion, epigenetic remodeling, and other metastasis-related processes promoted by its upregulation (Long *et al.,* 2022; Li *et al.,* 2021). Similar clinical studies have also revealed increased expression of HOTAIR in lymph-node bearing metastases and/or correlation with lymph-node and distant metastases.

Importantly, there was no significant sex difference in either circITCH or HOTAIR. This indicates that the observed variation in RNA expression was more related to tumor characteristics rather than to sex in this cohort. This interpretation is further supported by the absence of any major demographic differences between the patient and control groups. However, a larger number of patients would be required to assess if there is meaningful difference in the expression of circulating circITCH or HOTAIR according to age, sex, tumor location, molecular subtype or other patient parameters.

The ROC analysis revealed that both ncRNAs had high discriminatory ability between CRC patients and healthy controls. circITCH's results yielded an AUC of 0.889, had 83.3% sensitivity and 82.5% specificity, while HOTAIR's results yielded an AUC of 0.941, a 90.0% sensitivity, and an 87.5% specificity. Therefore, the performance of individual diagnosis was better with HOTAIR than with circITCH. Blood based investigations have shown that the circulating HOTAIR has good diagnostic potential as indicated above. Zhao *et al.* showed that significantly elevated HOTAIR was found in the serum of CRC patients and there was a strong correlation between circulating HOTAIR in serum and plasma, indicating its potential as a minimally invasive liquid biopsy tool. Their study further showed that it was possible to significantly enhance the discrimination between CRC and normal tissue by a combination of two lncRNAs: HOTAIR and another one (Zhao *et al.,* 2015).

The present results also demonstrated that the best performance was obtained with the combined circITCH + HOTAIR model, yielding an AUC of 0.969, sensitivity of 93.3% and specificity of 91.3%. The combined model is superior and can be explained by the fact that the two RNAs give complementary biological information. circITCH is an expression of a loss of a tumor-suppressive regulatory component, while HOTAIR shows activation of an oncogenic and metastasis-associated program. Thus, simultaneous assessment considers two directions of colorectal carcinogenesis, but does not rely on asingle biological signal. This is supported by the evidence that the use of combinations of circulating ncRNAs generally gives better discrimination than single RNA markers (Ghafouri-Fard *et al.,* 2021). For instance, the study by Zhao *et al.* showed that HOTAIR and CCAT1 in combination had an AUC of 0.954 and outperformed each individual marker (AUC 0.836 and 0.838, respectively) (Zhao *et al.,* 2015).

Remarkably, circITCH and HOTAIR showed better performance in the current diagnostic analysis compared to the conventional CEA and CA19-9. CEA achieved an AUC of 0.831 and CA19-9 an AUC of 0.748, compared with 0.889 for circITCH and 0.941 for HOTAIR. These results highlight one possible benefit of molecular biomarkers: the CEA and CA19-9 are relatively nonspecific tumor-associated products, whereas deregulated ncRNAs more directly reflect the transcriptional and regulatory abnormalities associated with cancer. However, the results of the present study should not, be viewed as a substitute for CEA and CA19-9 in clinical routine use. A more clinically realistic approach would be to determine the incremental diagnostic value of ncRNAs over known clinical parameters and conventional tumor markers.

Further evidence of potential clinical relevance was obtained using the metastatic versus non-metastatic ROC analysis. HOTAIR demonstrated higher discrimination for metastatic disease than circITCH (AUC 0.856 and 0.814, respectively) and CEA and CA19-9 showed lower performance. Results for HOTAIR are more favorable and are biologically more easily reconciled with the wealth of data published so far associating this lncRNA with EMT and metastasis. Wu *et al.* found HOTAIR to be a significant predictor of metastasis in colon cancer and showed it to be correlated with advanced stage, vascular invasion, lymph-node involvement and organ metastasis (Wu *et al.,* 2014; Wu and Xu, 2023). Meta-analytic evidence also shows in the gastrointestinal malignancies that the overexpression of HOTAIR is linked with lymph node metastasis, distant metastasis, increased invasion, and advanced clinical stage (Shen *et al.,* 2020).

The model circITCH + HOTAIR again achieved the highest AUC of 0.901, sensitivity of 86.7% and specificity of 82.2% for discrimination between metastasis and non-metastasis. The opposing behavior of the two biomarkers also suggests that they may be useful forcharacterizing the aggressiveness of the disease, this finding should be explored further. If the patient has a marked suppression of circITCH and a marked elevation of HOTAIR, it might be a molecular phenotype of more advanced malignant behavior. A model like this may be useful in addition to radiological and clinicopathological staging, but would require a prospective study to validate its use in the clinic.

The independent associations of these biomarkers with metastatic CRC were further confirmed using multivariate regression. Higher expression of HOTAIR was correlated with increased odds of metastasis (OR=2.31, 95% CI: 1.42–3.76), while higher expression of circITCH was correlated with decreased odds of metastasis (OR=0.21, 95% CI: 0.06–0.71). This is the opposite direction from the oncogenic and tumor-suppressive functions they were proposed to have, respectively. Otherwise, CEA was also independently associated with metastatic disease, while CA19-9 was no longer statistically significant. This latter finding suggests that there is some overlap between the apparent association of CA19-9 with metastasis and other markers. The persistence of circITCH and HOTAIR in the multivariate model indicates that each may provide additional data beyond that provided by CEA and CA19-9.

Our independent association of HOTAIR with mCRC is in line with previous evidence. Increased HOTAIR expression has been associated with metastatic potential and decreased survival in CRC on several occasions and experimental inhibition of HOTAIR decreases migratory and invasive properties (Song *et al.,* 2021; Wang *et al.,* 2018). In contrast, the protective effect of circITCH is similar to its known tumor-suppressive function in various cancers and its known regulation of the Wnt/β-catenin and other cancer-associated pathways.

Another intriguing implication of these is the use of serum. CircRNAs have a covalently closed form that renders them relatively stable, and circulating lncRNAs could be stabilized by being packaged in EVs and/or bound to RNA-binding proteins and other large molecular complexes. Both types of ncRNAs can therefore be isolated from peripheral blood and have been the subject of a lot of interest as part of liquid-biopsy approaches (Ji *et al.,* 2018). Circulating levels of HOTAIR have been shown to be detectable in CRC in previous studies and there was significant correlation between plasma and serum levels (Lin *et al.,* 2019). These properties could render serum circITCH and HOTAIR as good candidates for repeated, minimally invasive disease assessment.

This study had several strengths. It combined a multimarker assessment of the novel non-coding RNAs circITCH and HOTAIR with the conventional protein markers CEA and CA19-9, and included a thorough methodological validation of the qRT-PCR assay. Nevertheless, some limitations should be considered. First, the sample size was moderate and the participants were recruited from a single center, which may limit the generalizability of the findings. Second, functional analyses using in vitro and in vivo assays were not performed in this clinical study; therefore, the exact mechanisms of the circITCH/HOTAIR pathway remain to be elucidated.

Conclusion

The present study revealed that the expression of serum circITCH and lncRNA HOTAIR was different in CRC patients than in healthy individuals, where circITCH was significantly downregulated and HOTAIR was significantly upregulated. These expression changes increased in severity as the disease progressed. Both biomarkers had a good diagnostic ability, and HOTAIR had a better individual diagnostic performance

than circITCH, CEA and CA19-9. Importantly, the combined circITCH + HOTAIR model yielded the highest diagnostic accuracy for CRC patients versus healthy controls and enhanced CRC metastatic versus non-metastatic disease discrimination. Additionally, circITCH and HOTAIR were shown to be separately associated with metastatic CRC in the multivariable analysis.

The results of this study indicate that the rapidly measurable multi-biomarker approach of circulating circITCH and HOTAIR could serve as a minimally invasive strategy for CRC detection, disease staging, and metastatic potential, highlighting the potential of these molecules as novel biomarkers for CRC. However, larger multicenter studies with independent validation cohorts and longitudinal follow-up are needed before these biomarkers can be established for routine clinical use.

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Author contribution ASN: Conceptualization, methodology, investigation, data curation, writing original draft, writing review & editing, and agreement to publish; ERO: Validation, data curation, visualization, software, and writing review & editing.

Availability of data and materials The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declaration of generative AI in writing process During the preparation of this work, the author used ChatGPT (OpenAI) for limited assistance with manuscript organization and for checking analysis code. All study analyses, interpretation of results, and scientific conclusions were performed and verified by the author. Generative AI was not used to generate or alter the study data. The author reviewed all AI-assisted content and takes full responsibility for the content of the article.

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