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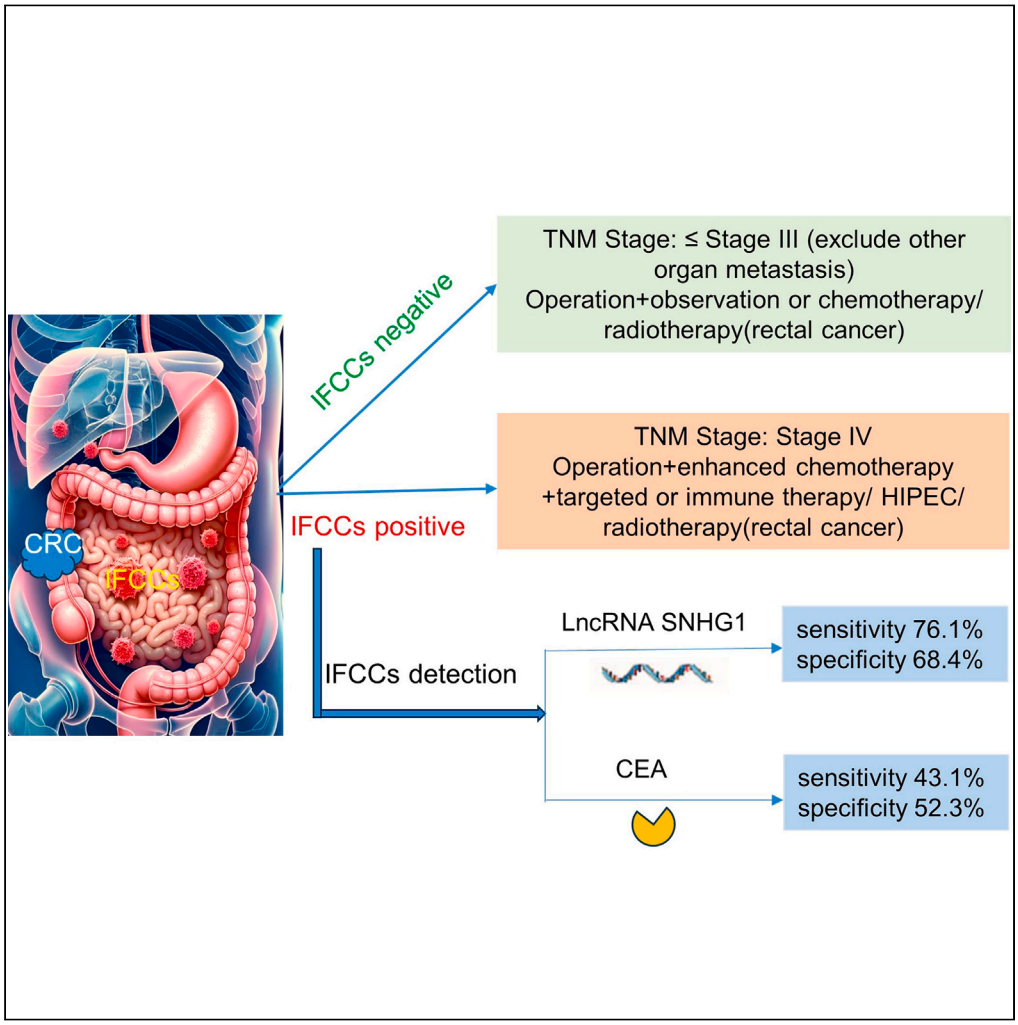

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Highlights

LncRNA SNHG1 shows high and stable expression in colorectal cancer (CRC)

SNHG1 outperforms CEA in detecting IFCCs across different TNM and T stages in CRC

SNHG1 has the potential to be an effective biomarker for IFCC detection in CRC

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Long noncoding RNA small nucleolar RNA host gene 1 as a potential novel biomarker for intraperitoneal free cancer cells in colorectal cancer

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SUMMARY

Colorectal cancer (CRC) is a prevalent cancer with intraperitoneal free cancer cells (IFCCs) playing a significant role in prognosis, especially during surgeries. The identification of IFCCs is crucial for determining the stage and treatment of patients with CRC. Existing methods for IFCC detection, such as conventional cytology, immunocytochemistry (ICC), and polymerase chain reaction (PCR), have limitations in sensitivity and specificity. This study investigates the potential of long noncoding RNA (lncRNA) SNHG1 as a biomarker for detecting IFCCs in patients with CRC. Testing on a cohort of 91 patients with CRC and 26 patients with gastrointestinal benign disease showed that SNHG1 outperformed CEA in distinguishing CRC cells and detecting IFCCs across different disease stages. SNHG1 demonstrated higher sensitivity (76.1% vs. 43.1%) and specificity (68.4% vs. 52.3%) than CEA for IFCC detection in patients with CRC, suggesting its promising role as a clinical method for identifying IFCCs in CRC.

INTRODUCTION

Colorectal cancer (CRC) is a malignancy with the third-highest incidence rate and the second-highest death rate globally.[1] The comprehensive treatment for CRC is based on surgery, radiotherapy and chemotherapy.[2–4] Although significant progress has been made in the treatment of CRC, it is notable that a substantial proportion of patients, ranging from 10 to 30%, continue to experience peritoneal recurrence following radical resection.[5,6] Intraperitoneal free cancer cells (IFCCs) can be detected in the peritoneal irrigation fluid during CRC surgeries, and positive IFCCs are considered to be the first step of CRC peritoneal spread.[7] Several studies have confirmed the association of positive IFCCs with recurrence and poor prognosis of CRC.[7–14] Furthermore, the efficacy of treatments targeting positive abdominal exfoliative cells, such as hyperthermic intraperitoneal chemotherapy (HIPEC), has illustrated the potential significance of IFCCs as a prognostic factor.[15] Consequently, the accurate identification of IFCCs positivity can enable more timely and precise therapeutic interventions, ultimately enhancing the patient's prognosis.

IFCCs in peritoneal lavage fluid have not been routinely tested clinically, and several studies have used a variety of assays to detect it. The most commonly used assays are cytology,[9,10] immunocytochemistry (ICC),[11,12] and reverse transcription-polymerase chain reaction (RT-PCR).[13,14] Various detection methods have their advantages and disadvantages, and there is no clinical gold standard for detecting IFCCs right now. Conventional cytology, renowned for its simplicity and high specificity,[7] is currently the predominant adjunctive diagnostic method employed in clinical practice. However, it is essential to note that numerous studies have reported the sensitivity of cytology to be less than 10%, which poses challenges in detection, even among patients in the advanced stages of the disease.[9,10,16–18] In order to improve the sensitivity and accuracy of diagnosis, ICC and RT-PCR were used to detect IFCCs in peritoneal lavage fluid. In ICC detection, carcinoembryonic antigen (CEA), Ra96, carbohydrate antigen (CA19-9) and cytokeratin 20 (CK20) were always chosen as the target to be evaluated by different monoclonal antibodies.[11,12,19] CK20, CEA mRNA or human mammaglobin-A (hMAM-A) and hMAM-B expression were the common detection targets in RT-PCR assay.[13,20,21] Although ICC assay improves the sensitivity of detecting IFCCs, it has been noted to diminish specificity and does not adequately address the limitations of conventional cytology.[12,19,22] In recent years, RT-PCR has emerged as a favored method for detecting IFCCs in numerous research studies. The quantitative amplification of DNA greatly enhances the efficiency of this assay after reverse transcription, allowing for the detection of even minute amounts of mRNA.[23] RT-PCR is widely regarded as the most sensitive assay to detect IFCCs, with CEA being the most commonly utilized and reliable biomarker for CRC cells to date.[13,24,25] The sensitivity and specificity of current biomarkers used for the detection and diagnosis of IFCCs from CRC are not always as satisfactory as desired, especially the insufficient sensitivity (Table 1).

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Table 1. The Sensitivity and Specificity of Existing Biomarkers used in IFCC detection of CRC

Biomarkers	Sensitivity	Specificity	Reference
CEA	40.0%	90.0%	Lloyd et al.[13]
	35.7%	87.5%	Hara et al.[26]
	66.7%	61.9%	Rossi Del Monte et al.[27]
	20.0%	92.5%	Ouchi et al.[25]
	35.3%	86.8%	Murono et al.[28]
CK20	26.7%	99.1%	Lloyd et al.[13]
	0.0%	50.0%	Altomare et al.[20]
	33.3%	92.8%	Rossi Del Monte et al.[27]
Matrilysin (MMP-7)	33.3%	83.6%	Lloyd et al.[13]
	100.0%	60.8%	Sica et al.[29]
K-ras	14.6%	95.5%	Kristensen et al.[21]
ephrin B4	0.0%	98.2%	Lloyd et al.[13]
laminin gamma2	0.0%	98.2%	Lloyd et al.[13]

However, it is important to highlight that positive IFCC detection has also been observed in a subset of patients with benign conditions and early-stage CRC,[26,28] which raises questions regarding the specificity of RT-PCR (CEA mRNA). Both *in vitro* and *in vivo* experiments have confirmed that CEA can be expressed in inflammatory cells.[30] Malignant or benign intestinal diseases are often accompanied by the inflammatory environment, which can lead to the non-specific increase of CEA expression in epithelial cells. The low specificity and potential for false positives can result in unnecessary intraperitoneal chemotherapy and heightened postoperative anxiety for patients. Over the years, various research teams have explored new technical methods to detect IFCCs, such as serosal stamp technique[31] and immunofluorescence for epithelial markers.[27] However, no breakthroughs have been achieved to date. Consequently, there has been a shift in focus toward the identification of more sensitive and specific biomarkers to enhance the accuracy and reliability of IFCC detection.

Long noncoding RNAs (lncRNAs), characterized by lengths exceeding 200 nucleotides, are non-coding RNAs that exert multiple functions through interactions with various cellular molecules.[32] Several lncRNAs have been identified to regulate CRC growth and development, and their aberrant expression often represents an abnormal or disease state.[33,34] Recent studies have reported the diagnostic potential of lncRNAs. Compared with TNM staging alone, combined detection of lncRNA GLCC1 expression levels can accurately predict the prognosis of patients with CRC.[35] LncRNA KRT7-AS and KRT7 expression among patients with CRC may be potential early predictors of lymph node status.[36] Notably, lncRNA NEAT1 has demonstrated high diagnostic potential, with an AUC value of 0.845, a sensitivity of 83.3% and a specificity of 83.3% to distinguish patients with CRC from healthy individuals.[37] The stability of lncRNAs also ensures reliable and reproducible detection results, as demonstrated by the stability of lncRNA-GC1 at room temperature for over 30 min post-sampling.[38] LncRNA SNHG1 was illuminated to form a regulatory network to confer an oncogenic function in CRC, suggesting to serve as a potential target for CRC diagnosis and treatment.[39] The expression of lncRNA CCAL is significantly increased in colorectal tissues following the sequence of normal-adenoma-carcinoma, and its high expression is associated with drug resistance and poor prognosis.[40] Furthermore, the expression level of lncRNA SNHG6 is closely associated with CRC progression and poor prognosis.[41]

In this study, we focused on three lncRNAs (SNHG1, CCAL, SNHG6) that have been identified as highly expressed in CRC and related to the disease's occurrence and development, as reported in high-quality literature. We used cell lines and patient tissue samples to validate their expression and select the most promising candidate for further comparison with CEA mRNA, a widely used biomarker in current research. We aimed to contribute to the development of more accurate and reliable diagnostic tools for detecting IFCCs in patients with CRC.

RESULTS

Small nucleolar RNA host gene 1 is expressed highly and stably in colorectal cancer cell lines and colorectal cancer tissues

We detected the expression of selected lncRNAs (SNHG1, CCAL, SNHG6) and CEA in CRC cell lines (SW620 and SW480) and norma colon cell lines (NCM460). The expression of all the selected lncRNAs and CEA mRNA was significantly higher in CRC cell lines than in normal cell lines ($p < 0.001$). (Figure 1A). Interestingly, the expression of all three lncRNAs was higher in the metastatic colon cancer cell line SW620 than in the primary lesion cell line SW480.

In the subsequent phase of our study, we evaluated the expression levels of the selected long noncoding RNAs (lncRNAs) - SNHG1, CCAL, and SNHG6 - along with CEA in CRC tissues and compared them with those in normal tissues. Our comparative analysis revealed the following observations among the 16 patients included in the study: SNHG1 expression was significantly elevated in CRC tissues compared to normal tissues in 15 patients, CCAL expression was found to be higher in CRC tissues than in normal tissues in 12 patients, SNHG6 expression demonstrated an increase in CRC tissues compared to normal tissues in 9 patients, while CEA expression levels were observed to be higher in CRC tissues than in normal tissues in 14 patients. (Figure 1B).

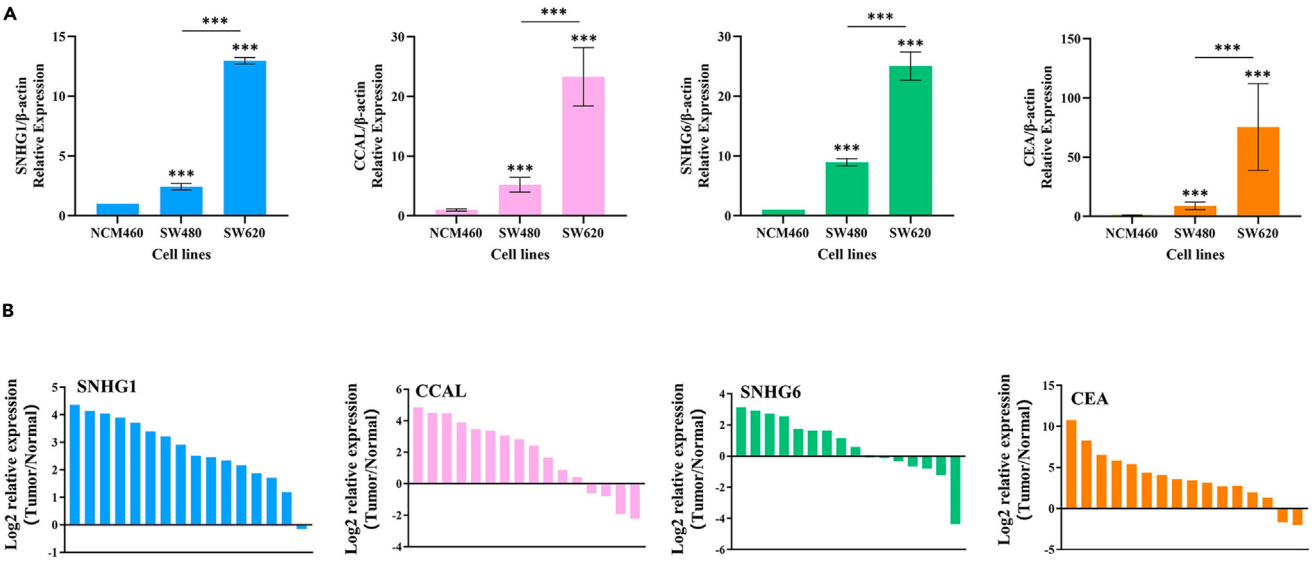

Figure 1. LncRNA and CEA Expression Profiles in Cell Lines and CRC Tissues

(A) The expression of LncRNA SNHG1, CCAL, SNHG6 and CEA mRNA across various cell lines (NCM460, SW480 and SW620). $n = 3$, ***$p < 0.001$.

(B) The relative expression of LncRNA SNHG1, CCAL, SNHG6 and CEA mRNA in CRC tumor tissues compared to normal tissues.

Notably, the expression of all selected lncRNAs was overall higher in tumors compared to normal tissues. Among these lncRNAs, we identified SNHG1 as a more stably expressed candidate for the follow-up study. We conducted a comparative analysis of SNHG1 expression levels in primary colon cancer tissues and liver metastases. Our findings revealed that both primary colon cancer foci and liver metastases exhibited elevated SNHG1 expression compared to normal tissues. Notably, the expression level of SNHG1 in liver metastases was slightly higher; however, this difference was not found to be statistically significant. (Figure S1).

We proceeded to compare SNHG1 with the currently utilized CEA mRNA to determine a more effective marker for the detection of IFCCs in patients with CRC.

Receiver-operating characteristic curves and cut-off values

Receiver-operating characteristic (ROC) curves were used to compare the accuracies of SNHG1/β-actin, CEA/β-actin ratio and determine the cut-off value by plotting sensitivity/specificity pairs for the two RNA ratios (Figure 2). The clinical value of SNHG1 and CEA detection was assessed based on the diagnostic data from 22 patients with positive cytology (Figure S2) and 26 control group patients with negative cytology (the detailed information was described in Table S4). The sensitivity and specificity of the SNHG1 assay, determined using the established cut-off value, were found to be 73.3% and 90.0%, respectively. In comparison, the sensitivity and specificity of the CEA assay were calculated to be 66.7% and 85.0%, respectively. These results suggest that the SNHG1 may offer improved diagnostic accuracy over the CEA in detecting IFCCs in patients with CRC.

Small nucleolar RNA host gene 1 can detect intraperitoneal free cancer cells better than carcinoembryonic antigen in patients with colorectal cancer with different TNM stages

The cohort comprised 12 stage I, 30 stage II, 39 stage III and 10 stage IV patients with CRC (Tables 2, S2, and S5). Collectively, the relative expression of CEA and SNHG1 were found to be significantly different in detecting IFCCs across various stages of patients with CRC ($p < 0.001$) (Figures 3A and 3B). However, in the subgroup analysis, we found that the relative expression of CEA did not exhibit significantly difference between stages I and II ($p > 0.05$), and the relative expression of SNHG1 was not significantly different in patients with stages III and IV ($p > 0.05$).

Upon plotting the positive cut-off values established in the pre-experimental phase, it was determined that the two detection methods for IFCCs did not exhibit statistically significant differences in certain subgroup comparisons when assessed based on the positivity rate. Specifically, the positivity rate of CEA could not distinguish the positivity rate of IFCCs well across different stages ($p = 0.137$) (Table S7). In contrast, the positivity rate of SNHG1 demonstrated a greater ability to distinguish the positivity rate of IFCCs among different stages ($p = 0.001$) (Table S6).

When examining each stage individually, the positivity rate of IFCCs as determined by CEA failed to consistently differentiate between stages, with the exception of stages II and IV. In contrast, the positivity rate of SNHG1 for detecting IFCCs in patients with CRC effectively distinguished each stage, save for a lack of differentiation between stages III and IV. While CEA positivity did not consistently increase across all stages, SNHG1 positivity exhibited an upward trend with the advancing stage. Although the difference in SNHG1 positivity was not

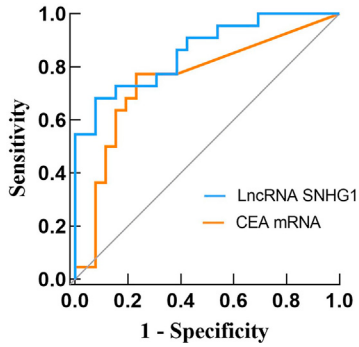

Figure 2. ROC curves for the detection of CRC cells using LncRNA SNHG1 and CEA mRNA
The area under the curve (AUC) for SNHG1 and CEA were 0.870 and 0.733, respectively.

statistically significant in distinguishing between stage III and IV patients ($p = 0.102$), the positivity rates for these stages were 51.3% and 80%, respectively.

In summary, SNHG1 demonstrated a superior capacity to detect IFCCs in patients with CRC across different TNM stages when compared to CEA. These findings suggest that SNHG1 may serve as a more reliable biomarker for the detection of IFCCs in patients with CRC.

Small nucleolar RNA host gene 1 can detect intraperitoneal free cancer cells better than carcinoembryonic antigen in patients with colorectal cancer with different T-stages

In CRC, the depth of local cancer infiltration greatly influences the presence of IFCCs, with the T stage reflects the local infiltration of the cancer. In patients with T3 and T4 CRC, where the cancer has invaded or perforated the serosal layer, the likelihood of cancer cell exfoliation increases substantially due to the movement and friction of the abdominal organs, consequently raising the positive rate of IFCCs. Therefore, we compared the IFCC positivity rates between SNHG1 and CEA detection in patients with CRC across various T-stages.

Our study cohort included 3 cases of T1, 10 cases of T2, 48 cases of T3 and 30 cases of patients with T4 CRC. Since the cancer cells in both T1 and T2 stages CRC did not exhibit deep infiltration, we grouped them together for analysis. Our findings revealed that the overall IFCC positivity rate for all enrolled patients with CRC for SNHG1 and CEA was 46.2% and 28.5%, respectively (Tables S8 and S9). When comparing positive IFCC rates, there is a significant difference between different stages by the detection of SNHG1 but not CEA. However, upon analyzing the relative expression levels, both SNHG1 and CEA demonstrated statistical differences across various stages (Figures 3C and 3D).

It is important to note that, at the relative expression level, SNHG1 was unable to differentiate between T3 and T4 stage cancers, while CEA could not differentiate between T1-2 and T3 stage cancers. These findings suggest that while both SNHG1 and CEA show potential as biomarkers for IFCC detection in patients with CRC, they each have limitations in distinguishing between certain T-stages.

Bayesian analysis of the sensitivity and specificity of two biomarkers

Based on reported studies of SNHG1 and CRC,[39,42–50] the pretest probability of the sensitivity of SNHG1 testing was set at 0.6 to 0.9, and the pretest probability of specificity was set at 0.714 to 0.9. On the basis of reported studies of CEA mRNA and CRC,[13,14,18,24–28] the pretest probability of the sensitivity of CEA testing was set at 0.33 to 0.667, and the pretest probability of specificity was set at 0.697 to 0.864.Based on the reported studies of IFCCs and CRC,[13,14,18,20,21,24–28] the pretest probability of positive rate sensitivity was set at 0.08 to 0.379. Using the DTAXG package in *R* language, we calculate the sensitivity and specificity without a gold standard using the *Bayesian* method (Table 3). The sensitivity of SNHG1 was higher than that of CEA (76.1% vs.43.1%, 95% confidence interval (95% CI), $p < 0.0001$), and the specificity of SNHG1 was also higher than that of CEA (68.3% vs.52.3%, 95% CI, $p < 0.0001$).

DISCUSSION

At present, radical resection remains the primary treatment for CRC.[2] Recurrence following curative surgery is commonly attributed to the IFCCs.[7] During CRC surgery, peritoneal lavage fluid can be used to detect IFCCs and assess the presence of the peritoneal dissemination of cancer. A multitude of studies have established a correlation between IFCCs and poor prognosis, with the results of IFCCs also serving to inform intraoperative chemotherapy and postoperative treatment strategies.[7,51,52]Conventional cytology, ICC and RT-PCR are the most common methods to detect IFCCs in clinical and scientific research. Numerous institutions have adopted peritoneal cytology as a valuable prognostic marker for CRC. However, it is difficult to use cytology alone to detect IFCCs in clinical practice. It is reported that the sensitivity of cytology is less than 10% and it remains difficult to detect IFCCs even in stage IV patients.[9,10,16–18] Low sensitivity often leads to the failure to find IFCC effectively, thus delaying treatment. Compared with cytology, ICC has shown improvements in sensitivity but has come at the cost of reduced specificity. Consequently, the detection rate of ICC remains low in some advanced-stage patients and does not effectively address the limitations of traditional cytology. In recent years, PCR technology, with its robust detection capabilities, has gained increasing favor

Table 2. Correlation between SNHG1, CEA and histopathological findings in colorectal cancer patients

		LncRNA SNHG1 evaluation		CEA mRNA evaluation	
Factor	All patients	positive	*p*-value	positive	*p*-value
No. of patients	91	42 (46.2%)		26 (28.5%)	0.010
Age (years)	58.2 ± 3.53				
Gender					
Male	65	31/65 (47.7%)	0.816	20/65 (30.8%)	0.609
Female	26	11/26 (42.3%)		6/26 (23.1%)	
Histology (differentiated/undifferentiated)					
G1–G2	66	32/66 (48.5%)	0.491	21/66 (31.8%)	0.310
G3–G4	25	10/25 (40.0%)		5/25 (20.0%)	
Depth of invasion					
T1–T2	13	1/13 (7.7%)	0.003	3/13 (23.1%)	0.103
T3	48	22/48 (45.8%)		10/48 (20.8%)	
T4	30	19/30 (63.3%)		13/30 (43.3%)	
Stage					
I	12	0/12 (0.0%)	<0.001	3/12 (25.0%)	0.085
II-III	69	34/69 (49.3%)		17/69 (24.6%)	
IV	10	8/10 (80.0%)		6/10 (60.0%)	
CEA mRNA evaluation					
Negative	65	24/65 (36.9%)	0.010		
Positive	26	18/26 (69.2%)			
LncRNA SNHG1 evaluation					
Negative	49			8/49 (16.3%)	0.010
Positive	42			18/42 (42.9%)	

among researchers for detecting IFCCs. Several studies have indicated that identify IFCCs that cytology may overlook, thereby significantly enhancing the detection efficiency.[26,53]

Various markers have been used for PCR detection, such as CK20, CEA or hMAM and hMAM-B expression.[13,20,21] To date, CEA mRNA has been mostly used in clinical studies to detect IFCCs in patients with CRC.[13,24,25] However, CEA mRNA's sensitivity ranges between 40 and 60% and its specificity is between 70 and 80%.[13,14,18,24–28] As the discovery of IFCCs is becoming increasingly important in the treatment decision of CRC, clinicians are in need of novel new biomarkers with higher sensitivity and specificity to detect IFCCs.

LncRNAs have been shown to play an important role in regulating the tumorigenesis and development of tumors, often exhibiting aberrant expression in tumors. High expression of a variety of lncRNAs and their detection efficiency have been reported in CRC, the primary focus of detection samples has been on tissue and blood samples.[35–37] To date, there have been no reports on the detection of IFCCs in CRC using lncRNAs. In pre-experiments utilizing both cell lines and patient tissues, we found that SNHG1 was stably and highly expressed in both colon cancer cell lines and patient tumor tissues. This high expression level is essential for ensuring the reliability and reproducibility of the assay results. Upon examining the peritoneal lavage fluid samples from patients, we observed a significant positive correlation between the detection rate of SNHG1 and the TNM stage and T stage of the tumor. In contrast, the relationship between CEA (carcinoembryonic antigen) levels and tumor stage is less clear-cut.

It is generally believed that as the tumor stage advances, there is an increased likelihood of cancer cells being shed during the operation. However, CEA detection results can sometimes contradict conventional clinical expectations. Jung et al. have demonstrated that CEA can be expressed in inflammatory cells both *in vitro* and *in vivo*.[30] The inflammatory environment associated with early stage malignant or even benign intestinal diseases can lead to the non-specific overexpression of CEA in epithelial cells. As a result, abnormal CEA expression in patients with early-stage CRC may lead to an unrepresentatively high positivity rate, which is not significantly different from that of stage III or T3 patients.

The detection rate of SNHG1 showed an increasing trend with the progression of the tumor stage, indicating that SNHG1 may possess a higher specificity compared to CEA. Additionally, we found fewer positive stage I patients tested by SNHG1 compared to CEA (0 vs. 3), further supporting this notion. Following radical resection, only about 5% of patients with AJCC stage I CRC will experience recurrence and metastasis several years later.[54] IFCCs is often considered as the initial step of peritoneal spread, and the detection of IFCCs serves an independent risk factor for recurrence and metastasis.[7–14] Notably, the results showing an excessive positive rate in stage I patients are inaccurate, potentially due to false positives caused by low specificity.

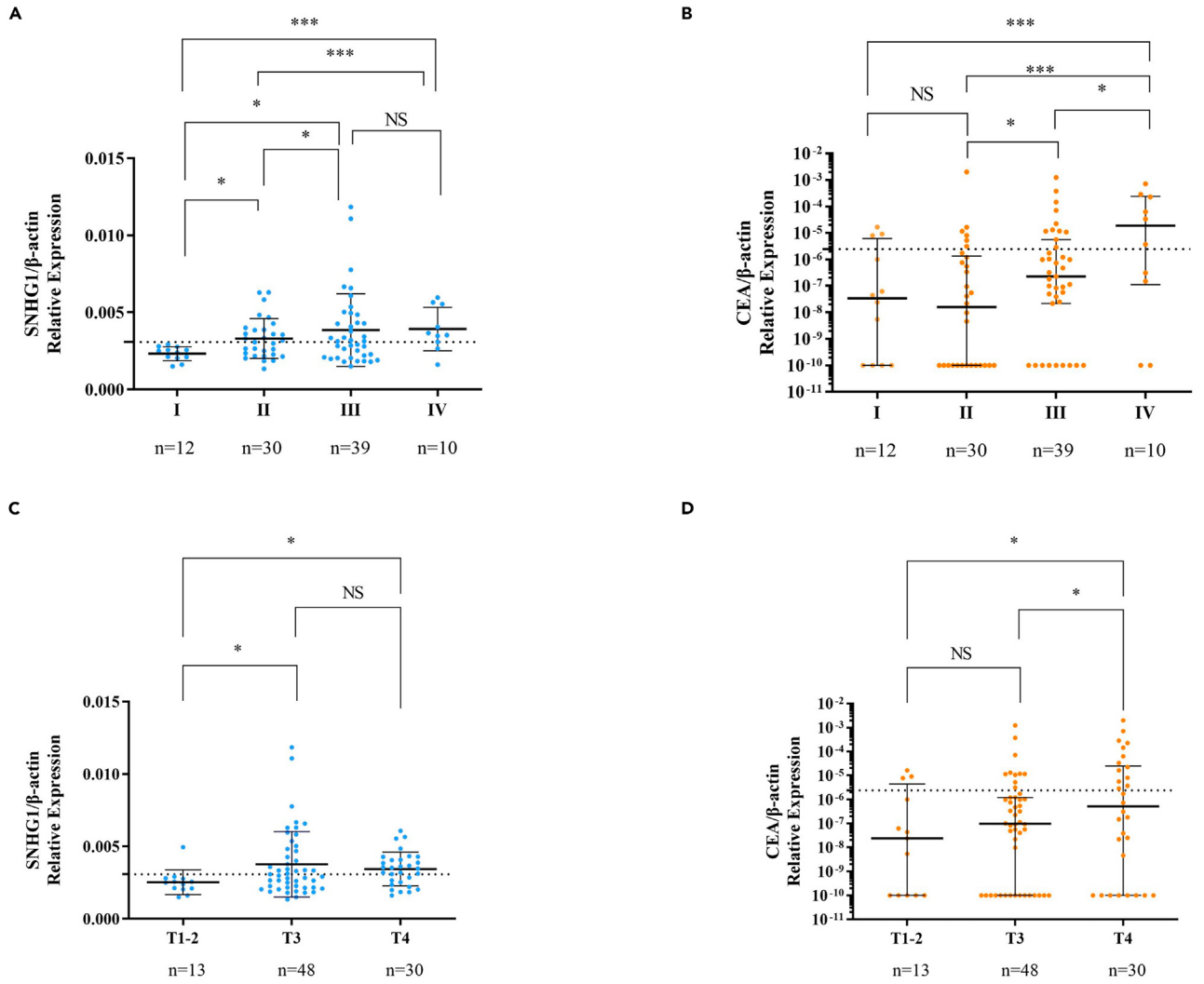

Figure 3. Relative expression of LncRNA SNHG1 and CEA in IFCCs found in the peritoneal lavage fluid of CRC patients across various TNM stages and T stages

(A) SNHG1 in the detection of the IFCCs in different TNM stages of CRC.

(B) CEA mRNA in detecting the IFCCs in different TNM stages of CRC.

(C) SNHG1 in detection of the IFCCs in different T stages of CRC.

(D) CEA mRNA in the detection of the IFCCs in different T stages of CRC. NS: no significant difference, *$p < 0.05$, ***$p < 0.001$.

However, there is a correlation between the SNHG1 method and the CEA assay. The results analyzed using the chi-square test demonstrate a favorable positive correlation between positive SNHG1 and positive CEA detection. Most patients who tested positive for CEA also t exhibited positive SNHG1 results, and SNHG1 can detect more cases of IFCCs that CEA failed to detect. This indicates that the high sensitivity of SNHG1 serves as a valuable complement to CEA, which has a sensitivity ranging from 40 to 60%.

Recurrence and metastasis of CRC following operations are influenced by various factors, such as tumor differentiation, tumor stages, tumor location, IFCCs positivity, surgeon's experience, the patient's compliance, and more.[55] Currently, there is no established gold standard for the diagnosing IFCCs. As a result, researchers have employed Bayesian models to estimate the sensitivity and specificity of a given test in the absence of a definitive gold standard. Our results indicate that SNHG1 outperforms CEA in terms of both sensitivity and specificity. While the estimated results may not be entirely accurate, they offer a solid theoretical foundation for further clinical testing and validation of SNHG1 as a potential biomarker for IFCCs in patients with CRC.

Conclusions

Long non-coding RNA SNHG1 has emerged as a promising biomarker with superior sensitivity and specificity compared to the conventionally used CEA mRNA in detecting IFCCs in patients with CRC. The enhanced sensitivity of SNHG1 allows for the identification of a greater number

Table 3. Bayesian analysis of the sensitivity and specificity with 95% CI of two biomarkers

	Positive rate	Se1	Se2	Sp1	Sp2
50%	0.2627971	0.7606617	0.4307666	0.6835393	0.5227116
2.5%	0.1297897	0.6051055	0.2853673	0.5897852	0.4362418
97.5%	0.4268576	0.8825851	0.5867525	0.7752889	0.6052403

Se1: The sensitivity of LncRNA SNHG1, Se2: The sensitivity of CEA mRNA, Sp1: The specificity of LncRNA SNHG1, Sp2: The specificity of CEA mRNA.

of positive cases, while its higher specificity helps in reducing the occurrence of false positives. These attributes suggest that SNHG1 has the potential to become a more effective biomarker for IFCC detection in patients with CRC, offering improved accuracy and reliability in clinical practice. Further research and validation studies are warranted to fully establish the role of SNHG1 in the diagnostic and prognostic evaluation of CRC.

Limitations of the study

This study included the relatively small sample size and the single-center design. In order to enhance the robustness and generalizability of the findings, future studies should consider involving multiple centers and increasing the sample size. This will help to ensure that the results can be more widely applicable and convincing for clinical implementation. Additionally, the study aims to establish the significance of SNHG1 in detecting IFCCs in patients with CRC. However, in order to fully elucidate the clinical implications of SNHG1 detection in CRC, it is essential to incorporate patient prognosis outcomes. These long-term follow-up results are expected to be available in the next two to three years, after which a comprehensive analysis can be conducted to evaluate the impact of SNHG1 detection on patient prognosis. Future research efforts should focus on addressing these limitations to validate the findings and determine the clinical utility of SNHG1 as a biomarker for IFCC detection in patients with CRC.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.110228.

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AUTHOR CONTRIBUTIONS

Y.W. and L.L. conducted most of the research and wrote the article. J.G., X.X., and Z.C. supervised and designed the study, W.J., X.X., and Z.C. revised the article. F.H., W.J., and L.L. collected the clinical data. Y.Z., a statistical analysis professional, was responsible for the statistical analysis of the data in this study. All authors read and approved the submitted version.

DECLARATION OF INTERESTS

The authors declare they have no conflict of interest.

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REFERENCES

1. Siegel, R.L., Miller, K.D., Fuchs, H.E., and Jemal, A. (2021). Cancer Statistics, 2021. CA. Cancer J. Clin. *71*, 7–33. https://doi.org/10.3322/caac.21654.
2. Vogel, J.D., Felder, S.I., Bhama, A.R., Hawkins, A.T., Langenfeld, S.J., Shaffer, V.O., Thorsen, A.J., Weiser, M.R., Chang, G.J., Lightner, A.L., et al. (2022). The American Society of Colon and Rectal Surgeons Clinical Practice Guidelines for the Management of Colon Cancer. Dis. Colon Rectum *65*, 148–177. https://doi.org/10.1097/DCR.0000000000002323.
3. You, Y.N., Hardiman, K.M., Bafford, A., Poylin, V., Francone, T.D., Davis, K., Paquette, I.M., Steele, S.R., Feingold, D.L., and Rectal, S.; On Behalf of the Clinical Practice Guidelines Committee of the American Society of Colon and Rectal Surgeons (2020). The American Society of Colon and Rectal Surgeons Clinical Practice Guidelines for the Management of Rectal Cancer. Dis. Colon Rectum *63*, 1191–1222. https://doi.org/10.1097/DCR.0000000000001762.
4. Watanabe, T., Muro, K., Ajioka, Y., Hashiguchi, Y., Ito, Y., Saito, Y., Hamaguchi, T., Ishida, H., Ishiguro, M., Ishihara, S., et al. (2018). Japanese Society for Cancer of the Colon and Rectum (JSCCR) guidelines 2016 for the treatment of colorectal cancer. Int. J. Clin. Oncol. *23*, 1–34. https://doi.org/10.1007/s10147-017-1101-6.
5. Lan, Y.T., Chang, S.C., Yang, S.H., Lin, C.C., Wang, H.S., Jiang, J.K., Chen, W.S., Lin, T.C., Chiou, S.H., and Lin, J.K. (2014). Comparison of clinicopathological characteristics and prognosis between early and late recurrence after curative surgery for colorectal cancer. Am. J. Surg. *207*, 922–930. https://doi.org/10.1016/j.amjsurg.2013.08.035.
6. Segelman, J., Granath, F., Holm, T., Machado, M., Mahteme, H., and Martling, A. (2012). Incidence, prevalence and risk factors for peritoneal carcinomatosis from colorectal cancer. Br. J. Surg. *99*, 699–705. https://doi.org/10.1002/bjs.8679.
7. Passot, G., Mohkam, K., Cotte, E., and Glehen, O. (2014). Intra-operative peritoneal lavage for colorectal cancer. World J. Gastroenterol. *20*, 1935–1939. https://doi.org/10.3748/wjg.v20.i8.1935.
8. Shang, A., Wang, S., Yang, Y., Li, L., Zhao, Z., Li, D., Guo, Y., and Wang, M. (2021). Effect and safety of intraoperative intraperitoneal chemotherapy on patients suffering from colorectal cancer. World J. Surg. Oncol. *19*, 84. https://doi.org/10.1186/s12957-021-02197-3.
9. Noura, S., Ohue, M., Seki, Y., Yano, M., Ishikawa, O., and Kameyama, M. (2009). Long-term prognostic value of conventional peritoneal lavage cytology in patients undergoing curative colorectal cancer resection. Dis. Colon Rectum *52*, 1312–1320. https://doi.org/10.1007/DCR.0b013e3181a745a4.
10. Fujii, S., Shimada, H., Yamagishi, S., Ota, M., Kunisaki, C., Ike, H., and Ichikawa, Y. (2009). Evaluation of intraperitoneal lavage cytology before colorectal cancer resection. Int. J. Colorectal Dis. *24*, 907–914. https://doi.org/10.1007/s00384-009-0733-z.
11. Bosch, B., Guller, U., Schnider, A., Maurer, R., Harder, F., Metzger, U., and Marti, W.R. (2003). Perioperative detection of disseminated tumour cells is an independent prognostic factor in patients with colorectal cancer. Br. J. Surg. *90*, 882–888. https://doi.org/10.1002/bjs.4129.
12. Vogel, I., Francksen, H., Soeth, E., Henne-Bruns, D., Kremer, B., and Juhl, H. (2001). The carcinoembryonic antigen and its prognostic impact on immunocytologically detected intraperitoneal colorectal cancer cells. Am. J. Surg. *181*, 188–193. https://doi.org/10.1016/s0002-9610(00)00555-9.
13. Lloyd, J.M., McIver, C.M., Stephenson, S.A., Hewett, P.J., Rieger, N., and Hardingham, J.E. (2006). Identification of early-stage colorectal cancer patients at risk of relapse post-resection by immunobead reverse transcription-PCR analysis of peritoneal lavage fluid for malignant cells. Clin. Cancer Res. *12*, 417–423. https://doi.org/10.1158/1078-0432.CCR-05-1473.
14. Katsuragi, K., Yashiro, M., Sawada, T., Osaka, H., Ohira, M., and Hirakawa, K. (2007). Prognostic impact of PCR-based identification of isolated tumour cells in the peritoneal lavage fluid of gastric cancer patients who underwent a curative R0 resection. Br. J. Cancer *97*, 550–556. https://doi.org/10.1038/sj.bjc.6603909.
15. Verwaal, V.J., van Ruth, S., de Bree, E., van Sloothen, G.W., van Tinteren, H., Boot, H., and Zoetmulder, F.A.N. (2003). Randomized trial of cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis of colorectal cancer. J. Clin. Oncol. *21*, 3737–3743. https://doi.org/10.1200/JCO.2003.04.187.
16. Nishikawa, T., Watanabe, T., Sunami, E., Tsuno, N.H., Kitayama, J., and Nagawa, H. (2009). Prognostic value of peritoneal cytology and the combination of peritoneal cytology and peritoneal dissemination in colorectal cancer. Dis. Colon Rectum *52*, 2016–2021. https://doi.org/10.1007/DCR.0b013e3181b4c46e.
17. Yamamoto, S., Akasu, T., Fujita, S., and Moriya, Y. (2003). Long-term prognostic value of conventional peritoneal cytology after curative resection for colorectal carcinoma. Jpn. J. Clin. Oncol. *33*, 33–37. https://doi.org/10.1093/jjco/hyg007.
18. Lee, I.K., Kim, D.H., Gorden, D.L., Lee, Y.S., Sung, N.Y., Park, G.S., Kim, H.J., Kang, W.K., Park, J.K., Ahn, C.H., et al. (2009). Prognostic value of CEA and CA 19-9 tumor markers combined with cytology from peritoneal fluid in colorectal cancer. Ann. Surg Oncol. *16*, 861–870. https://doi.org/10.1245/s10434-008-0294-3.
19. Yang, S.H., Lin, J.K., Lai, C.R., Chen, C.C., Li, A.F.Y., Liang, W.Y., and Jiang, J.K. (2004). Risk factors for peritoneal dissemination of colorectal cancer. J. Surg. Oncol. *87*, 167–173. https://doi.org/10.1002/jso.20109.
20. Altomare, D.F., Tedeschi, M., Rotelli, M.T., Bocale, D., Piscitelli, D., and Rinaldi, M. (2011). Lack of prognostic role of pre- and postoperative peritoneal cytology and cytokeratin PCR-expression on local recurrence after curative anterior resection for mid-low rectal cancer. Updates Surg. *63*, 109–113. https://doi.org/10.1007/s13304-011-0071-x.
21. Kristensen, A.T., Wiig, J.N., Larsen, S.G., Gierckskiy, K.-E., and Ekstrøm, P.O. (2008). Molecular detection (k-ras) of exfoliated tumour cells in the pelvis is a prognostic factor after resection of rectal cancer? BMC Cancer *8*, 213. https://doi.org/10.1186/1471-2407-8-213.
22. Schott, A., Vogel, I., Krueger, U., Kalthoff, H., Schreiber, H.W., Schmiegel, W., Henne-Bruns, D., Kremer, B., and Juhl, H. (1998). Isolated tumor cells are frequently detectable in the peritoneal cavity of gastric and colorectal cancer patients and serve as a new prognostic marker. Ann. Surg. *227*, 372–379. https://doi.org/10.1097/00000658-199803000-00009.
23. Green, M.R., and Sambrook, J. (2018). Analysis and Normalization of Real-Time Polymerase Chain Reaction (PCR) Experimental Data. Cold Spring Harb. Protoc. *2018*, pdb-top095000. https://doi.org/10.1101/pdb.top095000.
24. Nakamura, K., Iinuma, H., Aoyagi, Y., Shibuya, H., and Watanabe, T. (2010). Predictive value of cancer stem-like cells and cancer-associated genetic markers for peritoneal recurrence of colorectal cancer in patients after curative surgery. Oncology *78*, 309–315. https://doi.org/10.1159/000318862.
25. Ouchi, A., Kinoshita, T., Nakanishi, H., Komori, K., Oshiro, T., Yoshimura, M., Fujita, N., Hosoda, W., and Shimizu, Y. (2022). PCR-based quantitative detection of intraperitoneal free cancer cells for predicting locoregional recurrence after rectal cancer resection. ANZ J. Surg. *92*, 794–800. https://doi.org/10.1111/ans.17436.
26. Hara, M., Nakanishi, H., Jun, Q., Kanemitsu, Y., Ito, S., Mochizuki, Y., Yamamura, Y., Kodera, Y., Tatematsu, M., Hirai, T., and Kato, T. (2007). Comparative analysis of intraperitoneal minimal free cancer cells between colorectal and gastric cancer patients using quantitative RT-PCR: possible reason for rare peritoneal recurrence in

colorectal cancer. Clin. Exp. Metastasis *24*, 179–189. https://doi.org/10.1007/s10585-007-9067-9.

27. Rossi Del Monte, S., Ranieri, D., Mazzetta, F., Kazemi Nava, A., Raffa, S., Torrisi, M.R., and Ziparo, V. (2012). Free peritoneal tumor cells detection in gastric and colorectal cancer patients. J. Surg. Oncol. *106*, 17–23. https://doi.org/10.1002/jso.23052.
28. Murono, K., Kazama, S., Yamaguchi, H., Kawai, K., Ishihara, S., Sunami, E., Kitayama, J., Satoh, Y., Kurihara, M., Yatomi, Y., and Watanabe, T. (2015). Detection of carcinoembryonic antigen mRNA in peritoneal lavage by the transcription-reverse transcription concerted method indicates poor prognosis in patients with stage II and III colon cancer. Surgery *157*, 322–330. https://doi.org/10.1016/j.surg.2014.09.015.
29. Sica, G.S., Fiorani, C., Stolfi, C., Monteleone, G., Candi, E., Amelio, I., Catani, V., Sibio, S., Divizia, A., Tema, G., et al. (2015). Peritoneal expression of Matrilysin helps identify early post-operative recurrence of colorectal cancer. Oncotarget *6*, 13402–13415. https://doi.org/10.18632/oncotarget.2830.
30. Jung, R., Krüger, W., Hosch, S., Holweg, M., Kröger, N., Gutensohn, K., Wagener, C., Neumaier, M., and Zander, A.R. (1998). Specificity of reverse transcriptase polymerase chain reaction assays designed for the detection of circulating cancer cells is influenced by cytokines in vivo and in vitro. Br. J. Cancer *78*, 1194–1198. https://doi.org/10.1038/bjc.1998.653.
31. Uras, C., Altinkaya, E., Yardimci, H., Göksel, S., Yavuz, N., Kaptanoğlu, L., and Akçal, T. (1996). Peritoneal cytology in the determination of free tumour cells within the abdomen in colon cancer. Surg. Oncol. *5*, 259–263. https://doi.org/10.1016/s0960-7404(96)80030-2.
32. Bridges, M.C., Daulagala, A.C., and Kourtidis, A. (2021). LNCcation: lncRNA localization and function. J. Cell Biol. *220*, e202009045. https://doi.org/10.1083/jcb.202009045.
33. Han, D., Wang, M., Ma, N., Xu, Y., Jiang, Y., and Gao, X. (2015). Long noncoding RNAs: novel players in colorectal cancer. Cancer Lett. *361*, 13–21. https://doi.org/10.1016/j.canlet.2015.03.002.
34. Chen, L., He, M., Zhang, M., Sun, Q., Zeng, S., Zhao, H., Yang, H., Liu, M., Ren, S., Meng, X., and Xu, H. (2021). The Role of non-coding RNAs in colorectal cancer, with a focus on its autophagy. Pharmacol. Ther. *226*, 107868. https://doi.org/10.1016/j.pharmthera.2021.107868.
35. Tang, J., Yan, T., Bao, Y., Shen, C., Yu, C., Zhu, X., Tian, X., Guo, F., Liang, Q., Liu, Q., et al. (2019). LncRNA GLCC1 promotes colorectal carcinogenesis and glucose metabolism by stabilizing c-Myc. Nat. Commun. *10*, 3499. https://doi.org/10.1038/s41467-019-11447-8.
36. Chen, S., Su, T., Zhang, Y., Lee, A., He, J., Ge, Q., Wang, L., Si, J., Zhuo, W., and Wang, L. (2020). Fusobacterium nucleatum promotes colorectal cancer metastasis by modulating KRT7-AS/KRT7. Gut Microb. *11*, 511–525. https://doi.org/10.1080/19490976.2019.1695494.
37. Wu, Y., Yang, L., Zhao, J., Li, C., Nie, J., Liu, F., Zhuo, C., Zheng, Y., Li, B., Wang, Z., and Xu, Y. (2015). Nuclear-enriched abundant transcript 1 as a diagnostic and prognostic biomarker in colorectal cancer. Mol. Cancer *14*, 191. https://doi.org/10.1186/s12943-015-0455-5.
38. Guo, X., Lv, X., Ru, Y., Zhou, F., Wang, N., Xi, H., Zhang, K., Li, J., Chang, R., Xie, T., et al. (2020). Circulating Exosomal Gastric Cancer-Associated Long Noncoding RNA1 as a Biomarker for Early Detection and Monitoring Progression of Gastric Cancer: A Multiphase Study. JAMA Surg. *155*, 572–579. https://doi.org/10.1001/jamasurg.2020.1133.
39. Xu, M., Chen, X., Lin, K., Zeng, K., Liu, X., Pan, B., Xu, X., Xu, T., Hu, X., Sun, L., et al. (2018). The long noncoding RNA SNHG1 regulates colorectal cancer cell growth through interactions with EZH2 and miR-154-5p. Mol. Cancer *17*, 141. https://doi.org/10.1186/s12943-018-0894-x.
40. Ma, Y., Yang, Y., Wang, F., Moyer, M.P., Wei, Q., Zhang, P., Yang, Z., Liu, W., Zhang, H., Chen, N., et al. (2016). Long non-coding RNA CCAL regulates colorectal cancer progression by activating Wnt/β-catenin signalling pathway via suppression of activator protein 2α. Gut *65*, 1494–1504. https://doi.org/10.1136/gutjnl-2014-308392.
41. Xu, M., Chen, X., Lin, K., Zeng, K., Liu, X., Xu, X., Pan, B., Xu, T., Sun, L., He, B., et al. (2019). lncRNA SNHG6 regulates EZH2 expression by sponging miR-26a/b and miR-214 in colorectal cancer. J. Hematol. Oncol. *12*, 3. https://doi.org/10.1186/s13045-018-0690-5.
42. Qi, H., Wang, J., Wang, F., and Ma, H. (2017). Long non-coding RNA SNHG1 promotes cell proliferation and tumorigenesis in colorectal cancer *via* Wnt/β-catenin signaling. Pharmazie *72*, 395–401. https://doi.org/10.1691/ph.2017.7463.
43. Zhu, Y., Li, B., Liu, Z., Jiang, L., Wang, G., Lv, M., and Li, D. (2017). Up-regulation of lncRNA SNHG1 indicates poor prognosis and promotes cell proliferation and metastasis of colorectal cancer by activation of the Wnt/β-catenin signaling pathway. Oncotarget *8*, 111715–111727. https://doi.org/10.18632/oncotarget.22903.
44. Bai, J., Xu, J., Zhao, J., and Zhang, R. (2020). lncRNA SNHG1 cooperated with miR-497/miR-195-5p to modify epithelial-mesenchymal transition underlying colorectal cancer exacerbation. J. Cell. Physiol. *235*, 1453–1468. https://doi.org/10.1002/jcp.29065.
45. Avazpour, N., Hajjari, M., Kazemi Nezhad, S.R., and Tahmasebi Birgani, M. (2020). SNHG1 Long Noncoding RNA is Potentially Up-Regulated in Colorectal Adenocarcinoma. Asian Pac. J. Cancer Prev. *21*, 897–901. https://doi.org/10.31557/APJCP.2020.21.4.897.
46. Tian, T., Qiu, R., and Qiu, X. (2018). SNHG1 promotes cell proliferation by acting as a sponge of miR-145 in colorectal cancer. Oncotarget *9*, 2128–2139. https://doi.org/10.18632/oncotarget.23255.
47. Yang, H., Wang, S., Kang, Y.-J., Wang, C., Xu, Y., Zhang, Y., and Jiang, Z. (2018). Long non-coding RNA SNHG1 predicts a poor prognosis and promotes colon cancer tumorigenesis. Oncol. Rep. *40*, 261–271. https://doi.org/10.3892/or.2018.6412.
48. Fu, Y., Yin, Y., Peng, S., Yang, G., Yu, Y., Guo, C., Qin, Y., Zhang, X., Xu, W., and Qin, Y. (2019). Small nucleolar RNA host gene 1 promotes development and progression of colorectal cancer through negative regulation of miR-137. Mol. Carcinog. *58*, 2104–2117. https://doi.org/10.1002/mc.23101.
49. Zhao, Y., Qin, Z.S., Feng, Y., Tang, X.J., Zhang, T., and Yang, L. (2018). Long non-coding RNA (lncRNA) small nucleolar RNA host gene 1 (SNHG1) promote cell proliferation in colorectal cancer by affecting P53. Eur. Rev. Med. Pharmacol. Sci. *22*, 976–984. https://doi.org/10.26355/eurrev_201802_14379.
50. Huang, Q., Yang, Z., Tian, J.H., You, P.D., Wang, J., Ma, R., Yu, J., Zhang, X., Cao, J., Cao, J., and Wang, L.B. (2022). LncSNHG1 Promoted CRC Proliferation through the miR-181b-5p/SMAD2 Axis. J. Oncol. *2022*, 4181730. https://doi.org/10.1155/2022/4181730.
51. de Boer, N.L., Rovers, K., Burger, J.W.A., Madsen, E.V.E., Brandt-Kerkhof, A.R.M., Kok, N.F.M., de Wilt, J.H.W., de Reuver, P.H., Bos, A., de Hingh, I.H.J.T., and Verhoef, C. (2020). A population-based study on the prognostic impact of primary tumor sidedness in patients with peritoneal metastases from colon cancer. Cancer Med. *9*, 5851–5859. https://doi.org/10.1002/cam4.3243.
52. Rekhraj, S., Aziz, O., Prabhudesai, S., Zacharakis, E., Mohr, F., Athanasiou, T., Darzi, A., and Ziprin, P. (2008). Can intra-operative intraperitoneal free cancer cell detection techniques identify patients at higher recurrence risk following curative colorectal cancer resection: a meta-analysis. Ann. Surg Oncol. *15*, 60–68. https://doi.org/10.1245/s10434-007-9591-5.
53. Guller, U., Zajac, P., Schnider, A., Bösch, B., Vorburger, S., Zuber, M., Spagnoli, G.C., Oertli, D., Maurer, R., Metzger, U., et al. (2002). Disseminated single tumor cells as detected by real-time quantitative polymerase chain reaction represent a prognostic factor in patients undergoing surgery for colorectal cancer. Ann. Surg. *236*, 768–776. https://doi.org/10.1097/00000658-200212000-00009.
54. Amin, M.B., Greene, F.L., Edge, S.B., Compton, C.C., Gershenwald, J.E., Brookland, R.K., Meyer, L., Gress, D.M., Byrd, D.R., and Winchester, D.P. (2017). The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. CA. Cancer J. Clin. *67*, 93–99. https://doi.org/10.3322/caac.21388.
55. Thomson, W.H.F., Foy, C.J.W., and Longman, R.J. (2008). The nature of local recurrence after colorectal cancer resection. Colorectal Dis. *10*, 69–74. https://doi.org/10.1111/j.1463-1318.2007.01223.x.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Human abdominal lavage samples	Tongji Hospital, Huazhong University of Science and Technology	See method details
Critical commercial assays		
TRIzol	Invitrogen	Cat# 15596026CN
cDNA synthesis mix	Takara	Cat# 6215A
TB GreenPremix Ex Taq II FAST qPCR	Takara	Cat# CN830S
Experimental models: Cell lines		
SW480 Cell line	CCTCC	GDC0306
SW620 Cell line	ATCC	CCL-227
NCM460 Cell line	INCELL	CVCL0460
Software and algorithms		
R, v4.3.1	The R Foundation	https://www.r-project.org/
SPSS	IBM	IBM SPSS Statistics

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Xiangshang Xu (xsxu@tjh.tjmu.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Study participants

All patients were extensively informed and gave written consent for the investigations. The study was approved by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China) (TJ-IRB20230215). The clinical study was registered at Chinese Clinical Trial Registry (ChiCTR2400080490). 91 CRC patients (Table S2) who underwent laparoscopic operations between March 2023 and August 2023 in the Department of Gastrointestinal Surgery of Tongji Hospital were investigated. Patients with previous colorectal abdominal operations or other malignant diseases were excluded from the study. Patients enrolled in the study had not received any preoperative chemotherapy or radiotherapy. For comparative purposes, a control group consisting of 26 patients (Table S3) diagnosed with benign diseases was also included in the study.

METHOD DETAILS

Samples collection

Following laparoscopic exploration, abdominal lavage was conducted. 200 mL of saline solution was instilled into the abdominal cavity at the tumor site, and a minimum of 150 mL was subsequently aspirated for further analysis. All samples of the irrigation fluid were promptly stored in an ice box to maintain their integrity and then immediately transported to the laboratory for subsequent processing and analysis.

Cytology

After performing centrifugal enrichment on the cells present in the abdominal washings, a 0.1 mL aliquot was extracted for cytological examination. This sample was independently evaluated by two experienced pathologists. A positive cytology result was determined based on the observation of one or more anisotropic cells within the field of view during microscopic examination. (Figure S2).

RNA extraction and cDNA synthesis

Each peritoneal wash sample was centrifuged at 1,000 rpm for 5 min, and total RNA was extracted using the TRIzol method (Invitrogen, Carlsbad, CA) according to the manufacturer's procedure. Total RNA samples were stored at 80°C. After denaturation in DEPC-treated water at 70°C for 10 min, 1 μg of total RNA was used for cDNA synthesis using a cDNA synthesis mix (Takara, Tokyo).

Real-Time PCR primer design

Gene sequences were acquired from the NCBI database. Oligonucleotide primers for SNHG1, CCAL, SNHG6, CEA target genes and β-actin housekeeping gene were chosen with the assistance of the NCBI primer blast program. The primer sequences used throughout this study are described in Table S1. Oligonucleotide primers were purchased from Invitrogen.

PCR amplification

The QuantStudio 6&7 Real-Time PCR System (Appliedbiosystems, USA) performed RT-PCR with optimized PCR conditions. 1 μL of diluted template cDNA was mixed with TB Green Supermix 2 (Takara, Tokyo), each forward and reverse primer to a final reaction volume of 10 μL. The amplification reaction was carried out in a 384-well plate adapted to the instrument. All assays were repeated in three wells to avoid chance error. β-actin was introduced as an internal control to standardize the quantification of each target gene.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data analyses

Real-time quantitation was performed by using SYBR Green dye as the fluorescent signal, with the help of the QuantStudio 6&7 Real-Time PCR System software version 1.3 (Appliedbiosystems, USA), according to the manufacturer's manual. Quantitative values are obtained from the *Ct* number at which the increase in signal is associated with the exponential growth of PCR products. Target genes (*lncRNAs, CEA* mRNA) amplification was compared with the *β-actin* gene, and each sample was normalized based on its *β-actin* content. The target genes SNHG1 and CEA were tested for relative expression of cancer cell lines from colon cancer cell lines (SW480, SW620). A normal human colonic cell line (NCM460) was used as a negative control.

For data analysis, ROC curves were used to compare the diagnostic accuracies of lncRNA/β-actin, CEA/β-actin ratios. The determination of the optimal cut-off value was achieved by plotting sensitivity and specificity pairs for these RNA ratios. The clinical utility of the detection method was evaluated by examining the diagnostic data from 22 patients with positive cytology (Figure S2) and 26 patients with benign conditions.

Statistics

A cross-tabulation analysis of RT-PCR analysis was performed using the chi-square test for trend or Fisher's exact test. The Wilcoxon test was chosen for paired information where the distribution is not positively distributed. A *p*-value of 0.05 was considered statistically significant. By using DTAXG package in *R* language, the sensitivity and specificity are calculated by Bayesian parameter estimation. All data analysis and statistical analyses were done by Yujie Zhang, M.S. in Statistics, the software used in this study is SPSS 18.0.