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Expression of Long Non-Coding RNAs HNF1A-AS1 and MCM3AP-AS1 in Colorectal Cancer Patients and their Association with Chemotherapy

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ABSTRACT

Background & Objective: Colorectal cancer (CRC) is a common and multifactorial malignancy influenced by both genetic and environmental factors. Long non-coding RNAs (lncRNAs) have emerged as critical regulators of tumor initiation and progression. Previous studies have linked HNF1A-AS1 and MCM3AP-AS1 expression to clinicopathological features in several cancers. This case-control study aimed to evaluate the expression levels of these lncRNAs in CRC tissues before and after chemotherapy with capecitabine and celecoxib, compared with non-cancerous control tissues.

Materials & Methods: Tissue samples were collected from 98 CRC patients before and after chemotherapy and from 71 non-cancerous controls obtained during colonoscopy or surgery. Total RNA was extracted, reverse transcribed into complementary DNA (cDNA), and gene expression levels were quantified using real-time quantitative PCR (qRT-PCR).

Results: Both HNF1A-AS1 and MCM3AP-AS1 were significantly upregulated in CRC tissues before treatment compared with controls ($p = 0.0051$ and $p = 0.0393$, respectively). However, no significant differences in expression were observed between pre- and post-treatment samples ($p > 0.05$). ROC curve analysis revealed weak diagnostic performance for each lncRNA individually, whereas their combined analysis showed modest diagnostic value (AUC = 0.66). Chemotherapy was associated with an improvement in mean patient survival of approximately 16 months.

Conclusion: Chemotherapy with capecitabine and celecoxib did not significantly alter the expression of *HNF1A-AS1* or *MCM3AP-AS1* in CRC patients, despite improved survival outcomes. The combined assessment of these lncRNAs may have limited diagnostic utility; they do not appear to be suitable biomarkers for monitoring chemotherapy response.

Keywords: *Colorectal cancer, lncRNA MCM3AP-AS1, lncRNA HNF1A-AS1, chemotherapy*

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1. Introduction

According to global cancer statistics, colorectal cancer (CRC) is among the most frequently diagnosed malignancies of the gastrointestinal tract, ranking third in incidence and second in mortality worldwide (1). Patients with CRC rarely exhibit overt symptoms during early stages, but as the tumor progresses, they may have symptoms such as abdominal pain and hematochezia. There is a significant relationship between tumor development and the prognosis of patients, and this relationship directly affects treatment options and outcomes. Therefore, the diagnosis of CRC at the earliest possible period for treatment is very important (2, 3). Both non-modifiable and modifiable risk factors contribute to the development of CRC .Individual medical history, including factors such as gender, age, race, past adenomatous polyps, history of inflammatory bowel disease (IBD), and family history, falls into the category of non-modifiable risk factors. On the other hand, modifiable factors pertain to individual choices and behaviors that can be altered or controlled (4). By adjusting habits or lifestyle, individuals can lower their risk of CRC. The development of CRC involves four stages: initiation, promotion, progression, and metastasis. The liver is the most frequent site of metastasis, followed by the lung and bone. While it is challenging to determine the specific duration of each stage, several decades are likely necessary for the formation of CRC (5, 6). CRC advances as regional adenomas in the intestinal

epithelium transform into malignant tumors that have the potential to metastasize to the liver. Presently, chemotherapy is considered the preferred treatment approach for CRC. Among chemotherapy drugs, it is a reliable and successful treatment option, along with molecular targeting. However, colon cancer still poses a significant clinical challenge due to its asymptomatic progression, early-stage metastasis, and unfavorable prognosis (7). Chemotherapy regimens for CRC patients consist of various drugs, including fluorouracil (5-FU) combined with leucovorin (folinic acid), capecitabine, irinotecan, oxaliplatin, and trifluridine plus tipiracil. Typically, a combination of two or more of these drugs is administered to enhance the treatment's clinical effectiveness. Chemotherapy plays a crucial role in improving the quality of life and extending survival for individuals with advanced CRC. However, it is not without limitations. Patients receiving chemotherapy may experience various adverse effects, including hair loss, bone marrow suppression, nausea, vomiting, diarrhea, heightened susceptibility to infections, and neuropathy. Moreover, the emergence of multidrug resistance (MDR) presents a notable obstacle as it can result in cancer relapse and the spread of the disease. Drug resistance is responsible for treatment ineffectiveness in approximately 90% of individuals with metastatic cancer (8, 9). Among chemotherapy drugs, it is a reliable and successful treatment option for both breast cancer and CRC based on clinical trials. For individuals diagnosed with metastatic colorectal cancer (mCRC) and as a primary monotherapy for 30% of metastatic breast cancer cases, the response rate (RR) ranges from 25% to 27%. Celecoxib, a nonsteroidal anti-inflammatory drug (NSAID), exhibits higher selectivity in inhibiting COX-2 compared to COX-1. In the context of CRC, one of the roles of celecoxib is to impede the translocation of β-catenin to the nucleus, thereby preventing the activation of transcriptional activating factors (10). Despite their non-coding potential, lncRNAs play a role in many diseases, including cancer (11). Increasing evidence suggests that non-coding RNAs (ncRNAs) have significant regulatory functions in diverse biological processes, particularly in cancer development. Recent research has highlighted the involvement of multiple lncRNAs in modulating the onset and advancement of human CRC. These lncRNAs are crucial for the proliferation and migration of CRC cells, and their presence is linked to an unfavorable prognosis and the formation of colon tumors. By regulating various signaling pathways, these lncRNAs exert their influence on CRC progression and carcinogenesis (12). An increasing body of evidence indicates that lncRNAs play a significant role in the progression of CRC. These lncRNAs can epigenetically and transcriptionally regulate gene expression (13). Altered expression of certain lncRNAs has been identified in the tumor formation, development, and resistance to cancer therapies. These lncRNAs can function as oncogenes or tumor suppressors and have the potential to serve as diagnostic or prognostic markers (14). Additionally, they hold promise as therapeutic targets in cancer treatment .HNF1A antisense RNA 1 (HNF1A-AS1) is a lncRNA that originates from the antisense strand of the HNF1A gene cluster. It is a single-exon gene located on chromosome 12q24.31 and spans a length of 2.455 kb (15). Moreover, abnormal expression of HNF1A-AS1 is associated with various cancers (16). HNF1A-AS1 plays a crucial role in governing various biological processes, thereby serving as a significant regulator of cancer development. As a result of these attributes, HNF1A-AS1 is increasingly acknowledged as a promising biomarker for the diagnosis and prognosis of different cancers. Furthermore, it is considered an appealing target for therapeutic interventions (16). HNF1A-AS1, a long non-coding RNA (lncRNA) in CRC, acts as a prognostic indicator for a negative outcome and plays a role in cancer progression by activating the Wnt/β-catenin signaling pathway. HNF1A-AS1 is a bidirectional lncRNA associated with various tumor-related factors such as overall survival (OS), lymph node metastasis, and tumor stage (17). HNF1A-AS1 is additionally engaged in controlling diverse cellular processes in cancer cells through multiple mechanisms. These mechanisms encompass cell proliferation, apoptosis, invasion, migration, glycolysis, cellular resistance to chemotherapy, as well as resistance to radiation therapy (16). The lncRNA MCM3AP-AS1, which is the opposite-strand RNA of the MCM3AP protein-coding gene, is recognized as an oncogenic lncRNA. It is situated on chromosome 21. Multiple studies have provided evidence that MCM3AP-AS1 is expressed in different types of cancer and exerts diverse regulatory roles by influencing various downstream targets (18). MCM3AP-AS1 shows abnormal expression patterns in human cancers and exhibits a strong correlation with various clinical features, including tumor size, tumor-node-metastasis (TNM) stage, and pathological grade. Moreover, it exerts a significant influence on tumor progression by regulating various cellular functions. MCM3AP-AS1 is considered a promising biomarker for cancer diagnosis, prognostic evaluation, and treatment. It is predominantly localized in the chromatin and nucleoplasm within the cell (19).While the roles of HNF1A-AS1 and MCM3AP-AS1 in CRC progression—including cell proliferation, invasion, migration, and association with poor prognosis—are well established, their dynamic expression in response to chemotherapy and their potential as biomarkers for treatment response remain unclear. This case-control study aims to fill this gap by examining the expression levels of HNF1A-AS1 and MCM3AP-AS1 in CRC tissues before and after chemotherapy with capecitabine and celecoxib, compared to healthy controls, and by evaluating their diagnostic value.

2. Materials and Methods

2.1 Study Design

This study utilized a case-control design to investigate the regulatory roles of lncRNAs *HNF1A-AS1* and *MCM3AP-AS1* in CRC progression. Key elements of the design included sample collection, RNA extraction, cDNA synthesis, and real-time PCR analysis.

2.2 Setting

The study was conducted at the research center of Tehran University of Medical Sciences. Sample collection took place from October 2022 to March 2023. Recruitment, exposure, follow-up, and data collection were all completed within this period.

2.3 Participants

The study included 98 patients diagnosed with colon cancer and 71 individuals who were suspected of having colon cancer but whose pathology results were normal.

2.4 Sources and Methods of Case Ascertainment and Control Selection

Patients were selected on the based on their diagnosis and pathology results, and samples were collected during surgical procedures or colonoscopies. The rationale for selecting these cases and controls was to compare lncRNA expression levels between cancerous and normal tissues.

2.5 Matching Criteria

This study did not employ matched controls.

2.6 Variables

The primary outcomes were the expression levels of *HNF1A-AS1* and *MCM3AP-AS1*. The main predictor variables included the presence of CRC and chemotherapy treatment status. Potential confounders included patient demographics and clinical characteristics. Diagnostic criteria were based on pathology results confirming CRC.

2.7 Data Sources/Measurement

RNA was extracted from tissue samples using the TRIzol reagent method. The samples were then processed for cDNA synthesis and real-time PCR analysis. These methods ensured the comparability of assessment across patient groups.

2.8 Bias

To minimize bias, all samples were processed under identical conditions, and patient consent ensured voluntary participation. The use of RNase-free microtubes and stringent protocols for RNA extraction and cDNA synthesis further reduced technical variability.

2.9 Study Size

The sample size was calculated using G*Power 3.1 software to detect a moderate effect size (Cohen's $d = 0.5$) in lncRNA expression differences between groups, with $\alpha = 0.05$ and power = 0.80. This required a minimum of approximately 64 participants per group for unpaired comparisons.

The final enrollment of 98 CRC patients (providing paired pre- and post-treatment samples) and 71 controls exceeded this threshold, ensuring sufficient statistical power while considering patient availability during the collection period.

2.10 Quantitative Variables

Expression levels of the lncRNAs were quantified by real-time PCR. Expression levels were compared between CRC patients and controls, as well as before and after chemotherapy.

2.11 Collection of samples from patients

All patient specimens were collected between October 2022 and March 2023, in compliance with ethical principles and with the full consent of the patients. Sampling took place at the research center of Tehran University of Medical Sciences, where a total of 98 patients diagnosed with colon cancer and 71 individuals who were suspected of having colon cancer but whose pathology results were normal were enrolled. The surgeon collected samples from these patients during either surgical procedures or colonoscopies. Patient samples were obtained both before and after chemotherapy. These samples were then transferred into RNase-free microtubes and stored at −80 °C until extraction.

2.12 Total RNA extraction from tissue samples

Total RNA extraction was performed in a temperature-controlled, RNase-free room, and the environment was sterilized with 70% alcohol. The extraction was carried out using TRIzol reagent according to the manufacturer's instructions. Initially, the tissue was crushed, and 500 µl of TRIzol was added. The sample was then homogenized, and an additional 500 µl of TRIzol was added. The mixture was vortexed for 30 seconds and incubated at room temperature for 5 minutes. Next, 250 µl of chloroform was added, and the mixture was incubated for a further 5 minutes. After that, centrifugation was performed for 15 minutes, which resulted in the formation of three distinct phases. The aqueous (upper) phase was transferred to a microtube, and 350 µl of isopropanol was added, followed by vortexing, a 5-minute incubation, and centrifugation for 10 minutes. Subsequently, 1 ml of 70% ethanol was added, and the sample was centrifuged for 5 minutes. Finally, the RNA was dissolved in DEPC-treated water.

2.13 Synthesis of cDNA

Initially, 100 ng of RNA was obtained and measured using a Nanodrop device. The RNA was then transferred to a microtube that was free of RNase. Before the synthesis process, a treatment step was performed to eliminate impurities using DNase. To achieve this, 1 µl of DNase and 1 µl of DNase buffer were added to the microtube containing the RNA. The contents were gently mixed and incubated for 10 minutes at 37 degrees Celsius (with a lid temperature of 95 degrees Celsius in the RT machine). Subsequently, 1 µl of EDTA was added, and the mixture was incubated for an additional 10 minutes at 60 degrees Celsius. In the third step, 1 µl of oligo(dT), 1 µl of Random Hexamer primer, 1 µl of dNTP, 1 µl of Reverse Transcriptase enzyme, along with 3 µl of enzyme buffer and DEPC water, were added to reach a total volume of 15 µl. The mixture was subjected to two incubation stages: 50 degrees Celsius for 60 minutes and 72 degrees Celsius for 10 minutes. Finally, cDNA was synthesized.

2.14 Primer Design

The NCBI database was used for primer design, and BLAST was used to verify primer specificity. After final confirmation, the primers were ordered for synthesis from Sinaclon. Primer information is presented in Table 1.

Table 1. Real-time PCR primers information.

Gene	Primer sequence	Length(bp)	GC (%)	Tm (°C)
lncRNA MCM3AP-AS1 Forward	GCTCCTCTTCCAGTTACGGT	20	55	58.60
lncRNA MCM3AP-AS1 Reverse	GCGAGGCGAATTTTCTGAGTCT	22	50	59.40
LncRNA HNF1A-AS1 Forward	GAGTGTCCCTTCAGCCAGTC	20	60	61.40
LncRNA HNF1A-AS1 Reverse	TGGGAAGGAGAACAGTCCGA	20	55	59.35
β-actin Forward	AGAGCTACGAGCTGCCTGAC	20	60	61.40
β-actin Reverse	AGCACTGTGTTGGCGTACAG	20	55	59.35

2.15 Real-time PCR reaction

The reaction mixture (final volume 15 µL) consisted of 7.5 µL SYBR Green Master Mix, 0.3 µL each of forward and reverse primers (10 µM), 1 µL cDNA template, and sterile water to volume. All qRT-PCR reactions were performed in technical duplicates to ensure reliability and enable calculation of robust mean Ct values with standard deviations. No-template controls were included in each run, and melting curve analysis was performed to confirm amplification specificity and exclude primer dimers or contamination.

2.16 Thermal Cycling Conditions for Real-Time PCR

The amplification conditions for replicating the lncRNAs *HNF1A-AS1* and *MCM3AP-AS1* genes were as follows: an initial denaturation step at 95°C for 10 minutes, followed by 40 cycles, each cycle consisted of denaturation at 95°C for 30 seconds, annealing at 59°C for 30 seconds, and extension at 72°C for 30 seconds. A final elongation step at 72°C for 5 minutes was performed. To ensure accurate normalization of gene expression data, β-actin was employed as the reference housekeeping gene.

2.17 Statistical analysis

Expression data were analyzed using SPSS version 27 and GraphPad Prism 9 to compare lncRNA levels before and after chemotherapy in CRC patients and controls.

3. Result

3.1 Participants

Out of 200 individuals initially considered for the study, 169 were confirmed eligible. Specifically, 98 patients diagnosed with colon cancer and 71 individuals with normal pathology results were enrolled. All 169 participants completed the follow-up and were included in the analysis.

3.2 Reasons for Non-Participation

31 individuals were excluded due to either withdrawal of consent or incomplete data collection.

3.3 Flow Diagram

A flow diagram illustrating participant recruitment, eligibility screening, inclusion, follow-up, and analysis stages would be beneficial but is not provided in this text.

3.4 Descriptive Data

Demographic and clinical characteristics of the participants are summarized in Table 2.

The study included 98 CRC patients (mean age: 62.4 years, 54% male) and 71 controls (mean age: 60.1 years, 49% male). Exposure data included chemotherapy status, and potential confounders were patient age, sex, and stage of cancer.

3.5 Missing Data

There were no missing data for any variables of interest, as all enrolled participants completed the study.

3.6 Outcome Data

Expression levels of lncRNAs *HNF1A-AS1* and *MCM3AP-AS1* were measured in all samples.

Summary measures of exposure (mean expression levels) are provided in Table 2.

Table 2. Association between clinicopathological characteristics and relative expression levels of lncRNAs HNF1A-AS1 and MCM3AP-AS1 in colorectal cancer patients.

Characteristics	Number of patients	HNF1A-AS1 (Pre-treatment) Mean ± SEM	HNF1A-AS1 (Post-treatment) Mean ± SEM	p-value †	MCM3AP-AS1 (Pre-treatment) Mean ± SEM	MCM3AP-AS1 (Post-treatment) Mean ± SEM	† p-value
Age 55 =55>	 32 66	0.123 0.121	 0.111 0.095	0.407	0.139 0.148	0.137 0.123	0.957
Gender Men Women	 54 44	 0.124 0.119	 0.102 0.097	0.482	 0.134 0.158	 0.142 0.110	0.886
Tumor site Rectum Colon	 50 48	 0.126 0.117	 0.111 0.088	0.509	 0.156 0.134	 0.125 0.130	0.606
Tumor histology Adenocarcinoma Mucinous adenocarcinoma	 57 41	 0.113 0.133	 0.104 0.094	0437	 0.137 0.156	 0.113 0.147	0.903
Tumor differentiation Well/moderate Poor	 58 40	 0.141 0.093	 0.098 0.103	**0.030**	 0.148 0.141	 0.142 0.107	0.525
TNM stage (I,II) (III,IV)	 52 46	 0.131 0.112	 0.107 0.092	0.983	 0.143 0.148	 0.127 0.128	0.368
Lymph node metastasis Yes No	 39 59	 0.139 0.110	 0.111 0.092	0.959	 0.111 0.167	 0.119 0.133	0.191
Helicobacter pylori Yes No	 44 54	 0.104 0.136	 0.107 0.094	0.335	 0.145 0.145	 0.110 0.141	0.783

Note: p-values represent comparisons of lncRNA expression between clinical subgroups (e.g., male vs. female, early vs. advanced stage). These comparisons were performed separately for pre-treatment and post-treatment samples.
Values are presented as mean ± SEM of relative gene expression calculated using the $2^{-\Delta Ct}$ method, normalized to β-actin. †p-values indicate comparisons of lncRNA expression between subgroups within each clinicopathological category (e.g., male vs. female, stage I–II vs. III–IV), assessed using independent-samples statistical tests. p-value < 0.05 was considered statistically significant.

3.7 Main Results

Comparing the tumor tissue of patients before treatment with the control group, Figure 1(A) demonstrates a significant difference in the expression level of the lncRNA HNF1A-AS1 gene (p-value: 0.0051). Similarly, Figure 1(B) shows a significant difference in the expression level of the lncRNA MCM3AP-AS1 gene between the two groups (p-value: 0.0393). Comparing patients after treatment to the control group, Figure 1(C) indicates that there is no significant difference in the expression level of the lncRNA HNF1A-AS1 gene (p-value: 0.0934). Additionally, Figure 1(D) reveals that there is no significant difference in the expression level of the lncRNA MCM3AP-AS1 gene between the two groups (p-value: 0.2012). When comparing patients before and after treatment, Figure 1(E) shows no significant difference in the expression level of the lncRNA HNF1A-AS1 gene (p-value: 0.1907). Similarly, Figure 1(F) indicates that there is no significant difference in the expression level of the lncRNA MCM3AP-AS1 gene between the two groups (p-value: 0.2940).

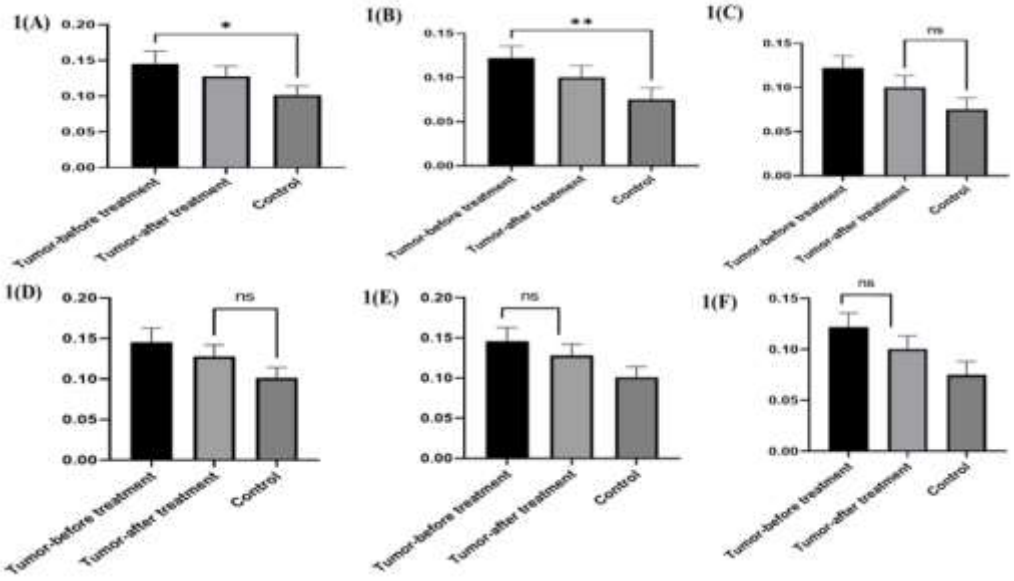

Figure 1. A. The level of lncRNAHNF1A-AS1 gene expression in the tumor tissue of patients before treatment with control people (Mean, SEM). **: significant at the probability level of 0.0051. B. The level of lncRNAMCM3AP-AS1 gene expression in the tumor tissue of patients before treatment with control people (Mean, SEM). **: significant at the probability level of 0.0393. C. The level of lncRNAHNF1A-AS1 gene expression in the tumor tissue of patients after treatment with control people (Mean, SEM). D. The level of lncRNAMCM3AP-AS1 gene expression in the tumor tissue of patients after treatment with control people (Mean, SEM). E. The level of lncRNAHNF1A-AS1 gene expression in the tumor tissue of patients before and after treatment (Mean, SEM). F. The level of lncRNAMCM3AP-AS1 gene expression in the tumor tissue of patients before and after treatment (Mean, SEM). (**Prepared by Authors, 2026**).

3.8 Category Boundaries for Continuous Variables

Expression levels were categorized into high and low based on median values for subgroup analyses.

3.9 Relative Risk Estimates

Relative risk estimates are not provided in this text.

3.10 ROC Curve Analysis

The ROC curve was used to check the diagnostic biomarker of lncRNA HNF1A-AS1 and lncRNAMCM3AP-AS1, and the larger the area under the curve, the higher the efficiency of the target gene as a biomarker. This chart is drawn based on sensitivity and specificity. According to Figure 2(A) drawn for lncRNA HNF1A-AS1, the area under the curve is 0.6258, SE: 0.04319, 95% CI, p-value: 0.0053. Therefore, they are considered weak diagnostic biomarkers in CRC, and according to Figure 2(B) drawn for lncRNA MCM3AP-AS1, the area under the curve is 0.5929, sensitivity 66.33, specificity 57.75, SE: 0.0431, 95%CI, p-value: 0.0053. Therefore, they are not considered diagnostic biomarkers in CRC. The prognostic potential of lncRNA HNF1A-AS1 and lncRNA MCM3AP-AS1 was evaluated using ROC curves. In Figure 2(C), the ROC curve for lncRNA HNF1A-AS1 had an area under the curve 0.5551, with a sensitivity of 41.84, specificity of 71.43, standard error (SE) of 0.04120, a 95% confidence interval (CI), and a p-value of 0.1828. Similarly, Figure 2(D) displayed the ROC curve for lncRNA MCM3AP-AS1, which exhibited an AUC of 0.5436, sensitivity of 55.10, specificity of 59.18, SE of 0.04130, 95% CI, and a p-value of 0.2913. Based on these findings, it can be concluded that lncRNA HNF1A-AS1 and lncRNA MCM3AP-AS1 do not serve as significant prognostic biomarkers in CRC. Figure 2(C) ROC curve drawn by GraphPad PRISM9 for lncRNA HNF1A-AS1 between patients before and after treatment. Figure 2(D) Using GraphPad PRISM9, a receiver operating characteristic (ROC) curve was generated to assess the performance of the lncRNA MCM3AP-AS1 as a prognostic biomarker. The ROC curve compared the expression levels of lncRNA MCM3AP-AS1 in patients before and after treatment. Relative expression levels were calculated using the $2^{-}\Delta Ct$ method, normalized to β-actin.

To explore potential diagnostic and prognostic biomarkers, the combined analysis of lncRNA HNF1A-AS1 and lncRNA MCM3AP-AS1 was conducted using the ROC curve. Figure 2(E) shows that the area under the graph was 0.66, with a sensitivity of 73.47, specificity of 52.11, standard error (SE) of 0.04253, 95% confidence interval (CI), and a p-value of 0.0002. These values indicate weak diagnostic biomarker potential in CRC. Similarly, Figure 2(F) demonstrates an AUC of 0.5814, sensitivity of 70.41, specificity of 43.88, SE of 0.04074, 95% CI, and a p-value of 0.0490. These findings suggest that lncRNA HNF1A-AS1 and lncRNA MCM3AP-AS1 are not considered prognostic biomarkers in CRC. Figure 2(E) ROC curve drawn by GraphPad PRISM9 for the combination of lncRNA HNF1A-AS1 and lncRNA MCM3AP-AS1 between patients before treatment and control people.

Figure 2 (F) ROC curve drawn by GraphPad PRISM9 for the combination of lncRNA HNF1A-AS1 and lncRNA MCM3AP-AS1 between patients before and after treatment.

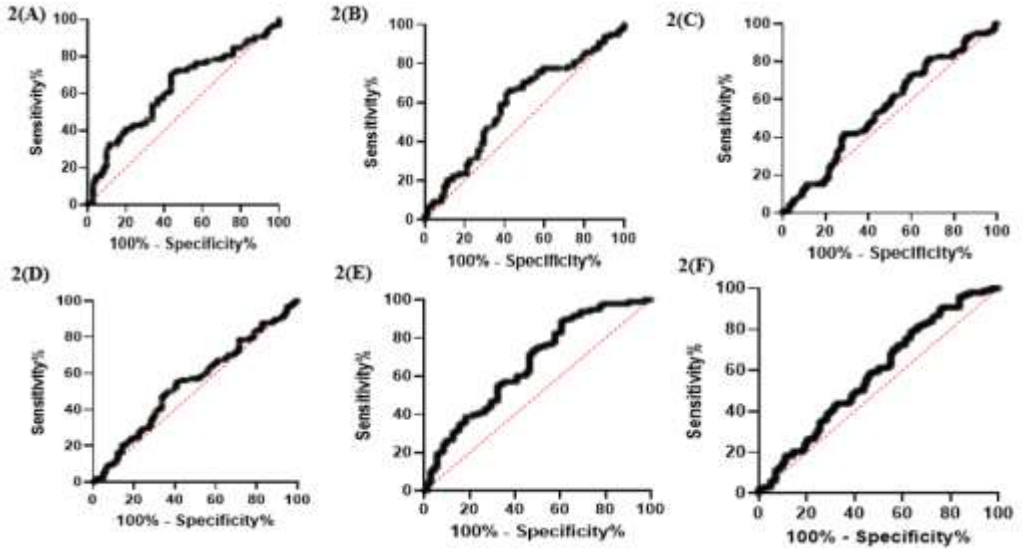

Figure 2. A. ROC curve drawn by GraphPad PRISM9 for lncRNA HNF1A-AS1 between patients before treatment and control people. AUC: 0.6258, Sensitivity: 70.41, Specificity: 56.34 SE:0.04319، 95%CI، P.value:0.0053. B. ROC curve drawn by GraphPad PRISM9 for lncRNA MCM3AP-AS1 between patients before treatment and control people. AUC :0.5929، Sensitivity: 66.33, Specificity :57.75 SE:0.04458، 95%CI، P.value:0.0395. C. ROC curve drawn by GraphPad PRISM9 for lncRNA HNF1A-AS1 between patients before treatment and after treatment. AUC:0.5551, Sensitivity: 41.84, Specificity :71.43 SE:0.04120, 95%CI ،P.value:0.1828. D. ROC curve drawn by GraphPad PRISM9 for lncRNA MCM3AP-AS1 between patients before treatment and after treatment. AUC:0.543, Sensitivity: 55.10, Specificity:59.18 SE:0.04130، 95%CI، P.value:0.2913. E. ROC curve for the combination of two biomarkers between pretreatment patients and control people. AUC: 0.66, Sensitivity: 73.47, Specificity: 52.11. F. ROC curve for the combination of two biomarkers between patients before and after treatment. AUC: 0.58, Sensitivity: 70.41, Specificity: 43.88. (**Prepared by Authors, 2026**).

3.11 The effect of chemotherapy on the survival of patients

The chemotherapy drug that the patients received was Xeloda or capecitabine at a dose of 825 mg/m2/bd and along with this chemotherapy, the drug celecoxib was also prescribed.The treatment response of the patients was CR, PR or NC, which was divided into two groups for CR analysis: group one and PR, NC of the second group and Kaplan-Meier was used for survival analysis. The results of the analysis showed that this treatment can increase the average survival by up to 16 months. As a result, this chemotherapy drug can improve the survival of patients by 16 months (Figure 3).

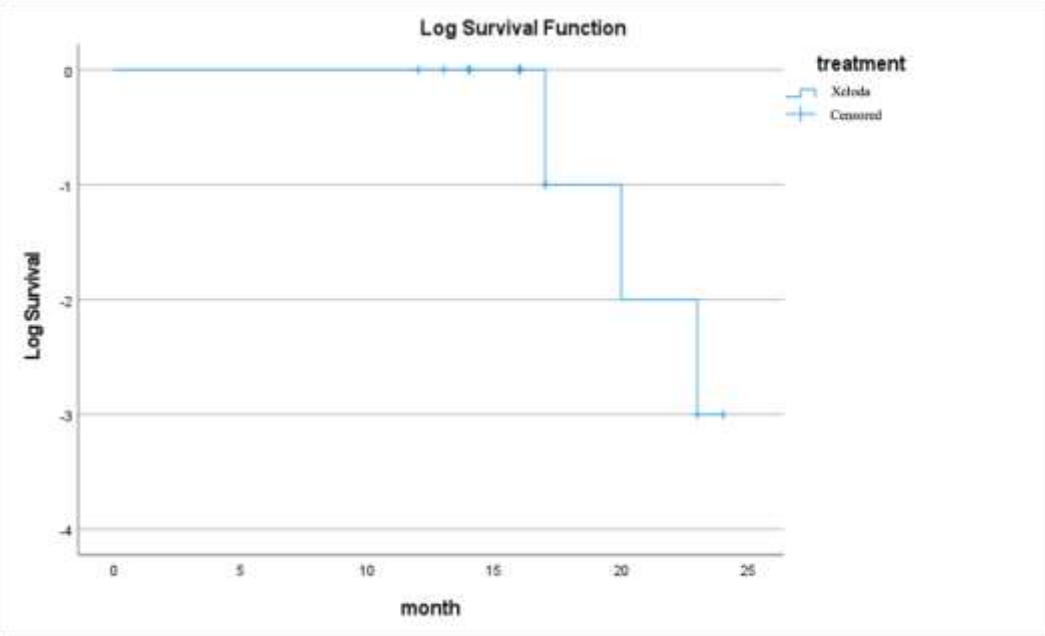

Figure 3. The impact of chemotherapy on the survival outcomes of patients. (**Prepared by Authors, 2026**).

4. Discussion

In the present study, we investigated the expression patterns of the lncRNAs *HNF1A-AS1* and *MCM3AP-AS1* in CRC tissues before and after chemotherapy with capecitabine and celecoxib, and compared them with non-cancerous control tissues. Our findings demonstrated that both lncRNAs were significantly upregulated in CRC tissues before treatment, whereas no significant changes in their expression were observed following chemotherapy. Despite the absence of post-treatment expression changes, chemotherapy was associated with a meaningful improvement in patient survival.

The observed overexpression of *HNF1A-AS1* in pre-treatment CRC tissues is consistent with a growing body of evidence supporting its oncogenic role in colorectal and other cancers. Multiple studies have reported elevated *HNF1A-AS1* expression in CRC tissues and cell lines, associating it with advanced TNM stage, lymph node metastasis, angiogenesis, and poor OS **(20, 21)**. Mechanistically, *HNF1A-AS1* has been shown to activate oncogenic pathways such as Wnt/β-catenin and ERK/MAPK signaling, thereby promoting tumor growth, invasion, and angiogenesis **(20, 22)**. Our findings reinforce these observations by demonstrating significantly higher HNF1A-AS1 expression in CRC tissues compared with controls.

Similarly, the elevated pre-treatment expression of *MCM3AP-AS1* observed in this study aligns with prior reports identifying this lncRNA as an oncogenic regulator in CRC and other malignancies. Previous studies have linked *MCM3AP-AS1* overexpression to increased tumor size, advanced stage, metastasis, and poor prognosis in patients with CRC **(23, 24)**. Functional studies suggest that *MCM3AP-AS1* contributes to tumor progression by regulating key molecular axes, including the *MCM3AP-AS1*/miR-19a-3p/FOXF2 pathway **(25)**. Our results support the clinical relevance of *MCM3AP-AS1* overexpression in CRC but suggest limited responsiveness to chemotherapy at the transcriptional level. An important finding of our study is the lack of significant change in *HNF1A-AS1* and *MCM3AP-AS1* expression following chemotherapy with capecitabine and celecoxib. This observation contrasts with some expectations that effective chemotherapy might downregulate oncogenic lncRNAs. However, similar findings have been reported for other lncRNAs, where expression levels remained stable despite clinical response, indicating that therapeutic benefit may occur through mechanisms independent of lncRNA transcriptional modulation **(14)**. It is also possible that these lncRNAs contribute to intrinsic tumor biology rather than directly mediating chemotherapy sensitivity, or that post-transcriptional regulation plays a more prominent role. The diagnostic performance of *HNF1A-AS1* and *MCM3AP-AS1* in our study was modest. Individually, both lncRNAs demonstrated weak diagnostic value (AUCs of 0.62 and 0.59, respectively), consistent with reports suggesting that single lncRNA biomarkers often lack sufficient sensitivity and specificity for clinical application **(12)**.

Notably, combining both lncRNAs improved diagnostic performance (AUC = 0.66),

supporting the concept that multi-marker panels may offer greater diagnostic accuracy than single biomarkers. Similar combinatorial approaches have been recommended in previous CRC biomarker studies **(13)**.

Despite their diagnostic potential, neither *HNF1A-AS1* nor *MCM3AP-AS1* demonstrated prognostic value for treatment response, as indicated by ROC analyses comparing pre- and post-treatment samples. This finding suggests that while these lncRNAs may be useful for distinguishing CRC tissue from non-cancerous tissue, they are not suitable markers for monitoring chemotherapy efficacy.

Importantly, chemotherapy significantly improved patient survival by approximately 16 months, indicating that clinical benefit can be achieved independently of changes in these lncRNA expression levels. Taken together, our findings suggest that HNF1A-AS1 and MCM3AP-AS1 play important roles in CRC tumor biology and may contribute to disease initiation and progression, but their expression appears relatively stable in response to capecitabine–celecoxib chemotherapy. This stability may reflect their involvement in fundamental oncogenic pathways rather than treatment-specific mechanisms. Future studies with larger cohorts, longitudinal sampling, and diverse chemotherapy regimens are needed to further clarify the temporal dynamics and clinical utility of these lncRNAs.

5. Conclusion

This study provides clinical evidence that HNF1A-AS1 and MCM3AP-AS1 are significantly upregulated in colorectal cancer tissues but remain transcriptionally stable following capecitabine–celecoxib chemotherapy. Although chemotherapy significantly improved patient survival, these lncRNAs did not demonstrate prognostic value for treatment response. Their combined analysis showed modest diagnostic potential, supporting their possible utility in CRC detection rather than treatment monitoring.

6. Declarations

6.1 Acknowledgments

Not Applicable

6.2 Ethical Considerations

The authors state that they have obtained appropriate institutional review board approval (no. 52/433991/2) and/or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, informed consent has been obtained from the participants involved in investigations involving human subjects.

6.3 Authors' Contributions

A.A., R.S. were involved in experimental assay, writing the article and data analysis. M.H., and E.A. were

involved in data collection. R.S. participated in the manuscript drafting and study design.

6.4 Conflict of Interest

Not applicable.

6.5 Fund or Financial Support

No funding was received for this manuscript.

6.6 Using Artificial Intelligence Tools (AI Tools)

The authors declare that no artificial intelligence (AI) tools or generative AI technologies were used in the writing, data analysis, or preparation of this manuscript.

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