Ind J Clin Biochem (July-Sept 2023) 38(3):331–337
https://doi.org/10.1007/s12291-022-01052-w

ORIGINAL RESEARCH ARTICLE

Impact of rs2107425 Polymorphism and Expression of lncH19 and miR-200a on the Susceptibility of Colorectal Cancer

Ebtsam Hamed Khalil[1] · Olfat G. Shaker[2] · Nabil A. Hasona[1]

Received: 27 January 2022 / Accepted: 6 May 2022 / Published online: 16 May 2022
© The Author(s), under exclusive licence to Association of Clinical Biochemists of India 2022

Abstract Cancer is the most common leading cause of mortality, making it a critical public health issue worldwide. Environmental and genetic abnormalities play a role in carcinogenesis, characterized by single nucleotide polymorphisms (SNPs) and abnormal gene expression. Also, non-coding RNA is a hot spot in cancer growth and metastasis. This study aimed to demonstrate the contribution of LncRNA H-19 rs2107425 to colorectal cancer (CRC) susceptibility and the correlation between miR-200a and LncRNA H-19 in patients with CRC. The current study was conducted on 100 participants, divided into 70 subjects with colorectal cancer and 30 age- and sex-matched healthy subjects. Patients with CRC experienced a significant elevation in WBC count, platelets, ALT, AST, and CEA. However, hemoglobin and albumin notably declined in patients with CRC compared with those in healthy controls. The expression of LncRNA H-19 and miR-200a increased in patients with CRC with a significant difference compared to healthy controls. Moreover, LncRNA H-19 and miR-200a expression significantly increased in stage III CRC compared to stage II CRC. As compared to carriers with the homozygous CC genotype, the frequency of rs2107425 CT and rs2107425 TT increased in patients with CRC. Our results indicate that the rs2107425 SNP of LncRNA H-19 may serve as a novel susceptibility marker for colorectal cancer. Moreover, miR-200a and LncRNA H-19 are prospective biomarkers of colorectal cancer.

Keywords Colorectal cancer · miR200a · lncH19 · rs2107425SNP

✉ Nabil A. Hasona
Drnabil80@yahoo.com; nabil.ahmed@science.bsu.edu.eg

Ebtsam Hamed Khalil
ebt23samhamed@gmail.com

Olfat G. Shaker
olfat.shaker@kasralainy.edu.eg

[1] Biochemistry Department, Faculty of Science, Beni-Suef University, Beni Suef, Egypt

[2] Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, Cairo, Egypt

Introduction

Colorectal cancer (CRC) is one of the most common and lethal disorders around the world [1] and is known as the third most common cancer as well as the fourth most deadly tumor worldwide [2]. It is responsible for 8.5% of all cancer deaths worldwide, emphasizing the importance of developing early diagnostic biomarkers [3].

Long noncoding RNA (LncRNA) is a group of genetic sequences that do not contribute to protein synthesis but can affect cellular activities such as chromatin remodeling, cell cycle advancement, and neoplasm formation [4].

LncRNA H19 was the first lncRNA discovered, with a 2.3 kb polyadenylated, spliced, and capped non-coding RNA encoded by its gene. It's on chromosome 11p15.5 in humans [5]. LncRNA H19 has been linked to a variety of cancers [6]. Anomalies in lncRNA H19 expression have been observed in a variety of cancers, implying that H19 plays an important role in cancer progression [7]. The H19 gene's single nucleotide polymorphisms (SNPs) may impact the gene's expression and function. Chu et al. [8] reported the association between three H19 polymorphisms (rs2839698, rs217727, and rs2107425) and cancer susceptibility. H19 also promotes epithelial-to-mesenchymal transition (EMT), a critical stage in cancer metastasis,

by acting as miRNA sponges and inhibiting the functions of related miRNA, according to Zhou et al. [9].

miR-200a, miR-200b, miR-200c, miR-141, and miR-429 are the five members of the miR-200 family. The miR-200 family is involved in cell transformation and tumorigenesis, cancer metastasis, tumor growth, angiogenesis, invasion, migration, and tumor cell survival within the circulation. miR-200 can be a potential diagnostic and prognostic tool for patients with cancer [10]. In recent years, single nucleotide polymorphisms (SNPs) of candidate genes have become the focal point of various studies on the genetic risk of cancer incidence; the expression of the host lncRNA may vary depending on the genetic variations of the non-coding RNA gene. However, there have been few investigations into the relationship between SNPs in lncRNAs and colon cancer risk. Xue et al. [11] reported that HOTAIR SNPs contributes to the susceptible to colorectal cancer. Also, Li et al. [12] stated that H19 rs2839689 increases the susceptibilities to colorectal cancer in the Chinese population. Moreover, Fu et al. [13] reported that SNP rs12982687 of UCA1 provide high susceptibility of CRC. Thus, the current study aimed to scrutinize the biochemical contribution of LncRNA H-19 rs2107425 to the susceptibility of CRC and the potential relationship between miR-200a and LncRNA H-19 in patients with colorectal cancer.

Subjects and Methods

The present ongoing study included 100 unrelated participants were analyzed, the age of all participants ranged from 29 to 62 years. The participants were divided into two main groups: Group I contained healthy controls and Group II consisted of individuals diagnosed with colorectal cancer (CRC), according to the colonoscopic examination and signs of CRC like significant unexplained weight loss and unexplained anemia; screening for colorectal cancer, and metastasis. The healthy participants were age, gender, and ethnicity matched to colorectal cancer patients.

Informed written consent was received from all the participants enrolled during this study. Characteristics of all enrolled participants, including age, gender, and family history, were collected. The study was performed with the approval of the Al-Kasr Al-Ainy Hospital's ethics committee at Cairo University, Egypt, and in accordance with the Helsinki Declaration (2008).

Regarding, All study participants underwent a thorough history and clinical examination, including routine laboratory tests like hemoglobin level, white blood cell count, Platelets, liver function profile, kidney function tests, and carcinoembryonic antigen (CEA) assay. Full colonoscopy; and imaging with abdominal ultrasound and computed tomography to stage CRC subjects. Patients who had previously received chemo- or radiation for CRC, were diagnosed with inflammatory bowel disease (IBD), had cancer at the other site at the time of selection, or had a history of recurrent malignancies were also ruled out.

All enrolled participants' venous blood samples (10 mL) were withdrawn and centrifuged at 4000 r/min for 10 min in serum separator tubes. Sera were stored at -80 °C until they were analyzed.

The QIAamp DNA Minikit (Qiagen, Valencia, CA) was used to extract genomic DNA from whole blood samples from all subjects according to the manufacturer's guidelines. The yield was determined using NanoDrop2000 (Thermo Scientific, USA). Genotyping was performed using real-time PCR with the TaqMan allelic discrimination assay using predesigned primer/probe sets for rs2107425 (C/T) (Applied Biosystems, USA). DNA amplification was performed in a 25 µl volume containing 12.5 µl TaqMan master mix, 1.25 µl primer/probe, 1 µl DNA, and 10.25 µl H_2O. Real-time PCR was performed employing a Rotor gene Q System (Qiagen) with the subsequent conditions: 95 °C for 10 min, followed by 45 cycles at 92 °C for 15 s and 60 °C for 90 s. Fluorescence was measured at the end of every cycle and therefore the endpoint.

Total RNA was extracted from serum by the miRNeasy extraction kit (Qiagen, Valencia, CA) using QIAzol lysis reagent according to the manufacturer's instructions.

Reverse transcription (RT) was applied to 60 ng of total RNA in a final volume of 20 µ l RT reactions using the RT2 first strand kit (Qiagen, Valencia, CA) in accordance with the manufacturer's guidelines. Before performing real-time PCR, the RT products were diluted in 50 µl RNAase-free water. Serum expression levels of the lncRNA H19 and miR-200a were evaluated using GAPDH, and SNORD68 as interior controls, using customized primers and the Maxima SYBR Green PCR kit (Thermo, USA) in line with the manufacturer's protocol.

The specificity of the target PCR product was confirmed by visualizing one peak within the melting curve analysis. qRT-PCR data analysis was accomplished based on the $2^{-\Delta\Delta ct}$ comparative expression method [14].

Statistical Analysis

The statistical software SPSS 21.0 was used to analyse the data (SPSS Inc., Chicago, IL, USA). The data is presented as a mean with standard error. One-way ANOVA has been used for descriptive statistics. The chi-square test was applied to compare the genotype frequencies for rs2107425. Hard-Weinberg equilibrium applied using an online calculator [15]. Pearson correlations coefficient test

were used to test relation between clinical and laboratory variables. $P < 0.05$ was considered statistically significant.

Results

The present ongoing study included 100 unrelated participants were analyzed. The participants were divided into 70 CRC patients and 30 healthy controls. Male CRC patients represented 61.43%, while female CRC patients represented 38.57%. Male healthy control participants constituted 46.67%, while female controls constituted 53.33%. According to the radiological and colonoscopy findings performed on the CRC patients, tumor sites in the cecum and ascending colon represented 20% of the cases, transverse and flexures represented 25.7%, descending and rectosigmoid tumor sites represented 41.4%, and rectal represented12.8%, as shown in Table 1.

Regarding biochemical and hematologic variables, there were a significant alterations in hemoglobin and total leukocyte count in CRC patients compared to the healthy control group ($P < 0.05$). Meanwhile, a significant alteration ($P < 0.05$) in liver enzymes (ALT, AST), total bilirubin, and albumin levels were observed in CRC patients compared with those in healthy controls. Moreover, CRC patients showed a significant elevation in creatinine and CEA levels as compared to the healthy control group. Additionally, this study shows a remarkable, statistically significant fold change elevation in miR-200a levels in CRC patients compared to healthy participants (mean fold change 6.66, $P < 0.05$), as shown in Table 2. Here also, LncH19 shows a highly remarkable fold change elevation in its expression level in the CRC patients compared to healthy subjects (mean fold change 9, $P < 0.05$). Moreover, the expression levels of LncRNA H-19 and miR-200a were significantly higher in participants with stage III than those in stage II ($P < 0.05$). Moreover, the other biochemical variables studied were numerically differed between stages II and III but didn't reach statistical significance ($P > 0.05$) (Table 2).

In this study, The patients with CRC had a statistically significantly lower distribution of the CC genotype than the healthy control participants (35.7% and 66.7%, respectively), a statistically higher distribution of the CT genotype than the healthy control participants (45.7% and 30%, respectively), and a statistically higher distribution of the TT genotype than the healthy control participants (18.6% and 3%, respectively) ($\chi^2 = 9.22$, $P < 0.05$) (Table 3). Moreover, the patients with CRC had a statistically higher distribution of T allele frequency (41.4% and 18.3%, respectively) and a lower percentage of C allele frequency than the healthy control participants (58.6% and 81.7%, respectively) ($\chi^2 = 9.92$, $P < 0.05$) (Table 3). Additionally, the average fold expression of miR-200a and LncRNA H19 for CT and TT genotypes of the rs2107425polymorphism was statistically significantly higher for CT and TT genotypes than for CC genotypes ($p < 0.05$) (Table 4).

Table 1 Demographic data and clinical-pathological features of the studied groups

Variables	Healthy control participants (n = 30)	CRC subjects (n = 70)
Age (range, year)	29–60	38–62
(mean ± SD)	42.85 ± 9.60	50.26 ± 6.63
Gender		
Male (n,%)	14 (46.67%)	43 (61.43%)
Female (n, %)	16 (53.33%)	27 (38.57%)
BMI (≤ 25)	23(76.6%)	53(75.71%)
(> 25)	7(23.4%)	17(24.29%)
Site of tumor		
Cecum and ascending	–	14(20%)
Transverse and flexures		18(25.7%)
Descending and rectosigmoid		29 (41.4%)
Rectal		9(12.8%)
Distant metastasis		
Present	–	14(20%)
Absent		56(80%)
Tumor stage		
Stage II	–	42(60%)
Stage III		28(40%)

CRC Colorectal cancer, *BMI* Body mass index

Table 2 Biochemical comparison between healthy control and CRC patients regarding laboratory investigations (mean ± SE)

Blood test	Healthy control participants (n = 30)	All CRC patients (n = 70)	Stage II (n = 42)	Stage III (n = 28)
Hemoglobin (g/dl)	12.48 ± 0.24	10.43 ± 0.36*	10.77 ± 0.55*	10.20 ± 0.47*
Leukocytes (× 10^3/mm^3)	6.57 ± 0.43	8.18 ± 0.27*	8.15 ± 0.35*	8.24 ± 0.44*
Platelets (× 10/mm^3)	296.67 ± 11.29	287.14 ± 12.69	281.74 ± 17.27	295.25 ± 18.58
ALT (IU/L)	12.83 ± 0.79	21.34 ± 0.54*	20.81 ± 0.76*	22.14 ± 0.72*
AST(IU/L)	15.27 ± 1.01	30.84 ± 0.81*	30.52 ± 1.16*	31.32 ± 1.06*
T. Bilirubin(mg/dl)	0.50 ± 0.02	0.77 ± 0.03*	0.74 ± 0.04*	0.80 ± 0.04*
Albumin (g/dl)	4.58 ± 0.05	3.48 ± 0.05*	3.47 ± 0.08*	3.44 ± 0.07*
Urea(mg/dl)	19.87 ± 0.85	19.57 ± 0.54	18.61 ± 0.65	20.21 ± 0.78
Creatinine (mg/dl)	0.65 ± 0.02	0.95 ± 0.02*	0.93 ± 0.02*	0.96 ± 0.03*
CEA(ng/ml)	0.83 ± 0.12	35.72 ± 4.82*	35.19 ± 6.36*	36.52 ± 7.48*
LncRNA H-19	0.99 ± 0.02	9.90 ± 0.70*	7.34 ± 0.40*	13.72 ± 1.10*
miR-200a	0.99 ± 0.01	7.58 ± 0.41*	6.51 ± 0.32*	9.20 ± 0.44*

Hb Hemoglobin, *ALT* Alanine transaminase, *AST* Aspartate transaminase, *CEA* Carcinoembryonic antigen, *LncRNA H-19* Long noncoding RNA H-19, *miR-200a* MicroRNA-200a, *SE* Standard error

*Significant from control participants ($P < 0.05$)

Table 3 Genotype and allele frequency of LncH19 rs2107425(C/T) gene single nucleotide polymorphism (SNP) between healthy control and CRC subjects

Groups	Genotype frequency			Allele frequency	
	CC	CT	TT	C	T
Healthy control	20 (66.7%)	9 (30%)	1 (3.3%)	49 (81.7%)	11(18.3%)
CRC subjects	25 (35.7%)	32 (45.7%)	13 (18.6%)	82 (58.6%)	58 (41.4%)
		χ^2 = 9.22	$P < 0.05$	χ^2 = 9.92	$P < 0.05$

Table 4 Expression fold of miR-200a and H19 regarding CRC patients(mean ± SE)

Variables	All CRC patients	Genotype		
		CC	CT	TT
miR-200a	7.58* ± 0.41	4.48* ± 0.39	8.31# ± 0.44	11.74# ± 0.61
Lnc RNA H-19	9.90* ± 0.70	5.51* ± 0.25	9.55# ± 0.45	19.20# ± 1.77

*Significant from control participants

#Significant from genotype CC of CRC patients

To assess the relationship between LncRNA H-19 and miR-200a with patients' clinical and laboratory variables, Pearson correlation analysis was conducted. LncRNA H-19 and miR-200a positively correlated with distant metastasis, stage of the tumor, WBC count, ALT, AST, total bilirubin, and CEA, and negatively with levels of hemoglobin and albumin. Additionally, a significant positive correlation was observed between the expression levels of LncRNA H-19 and miR-200a among studied participants (Table 5).

Discussion

Recent advancements in colorectal cancer diagnosis and therapy have improved health satisfaction; screening for CRC during an early stage, when it has been curable, can minimize both the disease's incidence and mortality [16]. Hence, there is a crucial need for noninvasive biomarkers to complement and improve the diagnosis and prognosis of colorectal cancer. This study explored the biochemical contribution of LncRNA H-19 rs2107425 to the susceptibility of colorectal cancer and the potential relationship between miR-200a and LncRNA H-19 in patients with colorectal cancer. Recently, the single nucleotide polymorphisms (SNPs) of lncRNA genes have been widely

Table 5 Correlation of serum H-19 and miR-200a expression levels with clinical and biochemical analysis of studied participants

Variables	miR-200a		LncRNA H-19	
	R	*P*	r	*P*
Site of tumor	-0.142	> 0.05	-0.074	> 0.05
Metastasis	-0.740	< 0.05	-0.623	< 0.001
Tumor Stages	0.520	< 0.05	0.603	< 0.001
Hemoglobin	-0.244	< 0.05	-0.195	< 0.05
WBCs	0.282	< 0.05	0.183	> 0.05
ALT	0.532	< 0.05	0.491	< 0.05
AST	0.677	< 0.05	0.622	< 0.05
Total bilirubin	0.498	< 0.05	0.413	< 0.05
Albumin	-0.695	< 0.05	-0.563	< 0.05
Creatinine	0.60	< 0.05	0.581	< 0.05
CEA	0.273	< 0.05	0.186	> 0.05
miR-200a	1	–	0.830	< 0.05
LncRNA H-19	0.830	< 0.05	1	–

r: Correlation coefficient; $P < 0.05$ was considered significant

confirmed to regulate the expression and function of lncRNAs, ultimately resulting in tumor susceptibility and a poor prognosis [17]. The LncRNA H19 has been widely renowned for its aberrant expression profile and role in carcinogenesis, and it is suggested to be a novel biomarker for cancer diagnosis[18].

Zhang et al. [19] and Ratti et al. [20], reported that the elevation of differential expression of lncRNA and miRNA are associated with an increased susceptibility of cancer formation, inflammation, and neurological diseases.

LncRNA H-19 expression level were up-regulated with a remarkable increase in patients with CRC compared with healthy controls. These findings are consistent with the results of [21–23], which reported that patients with CRC exhibited a noticeable elevation in LncRNA H-19 expression compared with healthy controls; and considered LncRNA H-19 as a novel CRC prognostic oncogenic biomarker. Additionally, the loss of imprinting of the gene has been suggested as a cause of H19 overexpression in CRC [18].

The miR-200a differential expression fold was assessed in patients with CRC and compared with that in healthy participants. All participants with CRC showed elevated miR-200a expression with a significant difference. Moreover, the differential expression fold of LncRNA H-19 and miR-200a were lower in participants with CRC with stage II than that in those with stage III, it were significant. This implies that the differential expression of LncRNA H-19 and miR-200a might be a biomarkers for predicting the progress of colorectal cancer.

Our findings support the study by Chen et al. [23], and previous studies of Yang et al. [24], which reported the impact of miR-200a and its oncogenic roles in tumor growth and metastasis. Additionally, Our findings have coincided with Carter et al. [25], who showed that miR-200a expression is frequently up-regulated in colorectal cancer samples compared to control ones.

GWAS has identified multiple independent single nucleotide polymorphisms (SNPs) associated with colorectal cancer risk. Previous studies has found links and relationships between cancer susceptibility and the three most prevalent SNPs in LncRNA H-19 (rs2839698 G > A, rs217727 G > A, and rs2107425 C > T) [12, 26, 27].

In the current study, we evaluate the association between *H19* rs2107425 polymorphism and CRC risk. To our knowledge, no previous research has looked into the link between rs2107425 in H19 and colorectal cancer risk in an Egyptian population. Our current study found that there was a remarkable association between H19 rs2107425 polymorphism and CRC, and the T allele of rs2107425 was significantly higher in CRC than in healthy control participants. Also, we found that the CC genotype has a higher percentage of controls compared to CRC participants. Both the mutant and heterozygote genotypes were noticed with increased frequency in CRC patients than in healthy control participants.

These findings agreed with the findings obtained by [27], who reported that T allele carriers have a significantly increased risk of developing lung cancer and that the functional SNP rs2107425 in H19 is highly likely to be involved in lung cancer development. In contrast, Chu et al. [8] reported that in the population studied, the H19 rs2107425 polymorphism was not linked to colon cancer susceptibility, and the frequency of the rs2107425 polymorphism in colon cancer cases and controls did not differ significantly. Verhaegh et al. [28], reported that H19 genetic polymorphisms (rs2839698 and rs2107425) might be associated with the susceptibility of bladder cancer in European Caucasians. Similarly, Yin et al. [29], observed that rs2107425 polymorphism may be able to influence the risk of lung cancer in Chinese female never smokers. All of these studies suggested that genetic variants of LncRNA H-19 may have a significant impact on many tumor susceptibilities.

Additionally, this study found a significant positive correlation between serum LncRNA H-19 and miR-200a, suggesting their concomitant expression in CRC. Additionally, we found a remarkable correlations between ncRNAs (LncRNA H-19 and miR-200a) and tumor stages, and distant metastases. Our data were consistent with previous studies [21, 30–32], which reported that LncRNA

H-19 and miR-200a were associated with distant metastases in cancers. Similarly, Becker et al. [33] reported that the over expression of miR-200a enhance the malignant transformation and acts as an oncomiR rather than a tumor suppressor by cooperating with an oncogene in malignant cell transformation.

To the best of our knowledge, This study is the first to detect the relationship between LncRNA H-19 rs2107425 to the susceptibility of colorectal cancer and the correlation between miR-200a and LncRNA H-19 in colorectal cancer. However, this study has some limitations, like the sample size, the selection of a single SNP for lncH19 (rs2107425), and the study of miR-200a of the miR-200 family. Therefore, further large-scale studies and clinical trials are needed. Nevertheless, our results implicate rs2107425 as genetic markers of CRC susceptibility that correlate with LncRNA H-19 and miR-200a expression, respectively. Serum LncRNA H, with rs2107425 and serum miR-200a, could predict the risk of CRC diagnosis among non-CRC groups, implications for stages of CRC screening, genetic counseling, and hold promise for large-scale application.

Funding The authors declare that they have received no funding for the research reported.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval The study was performed with the approval of the ethics committee of Al-Kasr Al-Ainy Hospital, Cairo University, Egypt, and the Helsinki Declaration (2008) was followed in the current research.

References

1. Fan Q, Liu B. Comprehensive analysis of a long noncoding RNA-associated competing endogenous RNA network in colorectal cancer. Onco Targets Ther. 2018;11:2453–66.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics. CA Cancer J Clin. 2019;69:7–34.
3. Zhang M, Li Y, Wang H, et al. LncRNA SNHG5 affects cell proliferation, metastasis and migration of colorectal cancer through regulating miR-132-3p/CREB5. Cancer Biol Ther. 2019;20(4):524–36.
4. Liao S, Yu C, Liu H, et al. Long non-coding RNA H19 promotes the proliferation and invasion of lung cancer cells and regulates the expression of E-cadherin, N-cadherin, and vimentin. Onco Targets Ther. 2019;12:4099–107.
5. Lu Y, Tan L, Shen N, et al. Association of lncRNA H19 rs217727 polymorphism and cancer risk in the Chinese population: a meta-analysis. Oncotarget. 2016;7(37):59580–8.
6. Hu JC, Lin CY, Wang SS, et al. Impact of H19 polymorphisms on prostate cancer clinicopathologic characteristics. Diagnostics. 2020;10(9):656.
7. Zhang LI, Zhou Y, Huang T, et al. The interplay of LncRNA-H19 and its binding partners in physiological process and gastric carcinogenesis. Int J Mol Sci. 2017;18(2):450.
8. Chu M, Yuan W, Wu S, et al. Quantitative assessment of polymorphisms in H19 lnc RNA and cancer risk: a met analysis of 13, 392 cases and 18, 893 controls. Oncotarget. 2016;7(48):78631–9.
9. Zhou W, Ye XL, Xu J, et al. The lncRNA H19 mediates breast cancer cell plasticity during EMT and MET plasticity by differentially sponging miR-200b/c and let-7b. Sci Signal. 2017;10:eaak9557.
10. Humphries B, Yang C. The microRNA-200 family: small molecules with novel roles in cancer development, progression and therapy. Oncotarget. 2015;6(9):6472–98.
11. Xue Y, Gu D, Ma G, et al. Genetic variants in lncRNA HOTAIR are associated with risk of colorectal cancer. Mutagenesis. 2014;30:303–10.
12. Li S, Hua Y, Jin J, et al. Association of genetic variants in lncRNA *H19* with risk of colorectal cancer in a Chinese population. Oncotarget. 2016;7:25470–7.
13. Fu Y, Zhang Y, Cui J, et al. SNP rs12982687 affects binding capacity of lncRNA UCA1 with miR-873-5p: involvement in smoking-triggered colorectal cancer progression. Cell Commun Signal. 2020;18:37.
14. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}$T Method. Methods (San Diego, Calif). 2001;25(4):402–8.
15. Equilibrium Hardy-Weinberg. Online calculator at: https://wpcalc.com/en/equilibrium-hardy-weinberg/.
16. Elshafei A, Shaker O, Abd El-motaal O, et al. The expression profiling of serum miR-92a, miR-375, and miR-760 in colorectal cancer: An Egyptian study. Tumor Biology. 2017;39(6):1–14.
17. Minotti L, Agnoletto C, Baldassari F, et al. SNPs and somatic mutation on long non-coding RNA. New Front Cancer Stud. 2018;7(4):34.
18. Chen S, Bu D, Ma Y, et al. H19 overexpression induces resistance to 1,25(OH)2D3 by targeting VDR through miR-675-5p in colon cancer cells. Neoplasia. 2017;19:226–36.
19. Zhang M, He P, Bian Z. Long noncoding RNAs in neurodegenerative diseases: pathogenesis and potential implications as clinical biomarkers. Front Mol Neurosci. 2021;14: 685143.
20. Ratti M, Lampis A, Ghidini M, et al. MicroRNAs (miRNAs) and long non-coding RNAs (lncRNAs) as new tools for cancer therapy: first steps from bench to bedside. Target Oncol. 2020;15(3):261–78.
21. Han D, Gao X, Wang M, et al. Long noncoding RNA H19 indicates a poor prognosis of colorectal cancer and promotes tumor growth by recruiting and binding to eIF4A3. Oncotarget. 2016;7(16):22159–73.
22. Tsang WP, Ng EK, Ng SS, et al. Oncofetal H19-derived miR-675 regulates tumor suppressor RB in human colorectal. Carcinogenesis. 2010;31:350–8.
23. Chen X, Li Y, Zuo C, et al. Long non−coding RNA H19 regulates glioma cell growth and metastasis *via* miR-200a-mediated CDK6 and ZEB1 expression. Front Oncol. 2021;11: 757650.
24. Yang W, Ning N, Jin X. The lncRNA H19 promotes cell proliferation by competitively binding to miR-200a and derepressing β-Catenin expression in colorectal cancer. Biomed Res Int. 2017;2767484:1–8.
25. Carter JV, O'brien SJ, Burton JF, et al. The microRNA-200 family acts as an oncogene in colorectal cancer by inhibiting the tumor suppressor RASSF2. Oncology. 2019;18:3994–4007.
26. Yang C, Tang R, Ma X, et al. Tag SNPs in long non-coding RNA H19 contribute to susceptibility to gastric cancer in the Chinese Han population. Oncotarget. 2015;6:15311–20.
27. Wang YC, Tsao SM, Li YT, et al. The Relationship between long noncoding RNA H19 polymorphism and the epidermal growth

factor receptor phenotypes on the clinic pathological characteristics of lung adenocarcinoma. Int J Environ Res Public Health. 2021;18(6):2862.
28. Verhaegh GW, Verkleij L, Vermeulen SH, et al. Polymorphisms in the H19 gene and the risk of bladder cancer. Eur Urol. 2008;54(5):1118–26.
29. Yin Z, Cui Z, Li H, et al. Polymorphisms in the H19 gene and the risk of lung Cancer among female never smokers in Shenyang, China. BMC Cancer. 2018;18(1):893.
30. Li W, Jiang X, Jin X, et al. Significant association between long non-coding RNA H19 polymorphisms and cancer susceptibility. Medicine. 2020;99(15): e19322.
31. Korpal M, Ell BJ, Buffa FM, et al. Direct targeting of Sec23a by miR-200s influences cancer cell secretome and promotes metastatic colonization. Nat Med. 2011;17:1101–8.
32. Gan L, Lv L, Liao S. Long non-coding RNA H19 regulates cell growth and metastasis via the miR-22-3p/Snail1 axis in gastric cancer. Int J Oncol. 2019;54:2157–68.
33. Becker LE, Takwi AA, Lu Z, et al. The role of miR-200a in mammalian epithelial cell transformation. Carcinogenesis. 2015;36(1):2–12.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.