Association of lncRNA-p53 regulatory network (lincRNA-p21, lincRNA-ROR and MALAT1) and p53 with the clinicopathological features of colorectal primary lesions and tumors

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Abstract. Colorectal cancer (CRC) is a common intestinal cancer with a high mortality rate. Early detection of this type of cancer is fundamental to the prevention of the disease, which results in improved survival rates. In the human colon tissue, transition from normal epithelium to adenoma is considered to be caused by unknown molecular incidents occurring over 5-10 years. The detection of CRC has proved problematic when in the early stages of disease. In addition, identifying suitable biomarkers for the detection of CRC progress in patients remains one of the most significant challenges. Long non-coding RNAs have been demonstrated to contribute to the promotion of CRC. The aim of the present study was to investigate the clinical and biological significance of long intergenic non-coding (linc)RNA-p21, lincRNA-regulator of reprogramming (ROR) and metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) in the colon tumor and polyp tissue, and the association that these have with the expression of p53 at the mRNA level. Neoplastic and paired adjacent normal tissue samples were obtained from 72 patients (46 polyps and 26 tumors). Reverse transcription-quantitative PCR was performed to determine the relative fold changes in the expression of lincRNA-p21, lincRNA-RoR, MALAT1 and p53 in the samples. A significant association was observed between the levels of MALAT1 and p53 in neoplasm tissues (R=0.073; P<0.05). The relative expression of the MALAT1 gene revealed a statistically significant difference between the different polyp types and number of polyps (P=0.0028 and 0.022, respectively). Adjuvant therapy in patients with tumors revealed an association between the levels of lincRNA-ROR and lincRNA-p21 expression (P=0.015 and 0.038, respectively). MALAT1 may be selected as an early detection biomarker for CRC. Furthermore, lincRNA-ROR and lincRNA-p21 may serve as prognostic and therapeutic biomarkers in patients with CRC.

Introduction

Colorectal cancer (CRC) is responsible for >1.8 million new colorectal cancer cases and 881,000 cases of CRC-associated mortality worldwide each year (according of GLOBOCAN 2018). It was also the third most commonly diagnosed cancer in both men and women in the United States, in 2017 (1,2). Evidence has suggested that different biological pathways are involved in CRC development, particularly the type that originated from the precursor lesions termed 'polyps' (3). Currently, histology is used to classify these lesions as adenomatous or serrated polyps (4). The adenoma subdivisions include villous, tubulovillous or tubuluar, and serrated polyps, which are then subdivided into hyperplastic polyps, sessile serrated polyps or traditional serrated adenomas (4).

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Early detection of CRC results in improved prognostic outcomes for patients, and late diagnosis leads to challenges in treatment (5,6). Currently, researchers are striving to identify highly specific novel biological markers for the non-invasive and easy diagnosis of CRC (7-9). Previous studies have indicated that long non-coding RNAs (lncRNAs) are involved in different biological processes of cells, particularly in gene expression via the regulation of transcription and post-transcriptional processing as well as chromatin modification. They can also potentially contribute to tumor progression and metastasis in different types of tissue (10-12). p53 mutations occur in 40-50% of sporadic cases of CRC, resulting in direct deactivation of the affected gene (13). Emerging evidence has suggested that lncRNAs may regulate the p53 gene or p53 targets (14). Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is a highly conserved lncRNA and is overexpressed in various types of human cancer (15). It has been revealed that p53 is a downstream mediator of MALAT1 activity, and the decrease in MALAT1 levels leads to the activation of p53 (16). In addition, the 1-kb sequence upstream of regulator of reprogramming (ROR) is a p53-binding site that induces ROR expression. ROR expression suppresses p53 to maintain cellular homeostasis (17). Cyclin-dependent kinase inhibitor 1 (CDKN1A), also known as p21, mediates p53-dependent growth arrest (18). LincRNA-p21 is induced by p53 and is expressed ~15 kb upstream of the CDKN1A (p21) on chromosome 17 (15).

Although there are preliminary data that support the involvement of the aforementioned lncRNAs on p53 regulation, their usage as predictive biomarkers in early detection and management of CRC is not currently well known in screening programs (14,15). Although detection of p53 at the protein level using immunohistochemistry could demonstrate this association, it could not determine the mediation of lncRNAs in this pathway.

Colon tissue homeostasis occurs in connection with the function of LincRNAs and other cellular regulatory systems. Understanding the associations between the p53 regulatory system and colon tissue-specific lincRNAs could aid in identifying the primary lesion and CRC tumor etiology. An increased knowledge of the aberrant expression levels of these lncRNAs and the p53 gene in the colon neoplasm could provide evidence for the potential biomarkers targeting future novel therapeutic approaches. The present study hypothesized that CRC-associated lincRNA-p21, lincRNA-RoR and MALAT1 may serve as prognostic and therapeutic targets in patients with CRC.

Materials and methods

Patients. A total of 125 volunteers with suspected colon polyps and CRC were subjected to colonoscopy. Non-Iranian patients and those who were negative for colon polyps and CRC were excluded (53 volunteers). The final number of patients included in the present study was 72. Finally, this cross-sectional study was performed on a total of 72 patients (46 polyps and 26 tumor tissues), who were referred to the Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences (Tehran, Iran) between August, 2014 and 2016, enrolled in the present study. The mean age ± standard deviation of the patients with polyps and tumors was 49.71±17.54 and 59.84±18.87 years, respectively.

The patients were of Iranian descent and provided written informed consent for the present study prior to the sampling procedure. Biopsies were performed during the colonoscopy and diagnoses were confirmed following evaluation by pathologists. Detailed clinicopathological parameters provided the following data on each polyp lesion and invasive colon cancer subject in patients with polyps [including age, body mass index (BMI), sex, smoking status, ethnicity, education, constipation, diarrhea, anemia, weight loss, bleeding from the anus, family history, polyp type, polyp location, number of polyps and polyp size] and patients with tumors (including age, sex, ethnicity, family history, tumor site, grade, tumor stage, tumor size, radiotherapy and adjuvant therapy) from patient records. The tumor stage was determined using Tumor-Node-Metastasis (TNM) classification of the Union for International Cancer Control (UICC) (19). The protocol for the present study conformed to the ethical guidelines of The Declaration of Helsinki (1975) as reflected by the approval from the Ethics Committee of the Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences (Date of approval: May 15, 2014, under the ethical code IR.SBMU.RIGLD.1393.Code 765). The non-tumorous tissue samples were provided with a 10 cm margin from the tumor tissue. The tissue samples were frozen in liquid nitrogen immediately following surgical removal and stored at -80˚C.

Reverse transcription-quantitative (RT-q) PCR and PCR analysis. RNA was extracted from 72 tissue samples (46 polyps and 26 tumor tissues) and paired adjacent normal tissues from the patients included in the present study using an RNeasy Mini kit (50) (Qiagen GmbH), according to the manufacturer's protocol. RNA integrity, quantity and quality were determined using 1% standard agarose gel electrophoresis and a NanoDrop™ 2000 system (NanoDrop Technologies; Thermo Fisher Scientific, Inc.). RNA was stored at -70˚C prior to use. cDNA was synthesized using 1 µg samples of RNA, and the RNAs Revert Aid RT Reverse Transcription kit (Thermo Fisher Scientific, Inc.), following the adjustment of the concentrations, according to the manufacturer's protocol. qPCR was performed using a PCR cycler (Rotor-Gene Q MDx; Qiagen GmbH). cDNA fragments were used as templates to amplify the lincRNA-p21, lincRNA-RoR, MALAT1 and p53 genes using SYBR® Premix Ex Taq™ (Takara Bio, Inc.), according to the manufacturer's protocol. The experimental protocol was performed as follows: i) Thermocycling conditions consisted of an initial activation step for 30 sec at 95˚C, 40 cycles at 95˚C for 5 sec and 60˚C for 35 sec; and ii) melting curve analysis. A duplicate, no template control, consisting of water, was included in every run for each primer pair to test for DNA contamination in buffers as well as solutions and to assess the primer-dimers.

To select the best normalizing gene, the previous study by Kheirelseid *et al* (20) was reviewed and the variation of Cq in several randomly selected samples using primers targeting B2M, GAPDH and β-actin was also compared. The results of these comparisons confirmed the use of B2M gene as a normalizer endogenous gene.
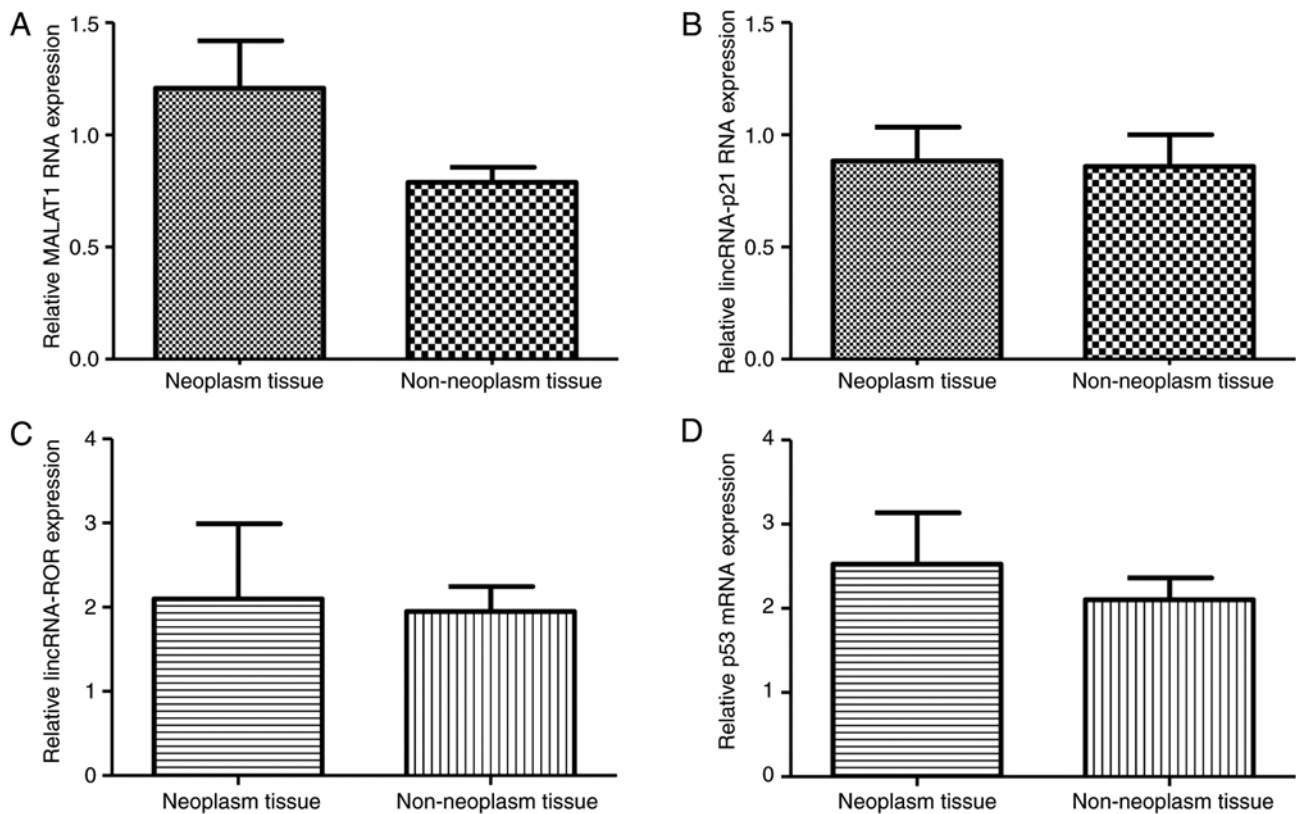
Figure 1. Relative expression of each gene between the neoplastic tissue and the adjacent non-neoplastic tissues. (A) Relative expression of MALAT1 between the neoplastic tissue and the adjacent non-neoplastic tissues (P=0.0675). (B) Relative expression of lincRNA-p21 between the neoplastic tissue and the adjacent non-neoplastic tissues (P=0.9). (C) Relative expression of lincRNA-ROR between the neoplastic tissue and the adjacent non-neoplastic tissues (P=0.8753) and (D) relative expression of p53 between the neoplastic tissue and the adjacent non-neoplastic tissues (P=0.5182). MALAT1, metastasis-associated lung adenocarcinoma transcript 1; lincRNA, long intergenic non-coding RNA; ROR, regulator of reprogramming; p53, tumor protein 53.

The primer sequences for lincRNA-p21, lincRNA-ROR, MALAT1, p53 and B2M, were as follows: lincRNA-p21: Forward: 5'-GGGTGGCTCACTCTTCTGGC-3', and reverse: 5'-TGGCCTTGCCCGGGCTTGTC-3';lincRNA-ROR:Forward, 5'-CCAGGACAATGAAACCAC-3', and reverse: 5'-AGGAGC CCAAAGTAACAG-3'; MALAT1: Forward, 5'-GGTAACGAT GGTGTCGAGGTC-3', and reverse: 5'-CCAGCATTACAGTTC TTGAACATG-3'; p53: Forward: 5'-TCTAGAGCCACCGTC CAGG-3', and reverse: 5'-ACGCTAGGATCTGACTGCG-3'; and B2M: Forward: 5'-TGCTGTCTCCATGTTTGATGTATCT-3' and reverse: 5'-TCTCTGCTCCCCACCTCTAAGT-3'. The $2^{-\Delta\Delta Cq}$ method was used to determine the expression fold changes (tumor vs. normal) (21). In addition, patients were divided into low-expression (≤median) and high-expression (>median) groups. The median cut-off value of 1.5 fold was used.

Statistical analysis. Data was plotted and statistical analysis was performed using GraphPad Prism (v.5.04; GraphPad Software, Inc.), and the significance was determined using paired t-test and one-way analysis of variance test, with a Tukey's multiple comparison post-hoc test in which P<0.05 was considered to indicate a statistically significant difference. Differences in clinicopathological outcome between groups were assessed using the SPSS software (v.19.0; IBM Corp.). The association between p53 and lncRNA expression was assessed via linear regression. The receiver operating characteristics (ROC) curve was constructed to describe diag-nostic specificity and sensitivity, and was performed using the GenEx program (v.6.1; MultiD Analsyses AB).

Results

General statistical information. CRC was characterized in 36.1% of the patients (26/72), and polyps were detected in 63.9% (46/72). In those patients with polyps in the evaluations (46 in total), the mean age of the patients was 49.71±17.54 years with a mean BMI of 25.74±3.42, 24 patients (52.2%) were male, 9/46 (25.7%) were smokers, 3/46 (8.6%) consumed alcohol and 9/46 (25.7%) had a positive family history for gastrointestinal polyps. The most common type of polyp was adenomatous polyp in 23 (50.0%) patients, followed by hyper-plastic and inflammatory polyps in 13 (28.3%) and 10 (21.7%) patients, respectively. The most common site of the polyps was the recto-sigmoid in 17 (37.0%) patients; followed by the transverse and descending colon, each in 9 (19.6%) patients; ascending colon in 6 (13.0%) and cecum in 3 (6.5%) patients. In the majority of cases [31 (67.4%) patients] the number of polyps was <5 and their size was 5-10 mm. The most common symptom in these patients was constipation (34.8%) followed by bleeding from the anus (32.6%), anemia (21.7%), diarrhea (19.6%) and weight loss (17.4%).

In those patients with colon cancer (26 cases in total), the mean age was 59.84±18.87 years, 18 patients (69.2%) were female, 5 (19.2)% were smokers and 5 (19.2%) had family

Table I. Evaluation between MALAT1, lincRNA-ROR and lincRNA-P21 expression in tumor tissue and polyp tissue.

Variables	MALAT1		LincRNA-ROR		LincRNA-p21	
	Low, n (%)	High, n (%)	Low, n (%)	High, n(%)	Low, n (%)	High, n (%)
Polyp tissue	34 (73.9)	12 (26.1)	37 (80.4)	9 (19.6)	32 (69.6)	14 (30.4)
Tumor tissue	15 (57.7)	11 (42.3)	18 (69.2)	8 (30.8)	13 (50.0)	13 (50.0)
P-value		0.156		0.282		0.1

MALAT1, metastasis-associated lung adenocarcinoma transcript 1; lincRNA, long intergenic non-coding RNA; ROR, regulator of reprogramming.
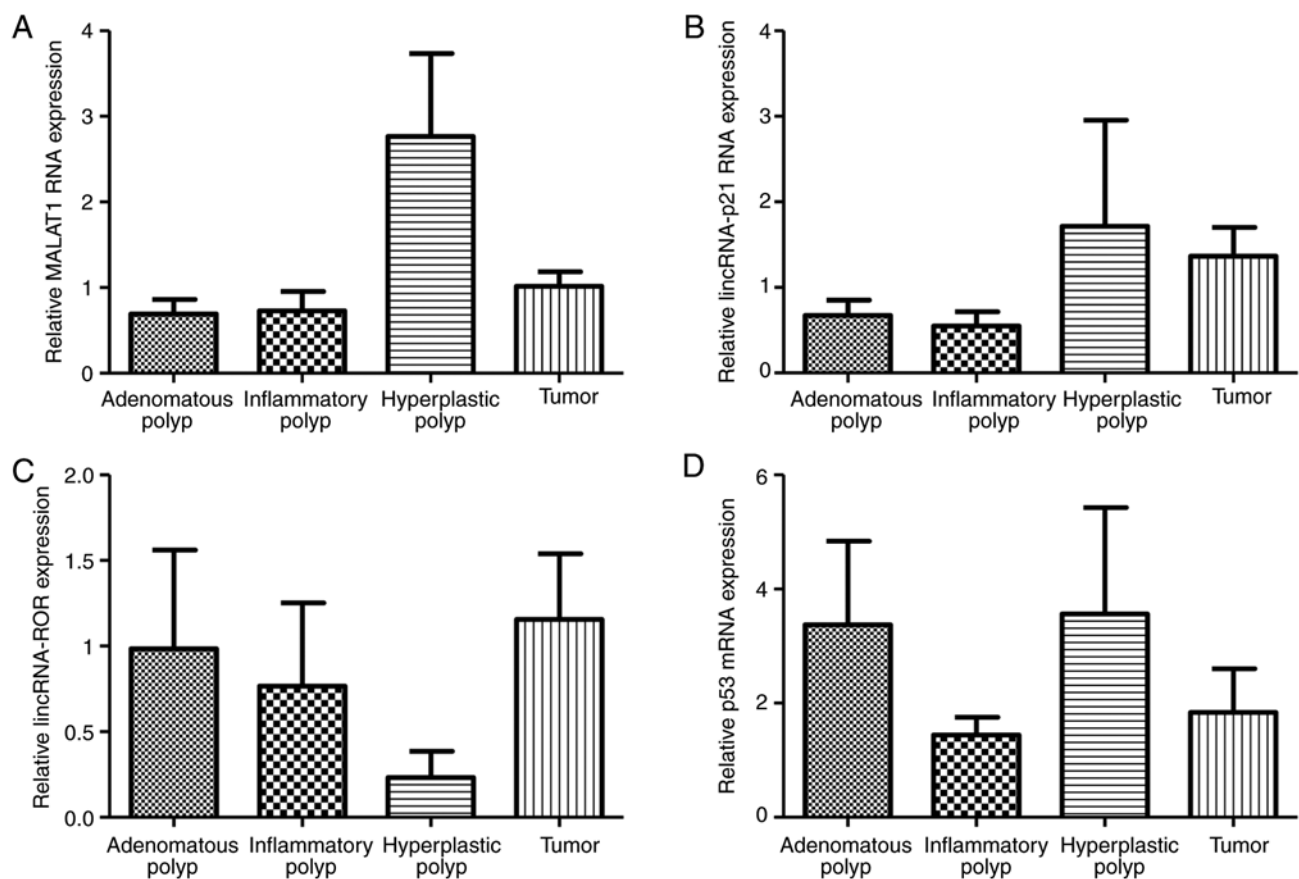

Figure 2. Relative mRNA expression between the different types of polyp (adenomatous polyp, inflammatory polyp and hyperplastic polyp) and colon cancer tissues. (A) Relative expression of MALAT1 between the different types of polyp and colon cancer tissues (P=0.0028). (B) Relative expression of lincRNA-p21 between the different types of polyp and colon cancer tissues (P=0.4402). (C) Relative expression of lincRNA-ROR between the different types of polyp and colon cancer tissues (P=0.5809). (D) Relative expression of p53 between the different types of polyp and colon cancer tissues (P=0.5837). MALAT1, metastasis-associated lung adenocarcinoma transcript 1; lincRNA, long intergenic non-coding RNA; ROR, regulator of reprogramming; p53, tumor protein 53.

history of colon cancer. In 16 patients (61.5%), the site of the cancer was the colon, whereas in 10 others (38.5%), it was the rectum. In 12, 10 and 4 patients, the tumor grade was determined as II, I and III, respectively. The tumor stage was III, I and II in 12, 10 and 4 cases, respectively. In 18 (69.2%) of the cases, the size of the tumor was ≥5 cm and 5 (19.2%) and 6 (23.1%) patients underwent radiotherapy and adjuvant therapy, respectively. The cancer patients' data were limited than those of the polyp patients, including BMI, consumed alcohol, constipation, bleeding from the anus, anemia, diarrhea and weight loss.

Expression of lncRNAs in the samples of patients. In order to investigate the expression of MALAT1, lincRNA-p21, lincRNA-ROR and p53 genes in the colon cancer tissues, a qPCR analysis was performed on 66 neoplastic and adjacent non-neoplastic tissues (46 with polyps and 20 with tumor tissue). The results indicated that there were no significant differences in the expression of MALAT-1, lincRNA-p21, lincRNA-ROR and p53 between the aforementioned tissues (Fig. 1A-D). The extent of the gene expression of the lncRNAs was compared between the polyp (adenomatous, inflammatory and hyperplastic types) and colon cancer tissues.

Table II. Association between clinicopathological characteristics and MALAT1, lincRNA-ROR and lincRNA-P21 expression in the 46 patients with colon polyps.

Variables	MALAT1			LncRNA-ROR			LncRNA-p21			p53		
	Low, n (%)	High, n (%)	P-value	Low, n (%)	High, n (%)	P-value	Low, n (%)	High, n (%)	P-value	Low, n (%)	High, n (%)	P-value
Age, years[a]			0.939			0.112			0.191			0.957
<50	12 (41.4)	4 (40.0)		15 (46.9)	1 (14.3)		14 (46.7)	2 (22.2)		5 (41.7)	11 (40.7)	
≥50	17 (58.6)	6 (60.0)		17 (53.1)	6 (85.7)		16 (53.3)	7 (77.8)		7 (58.3)	16 (59.3)	
BMI[a]			0.608			0.313			0.489			0.656
Underweight, ≤18.5	1 (5.0)	1 (16.7)		1 (4.3)	1 (33.3)		1 (4.8)	1 (20.0)		1 (16.7)	1 (5.0)	
Normal weight, 18.6-24.9	6 (30.0)	1 (16.7)		6 (26.1)	1 (33.3)		5 (23.8)	2 (40.0)		2 (33.3)	5 (25.0)	
Overweight, 25-29.9	11 (55.0)	4 (66.7)		14 (60.9)	1 (33.3)		13 (61.9)	2 (40.0)		3 (50.0)	12 (60.0)	
Obese, ≥30	2 (10.0)	-		2 (8.7)	-		2 (9.5)	-		-	2 (10.0)	
Sex			0.619			0.207			0.403			0.403
Male	17 (50.0)	7 (58.3)		21 (56.8)	3 (33.3)		18 (56.3)	6 (42.9)		6 (42.9)	18 (56.3)	
Female	17 (50.0)	5 (41.7)		16 (43.2)	6 (66.7)		14 (43.8)	8 (57.1)		8 (57.1)	14 (43.8)	
Smoking[a]			0.330			0.639			0.847			0.781
Yes	8 (29.6)	1 (12.5)		7 (24.1)	2 (33.3)		7 (25.0)	2 (28.6)		2 (22.2)	7 (26.9)	
No	19 (70.4)	7 (87.5)		22 (75.9)	4 (66.7)		21 (75.0)	5 (71.4)		7 (77.8)	19 (73.1)	
Ethnicity[a]			0.228			0.654			0.644			0.626
Turkish	2 (7.4)	1 (12.5)		3 (10.3)	-		2 (7.1)	1 (14.3)		-	3 (11.5)	
Persian	22 (81.5)	5 (62.5)		22 (75.9)	5 (83.3)		21 (75.0)	6 (85.7)		7 (77.8)	20 (76.9)	
Lurs	1 (3.7)	2 (25.0)		2 (6.9)	1 (16.7)		3 (10.7)	-		1 (11.1)	2 (7.7)	
Kurd	2 (7.4)	-		2 (6.9)	-		2 (7.1)	-		1 (11.1)	1 (3.8)	
Education[a]			0.865			0.817			0.865			0.492
Academic	13 (46.4)	3 (42.9)		13 (44.8)	3 (50.0)		13 (46.4)	3 (42.9)		5 (55.6)	11 (42.3)	
Non-academic	15 (53.6)	4 (57.1)		16 (55.2)	3 (50.0)		15 (53.6)	4 (57.1)		4 (44.4)	15 (57.7)	
Constipation			0.902			0.497			0.447			0.152
Yes	12 (35.3)	4 (33.3)		12 (32.4)	4 (44.4)		10 (31.3)	6 (42.9)		7 (50.0)	9 (28.1)	
No	22 (64.7)	8 (66.7)		25 (67.6)	5 (55.6)		22 (68.8)	8 (57.1)		7 (50.0)	23 (71.9)	
Diarrhea			0.768			0.099			0.833			0.308
Yes	7 (20.6)	2 (16.7)		9 (24.3)	-		6 (18.8)	3 (21.4)		4 (28.6)	5 (15.6)	
No	27 (79.4)	10 (83.3)		28 (75.7)	9 (100.0)		26 (81.3)	11 (78.6)		10 (71.4)	27 (84.4)	
Anemia			0.190			0.389			0.418			0.129
Yes	9 (26.5)	1 (8.3)		9 (24.3)	1 (11.1)		8 (25.0)	2 (14.3)		5 (35.7)	5 (15.6)	
No	25 (73.5)	11 (91.7)		28 (75.7)	8 (88.9)		24 (75.0)	12 (85.7)		9 (64.3)	27 (84.4)	
Weight loss			0.419			0.159			0.713			0.633
Yes	5 (14.7)	3 (25.0)		5 (13.5)	3 (33.3)		6 (18.8)	2 (14.3)		3 (21.4)	5 (15.6)	
No	29 (85.3)	9 (75.0)		32 (86.5)	6 (66.7)		26 (81.3)	12 (85.7)		11 (78.6)	27 (84.4)	

Table II. Continued.

Variables	MALAT1 Low, n (%)	MALAT1 High, n (%)	MALAT1 P-value	LncRNA-ROR Low, n (%)	LncRNA-ROR High, n (%)	LncRNA-ROR P-value	LncRNA-p21 Low, n (%)	LncRNA-p21 High, n (%)	LncRNA-p21 P-value	p53 Low, n (%)	p53 High, n (%)	p53 P-value
Bleeding from the anus			0.171			0.959			0.080			0.699
Yes	13 (38.2)	2 (16.7)		12 (32.4)	3 (33.3)		13 (40.6)	2 (14.3)		4 (28.6)	11 (34.4)	
No	21 (61.8)	10 (83.3)		25 (67.6)	6 (66.7)		19 (59.4)	12 (85.7)		10 (71.4)	21 (65.6)	
Family history of colon polyps[a]			0.958			0.639			0.246			0.136
Yes	7 (25.9)	2 (25.0)		7 (24.1)	2 (33.3)		6 (21.4)	3 (42.9)		4 (44.4)	5 (19.2)	
No	20 (74.1)	6 (75.0)		22 (75.9)	4 (66.7)		22 (78.6)	4 (57.1)		5 (55.6)	21 (80.8)	
Consumption of vegetables			0.126			0.976			0.509			0.509
Low	12 (46.2)	1 (14.3)		11 (39.3)	2 (40.0)		11 (42.3)	2 (28.6)		2 (28.6)	11 (42.3)	
High	14 (53.8)	6 (85.7)		17 (60.7)	3 (60.0)		15 (57.7)	5 (71.4)		5 (71.4)	15 (57.7)	
Consumption of red meat[a]			0.943			0.743			0.943			0.943
Low	4 (15.4)	1 (14.3)		4 (14.3)	1 (20.0)		4 (15.4)	1 (14.3)		1 (14.3)	4 (15.4)	
High	22 (84.6)	6 (85.7)		24 (85.7)	4 (80.0)		22 (84.6)	6 (85.7)		6 (85.7)	22 (84.6)	
Consumption of soda[a]			0.641			0.516			0.641			0.390
Low	13 (50.0)	4 (57.1)		15 (53.6)	2 (40.0)		13 (50.0)	4 (57.1)		2 (28.6)	15 (57.7)	
Moderate	3 (11.5)	-		3 (10.7)	-		3 (11.5)	-		1 (14.3)	2 (7.7)	
High	10 (38.5)	3 (42.9)		10 (35.7)	3 (60.0)		10 (38.5)	3 (42.9)		4 (57.1)	9 (34.6)	
Consumption of fast food[a]			0.385			0.605			0.346			0.922
Low	18 (69.2)	3 (42.9)		17 (60.7)	4 (80.0)		15 (57.7)	6 (85.7)		4 (57.1)	17 (65.4)	
Moderate	3 (11.5)	1 (14.3)		4 (14.3)	-		4 (15.4)	-		1 (14.3)	3 (11.5)	
High	5 (19.2)	3 (42.9)		7 (25.0)	1 (20.0)		7 (26.9)	1 (14.3)		2 (28.6)	6 (23.1)	
Exercise[a]			0.279			0.976			0.833			0.126
Yes	17 (65.4)	3 (42.9)		17 (60.7)	3 (60.0)		16 (61.5)	4 (57.1)		6 (85.7)	14 (53.8)	
No	9 (34.6)	4 (57.1)		11 (39.3)	2 (40.0)		10 (38.5)	3 (42.9)		1 (14.3)	12 (46.2)	
Type of polyp			0.024			0.409			0.434			0.727
Adenomatous	19 (82.6)	4 (17.4)		17 (73.9)	6 (26.1)		14 (60.9)	9 (39.1)		6 (26.1)	17 (73.9)	
Hyperplastic	6 (46.2)	7 (53.8)		12 (92.3)	1 (7.7)		10 (76.9)	3 (23.1)		4 (30.8)	9 (69.2)	
Inflammatory	9 (90.0)	1 (10.0)		8 (80.0)	2 (20.0)		8 (80.0)	2 (20.0)		4 (40.0)	6 (60.0)	
Location of polyp[a]			0.542			0.170			0.098			0.439
Cecum	2 (6.1)	1 (9.1)		2 (5.7)	1 (11.1)		2 (6.5)	1 (7.7)		2 (14.3)	1 (3.3)	
Ascending colon	5 (15.2)	1 (9.1)		3 (8.6)	3 (33.3)		2 (6.5)	4 (30.8)		1 (7.1)	5 (16.7)	
Transverse colon	8 (24.2)	1 (9.1)		9 (25.7)	-		9 (29.0)	-		2 (14.3)	7 (23.3)	
Descending colon	5 (15.2)	4 (36.4)		8 (22.9)	1 (11.1)		6 (19.4)	3 (23.1)		2 (14.3)	7 (23.3)	
Recto-sigmoid	13 (39.4)	4 (36.4)		13 (37.1)	4 (44.4)		12 (38.7)	5 (38.5)		7 (50.0)	10 (33.3)	

Table II. Continued.

Variables	MALAT1			LncRNA-ROR			LncRNA-p21			p53		
	Low, n (%)	High, n (%)	P-value	Low, n (%)	High, n (%)	P-value	Low, n (%)	High, n (%)	P-value	Low, n (%)	High, n (%)	P-value
Number of polyps			0.022			0.999			0.613			0.199
≤5	26 (76.5)	5 (41.7)		25 (67.6)	6 (66.7)		21 (65.6)	10 (71.4)		12 (85.7)	19 (59.4)	
5-10	4 (11.8)	6 (50.0)		8 (21.6)	2 (22.2)		7 (21.9)	3 (21.4)		1 (7.1)	9 (28.1)	
≥10	4 (11.8)	1 (8.3)		4 (10.8)	1 (11.1)		4 (12.5)	1 (7.1)		1 (7.1)	4 (12.5)	
Size of polyp[a]			0.563			0.225			0.537			0.836
≤5 mm	5 (20.0)	3 (37.5)		7 (25.9)	1 (16.7)		6 (22.2)	2 (33.3)		2 (22.2)	6 (25.0)	
5-10 mm	10 (40.0)	3 (37.5)		12 (44.4)	1 (16.7)		10 (37.0)	3 (50.0)		3 (33.3%)	10 (41.7%)	
≥10 mm	10 (40.0)	2 (25.0)		8 (29.6)	4 (66.7)		11 (40.7)	1 (16.7)		4 (44.4)	8 (33.3)	

[a]Data missing in 46 patients with colon polyp. MALAT1, metastasis-associated lung adenocarcinoma transcript 1; lincRNA, long intergenic non-coding RNA; ROR, regulator of reprogramming; p53, tumor protein p53; BMI, body mass index.

The level of expression of the MALAT1 gene revealed a statistically significant difference between the different polyp types and the tumor tissue (P=0.0028; Fig. 2A). However, there was no statistically significant difference identified in the expression of lincRNA-p21, lincRNA-RoR and p53 mRNA between the polyp and colon cancer tissues (P>0.05; Fig. 2B-D). Patients were divided into two groups, consisting of colon cancer tissue and colon polyps, and the association between the MALAT1, lincRNA-ROR and lincRNA-P21 genes was evaluated (Table I).

Associations between the expression of lncRNAs and clinical characteristics. In order to further evaluate the role of MALAT1, lincRNA-p21, lincRNA-RoR and p53 in colon cancer and polyps, the associations between the transcript levels of the genes and several clinicopathological features were also investigated (Tables II and III).

The MALAT-1 relative expression groups demonstrated a statistically significant difference between the polyp type and polyp number (Table II). The statistical analyses between these two groups revealed a significant association between the p53 transcript levels and family history (P=0.018). LincRNA-ROR and lincRNA-p21 expression was significantly associated with adjuvant therapy (P=0.015 and 0.038, respectively; Table III). No significant associations were identified between the transcript level groups and other clinicopathological variables (Tables II and III).

Relative expression of lncRNAs and p53 in individual samples. In order to determine whether any association was present between the expression of the LncRNAs and the p53 gene, the relative expression of these genes was compared in each set of the samples. A significant association was observed between the levels of MALAT1 and p53 in neoplastic tissues (R=0.073; P=0.034; Fig. 3A), but there was no significant association between the levels of lincRNA-ROR and p53 (R=0.006; P=0.56; Fig. 3B), or lincRNA-p21 and p53 (R=0.015; P=0.32; Fig. 3C).

Evaluation of MALAT-1, lincRNA-p21, lincRNA-ROR and p53 in neoplastic tissue as predictive CRC-associated biomarkers. To investigate the characteristics of MALAT-1, lincRNA-p21, lincRNA-ROR and p53 as potential biomarkers for CRC, the ROC curves and the area under the ROC curves (AUC) were generated and calculated for 66 samples from patients with CRC and healthy adjacent tissues. The ROC curves indicated a strong separation between the patients with CRC and the healthy adjacent tissue group, with an AUC of 0.603 [95% confidence interval (CI), 0.501-0.706; P<0.05] for MALAT1, 0.685 (95% CI, 0.595-0.775; P<0.001) for lincRNA-p21, 0.796 (95% CI, 0.723-0.868; P<0.001) for lincRNA-ROR and 0.643 (95% CI, 0.542-0.744; P<0.05) for p53 (Fig. 4A-D; Table IV).

Discussion

Colonoscopy is the gold standard method for the diagnosis of colon cancer, but the technique is invasive and expensive (22). Early detection of colorectal neoplasms is important since it decreases mortality and increases the survival rate in patients with CRC (23). Identifying a biomarker to detect cancer in

Table III. Association between clinicopathological characteristics and MALAT1, lincRNA-ROR and lincRNA-P21 expression in 26 patients with colon cancer tissue.

Variables	MALAT1 Low, n (%)	MALAT1 High, n (%)	P-value	LncRNA-ROR Low, n (%)	LncRNA-ROR High, n (%)	P-value	LncRNA-p21 Low, n (%)	LncRNA-p21 High, n (%)	P-value	p53 Low, n (%)	p53 High, n (%)	P-value
Age, years			0.664			0.877			0.352			0.940
<50	3 (20.0)	3 (27.3)		4 (22.2)	2 (25.0)		2 (15.4)	4 (30.8)		2 (22.2)	4 (23.5)	
≥50	12 (80.0)	8 (72.7)		14 (77.8)	6 (75.0)		11 (84.6)	9 (69.2)		7 (77.8)	13 (76.5)	
Sex			0.597			0.620			1			0.492
Male	4 (26.7)	4 (36.4)		5 (27.8)	3 (37.5)		4 (30.8)	4 (30.8)		2 (22.2)	6 (35.3)	
Female	11 (73.3)	7 (63.6)		13 (72.2)	5 (62.5)		9 (69.2)	9 (69.2)		7 (77.8)	11 (64.7)	
Ethnicity[a]			0.592			0.086			0.462			0.293
Turkish	3 (27.3)	3 (37.5)		2 (16.7)	4 (57.1)		2 (25.0)	4 (36.4)		2 (28.6)	4 (33.3)	
Persian	6 (54.5)	3 (37.5)		8 (66.7)	1 (14.3)		4 (50.0)	5 (45.5)		5 (71.4)	4 (33.3)	
Lurs	2 (18.2)	1 (12.5)		1 (8.3)	2 (28.6)		1 (12.5)	2 (18.2)		-	3 (25.0)	
Kurd	-	1 (12.5)		1 (8.3)	-		1 (12.5)	-		-	1 (8.3)	
Family history of colon cancer			0.261			0.619			0.135			0.018
Yes	4 (26.7)	1 (9.1)		3 (16.7)	2 (25.0)		1 (7.7)	4 (30.8)		4 (44.4)	1 (5.9)	
No	11 (73.3)	10 (90.9)		15 (83.3)	6 (75.0)		12 (92.3)	9 (69.2)		5 (55.6)	16 (94.1)	
Tumor site			0.851			0.420			1			0.648
Colon	9 (60.0)	7 (63.6)		12 (66.7)	4 (50.0)		8 (61.5)	8 (61.5)		5 (55.6)	11 (64.7)	
Rectum	6 (40.0)	4 (36.4)		6 (33.3)	4 (50.0)		5 (38.5)	5 (38.5)		4 (44.4)	6 (35.3)	
Grade			0.121			0.528			0.255			0.771
I	6 (40.0)	4 (36.4)		8 (44.4)	2 (25.0)		6 (46.2)	4 (30.8)		3 (33.3)	7 (41.2)	
II	5 (33.3)	7 (63.6)		7 (38.9)	5 (62.5)		4 (30.8)	8 (61.5)		5 (55.6)	7 (41.2)	
III	4 (26.7)	-		3 (16.7)	1 (12.5)		3 (23.1)	1 (7.7)		1 (11.1)	3 (17.6)	
Stage			0.338			0.057			0.231			0.771
I	6 (40.0)	4 (36.4)		9 (50.0)	1 (12.5)		7 (53.8)	3 (23.1)		3 (33.3)	7 (41.2)	
II	1 (6.7)	3 (27.3)		1 (5.6)	3 (37.5)		2 (15.4)	2 (15.4)		1 (11.1)	3 (17.6)	
III	8 (53.3)	4 (36.4)		8 (44.4)	4 (50.0)		4 (30.8)	8 (61.5)		5 (55.6)	7 (41.2)	
Size of tumor			0.165			0.671			0.395			0.492
<5 cm	3 (20.0)	5 (45.5)		6 (33.3)	2 (25.0)		5 (38.5)	3 (23.1)		2 (22.2)	6 (35.3)	
≥5 cm	12 (80.0)	6 (54.5)		12 (66.7)	6 (75.0)		8 (61.5)	10 (76.9)		7 (77.8)	11 (64.7)	
Radiotherapy			0.058			0.619			0.619			0.778
Yes	1 (6.7)	4 (36.4)		3 (16.7)	2 (25.0)		2 (15.4)	3 (23.1)		2 (22.2)	3 (17.6)	
No	14 (93.3)	7 (63.6)		15 (83.3)	6 (75.0)		11 (84.6)	10 (76.9)		7 (77.8)	14 (82.4)	

Table III. Continued.

Variables	MALAT1 Low, n (%)	MALAT1 High, n (%)	P-value	LncRNA-ROR Low, n (%)	LncRNA-ROR High, n (%)	P-value	LncRNA-p21 Low, n (%)	LncRNA-p21 High, n (%)	P-value	p53 Low, n (%)	p53 High, n (%)	P-value
Adjuvant therapy[a]			0.407			0.015			0.038			0.760
Yes	4 (36.4)	2 (20)		2 (13.3)	4 (66.7)		1 (9.1)	5 (50.0)		2 (33.3)	4 (26.7)	
No	7 (63.6)	8 (80.0)		13 (86.7)	2 (33.3)		10 (90.9)	5 (50.0)		4 (66.7)	11 (73.3)	

[a]Data missing in 26 patients with colorectal cancer. MALAT1, metastasis-associated lung adenocarcinoma transcript 1; lincRNA, long intergenic non-coding RNA; ROR, regulator of reprogramming; p53, tumor protein p53.

the early stages is, therefore, a major objective in this field. Although 70% of the human genome is transcribed into RNA, only a limited amount of RNAs encode proteins (24). The majority of lncRNAs have conserved sequences that lead to the concept that suggests lncRNA networks may possess a significant function in biological processes (25). For example, lncRNA regulation is involved in CRC progression, proliferation, apoptosis, differentiation, invasion and metastasis (26). The lncRNA function and expression patterns have been reported to be different in different cell types (27). Therefore, it is important to investigate lncRNA in the colon polyp and tumor to identify the potential biomarkers that can detect the disease in the early stages. More importantly, the molecular function and the biology of p53-regulated lncRNAs should be determined in the colorectal neoplastic tissue. In the present study, the expression of MALAT1, lincRNA-p21 and lincRNA-ROR was determined from the network of p53-regulated LncRNAs, as well as the clinicopathological features of colon polyps and tumor tissues. The results indicated that lincRNA-ROR expression did not reveal any association with radiotherapy sensitivity, but that a significant association did exist between the higher expression of lincRNA-ROR in patients receiving adjuvant therapy. According to a previous study by Yang *et al* (28) the knockdown of lincRNA-ROR improved sensitivity to radiotherapy in patients with CRC by preventing cell viability and promoting apoptosis. Further studies should be performed on the potential post-transcriptional regulatory mechanisms, with treatments other than radiotherapy. Indeed, previous studies have indicated that lincRNA-ROR did not regulate p53 protein levels in unstressed cells (from intracellular or extracellular stresses, e.g., DNA damage) and increased the level of lincRNA-ROR-suppressed p53; on the other hand, p53 activation can influence lincRNA-ROR expression by an autoregulatory negative feedback loop, which maintains cellular homeostasis (14,15). According to the results of the present study, there was no significant association between the expression of p53 and lincRNA-ROR in the colorectal neoplasm tissue; therefore, the present study hypothesized that this inconsistency could be due to the pathological diversity of the tissues of the patients. Gene expression analysis revealed no significant alteration of MALAT1 in the colon neoplastic tissue in comparison with the adjacent normal tissue. However, the present results demonstrate that the MALAT1 level was higher in patients with hyperplastic polyps when compared with those with other types of polyps and tumors. In addition, a association between MALAT1 expression and number of polyps was observed in the present study. MALAT1 is a functional lncRNA that was first reported in the invasive non-small cell carcinoma and overexpressed in a number of other types of cancer tissue, indicating that MALAT1 was associated with hyperproliferation, invasion and metastasis (15,29-31). To the best of our knowledge, however, the present study is the first to investigate the association between the MALAT1 RNA level and the different types of colon polyp and clinicopathological features.

CRC cases frequently progress from non-cancerous growths, called polyps, to malignant adenocarcinomas and distant metastases; therefore, CRC is known as a 'silent' disease, whose early diagnosis may aid the initiation of early

Table IV. Diagnosis value between neoplastic tissue and adjacent tissue.

Variables	MALAT1	LincRNA-ROR	LincRNA-p21	p53
Cut-off	-1.4142	-2.9084	-1.3672	-0.2797
Specificity, %	75	81.9	77.5	67.2
Sensitivity, %	50	63.9	59.2	53.4
AUC	0.603	0.796	0.685	0.643
95% CI	0.501±0.706	0.723±0.867	0.595±0.775	0.542±0.744

MALAT1, metastasis-associated lung adenocarcinoma transcript 1; lincRNA, long intergenic non-coding RNA; ROR, regulator of reprogramming; p53, tumor protein p53; AUC, area under the curve; CI, confidence interval.
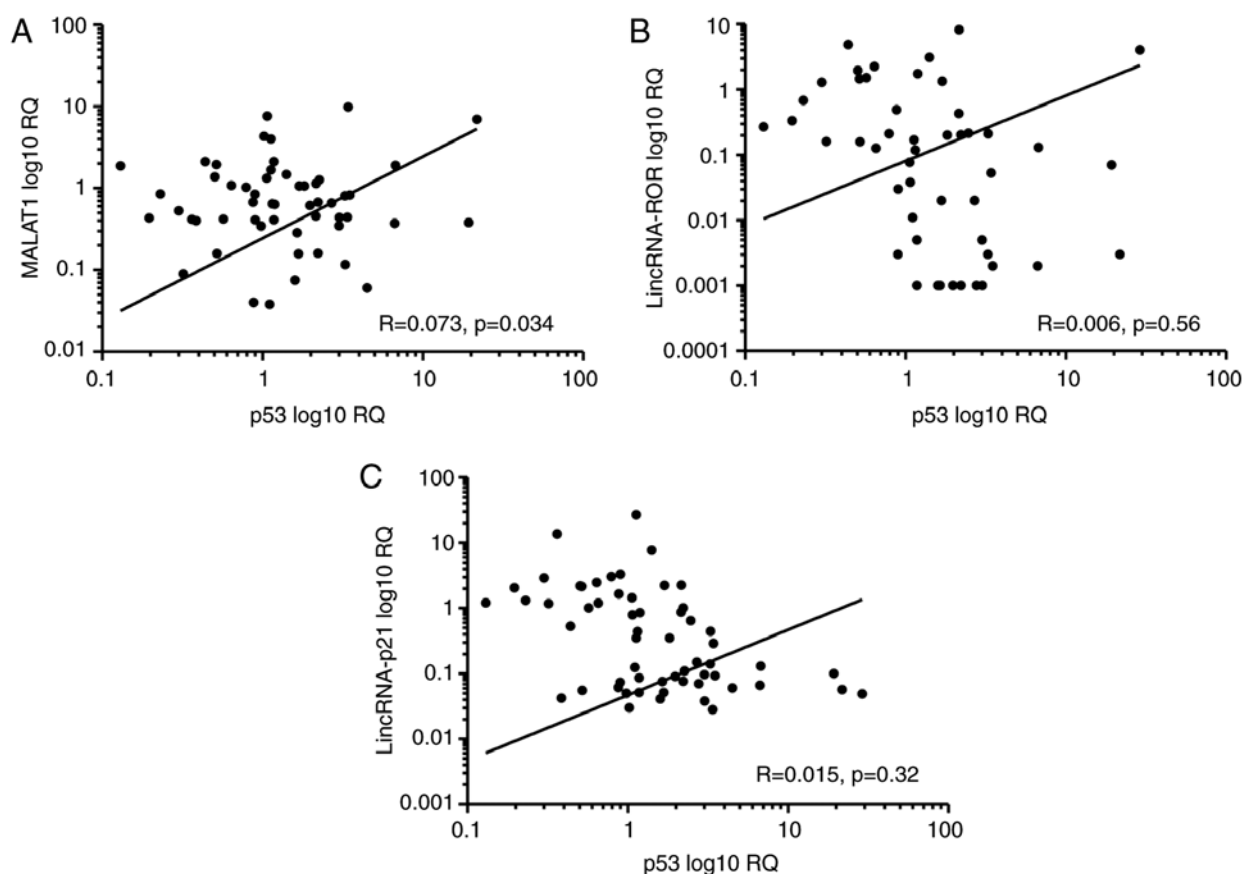
Figure 3. Association analysis between expression of each gene in tumor tissues compared with adjacent non-tumor tissues. (A) Association between p53 and MALAT1 expression in tumor tissues compared with adjacent non-tumor tissues. (B) Association between p53 and lincRNA-ROR expression in tumor tissues compared with adjacent non-tumor tissues. (C) Association between p53 and lincRNA-p21 expression in tumor tissues compared with adjacent non-tumor tissues. Association analyses were performed using a linear regression. The extent of RNA expression is presented as log10 transformed values. The R- and P-values are presented for each analysis. MALAT1, metastasis-associated lung adenocarcinoma transcript 1; lincRNA, long intergenic non-coding RNA; ROR, regulator of reprogramming; p53, tumor protein 53; RQ, relative quantification.

treatment and increase survival rates (32). The results of the present study provide further evidence of the importance of MALAT1 as an early CRC detector and as a prognostic marker. In neoplastic tissues, the analysis revealed that there was an association between the expression of MALAT1 and the p53 gene. Several studies support the potential role of MALAT1 in regulating cell proliferation, invasion and tumor formation in different types of tumor (33,34). According to a previous study by Tripathi *et al* (16), the destruction of MALAT1 led to the activation of p53 and its

targets. Nevertheless, the study of Tripathi *et al* (16) indicated that when MALAT1 was depleted in the HeLa, U2OS and WI-38-VA13 tumorigenic cell lines, possessing poor functional of p53, tumor suppressor p16 and retinoblastoma protein, the cells did not undergo G1 or G1/S arrest, and exhibited normal S-phase progression. Their data indicate cell cycle defects and the downregulation of the E2F target genes on MALAT1 depletion occurs only in specific cell lines (e.g., human dermal fibroblasts) (16). With regard to the function of MALAT1 in specific cells, the present study
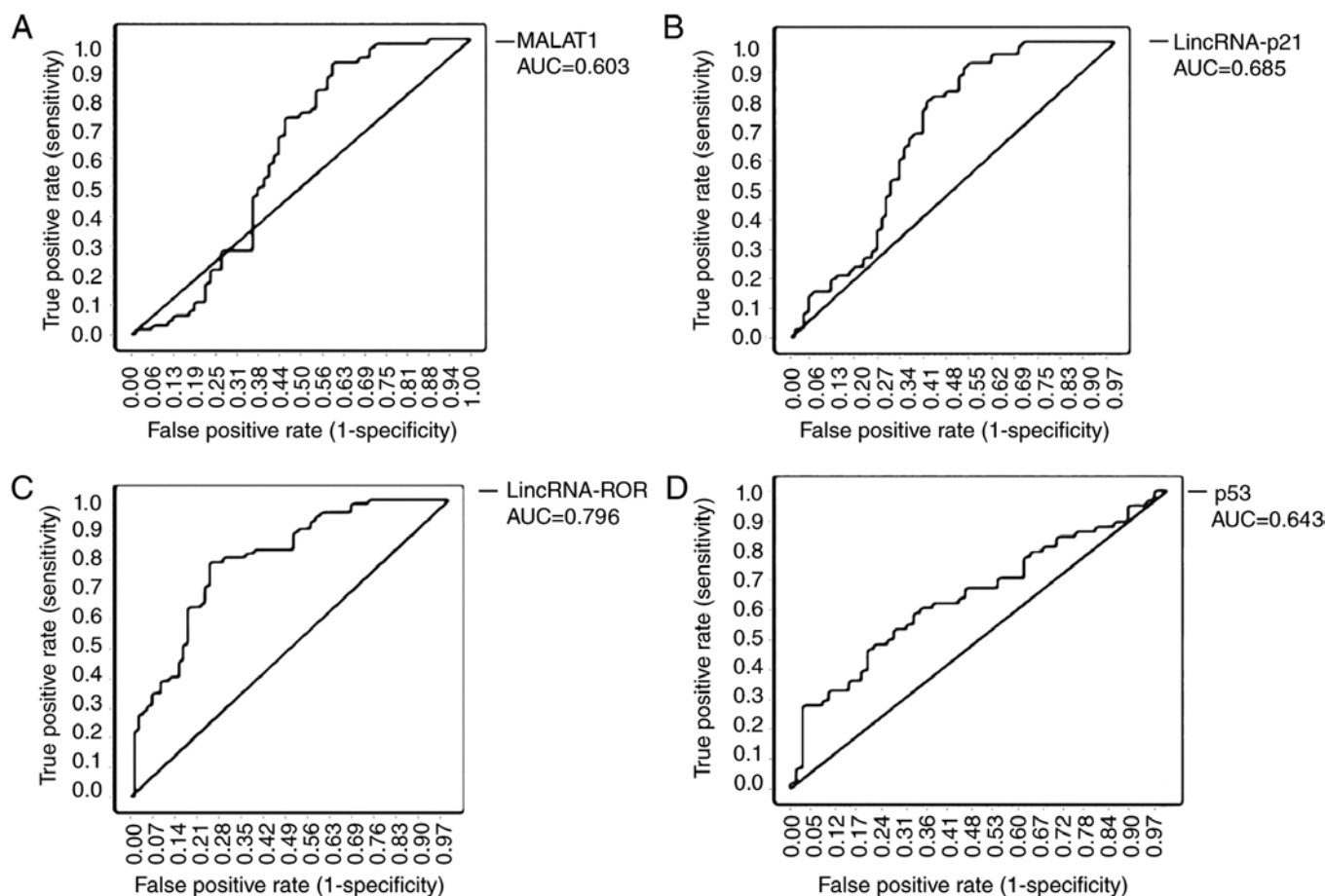
Figure 4. ROC curves of normalized lincRNA-p21, lincRNA-ROR, MALAT1 and p53 to distinguish primary lesion/tumor tissue from the adjacent normal tissue. (A) ROC curve of normalized MALAT1. (B) ROC curve of normalized lincRNA-p21. (C) ROC curve of normalized lincRNA-ROR. (D) ROC curve of normalized p53. The AUC was determined for each gene type and is presented in each graph. ROC, receiver-operating characteristic; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; lincRNA, long intergenic non-coding RNA; ROR, regulator of reprogramming; p53, tumor protein 53; AUC, area under the curve.

hypothesized that an interaction between MALAT1 and p53 exists in colorectal neoplasms.

Previous studies have assessed the expression of lincRNA-p21 with various types of cancer (18,35,36). However, few studies report the influence of lincRNa-p21 on CRC. In addition, further studies revealed that lincRNA-p21 was associated with malignancy progression, and contributed to the treatment and prognosis of the tumor (18,37,38). The results of the present study indicated that the relative expression of lincRNA-p21 was significantly associated with adjuvant therapy in the CRC tissue. A recent independent study by Wang *et al* (39), revealed that the increase in lincRNA-p21 inhibited the stability and/or translation of β-catenin. The inhibition of β-catenin leads to the suppression of the Wnt signaling pathway, which promotes cell apoptosis and increases the radiosensitivity for CRC (39). Another study by Zhao *et al* (40) indicated that lincRNA-p21 levels were significantly increased following surgical treatment in comparison with the time prior to surgery. Despite these results, further studies with a higher sample size are required to investigate the mechanism of this association between treatment models and the aberrant expression of lincRNA-P21.

In conclusion, the results of the present study suggest an interaction between lincRNA-ROR, MALAT1, lncRNA-p21 and certain clinicopathological features, which appear to serve an important role in tumorigenesis, development and influencing the response of cancer cells in treatments.

The results of the present study identified the association between the aberrant expression of lincRNA-p21 and lincRNA-ROR and adjuvant therapy in the CRC tissue. In addition, a association was observed between the MALAT1 level and the type of colon polyp, as well as the number of polyps in the patients. The results of the present study provide further evidence towards the importance of MALAT1 as both an early CRC detector and as a prognostic marker. Low sample size in this cross-sectional study affected the association, and thus, further studies on the same groups of patients are required in order to prove increased lncRNA levels in association with the increased risk of CRC development.

Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Authors' contributions

HAA, SI and VC conceived and designed the study. MA, VC and RM performed the experiments. VC, RM, SI, HAA, and MA contributed to the interpretation of the data. VC, and HAA wrote the original draft of the manuscript. SI, MA and HAA revised the manuscript. HAA acquired funding All authors read and approved the final manuscript.

Ethics approval and consent to participate

Ethical approval was obtained from the Ethics Committee of the Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Science, Tehran, Iran. The protocol conforms with the ethical guidelines of The Declaration of Helsinki (1975).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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