Original Article

Expression and Clinical Significance of Novel Long Noncoding RNA Fibroblast Growth Factor 10AS and FGF10 in Colorectal Cancer

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Abstract

Objective: Colorectal cancer (CRC) imposes great health burdens worldwide. Growth factors contribute to cell growth, differentiation, angiogenesis and, most importantly, tumour formation in many types of cancers. Natural antisense transcripts (NATs) are inclusively predicted to play a major role in cancer progression. The present study aims to evaluate the relationship of fibroblast growth factor 10 (*FGF10*) and novel long noncoding RNA (lncRNA) antisense *FGF10* (*FGF10AS*) expression with clinicopathologic features in CRC progression to designate a biomarker for CRC early detection.

Materials and Methods: This cross-sectional study was conducted on 100 CRC tumour and parallel adjacent normal tissues. We added 30 normal cases to enhance accuracy of the test. The expression levels of *FGF10* and *FGF10AS* were evaluated by real-time polymerase chain reaction (PCR). The findings were validated by measuring expression levels in the HT29 and SW480 cell lines. Immunohistochemistry analysis was performed systematically to evaluate FGF10 protein expression. The Mann-Whitney U test with Cox regression analysis were applied. $P<0.05$ were designated as significant.

Results: A significant increase in expression was observed in *FGF10* ($P<0.001$) along with a significant decrease in *FGF10AS* ($P<0.02$) in the tumour tissues in comparison with the adjacent normal tissues. Upregulation of *FGF10* and downregulation of *FGF10AS* expression were strongly correlated with the Tumour, Node, Metastasis (TNM) stage ($P<0.007$ and $P<0.004$), vascular invasion ($P<0.03$ and $P<0.01$), lymph invasion ($P<0.02$ and $P<0.04$), and differentiation ($P<0.01$ and $P<0.02$), respectively. Moreover, the area under the receiver operating characteristic (ROC) curve for the prognostic value of *FGF10* was about 0.84 (95% confidence interval [CI]: 0.771-0.912). Linear regression analysis confirmed a negative correlation between *FGF10* expression and its antisense transcript ($r=-0.02$).

Conclusion: The relationship between the expression levels of *FGF10* and *FGF10AS* in tumour tissues and adjacent normal tissues indicated that sense and antisense *FGF* RNAs could be remarkable prognostic biomarkers for achieving effective and primitive treatment.

Keywords: Biomarker, Colorectal Cancer, Fibroblast Growth Factor 10, Long Noncoding RNA

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Introduction

Colorectal cancer (CRC) imposes great health burdens worldwide because of its late presentation of clinical features, rapid lymph metastasis, simple recurrence, and tumour growth (1, 2). Following the diagnosis of CRC, the patient's survival is dependent on the stage of this cancer. Five-year survival rates of >90%, 70%, and < 10% have been predicted in patients diagnosed at an early stage, patients with regional extension, and those with metastatic outcomes, respectively (2, 3). Accordingly, the development of diagnostic and prognostic biomarkers for early detection of CRC can help increase the lifespan of patients with CRC (4, 5).

Fibroblast growth factors (FGFs) include complicated intracellular signalling pathways. Generally, heparin/heparan sulphate proteoglycans (HSPGs) bind as a cofactor with ligand/receptor (FGF/FGFR) and stimulate triplex FGF-FGFR-HS complex configuration to initiate phosphorylation of the tyrosine domain. Coupled activated receptors start a signalling cascade of RAS-RAF-MAPK, PI3K-AKT, STAT, and PLC (6-8). Alterations in any of the mentioned FGF signalling pathways result in many

types of cancers. Deregulation can happen at the level of gene/protein expression of ligands or receptors, and lead to transcriptional activity or gene amplification variations (9) that interact with four different FGF receptors (FGFRs) and start a signalling cascade (1, 10). The FGF family is categorised as either intracrine, paracrine, or endocrine according to the mechanism of action (6). The largest FGF family, the paracrine family, is known for its therapeutic potential in angiogenesis, cytoprotection, and tissue repair. Research has shown that paracrine FGFs are overexpressed in cancer and their deregulation enhances proliferation and angiogenesis, which correlate with cancer improvement (7, 11).

FGF10 is deemed to function as an androgen mediator (12, 13). Thus, it can play a role in cancer pathogenesis by promoting epithelial cell proliferation (7). *FGF10* overexpression is correlated with the progression of several cancers, including prostate, breast, and pancreatic cancers (1, 14-16).

According to genome sequencing, DNA sequence transcriptions are estimated for more than 90% of the whole genome sequence and for only around 2% of transcription code proteins. Therefore, most transcriptions are noncoding RNAs (ncRNAs). Long noncoding RNA (lncRNA) is a class of non-protein coding RNA transcriptions that have lengths of more than 200 nucleotides. Research shows that deregulation of lncRNAs is responsible for several biological functions in cancers, including cell proliferation, apoptosis, chemoresistance, and metastasis (17, 18).

Natural antisense transcripts (NATs) are abundant in the human genome and, on average, 38% of the genomic locus in cancer cells express sense-antisense couples. Generally, NATs develop as an alternate strand of the sense strand in numerous protein coding regions, and they frequently overlap with mRNA in regulatory regions and gene promoters (19). NATs may impress their sense transcripts whether by activating or suppressing of targets. Sense and antisense convergency may bring out tail-to-tail transcriptional encountering, since, sense strand suppression precede (20). Moreover, several NATs that are expressed by cancer-related gene loci have compatible expressions with the oncogenic or tumour-suppressor sense participant genes. Previous findings have also indicated that the NAT survival significance in cancer cases implies their potential involvement in the molecular pathology of advanced breast cancer and metastasis (21).

The relevance of *FGF14-AS2* mRNA expression with clinical characteristics and prognostic value of *FGF14-AS2* was calculated in breast cancer. It was deduced that *FGF14-AS2* is a contingent novel marker that can be used as an object for breast cancer treatment. The recognition of cancer-correlated lncRNAs and consideration of related biological roles could reveal a new horizon of cancer progression and formation. Hence, biomarker prospect generation is an immediate action that should be under consideration for the patients' cancer classification and adequate therapy to prevent over or under treatment (22).

The performance of novel *FGF10AS* against a related sense gene, *FGF10,* and its functional role as a prognostic biomarker for CRC remains unknown. Therefore, we intended to compare the expression levels of *FGF10* and linked lncRNA *FGF10AS* in CRC tissues and adjacent normal tissues. We investigated their relationships with clinicopathologic features in 100 selected patients to determine possible beneficial targets for early detection of CRC and lessen the mortality rate of this cancer.

Materials and Methods

Sample preparation

In this cross-sectional investigation, fresh surgical samples from 100 patients diagnosed with CRC were selected among 500 fresh samples of patients who underwent surgery at Taleghani Hospital and Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences (Tehran, Iran) from 2016 to 2019.

A total of 30 normal cases that had no symptoms of CRC were assessed to improve the validity of the tests. Written informed consents were received from all participants before the samples were categorised. The informed consent process was conducted in accordance with the Declaration of Helsinki (23) and the study was approved by the Institutional Research Ethics Committee of Taleghani Hospital (IR.SBMU.RIGLD.REC.1396.180).

Selected patients who entered the study were diagnosed with adenocarcinoma and referred for surgery. Clinical data and pathology reports were available and none of the patients had received chemotherapy or radiotherapy prior to surgery. Qualification of the specimen was also considered. The samples were placed in liquid nitrogen immediately after tumour resection and maintained until use for RNA extraction. Validation of the diagnosis cohort was confirmed by two experienced pathologists. The tumour, node, metastasis (TNM) stage was determined according to the guidelines of the Seventh American Joint Committee on Cancer and Union for International Cancer Control (1).

Cell culture

The SW480 and HT29 cell lines were purchased from the National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran. The cells were preserved in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Germany) that consisted of foetal bovine serum 10% (Gibco, Germany), penicillin (107 U/L), and streptomycin (10 mg/L) at 37°C in a humidified chamber with 5% CO_2. Cell lines were seeded in 75 cm^2 flasks at 2.4×10^4 cells per cm^2. The

flask medium was changed every two days beginning the day after seeding. The intended outcome was 80-90% confluency at day 6-7 of culture (24). Passage-3 cultured cell lines were used for extraction.

RNA extraction and real-time quantitative polymerase chain reaction assay

The pathologically confirmed tissues and cultured cell lines underwent RNA extraction with TRIzol (Life Technologies, Paisley, UK) according to a conventional procedure in the user's manual. Briefly, homogenisation of the tissue samples was performed in 1 mL of TRIzol reagent, after which 0.2 mL of chloroform was added to extract the proteins. The extracted RNA was precipitated with 0.5 mL of isopropyl alcohol and quantified by a NanoDrop spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA) after dissolving in distilled water (dH_2O) (25). Integrity of the RNA was confirmed using Qiaxcel (Qiagen, Germany).

cDNA synthesis was done with 500 ng of mRNA and a Takara Kit (PrimeScript™ RT Reagent Kit, China). The real-time quantitative polymerase chain reaction **(**RT-PCR) assay was performed using 75 ng of synthesized cDNA, 10 µL of 2× SYBR Green Premix Ex Taq™ (Takara, China), and 200 nM of forward and reverse primers in a LightCycler® instrument (Roche Diagnostics, Germany), according to the manufacturer's protocols. *β-globin* and *GAPDH* were used as internal controls. The expressions of specific primers for *FGF10* and *FGF10* antisense were determined for RT-PCR with Primer 3 and an IDT analyser. Table 1 lists the primer sequences and their RT-PCR product lengths.

The primer efficiency was determined by LinRegPCR software, which indicated approximately 95% efficiency for each primer. In general, a 20 µL volume that contained 0.5 mM of each primer, 10 µL of SYBR Green, and 0.5 µL of cDNA was assessed according to the setup thermal index amplification. Then, mRNA from the samples was quantified with reference to a standard curve constructed with LightCycler®. The real-time PCR assay was performed at least twice for each sample.

Immunohistochemistry

We randomly chose 50 tissue specimens for FGF staining according to higher and lower than the estimated relative quantification (RQ) median (12.39) for *FGF10*. The first paraffin separation was done at 55°C, then, after 20 minutes, three washes with xylene. Next, the samples were rehydrated in ethanol concentrations of 100%, 95%, and 80%. Subsequently, 3% hydrogen peroxide was applied to block any endogenous peroxidase activity. The prepared slides were heated in citrate acidic buffer (pH=6.0) in an oven for 10 minutes through antigen retrieval. Primary antibody (1:100) incubation at 4°C was done for two hours, then each slide was incubated for 30 minutes in biotin labelled with secondary antibody (Abcam, ab80064, Cambridge, UK) and tracked with incubation in streptavidin-peroxidase (Dakocytomation, Inc., CA, USA). The Diaminobenzidine substrate was added to the slides, and they were visualized under a microscope. Finally, the cutting segments were stained with haematoxylin and dehydrated. For checking the negative control, the intended segment was incubated with phosphate-buffered saline (PBS) instead of primary antibody, followed by the rest of the previously mentioned procedures. The positive control was brain-skin tissue segments, which had high *FGF10* expression (1).

Immunohistochemistry scoring

The stained slides were evaluated by two experienced pathologists who were not aware of the patient's clinical characteristics. Four area of the slides were randomly chosen and visualized with an optical microscope. The *FGF10* score was determined to be the positive cell product multiplied by staining intensity. The staining scoring was described as: 0 for negative staining and 1 for positive staining. The scoring set for allocating to the positive cell percent was: 0 (less than 10% positive cells); 1 (10% to 30% positive cells); 2 (30% to 50% positive cells); and 3 (>50% positive cells) (1).

Table 1: Primer sequences with real-time quantitative polymerase chain reaction (RT-PCR) product lengths

Gene	Primers sequence (5′–3′)	Primer length (bp)	Product length (bp)	Accession Num/ Gene ID
FGF10	F: TGTGCGGAGCTACAATCACC	20	136	HGNC:3666/ 2255
	R: CAGGATGCTGTACGGGCAGT	20		
FGF10AS	F: GCCTGCACCTTTGTAACTGG	20	158	HGNC:49382/101927075
	R: TGCTTCTTGCTTGCATCTGT	20		
B2M	F: TGCTGTCTCCATGTTTAGTGTATCT	25	86	HGNC:914/ 567
	R: TCTCTGCTCCCCACCTCTAAGT	22		
GAPDH	F: GCTCTCTGCTCCTCCTGTTC	20	115	HGNC:4141/ 2597
	R: ACGACCAAATCCGTTGACTC	20		

Statistical analysis

The collected data were analysed by SPSS 23.0 (IBM Corporation, Armonk, NY, USA) and Prism 8.02. The relationships between *FGF10* and *FGF10AS* expression levels and clinicopathologic characteristics were investigated using the Mann-Whitney U test, one-way ANOVA (Kruskal-Wallis test), and Pearson's correlation test. The Kaplan-Meier method was utilized to plot the patients' survival curve. Cox regression for univariate and multivariate analyses were performed to assess *FGF10* and *FGF10AS,* as a substantial biomarker in CRC. Spearman's rank correlation for linear regression was tested. The area under the receiver operating characteristic (ROC) curve was determined by distinguishing stages I and II from stages III and IV in favourable sense and antisense. P<0.05 were considered statistically significant.

Results

Relationship between fibroblast growth factor 10 (FGF10/FGF10AS) and clinicopathologic parameters

To determine the mRNA levels of expression in *FGF10* and *FGF10AS* in CRC patients, fresh tumour tissues and adjacent normal tissues were analysed by quantitative RT-PCR (qRT-PCR). The results demonstrated *FGF10* overexpression in tumour tissues with a mean RQ of 12.39-fold and *FGF10AS* downregulation with a mean RQ of 0.67-fold.

Next, we analysed the correlation between *FGF10* expression in tumour tissues and clinicopathologic data of 100 CRC patients. The data were analysed by the Mann-Whitney U test and showed that *FGF10* gene expression had a marginally significant correlation with the patient's sex (P<0.058). Men appeared to be more prone to CRC than females, which is in line with the results of previous studies (26).

There was a significant relationship was between *FGF10* expression and age (P<0.04) and tumour size (P<0.02). The relation between *FGF10AS* expression and age of the CRC patients was considerably significant (P<0.035).

According to the pathological data, lymph node metastasis was found in 82% of these patients, which indicated that lymph invasion is the earliest and most prevalent metastatic pathway in CRC. Expression analysis data show a significant relation between *FGF10* and *FGF10AS* with lymphatic invasion (P<0.02 and P<0.04, respectively). *FGF10* had significant overexpression in patients with distant metastasis compared to those without distant metastasis (P<0.03), but there was no significant reduction detected in *FGF10* antisense. Therefore, *FGF10* may initiate tumour invasion and metastasis. The TNM stage data obtained by the Kruskal-Wallis test showed that *FGF10* overexpression and downregulation of the relevant antisense were significantly related to poor prognosis of CRC (P<0.007 and P<0.004, respectively). The pattern of *FGF10* gene and dependent antisense expression had a significant correlation with poor tumour differentiation (P<0.014 and P<0.027, respectively), which implied that *FGF10* and *FGF10AS* are potentially strong boosters of CRC progression via epithelial-mesenchymal transition (EMT). Surprisingly, vascular invasion had a significant correlation with an increase in *FGF10* (P<0.03) and decrease in *FGF10AS* (P<0.01) gene expression in tumour tissues compared to adjacent normal tissues. Both lymph and blood vessel invasion appeared to be main prognostic indicators in patients suffering from CRC, with significance in early and advanced lesions.

FGF10 and FGF10AS prognostic value in colorectal cancer samples

Our results underscored the clinical potential of *FGF10* and its related antisense for early detection of CRC cases. The Kaplan-Meier survival curves were plotted to analyse the 36-month survival rate for *FGF10* and the long noncoding antisense. According to the Kaplan-Meier test, *FGF10* expression had a significant correlation with the overall three-year survival rate in CRC (P<0.0001, Fig.1A). The overall survival of patients with downregulated *FGF10AS* was notably shorter than those with *FGF10AS* overexpression (P<0.064, Fig.1B).

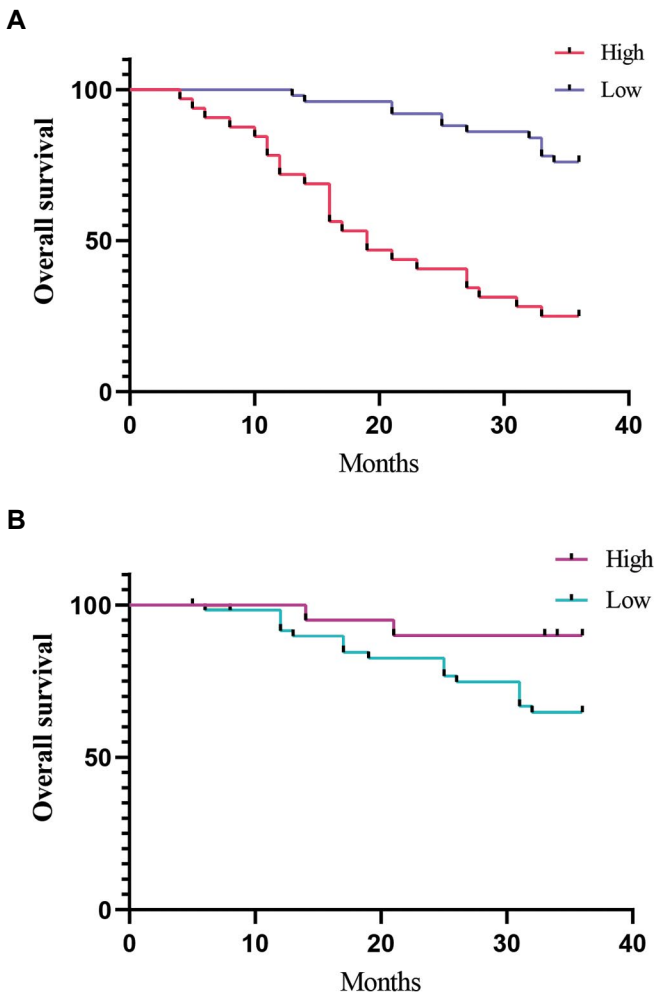


Fig.1: Overall survival of colorectal cancer (CRC) patients by Kaplan-Meier analysis. **A.** Patients with high fibroblast growth factor 10 (*FGF10*, P<0.0001) and **B.** Low antisense *FGF10* (*FGF10AS*, P<0.048), expression had a shorter overall survival than those with low *FGF10* and high *FGF10AS* expression.

ROC curve analysis was performed to have a more detailed investigation of the role of *FGF10* expression as a considerable biomarker in CRC. The level of *FGF10* expression showed an area under the ROC curve of 0.821 (*P<0.0001, 95% confidence interval [CI]: 0.756-0.886) at a cut-off CT value of 28.02 with an 80.52% sensitivity and 69.74% specificity for distinguishing CRC and adjacent normal tissues (data not shown) Then, we identified stages I-II and III-IV for predicting CRC invasion, with 80.77% sensitivity and 73.33% specificity at a cut-off RQ of 9.234 (Area Under Curve [AUC]=0.855, 95% CI: 0.768-0.942, P<0.0001) in *FGF10* and the estimated area under the ROC curve plotted for *FGF10AS* was 0.632, with 65.38% sensitivity and 60% specificity at a cut-off RQ of 0.375 (95% CI: 0.498-0.766, P<0.064) which was not significant (Fig.2A, B).

Further univariate and multivariate Cox regression analysis was performed by inserting several estimated significant clinical features. The expression level median of sense *FGF10* and its antisense were calculated and cases were classified in to low and high expression groups. The outcome of Cox analysis showed no significant P value to interpret *FGF10* and *FGF10AS* as independent diagnostic factors in CRC (Table 2).

FGF10 and its antisense correlation

The gene with its antisense sequences and all informed data were obtained from the National Centre for Biotechnology Information and University of California Santa Cruz (UCSC) genome browser. According to the transcript summary of the ensemble genome database (www.https://asia.ensembl.org) *FGF10* is located on a reverse strand and its antisense is placed on the opposite strand (Fig.3A). Thus, ncRNA transcripts overlap the 5′UTR of the protein coding gene on the similar strand (Fig.3B).

Linear regression analysis confirmed a negative correlation between *FGF10* and its antisense transcript; however, this finding was not significant (r=-0.02, P<0.8, Fig.3C).

Table 2: Univariate and multivariate analyses of FGF10 and FGF10AS and clinical characteristics in CRC patients' samples

	Univariate analysis			Multivariate analysis		
Parameters	**HR**	**95% CI**	**P value**	**HR**	**95% CI**	**P value**
Sex						
Female	1	0.33 - 1.47	0.34	1	0.31 - 1.56	0.38
Male	0.69			0.70		
Age (Y)						
<50	1	0.86 - 3.56	0.13	1	0.87 - 3.86	0.11
≥50	1.74			1.83		
Stage						
I and II	1	0.57 - 2.45	0.63	1	0.46 -2.24	0.94
III and IV	1.19			1.02		
Differentiation						
Well+MOD	1	0.60 - 2.38	0.59	1	0.51 - 2.11	0.91
POOR	1.20			1.03		
Location						
Left	1	0.43 - 1.54	0.53	1	0.37 - 1.51	0.42
Right	0.81			0.75		
FGF10 expression						
Low	1	0.74 - 2.67	0.29	1	0.63 - 2.64	0.47
High	1.41			1.29		
FGF10AS expression						
High	1	1.29 - 0.35	0.23	1	1.71 - 0.41	0.63
Low	0.67			0.83		

FGF10; Fibroblast growth factor 10, *FGF10AS*; Antisense *FGF10*, CRC; Colorectal cancer, HR; Hazard ratio, and CI; Confidence interval.

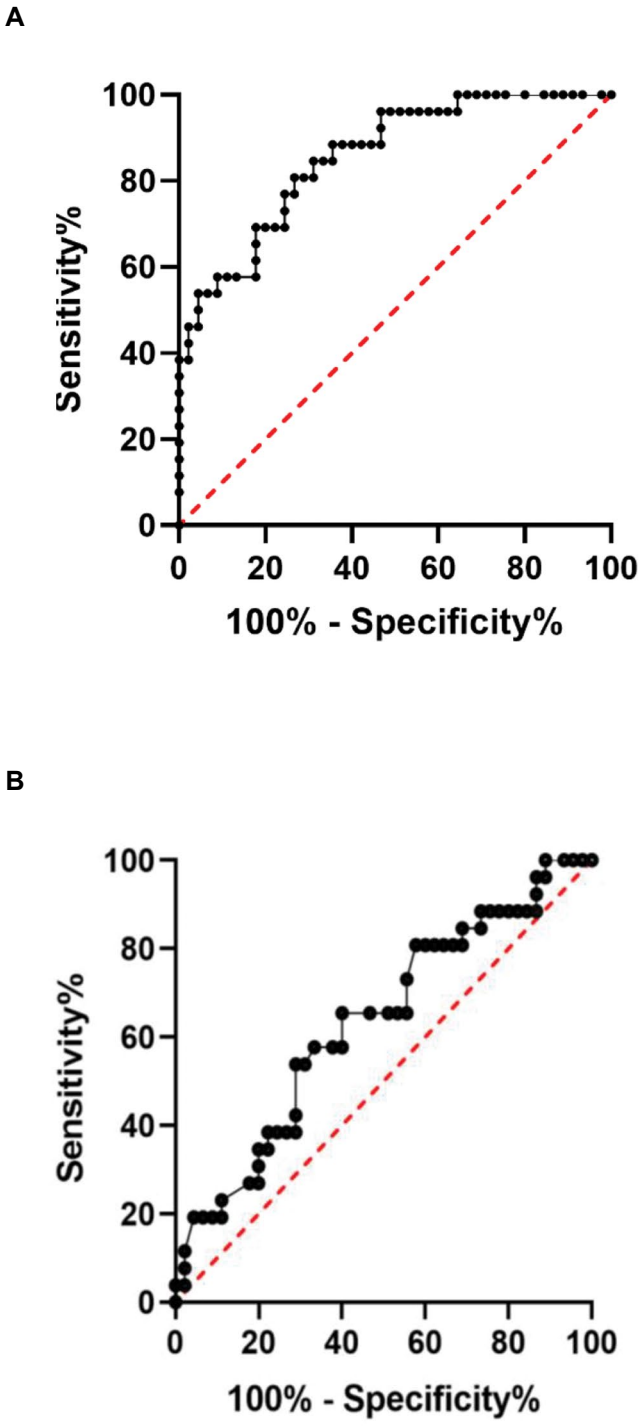


Fig.2: Distinction between early or late stage of disease by plotting ROC curves. **A.** In order to predict cancer invasion in fibroblast growth factor 10 (*FGF10*), the ROC curve was drawn (0.855), with 80.77% sensitivity and 73.33% specificity at a cut-off RQ of 9.234. Stages I and II could be distinguished from stages III and IV (P<0.0001). **B.** The area under the ROC curve was estimated to be 0.632, with 65.38% sensitivity and 60% specificity at a cut-off RQ of 0.375. Stages I and II could be distinguished from stages III and IV with antisense *FGF10* (*FGF10AS*, P<0.064). ROC; Receiver operating characteristic curve and RQ; Relative quantification.

FGF10 overexpression confirmation in the tumour tissues and cancer cell lines

For subsequent validation, the *FGF10* and *FGF10AS* mRNA expression levels were evaluated in normal/normal (N/N) tissues, and in the HT29 and SW480 cell lines. *FGF10* was significantly overexpressed while *FGF10AS* was mainly downregulated in tumour tissues and the selected cell lines compared to the corresponding adjacent normal and N/N tissues (P<0.0001).

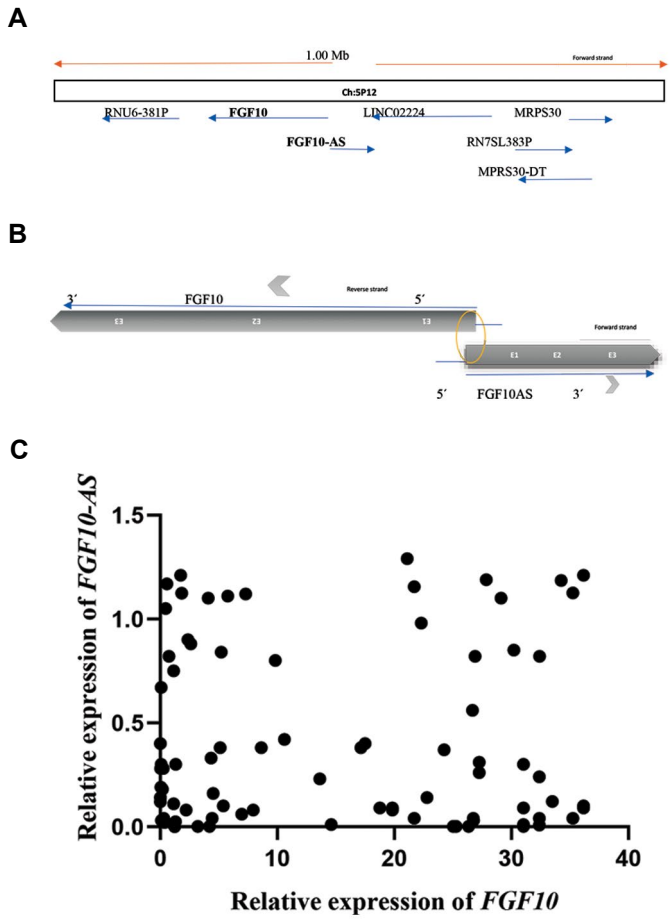


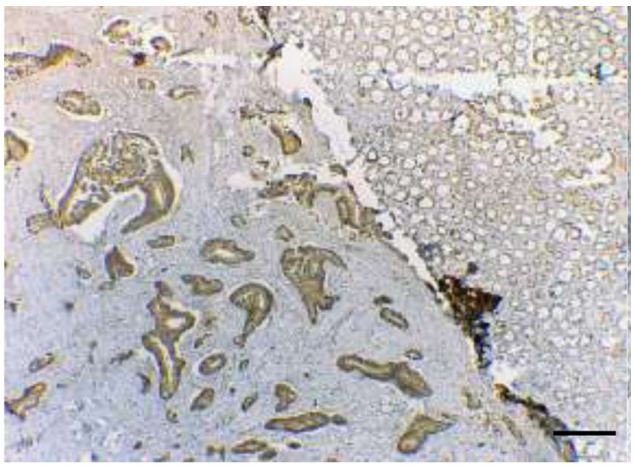
Fig.3: Sense and antisense chromosomal positions. **A.** Fibroblast growth factor 10 (*FGF10*) and its related antisense (*FGF10AS*) genomic location in ch:5p12 according to the Ensembl genome browser. **B.** Comparative correlation of FGF10 and FGF10AS. "E" display exons. The yellow elliptic represents an overlapping zone of sense and antisense. The direction of transcription is marked by arrows. **C.** Pearson's correlation analysis shows a negative relation between *FGF10* and *FGF10AS* in colorectal cancer (CRC) tissues (r=-0.02, P<0.8).

In our final observation, *FGF10* was generally perceived in the cytoplasm of the CRC cells. The specimens were divided into high and low expression groups according to immunohistochemistry scoring, which was the result of protein staining intensity in the positive cells (Fig.4A, B). *FGF10* expression levels were higher in patients who had positively stained samples compared to those who had specimens that stained negative for FGF10 in the protein level of expression, which suggested that the FGF10 protein may confirm an impressive role in CRC tumourigenesis or progression.

The results showed that *FGF10AS* downregulated in CRC tissues and in the selected cell lines, and this was correlated with poor prognosis in the CRC cases.

However, there were no significant correlations with other clinicopathologic parameters.

A



B

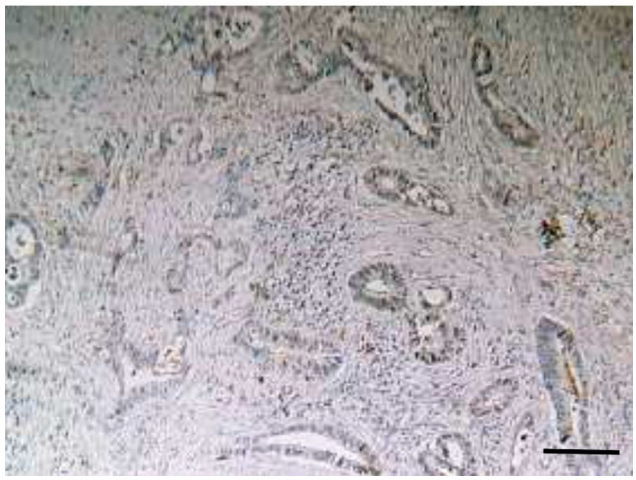


Fig.4: Representative immunohistochemical staining of fibroblast growth factor 10 (FGF10) in colorectal cancer (CRC) tissues. **A.** Positive FGF10 staining (x10) and **B.** Negative FGF10 staining (x10) (scale bar: 50 µm).

Discussion

In the current study, we sought to define the expression level of *FGF10* and its antisense to evaluate their diagnostic and prognostic values in CRC. The results showed remarkable upregulation of *FGF10* in CRC tumour tissues compared to the adjacent normal tissues. We demonstrated that the overexpression of *FGF10* was significantly related to lower overall survival according to the Kaplan-Meier test.

Previous studies have confirmed that tumour cells secrete different types of proteins, particularly growth factors, such as EGF, VEGF, TGF, HGF and FGF to meet the growth requirements. Autocrine and paracrine secretion is the main kind of tumour advancement (27). We illustrated that elevated *FGF10* expression could show undesirable prognosis in CRC, and this finding could open new horizons for studying the rest of the paracrine FGF family in CRC. Based on the findings, we can claim that *FGF10* overexpression may motivate FGFR2 sequentially and that *FGF10* may play a potential role in CRC treatment. FGF*10* is the most important ligand of FGFR2 in the paracrine FGF family, which can act as a promoter of FGFR2 and activate a downstream signalling cascade that consists of the RAS/MAPK/ERK signalling pathway in various cancers. The findings indicated that *FGF10* regulation played an important role in cell viability, proliferation, immortality and apoptosis. *FGF10* activates cancer cell proliferation by boosting G1-to-S phase transition and prepares the cells for synthesis and mitosis (28, 29).

The results of studies of FGF10 and its related receptor, FGFR2b, in mouse foetuses show that FGF10-FGFR2b signalling represent a main role in mammary gland formation (30-32). *FGF10* gene transcription is elevated by 10% in breast carcinomas (33). *In vivo* experiments have shown that upregulated *FGF10* triggers adenocarcinoma expansion in mouse prostate epithelial cells, which is reflected primarily through activation of epithelial FGFR1 (34). A similarly elevated stromal *FGF10* expression was accompanied by upregulation of cancer cell FGFR2b and correlated with poor prognosis (35, 36).

Likewise, *FGF10* overexpression was shown in 3% of gastric cancer patients (37). Immunohistologic analysis in patients with gastric adenocarcinoma shows that high *FGF10* expression is interrelated with poor prognosis. Recent reports that *FGF10* overexpression was likely present in 6% of gastric adenocarcinoma cases (35, 38). It is proposed that tumour-associated macrophages secrete FGF10, which may have a key role in the promotion of lung tumourigenesis (37). However, the role of FGF10 in initiating CRC and progression is not totally elucidated.

Increased *FGF10* expression correlated with the promoted *EMT* gene expression in colon cancer cells in a co-culture model. The important role of *FGF10* in type-III EMT of cancer cells and initiation of metastasis through several signalling pathways has been proposed (28, 39). In this regard, inhibitor molecules targeting FGFR2 function as anticancer agents in both *in vivo* and *in vitro* experiments. Therefore, this could support this idea that blockage of the FGF10-FGFR2 signalling pathway could be a major therapeutic drug goal. However, further research should be conducted to determine the molecular mechanisms, such as secretion of FGF10 and FGF10 oncogenic pathways.

Based on previous findings, lncRNAs can play a crucial role in CRC progression (40). In this study, we observed that the expression of a novel lncRNA, *FGF10* antisense, decreased in CRC tissues and cell lines compared to normal controls. Therefore, it could not compensate for the high expression of *FGF10* in tumour tissues and, subsequently, more growth and progression were detected. In breast cancer lncRNA, *FGF14AS2* is reported as one of the downregulated lncRNAs. *FGF14AS2* is a possible unprecedented marker and a target for gene therapy in

breast cancer treatment (22). In addition, our study results showed that *FGF10AS* might be a potential, promising molecular drug target in the future.

Strong evidence shows that *FGF10* can increase cancer progression; therefore, anti-*FGF10* therapy can diminish cancer growth. Based on the present study, we assume that an inhibitor, which can prevent FGF10 and FGFR2 association with competitive function could possibly be a very helpful drug in CRC therapy. We hope the newly recommended genes in our study can help to locate a new effective molecular drug target that can overcome CRC progression.

Conclusion

Upregulation of *FGF10* and downregulation of *FGF10AS* were ordinary occurrences in the CRC tissue samples. This observation was correlated with poor clinicopathologic features like TNM stage, lymphovascular invasion and differentiation in both *FGF10* and *FGF10AS*. Although *FGF10* and *FGF10AS* were not detected as independent prognostic biomarkers in this study, lnc-RNA comprehension enhancement in cancer will shed light on disease aetiology improving CRC early detection and finally better therapeutic strategies will be ahead of patients. *FGF10AS* and *FGF10* are attractive biomarkers for improving the survival rate of CRC patients.

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Authors' Contributions

All authors contributed to the study conception and design. L.R.; Preparation material, data collection and analysis, written the first draft. E.N.M., M.H.; Formal analysis and investigation. S.Y.S., M.H.; The supervision of the experiments. H.A.A.; Edited the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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