RESEARCH ARTICLE

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LncRNA ANRIL Promotes Glucose Metabolism and Proliferation of Colon Cancer in a High-Glucose Environment and is Associated with Worse Outcome in Diabetic Colon Cancer Patients

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Abstract

Background: The potential involvement of type 2 diabetes mellitus (T2DM) as a risk factor for colon cancer (CC) has been previously reported. Epigenetic changes, such as deregulation of long non-coding RNA (lncRNA) and microRNA (miR), have been linked to the advancement of CC; however, the effects of high glucose levels on their deregulation and, in turn, colon cancer remain unexplored. **Methods:** Fifty patients had a dual diagnosis of CC and T2DM, and 60 patients with CC without diabetes mellitus were included in the study. qRT-PCR was used to examine the expression of lncRNA ANRIL and miR-186-5p in tissue samples. ANRIL, miR-186-5p, and their downstream target genes HIF-1α, PFK, HK, Bcl-2, and Bax were also determined in CC cell lines under various glucose conditions. Glucose uptake, lactate production and cells proliferation were estimated in CC cell lines. **Results:** A significant upregulation of ANRIL expression levels ($p<0.001$) and a significant downregulation of miR-186-5p expression ($p<0.001$) in diabetic colon cancer specimens compared to those in non-diabetic colon cancer group were observed. MiR-186-5p expression levels were inversely correlated with ANRIL expression levels, blood glucose levels and HbA1c%. Concerning in vitro model, a significant upregulation of ANRIL, downregulation of miR-186-5p, upregulation of HIF-1α, glycolytic enzymes and activation of antiapoptotic pathway was detected in higher glucose concentrations than lower one. There was a significant increase of glucose uptake, lactate accumulation and proliferation of the Caco2 and SW620 cell lines in a dose dependent manner of glucose concentrations. Moreover, a significant positive correlation between glucose uptake and ANRIL expression was shown. **Conclusions:** A high-glucose environment can increase the tumor-promoting effect of ANRIL. ANRIL can promote glucose metabolism and colon cancer proliferation by downregulating miR-186-5p with subsequent upregulation of glycolysis enzymes expression and inhibition of apoptosis.

Keywords: Colon cancer- miR-186-5p- ANRIL- Glycolysis- Type 2 DM- Hyperglycemia

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Introduction

Diabetes and cancer are two of the most common causes of death worldwide. Epidemiologic evidence suggests an association between diabetes mellitus (DM) and an increased risk of many types of cancer [1]. Colon cancer (CC) is one of the most prevalent malignant tumours of the digestive system, with substantial morbidity and mortality rates, accounting for approximately 10% of cancer-related fatalities [2]. Colon cancer is the second most prevalent cancer-related cause of death worldwide [3]. Multiple observational studies, but not all, have suggested that diabetes increases the risk of CC considerably [4, 5]. The excess glucose permits cancer cells to have higher energetic and biosynthetic needs [6]. As a result, a deeper understanding of the underlying molecular mechanisms involved in the progression of CC and its relation with diabetes is highly indicated.

Unlike normal cells, which get most of their energy from mitochondrial oxidative phosphorylation, cancer cells get most of their energy from aerobic glycolysis, which provides constant energy and nutrients to promote uncontrolled multiplication; this reprogramming is known as the Warburg effect. The 'Warburg effect,' which was first observed by Warburg 90 years ago, is the most common malignant metabolic characteristic. It asserts that

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glycolytic fermentation the process of converting glucose to lactic acid is a prominent metabolic pathway for tumour cells under aerobic conditions [7]. Many oncogenes and tumour suppressors exert their effects through regulation of glycolysis [8].

Long non-coding RNAs (lncRNAs) are transcripts with a length of more than 200 nucleotides but no evident protein-coding function. They've gotten a lot of press as emerging regulators in a variety of biological processes [9]. ANRIL, also known as CDKN2B-AS, is a long-chain non-coding RNA that is transcribed from the CDKN2A/B gene cluster on the Chr9p21 chromosome and is involved in tumour formation. ANRIL expression levels have been found to be considerably elevated in lung cancer, liver cancer, and esophageal squamous cell carcinoma in previous investigations, suggesting that ANRIL may operate as an oncogene [10]. Meanwhile, recent research has found that the lncRNA ANRIL is substantially expressed in cells treated with high hyperglycemia (HG) [11-13].

It is well known that lncRNAs serve as a miRNA "sponge," modulating functional target mRNAs in an indirect manner to regulate physiological activity [14]. LncRNAs and miRNAs have a complementary pairing sequence at their 3'-UTR, which allows for molecular-level binding and counteraction [14]. Zhang et al. predicted ANRIL target miRNAs, indicating that miR-186 is targeted by seven complementary binding sites. They found that the ANRIL/miR-186 axis is involved in the carcinogenesis of cervical cancer [15].

MiR 186-5p has been studied in a variety of cancers, including hepatocellular carcinoma. [16], non small cell lung cancer [17] ovarian cancer [18], prostate cancer [19], osteosarcoma [20]. MiR-186-5p was rarely studied in CC.

Hypoxia inducible factor-1 (HIF-1) is one of miR-186's target genes [21]. HIF-1 regulates transcription of a wide range of target genes and modifies hypoxia adaption as the active subunit of HIF-1. HIF-1 plays a key function in aerobic glycolysis and carcinogenesis by altering glycolytic pathway rate limiting enzymes and programmed cell death [22].

Although a link between T2DM and CC has been established, the impact of blood glucose levels on the clinicopathological characteristics of CC has yet to be investigated. In addition, the biochemical mechanism by which hyperglycemia promotes CC prognosis is unknown. The expression of ANRIL and miR-186-5p in colon cancer specimens from type 2 diabetes individuals was investigated in this study. We also looked into whether a high-glucose environment could help the ANRIL/ miR-186-5p axis promote tumour growth. The *miR 186-5p* downstream targets were investigated further.

Materials and Methods

The study included a total of 110 participants with colon cancer. All colon cancer diagnoses were made using ultrasonic or CT scan results, as well as endoscopic biopsy or surgical resection. There was no preoperative radiation, chemotherapy, or other anticancer treatment. The American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) 2009 staging classification for colorectal cancer (7th edition) was used to classify cancer colon patients using the TNM system. Patients with familial adenomatous polyposis, various acute and chronic infectious inflammations, cardiovascular and cerebrovascular illnesses, liver and renal function impairment, and other stress conditions were excluded from the study.

The 110 cancer colon patients were divided into 2 groups; 50 patients who had a dual diagnosis of CC and T2DM, and 60 patients with CC without DM. The criteria to identify T2DM patients was a prescription for diabetic medications prior to the cancer colon diagnosis (all type2 DM patients treated with metformin as an oral hypoglycemic drug) and all of them had poor glycemic control (HbA1c > 7.5%). Among the nondiabetic patients, the medication history, blood glucose values, HbA1c values and clinical symptoms were reviewed to ensure that they were, indeed, non-diabetics. The clinicopathological features of colon cancer patients are summarized in Table 1. Written informed consent was obtained from all participants. The study was approved by the research ethical committee of Faculty of Medicine, Zagazig University (ZU-IRB#9852/4-1-2022). The study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Specimen collection

All 110 patients who underwent surgical resection had tumour tissues and nearby healthy tissues within 50 mm of the tumours retrieved. Colon tumour tissue and normal tissue from people without diabetes and colon tumour tissue and normal tissue from patients with T2DM were split into four groups. Following resection, tissue samples were frozen in liquid nitrogen and kept at -80 °C until RNA extraction.

Cell lines and cell culture

The American Type Culture Collection provided the colon cancer cell line Caco2, SW620, and normal colon cells FHC (ATCC, Manassas, VA, USA). The cells were grown in DMEM media with low glucose content (1.0 g/L, 5.6 mmol/L). In addition, 10 percent foetal bovine serum (FBS) (Lonza Bioproducts), penicillin 100 U/mL, and streptomycin 100 mg/mL (Lonza Bioproducts) were added to the media, which was incubated at 37 °C in a humidified chamber with 5% CO_2.

Caco2, SW620, and FHC colon cancer cell lines were planted in 6-well plates as indicated above. All cell lines were cultured for 24 hours (3 wells per treatment) with different doses of D-(+)-glucose [5 Mm (90 mg/dL), 10 mM (181 mg/dL), and 15 mM (271 mg/dL)].

Glucose uptake and lactate production detection

The Glucose Test Kit was used to perform the glucose uptake experiment (SPINREACT, Girona, Spain). Caco2, SW620, and FHC cells were seeded at a density of 106 cells per well in 6-well plates and incubated at 37 °C for 48 hours, with the medium at 0 h collected as the background glucose concentration. Cellular glucose uptake was

defined as a decrease in medium glucose concentration. (Background concentration – reading concentration)/ protein concentration = glucose uptake.

Lactate Assay Kit was used to assess extracellular lactic acid generation in the cell culture medium (Eton Bioscience Inc., San Diego, CA). Lactate dehydrogenase converts lactate to pyruvate, which is used to determine lactate. The results were adjusted to account for the protein concentration.

Cell viability Assay

As previously disclosed, cancer colon cell lines Caco2, SW620, and FHC were planted in 96-well plates in low glucose DMEM. For 24 hours, cells were treated in varied D-(+)- glucose concentrations (5, 10, or 15 mM) (4 wells per treatment). To determine cell viability, cells were incubated with MTT (3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) (Sigma; St. Louis, MO) for 4 hours at 37°C, after which the MTT solution was removed and DMSO was added to each well, and the absorbance at 570 nm was measured using a microplate reader.

Real time PCR for expression of ANRIL and miR-186-5p in colon cancer specimens and cell lines

Total RNA was extracted from tissue homogenate and cell lines with using the RNeasy Mini kit including DNase I digestion, as directed by the manufacturer's protocol (QIAGEN, Germany). By using Quantiscript reverse transcriptase (QuantiTect Reverse Transcription Kit, QIAGEN, Germany) the RNA was reverse transcribed.

In a total volume of 20 μL, quantitative real-time PCR analysis was carried out with 5 μL of the cDNA, 100 pmol/ μL of each primer (0.5 μL each; Biolegio, Gelderland, The Netherlands), 10 μL of EvaGreen PCR Master mix (Jena Bioscience, Jena, Germany), and 4 μL PCR-grade water. The endogenous control of miRNA was U6, while the reference gene related with ANRIL was GAPDH. The primer sequences utilised are shown in Table 2. One cycle of initial denaturation at 95 °C for 3 min was followed by 40 cycles of 95 °C for 15 s and 60 °C for 30 s. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression levels of miRNA and mRNA.

Real time PCR for expression of the related genes in colon cancer cell lines

Expression of HIF-1α and their target genes; rete limiting enzymes of aerobic glycolysis (PFKP and HK2) and programmed cell death genes (BAX and Bcl-2) were analysed in Caco2 and SW620 cell lines to study the mechanistic effects of ANRIL/miR-186-5p axis in presence of different concentrations of glucose. The methods used were as mentioned above. Table 2 contains the primers sequences.

Western blot analysis for HIF-1α, BAX and Bcl2

The cells were lysed in RIPA buffer (Beyotime). Bradford protein assay kit (Cat #SK3041, Bio basic inc., Ontario, Canada) was employed for quantitative protein analysis. Samples proteins were then separated on a polyacrylamide gel (TGX Stain-Free FastCast Acrylamide Kit) and transferred to a PVDF membrane (Millipore, Burlington, USA). PVDF blot was incubated in 5% nonfat dry milk, Tris-HCL, 0.1% Tween 20 for 1 h. Then the membranes were incubated with primary antibodies against HIF-1α (1:1000, (Dallas, TX, USA), BAX (1:5000, LTD, Hangzhou, China) or Bcl2 (1:5000, LTD, Hangzhou, China) overnight at 4°C.IgG secondary antibody (1:10000) was incubated for 2 h at room temperature. After being washed twice in 1 × TBS-T, densitometric analysis of the immunoblot was performed to quantify the amounts of HIF-1α, Bcl2 and BAX in all studied samples against control sample β-actin by protein normalization on the ChemiDoc MP imaging system (version 3) produced by Bio-Rad (Hercules, CA).

Statistical analysis

The study's findings were reported as mean, standard deviation (SD), and percentages when applicable. The t-test and ANOVA were used to compare the groups. For nonparametric values, the Chi square test was utilized. When P values were less than 0.05, differences were considered significant. SPSS software (SPSS Inc., Chicago, IL, USA) version 17 was used to analyse the study data.

Results

Demographic data and clinicopathological features

In terms of gender, age, and distant metastasis, there was no significant difference between diabetic and non-diabetic colon cancer patients. Patients with diabetes mellitus had significantly higher blood glucose and HbA1c levels than those without diabetes ($P< 0.001$). Furthermore, the diabetic cancer colon group and non-diabetic cancer colon patients had substantial variations in tumour differentiation, primary tumour invasion, lymph node metastasis, and TNM stage (Table 1).

Glucose uptake and lactate production results

In the Caco2 and SW620 colon cancer cell lines, glucose uptake and lactate buildup increased in a dose-dependent way as glucose concentrations increased. In the three distinct glucose concentrations, there was also a substantial positive link between glucose uptake and ANRIL expression (Figure 1a).

Effects of differing glucose concentrations on colon cancer cell proliferation

The influence of glucose supplementation on colon cancer cell proliferation was investigated using three D-(+)-glucose concentration thresholds (a baseline concentration of 5 mM and two higher doses of 10 mM and 15 mM). When 10 mM D-(+)-glucose was administered, the proliferation rate of Caco2 and SW620 increased considerably at 24 hours (both $p <0.001$), compared to 5 mM D-(+)-glucose administration. Caco2 and SW620 proliferation rates increased considerably after 24 hours after 15 mM D-(+)-glucose administration compared to 5 mM D-(+)-glucose administration (both $p <0.001$) and 10 mM concentration (both $P <0. 001$). At 24 hours, the proliferation rate of FHC cells was unaffected by different

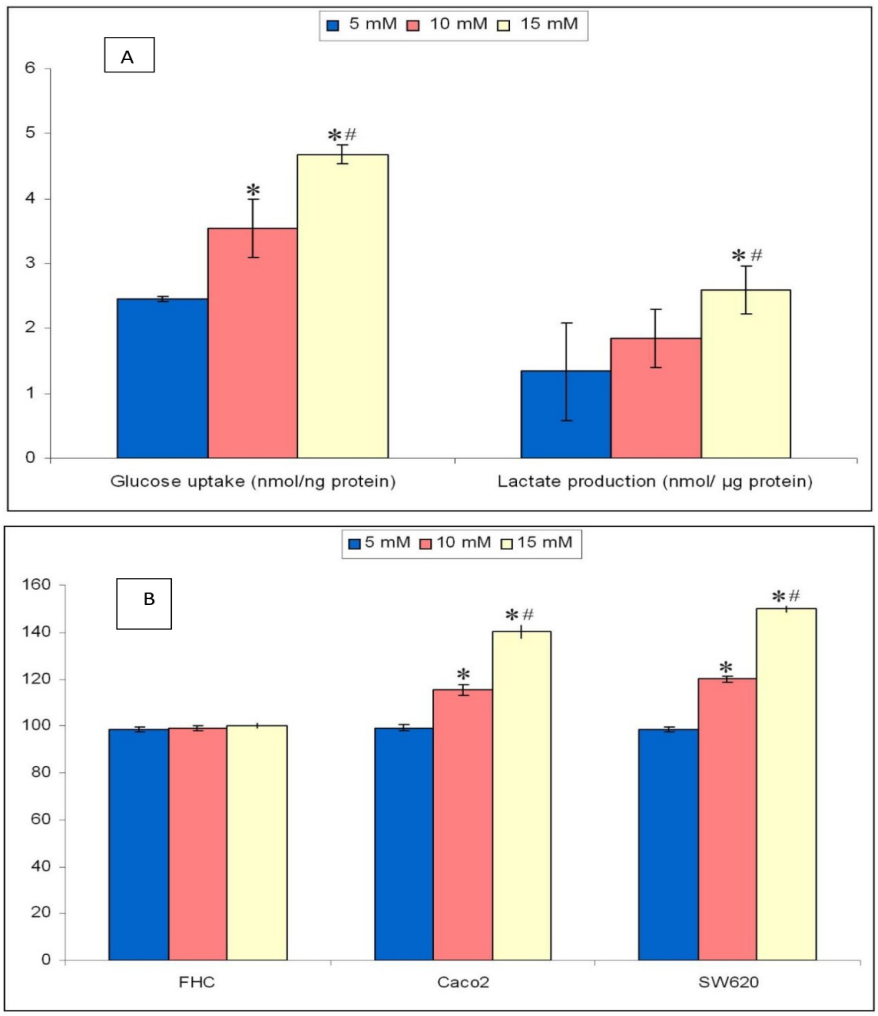

Figure 1. (A) Glucose uptake and lactate production results *p<0.05 when compared to 5 mM D-(+)-glucose concentrations # p<0.05 when compared to 10 mM D-(+)-glucose concentrations (B) Effects of different glucose concentrations on colon cancer cell proliferation.*p<0.05 when compared to 5 mM D-(+)-glucose concentration # p<0.05 when compared to 10 mM D-(+)-glucose concentration

glucose concentrations (Figure 1b).

Expression of ANRIL and miR-186-5p in colon cancer specimens

ANRIL expression was up to 4.5 times higher in diabetes colon cancer samples compared to non-diabetic colon cancer samples (p<0.001), but miR-186-5p expression was reduced in diabetic colon cancer samples compared to non-diabetics (p<0.001) (Table 1). Furthermore, ANRIL RNA expression was considerably higher in tumour tissues of colon cancer patients with or without DM than in the distal normal intestinal mucosa (both P<0.001). When compared to normal colon tissues, MiR-186-5p expression was downregulated in tumour tissues of colon cancer patients with or without DM (both p<0.05).

Expression of ANRIL mRNA in colon cancer cell lines cultured under different glucose concentrations

Under varied glucose concentrations, there was a significant increase of ANRIL expression levels in Caco2 and SW620 colon cancer cell lines when compared to normal colon cells (FHC) (each P<0.001). When we evaluated ANRIL expression in Caco2 cells between different glucose concentrations, we discovered a substantial dose-dependent increase (p0.001). This was also true for SW620 (p<0.001) (Figure 2a).

Expression of miR-186-5p mRNA in colon cancer cell lines cultured under different glucose concentrations

When comparing Caco2 and SW620 colon cancer cell lines to normal colon cells (FHC) under different glucose concentrations, there was a significant downregulation of miR-186-5p expression levels (p=0.005, 0.002 for 5 mM of D-(+)-glucose, respectively, both p<0.001 for 10 mM of D-(+)-glucose, both p<0.001 for 15 mM of D-(+)-glucose. When we evaluated the expression of miR-186-5p in Caco2 cells at different glucose concentrations, we discovered that it decreased significantly in a dose-dependent way (p<0.001). This was also true for SW620 (p=0.007) (Figure 2b).

Correlation between diabetes biomarkers & ANRIL and miR-186-5p expression of colon cancer specimens

The expression levels of ANRIL were found to have substantial positive relationships with blood glucose

Table 1. Clinicopathological Features of Colon Cancer Patients

	Colon cancer patients with T2DM N=50	Colon cancer patients without T2DM N=60	P value
Gender			
Male	27	33	0.93
Female	23	27	
Age			
>45 years	37	40	0.53
≤ 45 years	13	20	
Differentiation			
Low-moderate	28	46	0.036
High	22	14	
Primary tumour invasion			
T1+T2	4	21	0.002
T3+T4	46	39	
Lymph metastasis			
N0	30	52	0.003
N1-N2	20	8	
Distant metastasis			
M0	46	53	0.75
M1	4	7	
TNM stage			
I, II	26	48	0.004
III, IV	24	12	
Laboratory investigations			
Blood glucose (mg/dL)	200.24±24.30	94.12±12.34	<0.001
Hb A1c	8.15±1.25	5.43±1.23	<0.001
Diabetes duration (years)	7.20±3.41		
ANRIL expression	4.56±1.21	1.01±0.23	<0.001
miR-186-5p expression	0.65±0.16	1.02±0.31	<0.001

levels and HbA1c percent in all 110 colon cancer patients (r =0.63, p<0.001 and r= 0.29, p0.005, respectively). MiR-186-5p expression, on the other hand, was found to be adversely linked with both blood glucose levels and HbA1c percent (r =-0.69, p<0.001 and r=- 0.40, p<0.001, respectively). Furthermore, ANRIL levels were negatively linked with miR-186-5p expression (r=-0.71, p<0.001).

Expression of miR-186-5p related genes in Coca2 cell line under different glucose concentrations

HIF-1 mRNA levels increased 4.5 fold in 10 mM of D-(+)-glucose (P = 0.01) and 5.5 fold in 15 mM glucose (p=0.003), PFKP mRNA levels increased 2 fold in 10 mM of D-(+)-glucose (P = 0.001) and 3 fold in 15 mM of D-(+)-glucose (P = 0.006), and HK2 High glucose concentrations enhanced Bcl-2 expression in 10 mM D-(+)-glucose (P = 0.13) and 15 mM D-(+)-glucose (P = 0.008), but decreased BAX expression in 10 mM D-(+)-glucose (P = 0.02) (Figure 3a).

Expression of miR-186-5p related genes in SW620 cell line under different glucose concentrations

The HIF-1α mRNA levels increased 3.3 fold in 10 mM glucose (P = 0.003) and 4.7 fold in 15 mM glucose (p<0.001) compared with 5 mM of D-(+)-glucose, the PFKP mRNA levels increased 3 fold in 10 mM of D-(+)-glucose (P < 0.001) and 5 fold in 15 mM of D-(+)-glucose (P < 0.001), and the HK2 mRNA levels increased 4 fold in 10 mM of D-(+)-glucose (P = 0.005) and 5 fold in 15 mM of D-(+)-glucose (P =0.002).

The apoptotic markers showed significant upregulation of Bcl-2 expression in 10 mM of D-(+)-glucose (P = 0.02) and in 15 mM of D-(+)-glucose (P = 0.002) when compared to 5 mM of D-(+)-glucose. Also there was significant downregulation of BAX expression in 10 mM of D-(+)-glucose (p=0.001) and in15 mM of D-(+)-glucose (P <0.001) (Figure 3b).

Western blot analysis results

The protein expression levels of HIF-1α and Bcl2 increased gradually in 10 mM glucose and in 15 mM glucose compared with 5 mM of D-(+)-glucose in both Coca2 and SW620 cell line while BAX proteins levels decreased gradually in 10 mM glucose and in 15 mM glucose compared with 5 mM of D-(+)-glucose (Figure 4).

Discussion

Diabetes is characterised by hyperglycemia, which is also a risk factor for cancer progression. High hyperglycemia has been linked to a number of direct and indirect processes that work together to enhance CC

Table 2. Primer Sequences for the Genes Studied

	Forward	Reverse
ANRIL	5′-TGCTCTATCCGCC AATCAGG-3′	5′-GGGCCTCAGTGGCACATACC-3′
miR-186-5p	5'-TCA AAG AAT TCT CCT TTT GGG CT-3'	5'-CGC TTC ACG AAT TTG CGT GTC AT-3'
U6	5′-CGCTTCGGCAGCACATATAC-3′	5′-TTCACGAATTTGCGTGTCATC-3′
GAPDH	5′-GCAAATTCCATGGCACCGT-3′	5′-TCGCCCCACTTGATT TTGG-3′
HIF-1α	5′-CATAAAGTCTGCAACATGGAAGGT-3′	5′-ATTTGATGGGTGAGGAATGGGTT-3′
PFKP	5′-GGAGTGGAGTGGGCTGCTGGAG-3′	5′-CATGTCGGTGCCGCAGAAATCA-3′
HK2	5’-GAGGTCATGGAGCACAGGTT-3′	5’-CTGGTCCAGCTCCAGTAAGC-3’
BAX	5′-GGTTGTCGCCCTTTTCTA-3′	5′-CGGAGGAAGTCCAATGTC-3′
Bcl2	5′-GATGTGATGCCTCTGCGAAG-3′	5′-CATGCTGATGTCTCTGGAATCT-3′

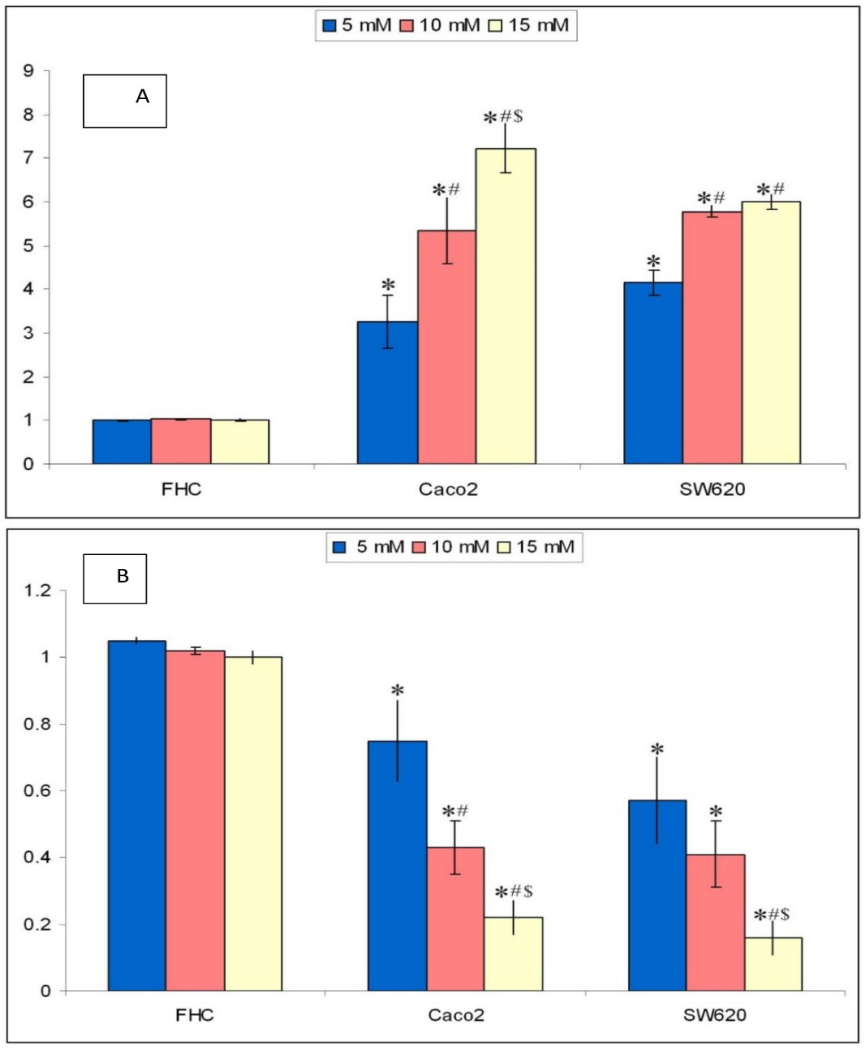

Figure 2. (A) Expression of ANRIL mRNA in colon cancer cell lines cultured under different glucose concentrations *p<0.05 when compared to FHC cell line # p<0.05 when compared to 5 mM D-(+)-glucose concentrations $ p<0.05 when compared to 10 mM D-(+)-glucose concentration (B) Expression of miR-186-5p mRNA in colon cancer cell lines cultured under different glucose concentrations * p<0.05 when compared to FHC cell line # p<0.05 when compared to 5 mM D-(+)-glucose concentration $ p<0.05 when compared to 10 mM D-(+)-glucose concentration

development [23]. Although deregulation of lncRNA has been linked to the advancement of colon cancer, the consequences of high glucose levels on their deregulation and, as a result, colon cancer are unknown.

High glucose (HG) may provide a favourable molecular environment for CC tumorgenesis in T2DM patients, according to the findings of this study. The current study found substantial differences in tumour differentiation, primary tumour invasion, lymph node metastasis, and TNM stage between diabetic and non-diabetic cancer colon patients, implying that diabetic patients have a worse prognosis. When compared to patients without hyperglycemia, Ramteke et al found that hyperglycemic cancer patients have a higher rate of metastasis and poorer outcomes [24]. In comparison to the normal glucose group, Cui et al found that CC patients in the HG group had bigger tumour sizes and lesser tumour differentiation. In addition, the HG group had a greater ratio of patients and more advanced TNM staging and more ulcerative CRC gross type [25].

According to the current study, one of the mechanisms of poorer clinical outcome of CC was deregulation of ANRIL and its taget; miR 186-5p. We found an upregulation of ANRIL expression levels and downregulation of *miR 186-5p* in diabetic colon cancer specimens in relation to those in non-diabetic colon cancer. In the current research there were significant positive correlations between the expression levels of ANRIL with blood glucose levels and HbA1c% and negative correlation between *miR 186-5p* and these diabetic biomarkers indicating that a high-glucose environment can enhance the tumor-promoting effect of ANRIL.

Human cancer and metabolic disease risk are linked to the CDKN2A/B genetic locus. Despite the fact that the locus contains multiple key protein-coding genes, studies suggest that a lesser-known antisense lncRNA encoded at this locus, dubbed ANRIL, may play a role in illness [10]. Because of its frequent overexpression in various malignancies and associated with a poor prognosis, ANRIL is considered an oncogene [26]. ANRIL RNA expression was considerably higher in tumour tissues of colon cancer patients with or without DM than in the distal

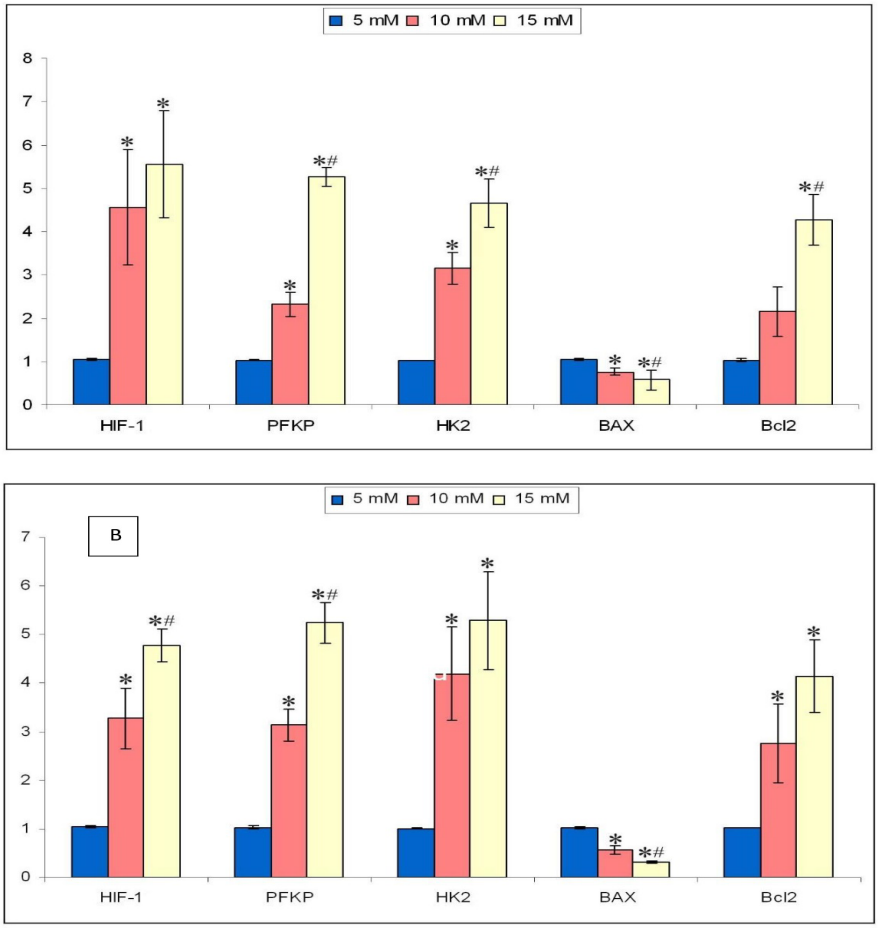

Figure 3. (A) Expression of miR-186-5p related genes in Coca2 cell line under different glucose concentrations *p<0.05 when compared to 5 mM D-(+)-glucose concentrations #p<0.05 when compared to 10 mM D-(+)-glucose concentrations (B) Expression of miR-186-5p related genes in SW620 cell line under different glucose concentrations *p<0.05 when compared to 5 mM D-(+)-glucose concentrations #p<0.05 when compared to 10 mM D-(+)-glucose concentrations

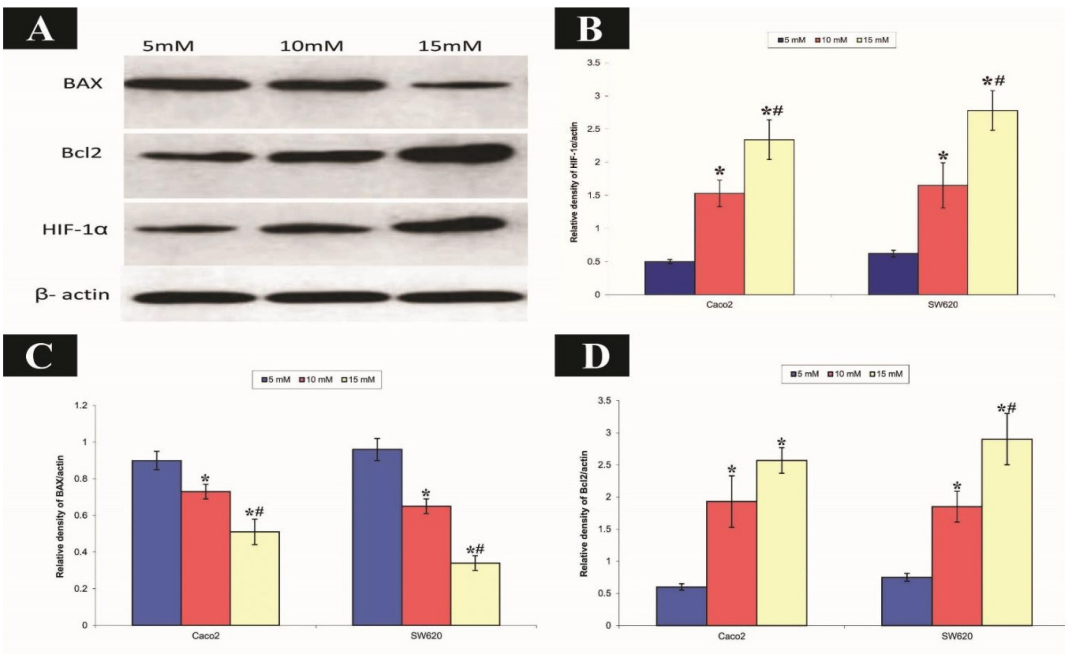

Figure 4. Expression of miR-186-5p related genes in Caco2 and SW620 cell line under different glucose concentrations (A) A representative western blot of HIF-1α, Bcl2 and BAX expression in 5mM, 10 mM, and 15mM glucose concentrations (B) Relative levels of HIF-1α (C) Relative levels of BAX (D) Relative levels of Bcl2 in Caco2 and SW620 cell line under different glucose concentrations. *p<0.05 when compared to 5 mM D-(+)-glucose concentrations #p<0.05 when compared to 10 mM D-(+)-glucose concentrations

normal intestinal mucosa in the current investigation. In addition, when compared to normal colon cells, ANRIL expression levels in Caco2 and SW620 colon cancer cell lines were much higher (FHC).

Previous research had found that ANRIL was substantially expressed in cancer tissues and cell lines from colon cancer patients [27, 28]. Although Gan et al. found considerable ANRIL overexpression in colon cancer tissues; they found no link between ANRIL lncRNA expression and colon cancer prognosis [27]. The new functions of ANRIL, which can enhance cell proliferation and transformation via stem-like cancer cell induction, have been established. ANRIL can also reprogram glucose metabolism to satisfy urgent energy needs by boosting glucose uptake for glycolysis to produce more ATP, which is regulated by the mTOR signal pathway [29]. ANRIL was shown to be substantially expressed in colon cancer cell lines, particularly the SW480 cell line, according to a recent study [30].

A previous study used a bioinformatics tool to determine target miRNAs for ANRIL, demonstrating the targeting of miR-186 with seven complementary binding sites. The luciferase reporter test verified their prediction. MiR-186 could reverse the action of ANRIL in cervical cancer cells in rescue tests, showing a physiological antagonism between ANRIL and miR-186 [15]. There was a negative connection between ANRIL and miR-186-5p expression in this investigation. A subsequent research in multiple myeloma [31] confirmed these findings.

In our study, miR-186-5p RNA expression was considerably lower in tumour tissues of colon cancer patients with or without DM than in the distal normal intestinal mucosa. In addition, when compared to normal colon cells (FHC), the expression of miR-186-5p was significantly reduced in Caco2 and SW620 colon cancer cell lines. This was similar to Li et al., who found that colorectal cancer cell lines (HT116, H29, SW620, and LoVo) have lower miR-186-5p expression than normal colonic epithelial cell line NCM460. Overexpression of MiR-186-5p in colorectal cancer cell line LoVo suppresses proliferation, metastasis, and epithelial-to-mesenchymal transition (EMT) [32]. MiR-186 was also found to be down-regulated in colon cancer tissues and highly metastatic SW620 and LoVo cells, according to Chen et al [33].

Concerning in vitro study of the current research, miR-186-5p expression was inhibited through the overexpressed ANRIL gene in a high glucose induced colon cancer cell lines. The impact of the HG environment on ANRIL expression has been investigated in many cell types. ANRIL was upregulated in human retinal endothelial cells HRECs and diabetic mouse retinal tissues in response to high glucose and diabetes [11]. In HG-induced podocytes, LncRNA ANRIL was also substantially expressed [12]. When HG-treated mouse mesangial cells (SV40-MES13 cells) were compared to regular glucose-treated SV40-MES13 cells and osmotic control-treated SV40-MES13 cells, ANRIL expression was considerably higher. In HG-treated SV40-MES13 cells, knocking down Lnc-ANRIL decreased cell growth and accelerated cell death [13]. In HG-induced cardiomyocytes, however, Liu et al found low expression of miR-186-5p [34]. In AC16 cardiomyocytes, Jiang et al confirmed that downregulation of miRNA-186-5p causes HG-induced cytotoxicity and death [35].

Multiple targets controlled by a single miR can function in concert to regulate the same biological process. Bioinformatics prediction identified HIF-1 as the potential target gene of miR-186 (http://www.microrna.org/microrna/home.do). Through miR-186 introduction and ablation, the negative regulatory effect of miR-186 on HIF-1 was confirmed. The direct binding of miR-186 to HIF-1 was validated using a luciferase reporter experiment [21]. That was validated by other investigations. HIF-1 was a miR-186-5p downstream target [36, 37].

Downregulation of HIF-1 mediated by miR-186 is accompanied by a decrease in intracellular glucose and lactate levels in osteosarcoma cells, showing that miR-186 may restrict glycolysis via HIF-1 suppression [37]. In gastric cancer cell lines MKN45 and SGC7901, MiR-186 inhibits cell growth mediated by HIF-1 [38]. We discovered that downregulation of miR-186-5p facilitated increase of HIF-1 expression in the current study. HIF-1 promotes the synthesis of glucose transporters (Glut) and glycolytic enzymes, which improves glycolysis in cancer cells [22].

Hexokinase, an HIF-1 target gene, is one of the glycolytic pathway's rate-limiting enzymes [39]. There are four subtypes of hexokinase in mammals; type II is insulin-sensitive and overexpressed in poorly differentiated tumour tissues [40]. PFK-1 (phosphofructokinase 1), a possible transcriptional target of HIF-1, is another rate-limiting enzyme in the glycolytic process. PFK-1 is a tetrameric enzyme that comes in three different isoforms: platelet (PFKP), muscle (PFKM), and liver (PFKL), with PFKP being the most common in tumours [41]. We demonstrated that high glucose circumstances can promote glucose uptake, lactate accumulation and cell proliferation in two colon cancer cell lines through a series of in vitro investigations.

The capacity of cancer cells to alter glucose metabolism is one of their most dangerous characteristics. Normal metabolism produces enough energy to keep things running smoothly. The Warburg effect occurs when cancer cells shift to an inefficient glycolytic mode due to high energy demands, driving a large flux of resources into glycolysis rather than oxidative phosphorylation (OXPHOS). Cancer cells boost the synthesis of glycolytic intermediates by upregulating glycolysis. [42]. Cancer cells ingest more glucose than normal cells for this reason, giving them a selective advantage in nutrient-limited environments. These constraints do not exist in hyperglycemia since glucose is readily available. As a result, hyperglycemia stimulates glycolysis in a variety of cancer cells [43]. It improves metabolic reprogramming by increasing the expression of glycolytic enzymes such hexokinase-II (HK-2) and pyruvate kinase M. Lactate produced as a consequence of this rewiring is also used by tumour cells as a shuttle and energy source in areas where blood and oxygen cannot reach due to poor angiogenesis [44].

Under hyperglycemic conditions, normal cells develop slowly. In cancer, however, this process is reversed. Hyperglycemia provides the extra energy needed for cell proliferation. In vitro and under vivo, cancer cells multiply faster in high glucose environments with little apoptosis [45]. Studies in cancer cells show that hyperglycemia promotes cancer cell proliferation by oncogene or metabolic and molecular changes [24]. A recent study found that colon cancer cells cultivated with high glucose had better proliferation ability than colon cancer cells cultured with low glucose [46].

In tumerigenesis, the anti-apoptotic impact is extremely important. This study looked at the levels of expression of apoptosis regulating genes including Bcl-2 and Bax in cancer colon cell lines. The results showed that as the glucose concentration in cancer cell lines increased, Bcl-2 expression increased and Bax expression dropped. The elevated production of HIF- is responsible for hyperglycemia's anti-apoptotic action [21, 47].

In conclusions, to our knowledge, the current findings are the first to show that a high-glucose environment can improve ANRIL's tumor-promoting function. ANRIL can promote colon cancer cell proliferation and change glucose metabolism by downregulating miR-186-5p, which leads to increased production of glycolysis enzymes and apoptosis inhibition. T2DM colon cancer patients exhibited a worse prognosis than those without diabetes, which could be because of their increased ANRIL expression.

Author Contribution Statement

H.M., S.S., N.M., M.A., A.F., H.A. and W.S. shared the design of the work, analysis and interpretation of data. They have approved the submitted version.

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Ethics approval

The study was approved by the research ethical committee of Faculty of Medicine, Zagazig University (ZU-IRB#9852/4-1-2022).

Data availability

Derived data supporting the findings of this study are available from the corresponding author [Shalaby SM] on request.

Conflict of interest

None.

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