Shahid Bahonar University of Kerman
Agricultural Biotechnology Journal
Iranian Biotechnology Society
Print ISSN: 2228-6705 Online ISSN: 2228-6500

Genetic study of miRNA binding site free energy changes and their relationship to histological features in colorectal cancer

Shaimaa A. Al-Oubaidy
* Corresponding author. Department of Human Anatomy and Histology, Medical College, University of Babylon, Iraq. E-mail: shaimaaaloubaidy2010@gmail.com

Asmaa Mohammed Mekkey
Department of Human Anatomy and Histology, Medical College, University of Babylon, Iraq. E-mail: med.asmaa.mohm@uobabylon.edu.iq

Abstract

Objective

Histological changes in colorectal cancer are an essential feature influenced by different factors, and they form an important key role in classification and diagnosis of the disease. Colorectal cancer is the most common cancer type that increased in last year among Iraqi population. **T**his study aimed to estimate the association between histological characterization of colorectal cancer and the impact of mi-RNA free energy changings on their binding sites.

Materials and methods

A cross-sectional study was suggested to achieve the study's goal, histological features and characterization were used along with the analysis of changing in free energy of some mi-RNA (miR-125a-3p, miR-383-5p, miR-148a, miR-148b, miR-365a, miR-26a, miR-130a and miR-27) binding with their target sequences by PCR sequencing and *in silico* miRNA-mRNA hybridization prediction.

Results

The results of these histological features demonstrated that adenocarcinoma was more abundant in this study (75%), about 58% of cases were well differentiated. The glands infiltrate the muscular layer but did not infiltrate the pericolonic fat. The free energy of CRC study samples distribution according to a Binding threshold of ΔG >-15 kcal/mol, a significant change in miR-148b was observed while other mi-RNA varied non-significantly between patient and control group. High energy levels were reported for miR-125a-3p, miR-383-5p, miR-148a, miR-365a and miR-26a in mucinous adenocarcinoma though non-significant. Variation in free energy levels was observed in different stages but non-significant. In the grade categories, significant elevation in miR-27 free energy was seen in well differentiation. All free energy values were non-significantly with respect to metastasis. In all types of mi-RNA, free energy levels were non-significantly changes across the N categories. Significant association was found between miR-365a and miR-148b while an inverse correlation was observed in the control group. On the other hand, in the

control group significant association between miR-130a and miR-383-5p whereas an inverse correlation was found in the patient group.

Conclusion

The findings demonstrated that the binding free energy was greater than -15 kcal/mol in almost all samples, suggesting that the miRNA-mRNA complexes were comparatively stable. miR-27 was found to have a significant impact on the CRC subtype, indicating a role in tumor classification, while miR-148b showed a significant tumor suppressive effect. Modified correlation patterns between patient and control groups' mi-RNA types suggest a power regulatory disturbance that could be useful for CRC diagnosis or treatment.

Keywords: binding sites, colorectal cancer, free energy, histological characterization, mi-RNA

Paper Type: Research Paper.

Citation: Al-Oubaidy, S. A., & Mekkey, A. M. (2026). Genetic study of miRNA binding site free energy changes and their relationship to histological features in colorectal cancer. *Agricultural Biotechnology Journal,* 18(3), 137-154.

Agricultural Biotechnology Journal, 18(3), 137-154. DOI: 10.22103/jab.2026.26904.1863

Received: January 03, 2026. Received in revised form: February 27, 2026.

Accepted: February 28, 2026. Published online: June 30, 2026.

Publisher: Shahid Bahonar University of Kerman & Iranian Biotechnology Society.

Introduction

Various clinical, histopathological, and molecular characteristics have generally been identified as important indicators of colorectal cancer. These characteristics' potential clinical benefits could be independent or dependent (Mahan, 2016). The histopathological markers have been used extensively for malignancy classification to distinguish subtypes, moreover, the biological features of tumors could be used to predict disease status and select appropriate therapeutic strategies. Furthermore, the molecular technology has discovered new biomarkers along with histological and molecular approach (Zaha, 2014). Key in molecular mechanisms in carcinogenesis, like aberrant cell proliferation and angiogenesis can be affected by different signaling pathways (Ferlay et al., 2010). In the United States, American Cancer society evaluate a new cancer cases and deaths in the United States about an estimate 2,041,910 new cases and approximately 618,120 cancer deaths are projected for 2025 (Siegel et al., 2025). This society reported that the CRC is the third most common cancer in both sexes. Signs and symptoms colorectal cancer include bowel habits changing, rectal bleeding, constipation (or some cases diarrhea), unexplained weight loss and abdominal discomfort, most CRCs begin as a polyp which can be removed by colonoscopy (Pawlina, 2023; Torre et al., 2012). The CRC incidence is three times higher in developed countries (Bray et al., 2024). Evidences suggested that CRC imposes

global burden that could be associated with population growth and aging, socioeconomic status, life styles, diet and habits including smoking, unhealthy diet and exercise (Bray and Møller, 2006; Nowatzki et al., 2011; Mahan, 2016). The CRC risk factors including diet and smoking could be modified in contrast to non-modifiable genetic factors (Bognár et al., 2006; Azzoni et al., 2007; Ferlay et al., 2011). One of the crucial genetic factors is miRNAs which regulate about 60% of protein encoding genes (Friedman et al., 2009). miRNA play a major role in the histological changes and pathogenesis of CRC (Liu et al., 2016). They impact in the prognosis of cancer via gene transcription, expression, processing, and target selection (Wu et al., 2008; Ryan et al., 2010; Gong et al., 2012). A meta-analysis showed significant effect of different types of miRNA in CRC like miR-27a rs895819 (Liu et al., 2016), miR-146a and in miR-196a2 (Xu and Tang, 2016) in miR-146a, hsa-miR-149, and hsa-miR-196a2 (Liu et al., 2015). A previous article showed polymorphisms in microRNA gene let-7, miR-149, miR-603, miR-34b/c, and miR-146a gene SNPs were associated with CRC (Rong et al., 2017). Moreover, the study of cancer is very vital due to the high rate of misdiagnosis (10-20%) that can threaten the lives of the elderly (Mortazavi et al., 2005; Mohammadabadi et al., 2009). This research is of fundamental importance for improving more accurate diagnostic methods, developing more effective treatments, increasing the quality of life of patients, and better understanding the social supports needed (Heidarpour et al., 2011; Alavi et al., 2022). The study of cancer and biomarkers is one of the most vital research areas in modern medicine, playing a key role in increasing patient survival rates, early diagnosis, and personalizing treatments (Mohammadabadi Mozafari, 2018). Biomarkers are molecules (such as proteins, DNA, or RNA) that indicate the presence of an abnormal process or a disease, such as cancer, in the body and can be measured in blood, tissue, or other body fluids (Zarrabi et al., 2020). Thus, the aim of this study was to investigate the possibility of free energy changes of some miRNA binding in the histological and tumor characterization of colorectal cancer.

Materials and methods

Sample collection: Colorectal cancer patients attended to a private lab for diagnosis tumor during (2023-2024). All samples were collected prior treatment, and embedded tissue was used in the present study.

Ethical approval: This study was conducted according standard procedures, with informed consent obtained from each patient. Ethical approval was granted by the University of Babylon, College of Science, Department of Biology and Biotechnology (B240505 dated 10/5/2023).

Histological examination: Tumor tissue sections from the FFPE blocks were studied for colorectal cancer characterization by hematoxylin - eosin (H&E) staining. Samples were categorized according to tumor subtype, stage, grades, metastasis, lymph node, TNM system and sex.

DNA extraction: Whole genomic was extracted via DNA extraction kit purchased from Favorgen (FavorPrep™ Tissue Genomic DNA Extraction Kit).

miRNA binding site sequencing: Some miRNAs binding sites types were included in this investigation (Table 1). Binding sites were amplified using specific primers, for IL-23 UTR to produce 249 bp, and for ACVR1 UTR to produce 398 bp. The amplification conditions of these

target loci were as standard condition with annealing temperatures 58 ºC and 57 ºC for IL-23 UTR and ACVR1 UTR, respectively. PCR products were visualized using 1% agarose gel electrophoresis. The products were sequenced via Macrogen Company (Korea) using forward strand.

Data analysis: Histology characterization was conducting using microscope with hematoxylin and eosin (H&E) staining. Data were represented as mean±SD and descriptive data were presented as a percentage %. Statistical analysis was performed using SPSS software (V26), applying independent sample t test. ANOVA one way with p value less than 0.05 was considered statistically significant.

Free energy and miRNA-RNA hybridization: The target sequences were in silico transcripted using Transcription and Translation Tool (https://biomodel.uah.es/en/lab/cybertory/analysis/trans.htm). Free energy and miRNA-RNA hybridization was implemented using BiBiserve (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid) (Rehmsmeier et al., 2004).

The ΔG < 0 (Negative Free Energy): This energy refers to a spontaneous and stable interaction between miRNA and mRNA. Lower (more negative) ΔG values mean stronger binding and effective gene regulation.

ΔG > 0 (Positive Free Energy): This energy indicates an unstable interaction, or the miRNA is unlikely to bind and regulate the target efficiently. While, when Threshold Binding is < -15 kcal/mol indicates a strong and biologically relevant interaction (Wang et al., 2020; Ghoshal et al., 2016).

Table 1. Types of miRNA and their binding sites in the present study

miRNA	Binding sites	Reference
miR-148a	ACVR1 (UTR)	
miR-148b		
miR-365a		(Karimzadeh et al., 2020)
miR-26a		
miR-27a		
miR-130a		
miR-125a	IL-23 (UTR)	
miR-383-5p		

Results

This study designed as a continuation of previous investigations that examined genetic diversity of some miRNA binding sites. In this research, different mi-RNA including miR-125a-3p, miR-383-5p, miR-148a, miR-148b, miR-365a, miR-26a, miR-130a and miR-27 were studied. The histological features of the study samples are shown in Figures 1 and 2. In Figure 1 distribution of study samples according to type of CRC, grade, lymph number, metastasis and stage were clarified. The results showed that adenocarcinoma was the more prevalent type (75%), about 58% of cases were classified as well differentiation. The most samples fell under stage III (61.1%), with regard to lymph node involvement (N classification). 47% of samples were

categorized as N0 and according to metastasis classification. 61% of study were identified as MX. Regarding the microscopic histological changes, the tops left of Figure 2 shows glandular structure with normal organization lost in adenocarcinoma. While, the top right has fibrotic stroma surrounding clusters of malignant cells in invasive carcinoma. The lower figures showed disorganization and crowded glandular structure in adenocarcinoma. In general, the slides show well-formed atypical glandular architecture with hyperchromatic nuclei and high Nuclear/Cytoplasmic ratio associated with desmoplastic reaction. The glands infiltrate the muscular layer but do not invade the pericolonic fat.

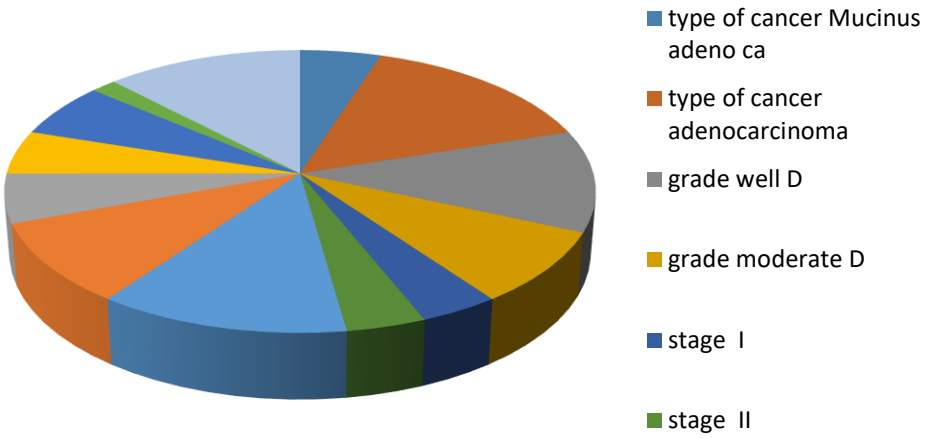

Figure 1. The colorectal cancer samples distribution according to histological features

The free energy of CRC study samples distribution according to Threshold for Binding a ΔG>-15 kcal/mol suggests a strong and biologically relevant interaction (Figure 3). The effect of free energy of miRNAs-RNA in histological characterization in CRC samples is demonstrated in Table 2. The differences between patient and control showed a significant change in miR-148b while other types varied non-significantly. The effect of sex on some miRNAs-RNA free energy showed non-significant differences. In women miR-125a-3p, miR-383-5p and miR-27 exhibited higher free energy values than men. While, miR-148b showed lower energy in women compared to men (Table 3). Two types of CRC were observed in this study (mucinous and adenocarcinoma). Higher free energy was reported for miR-125a-3p, miR-383-5p, miR-148a, miR-365a and miR-26a in mucinous adenocarcinoma. While, other types were lower than adenocarcinoma type (Table 4). All changes were non-significant. Regarding the stages of CRC (I, II and III), variations in free energy levels were observed among different stages. Overall, all changes were non-significant (Table 5). In the grade categories, significant elevation in miR-27 free energy was observed in well differentiation samples, while changes in other types were non-significant (Table 6). Table 7 illustrated free energy levels according to metastasis status. The significant values of all variations were greater than 0.05. Thus, all changes were non-significantly across metastasis

categories. In all types of mi-RNA, free energy levels showed non-significant changes across the three lymph node categories of N (N0, N1 and N2). Slight changes were reported in Table 8.

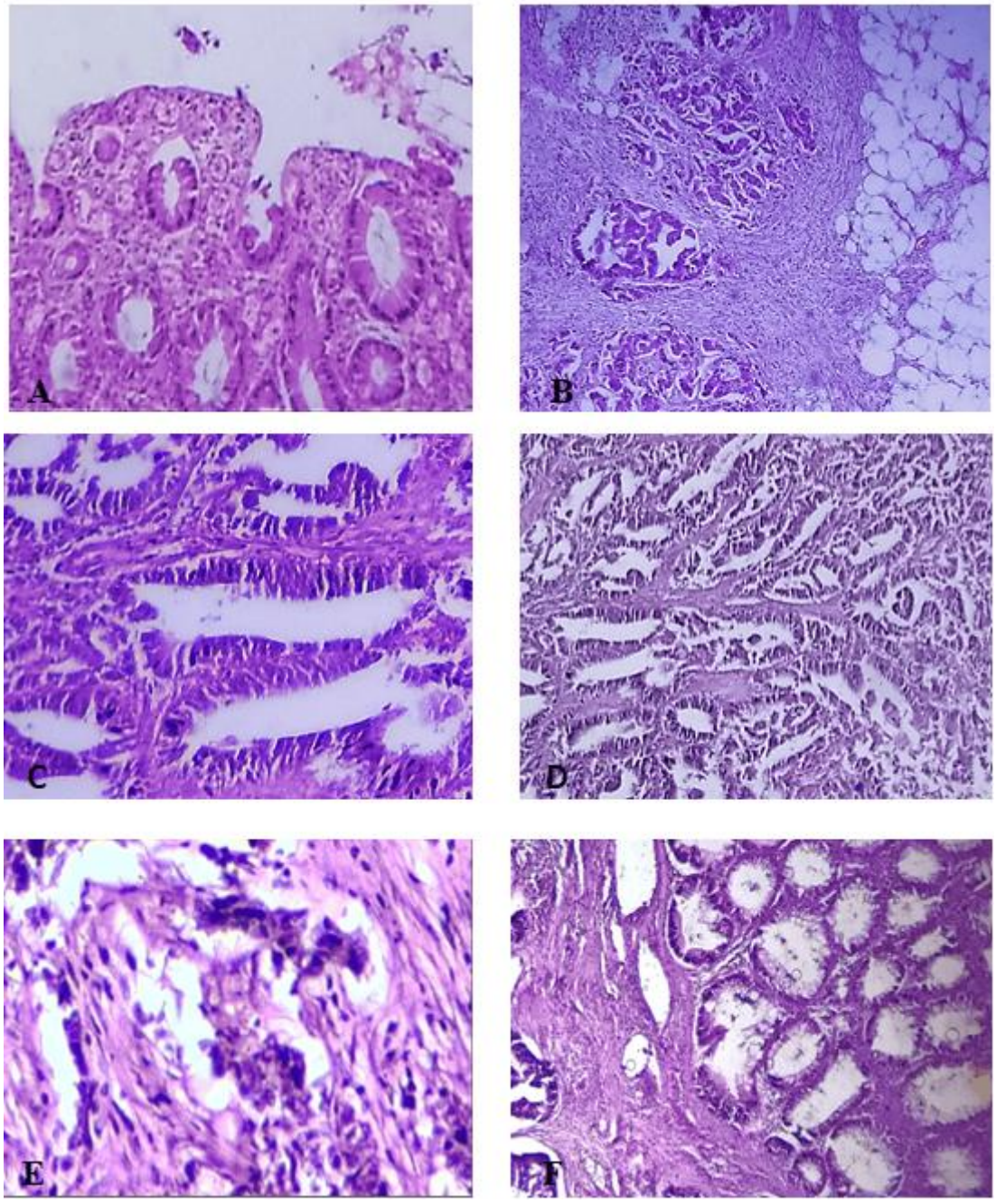

Figure 2. Histological features of colorectal cancer slides with hematoxyline and eosin staining, different panels demonstrate carrying tissue structure

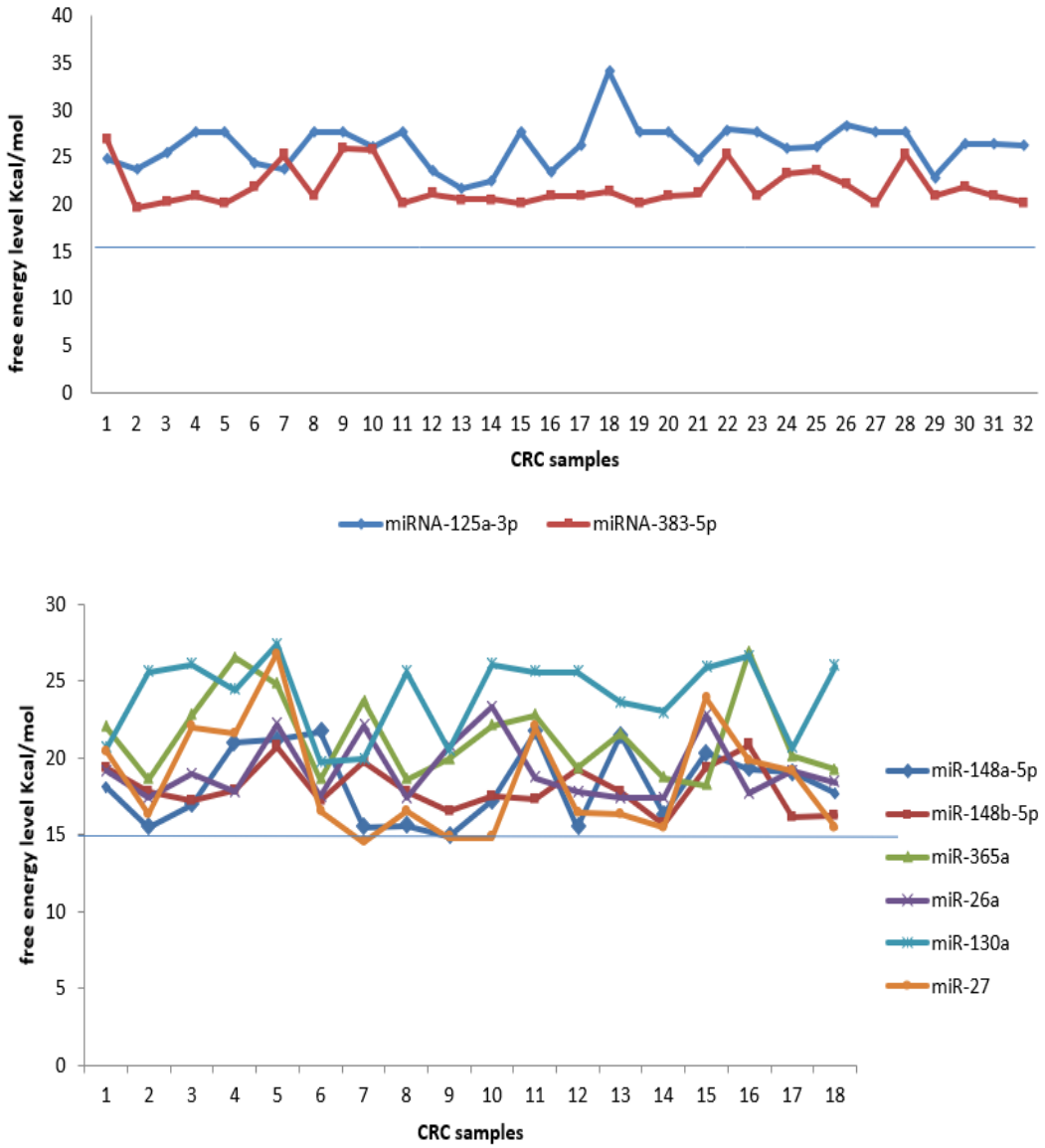

Figure 3. The free energy of CRC study samples distribution according to Threshold for Binding a ΔG = -15 kcal/mol

Table 2. Free energy variation of selected miRNAs-RNA interaction in study samples (CRC v control group)

Types	CRC cases	Control group	p
miR-125a-3p	-23.29±8.521	-25.87±2.62	0.184
miR-383-5p	-21.31±4.317	-21.71±1.766	0.685
miR-148a	-18.27±2.411	-17.66±1.633	0.341
miR-148b	-18.00±1.49	-16.82±1.917	0.033*
miR-365a	-21.40±2.70	-20.08±1.61	0.057
miR-26a	-19.16±2.02	-19.70±2.32	0.427
miR-130a	-24.12±2.57	-23.46±3.08	0.461
miR-27	-18.42±3.51	-17.49±2.14	0.306

Table 3. the effect of CRC samples sex (men Vs women) on the variation of free energy in selected miRNAs-RNA interaction

Types	men	women	p
miR-125a-3p	-22.88±9.15	-24.10±7.822	0.696
miR-383-5p	-20.84±5.11	-21.89±2.042	0.521
miR-148a	-18.32±2.38	-18.16±3.00	0.905
miR-148b	-18.18±1.42	-17.58±1.91	0.472
miR-365a	-21.29±3.00	-21.56±2.39	0.861
miR-26a	-19.17±2.13	-19.34±2.10	0.886
miR-130a	-24.01±2.70	-24.12±2.70	0.942
miR-27	-18.26±3.05	-19.08±5.17	0.680

Table 4. Types of CRC (mucinous and adenocarcinoma) and their effect on the free energy variation of selected miRNAs-RNA interactions

Types	Mucinus adeno carcinoma	adeno carcinoma	p
miR-125a-3p	-25.86±1.719	-22.43±9.82	0.309
miR-383-5p	-22.03±2.42	-20.87±4.85	0.504
miR-148a	-19.30±1.73	-17.62±2.73	0.170
miR-148b	-17.67±1.348	-18.23±1.66	0.464
miR-365a	-20.97±2.879	-21.61±2.82	0.645
miR-26a	-19.74±2.352	-18.89±1.89	0.410
miR-130a	-23.32±2.905	-24.50±2.45	0.371
miR-27	-18.84±3.384	-18.26±3.88	0.751

Table 5. the effect of stages (I, II and III) on the variation in free energy of selected miRNAs-RNA interactions

Types	I	II	III	P
miR-125a-3p	-26.47±1.91	-21.91±9.81	-22.56±9.77	0.727
miR-383-5p	-22.07±1.96	-17.98±7.94	-21.85±2.31	0.126
miR-148a	-15.60±0.001	-19.40±2.25	-18.22±2.53	0.435
miR-148b	-17.80±0.01	-18.50±1.99	-17.92±1.55	0.851
miR-365a	-18.60±0.02	-22.53±4.16	-21.31±2.55	0.494
miR-26a	-17.40±0.09	-19.50±3.29	-19.29±1.90	0.680
miR-130a	-25.60±0.07	-24.13±3.84	-23.91±2.55	0.840
miR-27	-16.50±0.09	-17.03±2.54	-18.94±3.87	0.631

Table 6. The effect of CRC grade (well differentiation vs moderate differentiation) in the free energy variation of selected miRNAs-RNA interactions

Types	Well differentiation	Moderate differentiation	p
miR-125a-3p	-23.93±8.08	-22.40±9.58	0.607
miR-383-5p	-20.77±5.28	-21.84±2.20	0.494
miR-148a	-19.01±2.26	-16.80±2.39	0.072
miR-148b	-17.86±1.78	-18.31±0.93	0.574
miR-365a	-21.71±3.08	-20.66±2.11	0.467
miR-26a	-19.21±1.79	-19.23±2.71	0.988
miR-130a	-23.86±2.82	-24.40±2.37	0.697
miR-27	-19.83±3.72	-15.80±0.89	0.02*

Table 7. The effect of CRC metastasis status (M0, M1 and MX) on the variation in free energy of selected miRNAs-RNA interactions

Types	M0	M1	MX	P
miR-125a-3p	-25.80±1.80	-20.06±17.83	-22.48±9.33	0.476
miR-383-5p	-22.09±2.63	-23.50±3.11	-20.47±5.07	0.463
miR-148a	-18.55±2.72	-21.70±0.006	-17.74±2.23	0.309
miR-148b	-18.55±1.37	-17.30±0.02	-17.71±1.67	0.505
miR-365a	-22.42±2.98	-22.80±0.02	-20.48±2.58	0.335
miR-26a	-19.31±2.05	-18.70±0.05	-19.21±2.27	0.966
miR-130a	-23.38±3.23	-25.60±0.009	-24.35±2.30	0.656
miR-27	-19.72±4.25	-22.00±0.09	-17.27±2.88	0.244

Table 8. The effect of CRC lymph node involvement (N0, N1 and N2) on the variation in free energy of selected miRNAs-RNA interactions

Types	N0	N1	N2	P
miR-125a-3p	-22.75±8.74	-23.71±8.51	-23.84±9.54	0.943
miR-383-5p	-20.63±5.86	-20.66±0.40	-22.90±2.67	0.454
miR-148a	-18.74±2.78	-17.06±2.12	-18.75±2.16	0.461
miR-148b	-18.52±1.45	-17.72±1.53	-17.25±1.69	0.362
miR-365a	-22.51±3.31	-19.92±1.72	-20.60±1.50	0.211
miR-26a	-19.34±2.44	-19.74±2.02	-18.30±1.03	0.593
miR-130a	-24.54±2.81	-24.82±2.42	-21.95±1.57	0.179
miR-27	-18.75±4.09	-18.52±4.138	-17.85±2.32	0.925

The correlation among mi-RNA free energy level was analyzed. The results showed significant positive association between miR-125a-3p and miR-383-5p in patients. While, in the control there was a weak positive association. Significant positive association was also found between miR-27 and miR-148a in patients. While, a weak positive association was seen in the control group. Furthermore, a significant association between miR-365a and miR-148b was found. Whereas, an inverse association was reported in the control group. On the other hand, a significant association was seen between miR-130a and miR-383-5p in the control group. Whereas, in the patients an inverse correlation was found. Other changes were non-significant (Table 9).

Discussion

Some factors related to histopathology characterization of CRC have been studied, including genetic factors, mitochondrial function, the duration of disease, treatment types and the inflammation activities which may vary from case to case. Moreover, mutations in genes related to CRC progression (for example, APC, KRAS, TP53 and some repair genes) have been reported, affecting tumor behavior, growth rate metastasis and treatment response (Armaghany et al., 2012). Notably, mitochondrial dysfunction contributed in CRC histological changes. Alteration in DNA, bioenergetics, and ROS expression play a role in malignant transformation and chemo resistance of CRC cells. The mitochondrial dysfunction may influence cell death and metastasis (Kong et al., 2014; Hertweck and Dasgupta, 2017; Wu et al., 2024). The duration of disease can impact histological features, as prolong exposure to inflammation in cancer microenvironment

leads to tumor prognosis and development (Tripathi et al., 2025). The tumor microenvironment is characterized by inflammatory cytokines, immune cells and oxidative stress which enhance histological changes, as a consequence of diversity of immune response in each case. Tissue infiltration and tumor architecture can be changed (Fu et al., 2021; Martínez-Hernández et al., 2024).

Table 9. The correlation coefficients among the free energy levels of mi-RNAs in study samples (blue for cases and pink for control)

Types of mi-RNAs		miR-125a-3p	miR-383-5p	miR-148a	miR-148b	miR-365a	miR-26a	miR-130a	miR-27
miR-125a-3p	R		0.767**	-0.252	-0.091	0.033	0.239	-0.226	-0.031
	P		0.000	0.298	0.710	0.894	0.324	0.353	0.899
miR-383-5p	R	0.056		0.037	0.381	0.349	0.154	-0.243	0.140
	P	0.811		0.893	0.145	0.186	0.570	0.365	0.604
miR-148a	R	0.267	-0.301		0.166	0.349	-0.024	0.093	0.596**
	P	0.300	0.240		0.496	0.143	0.923	0.705	0.007
miR-148b	R	0.159	-0.228	0.067		0.511*	0.306	0.268	0.451
	P	0.543	0.380	0.763		0.025	0.203	0.268	0.053
miR-365a	R	-0.210	0.153	0.108	-0.294		0.129	0.208	0.422
	P	0.435	0.571	0.634	0.173		0.600	0.393	0.072
miR-26a	R	0.111	-0.394	0.147	0.012	0.021		0.015	0.237
	P	0.683	0.131	0.515	0.955	0.927		0.952	0.329
miR-130a	R	0.280	0.505*	-0.197	-0.160	-0.088	-.0065		0.388
	P	0.294	0.046	0.380	0.466	0.696	0.768		0.101
miR-27	R	0.210	-0.086	0.171	0.377	-0.209	-0.020	-0.042	
	P	0.453	0.761	0.460	0.084	0.364	0.930	0.854	

**. significant at the 0.01 level (2-tailed), *. significant at the 0.05 level (2-tailed).

In the present study the regulation of inflammatory gene expression by mi-RNA free energy showed low effect on histological changes, except miR-27 which had a significant effect in the type of CRC. However, the correlation showed difference among free energy mi-RNAs types and this may be contributed in histology changes in addition to other factors. Literally, the tumor treatment like chemotherapy, radiation and target therapy can stimulate histological changes for instance; some medications induce necrosis or fibrosis (de Sousa et al., 2023). In this study the samples were collected from patients before receiving any types of treatment, thus all histological changes were attributed to other factors. Furthermore, other factors like angiogenesis, extracellular matrix remodeling and infiltration of immune cell are involved in the tumor microenvironment leading to histopathological changes which vary from cases to case. Free energy can be used to predict the stability of a biological system. If the binding of a miRNA to a target mRNA is predicted to be stable, it implies strong interaction. As a result of difficult free energy measured, its changing during reactions is often considered, a negative ΔG indicate that less energy is required for the reaction to proceed which led to increased stability. The hybridization between mi-RNA and target sequence, both high and low free energy regions can be inferred and the overall free energy can be used as an indicator of how strongly bound they are

bound (Yue et al., 2009). The results showed a significant association between the free energy of miR-148b and CRC, while other types showed non-significant differences. The present mi-RNA free energy included miR-148a, miR-148b, miR-365a, miR-26a, miR-27a, miR-130a, miR-125a, and miR-383-5p. These types have been implicated in the progression, development, diagnosis and treatment of cancer. The function of these mi-RNA is tumor suppressive or oncogenic depending on their target mRNA (Smolarz et al., 2022). Investigations found that miR-148a function as a tumor suppresser, its down regulation in CRC which targeted DNA methyletransfrase 1 causes reduction in tumor growth and metastasis and associated with epithelial-mesenchymal transition and resistance to chemotherapy (Shi et al., 2018). The miR-148b has also down regulation in CRC. Its decreased expression is associated with advance tumor stage and poor prognosis (Song et al., 2012). The miR-365a act as oncogene that upper regulated in CRC contributing to metastasis and tumor growth (Hong et al., 2020). Moreover, miR-26a is a tumor suppresser that down regulation in CRC leads to increased stemness and chemoresistance (Murayama and Gotoh, 2019). miR-27a is upper regulated in CRC and it's considered an oncomiR that linked to angiogenesis, invasion, and poor prognosis (Barisciano et al., 2020). Furthermore, a dual role of miR-130a has been observed. High expression is linked to poor survival and increased metastasis (Chen et al., 2017). Down regulation of miR-125a also reported in CRC which enhanced invasion and metastasis (Yang et al., 2019). Finally, miR-383-5p is poorly expressed in CRC which correlate with poor prognosis and metastasis (Bilegsaikhan et al., 2018). In this study, the ACVR1 and IL-23R binding site were targeted to study free energy of some mi-RNAs mRNA hybridization. The previous investigations indicated that these genes are related to inflammation in CRC. The difference in free energies between the two alleles of target sequence was studied as ΔΔG. Therefore, the impact of genetic variant in binding capacity was classified in two classes including increase in binding (for negative ΔΔG) and decreased binding (for positive ΔΔG) (Karimzadeh et al., 2020). The changes in correlations among mi-RNA in CRC cases and control can be considered important evidence of the influence of free energy effect on CRC and histological changes. In general, the free energy values of CRC samples were classified as a stronger binding and effective in gene regulation because $\Delta G < 0$ indicates a spontaneous and stable interaction between miRNA-mRNA. Lower (more negative) ΔG values correspond to stronger binding and more effective gene regulation, which may explain the low number of significant associations between free energy changes and various histological changes (Karimzadeh et al., 2020; Ahmed and Yalda, 2024). Finally, further researches should be conducted to identify the various contributing factors and understand how they interact to influence tumor progression and development.

Conclusion: The current research focuses on important cellular mechanism in CRC, particularly, the interaction between some mi-RNAs and their mRNA target sequence. A key aspect examined is the free energy of miRNA-mRNA binding, which reflect the stability of these interactions and may influence cellular energy dynamics within tumor environments. The results revealed that in nearly all samples, the binding free energy exceeded -15 kcal/mol, indicating relatively stable miRNA-mRNA complexes. Among mi-RNAs studied, miR-148b demonstrated a significant tumor suppressive effect, aligning with previous reports of its role in inhibiting

cancer progression. Additionally, miR-27 was found to valuably impact CRC type, suggesting role in tumor classification, notably, changes in the correlation pattern among mi-RNAs types were observed between patients and control group, indicate to abrader regulatory disruption that may have diagnostic or therapeutic value in CRC.

Author Contributions

S. A. A.: Writing-preparation of the first draft, writing-review, visualization, and funding acquisition; and A. M. M.: conceptualization, methodology, software, validation, formal analysis, investigation, resources, and data curation.

Data availability statement

The data of this research article are available from the authors upon reasonable request.

Acknowledgements

The authors thanks all the staff of Department of Human Anatomy and Histology, Medical College, University of Babylon, Iraq for their supporting to generate this research paper.

Ethical considerations

This study was conducted according standard procedures, with informed consent obtained from each patient. Ethical approval was granted by the University of Babylon, College of Science, Department of Biology and Biotechnology (B240505 dated 10/5/2023). There was no scientific misconduct, plagiarism, fabrication, or falsification in the study's execution.

Funding

This research is supported by the Department of Human Anatomy and Histology, Medical College, University of Babylon, Iraq.

Conflict of interest

The authors declare that there is no conflict of interest related to this research.

References

Ahmed, H. S., & Yalda, M. I. (2024). The receptor tyrosine kinase EphA2 and integrin-linked kinase expression in colorectal cancer in relation to the severity of the tumor. *Medical Journal of Babylon*, *21*(Suppl. 1), S1–S7. https://doi.org/10.4103/mjbl.mjbl_221_23

Alavi, M., Mozafari, M. R., Ghaemi, S., Ashengroph, M., Hasanzadeh Davarani, F., & Mohammadabadi, M. (2022). Interaction of epigallocatechin gallate and quercetin with spike glycoprotein (S-glycoprotein) of SARS-CoV-2: In silico study. *Biomedicines*, *10*(12), Article 3074. https://doi.org/10.3390/biomedicines10123074

Armaghany, T., Wilson, J. D., Chu, Q., & Mills, G. (2012). Genetic alterations in colorectal cancer. *Gastrointestinal Cancer Research*, *5*(1), 19–27. https://pmc.ncbi.nlm.nih.gov/articles/PMC3348713/

Azzoni, C., Bottarelli, L., Campanini, N., Di Cola, G., Bader, G., Mazzeo, A., Salvemini, C., Morari, S., Di Mauro, D., Donadei, E., Roncoroni, L., Bordi, C., & Sarli, L. (2007). Distinct molecular patterns based on proximal and distal sporadic colorectal cancer: Arguments for different mechanisms in the tumorigenesis. *International Journal of Colorectal Disease*, *22*(2), 115–126. https://doi.org/10.1007/s00384-006-0093-x

Barisciano, G., Colangelo, T., Rosato, V., Muccillo, L., Taddei, M. L., Ippolito, L., Chiarugi, P., Galgani, M., Bruzzaniti, S., Matarese, G., Fassan, M., Agostini, M., Bergamo, F., Pucciarelli, S., Carbone, A., Mazzoccoli, G., Colantuoni, V., Bianchi, F., & Sabatino, L. (2020). miR-27a is a master regulator of metabolic reprogramming and chemoresistance in colorectal cancer. *British Journal of Cancer*, *122*(9), 1354–1366. https://doi.org/10.1038/s41416-020-0773-2 (Erratum published 2020, *British Journal of Cancer*, *122*(10), 1576. https://doi.org/10.1038/s41416-020-0855-1)

Bilegsaikhan, E., Liu, H. N., Shen, X. Z., & Liu, T. T. (2018). Circulating miR-338-5p is a potential diagnostic biomarker in colorectal cancer. *Journal of Digestive Diseases*, *19*(7), 404–410. https://doi.org/10.1111/1751-2980.12643

Bognár, G., Ledniczky, G., István, G., & Ondrejka, P. (2006). A colorectalis adenocarcinomák áttétképzésének molekuláris alapjai [Molecular mechanisms in development of colorectal cancer metastasis]. *Magyar Sebészet*, *59*(5), 342–349. https://pubmed.ncbi.nlm.nih.gov/17201342/

Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R. L., Soerjomataram, I., & Jemal, A. (2024). Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, *74*(3), 229–263. https://doi.org/10.3322/caac.21834

Bray, F., & Møller, B. (2006). Predicting the future burden of cancer. *Nature Reviews Cancer*, *6*(1), 63–74. https://doi.org/10.1038/nrc1781

Chen, W., Tong, K., & Yu, J. (2017). MicroRNA-130a is upregulated in colorectal cancer and promotes cell growth and motility by directly targeting forkhead box F2. *Molecular Medicine Reports*, *16*(4), 5241–5248. https://doi.org/10.3892/mmr.2017.7257

de Sousa, I. V. F., Lopes, J. M. D., Nogueiro, J. P. M., Costa, T. R., Barbosa, L. E. R., & Aral, M. M. M. (2023). Histological tumor response predicts clinical outcome in patients with colorectal peritoneal metastasis treated with preoperative chemotherapy followed by cytoreduction and HIPEC. *Pleura and Peritoneum*, *8*(1), 37–44. https://doi.org/10.1515/pp-2022-0117

Ferlay, J., Parkin, D. M., & Steliarova-Foucher, E. (2010). Estimates of cancer incidence and mortality in Europe in 2008. *European Journal of Cancer*, *46*(4), 765–781. https://doi.org/10.1016/j.ejca.2009.12.014

Friedman, R. C., Farh, K. K.-H., Burge, C. B., & Bartel, D. P. (2009). Most mammalian mRNAs are conserved targets of microRNAs. *Genome Research*, *19*(1), 92–105. https://doi.org/10.1101/gr.082701.108

Fu, T., Dai, L.-J., Wu, S.-Y., Xiao, Y., Ma, D., Jiang, Y.-Z., & Shao, Z.-M. (2021). Spatial architecture of the immune microenvironment orchestrates tumor immunity and therapeutic

response. *Journal of Hematology & Oncology*, *14*, Article 98. https://doi.org/10.1186/s13045-021-01103-4

Ghoshal, A., Shankar, R., Bagchi, S., Grama, A., & Chaterji, S. (2015). MicroRNA target prediction using thermodynamic and sequence curves. *BMC Genomics*, *16*, Article 999. https://doi.org/10.1186/s12864-015-1933-2 (Erratum published 2016, *BMC Genomics*, *17*, Article 216. https://doi.org/10.1186/s12864-016-2367-1)

Gong, J., Tong, Y., Zhang, H.-M., Wang, K., Hu, T., Shan, G., Sun, J., & Guo, A.-Y. (2012). Genome-wide identification of SNPs in microRNA genes and the SNP effects on microRNA target binding and biogenesis. *Human Mutation*, *33*(1), 254–263. https://doi.org/10.1002/humu.21641

Heidarpour, F., Mohammadabadi, M. R., Zaidul, I. S. M., Maherani, B., Saari, N., Hamid, A. A., Abas, F., Manap, M. Y. A., & Mozafari, M. R. (2011). Use of prebiotics in oral delivery of bioactive compounds: A nanotechnology perspective. *Pharmazie*, *66*(5), 319–324. https://doi.org/10.1691/ph.2011.0279

Hertweck, K. L., & Dasgupta, S. (2017). The landscape of mtDNA modifications in cancer: A tale of two cities. *Frontiers in Oncology*, *7*, Article 262. https://doi.org/10.3389/fonc.2017.00262

Hong, Y.-G., Xin, C., Zheng, H., Huang, Z.-P., Yang, Y., Zhou, J.-D., Gao, X.-H., Hao, L., Liu, Q.-Z., Zhang, W., & Hao, L.-Q. (2020). miR-365a-3p regulates ADAM10-JAK-STAT signaling to suppress the growth and metastasis of colorectal cancer cells. *Journal of Cancer*, *11*(12), 3634–3644. https://doi.org/10.7150/jca.42731 (Erratum published 2023, *Journal of Cancer*, *14*(2), 318–321. https://doi.org/10.7150/jca.79828)

Karimzadeh, M. R., Zarin, M., Ehtesham, N., Khosravi, S., Soosanabadi, M., Mosallaei, M., & Pourdavoud, P. (2020). MicroRNA binding site polymorphism in inflammatory genes associated with colorectal cancer: Literature review and bioinformatics analysis. *Cancer Gene Therapy*, *27*(10–11), 739–753. https://doi.org/10.1038/s41417-020-0172-0

Kong, Y., Trabucco, S. E., & Zhang, H. (2014). Oxidative stress, mitochondrial dysfunction and the mitochondria theory of aging. *Interdisciplinary Topics in Gerontology*, *39*, 86–107. https://doi.org/10.1159/000358901

Liu, F., Dear, K., Huang, L., Liu, L., Shi, Y., Nie, S., Liu, Y., Lu, Y., & Xiang, H. (2016). Association between microRNA-27a rs895819 polymorphism and risk of colorectal cancer: A meta-analysis. *Cancer Genetics*, *209*(9), 388–394. https://doi.org/10.1016/j.cancergen.2016.08.003

Liu, X. X., Wang, M., Xu, D., Yang, J. H., Kang, H. F., Wang, X. J., Lin, S., Yang, P. T., Liu, X. H., & Dai, Z. J. (2015). Quantitative assessment of the association between genetic variants in microRNAs and colorectal cancer risk. *BioMed Research International*, *2015*, Article 276410. https://doi.org/10.1155/2015/276410

Mahan, L. K. (2016). *Krause's food & the nutrition care process* (14th ed.). Elsevier.

Martínez-Hernández, R., Sánchez de la Blanca, N., Sacristán-Gómez, P., Serrano-Somavilla, A., Muñoz De Nova, J. L., Sánchez Cabo, F., Heyn, H., Sampedro-Núñez, M., & Marazuela, M. (2024). Unraveling the molecular architecture of autoimmune thyroid diseases at spatial

resolution. *Nature Communications*, *15*(1), Article 5895. https://doi.org/10.1038/s41467-024-50192-5

Mohammadabadi, M. R., & Mozafari, M. R. (2018). Enhanced efficacy and bioavailability of thymoquinone using nanoliposomal dosage form. *Journal of Drug Delivery Science and Technology*, *47*, 445–453. https://doi.org/10.1016/j.jddst.2018.08.019

Mohammadabadi, M. R., El-Tamimy, M., Gianello, R., & Mozafari, M. R. (2009). Supramolecular assemblies of zwitterionic nanoliposome-polynucleotide complexes as gene transfer vectors: Nanolipoplex formulation and in vitro characterization. *Journal of Liposome Research*, *19*(2), 105–115. https://doi.org/10.1080/08982100802547326

Mortazavi, S. M., Mohammadabadi, M. R., & Mozafari, M. R. (2005). Applications and in vivo behaviour of lipid vesicles. In M. R. Mozafari (Ed.), *Nanoliposomes: From fundamentals to recent developments* (pp. 67–76). Elsevier.

Murayama, T., & Gotoh, N. (2019). Drug resistance mechanisms of cancer stem-like cells and their therapeutic potential as drug targets. *Cancer Drug Resistance*, *2*(3), 457–470. https://doi.org/10.20517/cdr.2019.36

Nowatzki, J., Moller, B., & Demers, A. (2011). Projection of future cancer incidence and new cancer cases in Manitoba, 2006-2025. *Chronic Diseases in Canada*, *31*(2), 71–78. https://pubmed.ncbi.nlm.nih.gov/21466757/

Pawlina, W. (2023). *Histology: A text and atlas: With correlated cell and molecular biology* (9th ed.). Wolters Kluwer.

Rehmsmeier, M., Steffen, P., Hochsmann, M., & Giegerich, R. (2004). Fast and effective prediction of microRNA/target duplexes. *RNA*, *10*(10), 1507–1517. https://doi.org/10.1261/rna.5248604

Rong, G. Q., Zhang, X. M., Chen, B., Yang, X. D., Wu, H. R., & Gong, W. (2017). MicroRNA gene polymorphisms and the risk of colorectal cancer. *Oncology Letters*, *13*(5), 3617–3623. https://doi.org/10.3892/ol.2017.5885

Ryan, B. M., Robles, A. I., & Harris, C. C. (2010). Genetic variation in microRNA networks: The implications for cancer research. *Nature Reviews Cancer*, *10*(6), 389–402. https://doi.org/10.1038/nrc2867 (Erratum published 2010, *Nature Reviews Cancer*, *10*(7), 523)

Shi, H., Chen, X., Jiang, H., Wang, X., Yu, H., Sun, P., & Sui, X. (2018). miR-148a suppresses cell invasion and migration in gastric cancer by targeting DNA methyltransferase 1. *Oncology Letters*, *15*(4), 4944–4950. https://doi.org/10.3892/ol.2018.7907

Siegel, R. L., Kratzer, T. B., Giaquinto, A. N., Sung, H., & Jemal, A. (2025). Cancer statistics, 2025. *CA: A Cancer Journal for Clinicians*, *75*(1), 10–45. https://doi.org/10.3322/caac.21871

Smolarz, B., Durczyński, A., Romanowicz, H., Szyłło, K., & Hogendorf, P. (2022). miRNAs in cancer (review of literature). *International Journal of Molecular Sciences*, *23*(5), Article 2805. https://doi.org/10.3390/ijms23052805

Song, Y., Xu, Y., Wang, Z., Chen, Y., Yue, Z., Gao, P., Xing, C., & Xu, H. (2012). MicroRNA-148b suppresses cell growth by targeting cholecystokinin-2 receptor in colorectal cancer. *International Journal of Cancer*, *131*(5), 1042–1051. https://doi.org/10.1002/ijc.26485

Torre, L. A., Bray, F., Siegel, R. L., Ferlay, J., Lortet-Tieulent, J., & Jemal, A. (2015). Global cancer statistics, 2012. *CA: A Cancer Journal for Clinicians*, *65*(2), 87–108. https://doi.org/10.3322/caac.21262

Tripathi, S., Sharma, Y., & Kumar, D. (2025). Unveiling the link between chronic inflammation and cancer. *Metabolism Open*, *25*, Article 100347. https://doi.org/10.1016/j.metop.2025.100347

Wang, Y., Li, Y., Ma, Z., Yang, W., & Ai, C. (2010). Mechanism of microRNA-target interaction: Molecular dynamics simulations and thermodynamics analysis. *PLoS Computational Biology*, *6*(7), Article e1000866. https://doi.org/10.1371/journal.pcbi.1000866

Wu, M., Jolicoeur, N., Li, Z., Zhang, L., Fortin, Y., L'Abbe, D., Yu, Z., & Shen, S. H. (2008). Genetic variations of microRNAs in human cancer and their effects on the expression of miRNAs. *Carcinogenesis*, *29*(9), 1710–1716. https://doi.org/10.1093/carcin/bgn073

Wu, Z., Xiao, C., Long, J., Huang, W., You, F., & Li, X. (2024). Mitochondrial dynamics and colorectal cancer biology: Mechanisms and potential targets. *Cell Communication and Signaling*, *22*(1), Article 91. https://doi.org/10.1186/s12964-024-01490-4

Xu, L., & Tang, W. (2016). Associations of polymorphisms in miR-196a2, miR-146a and miR-149 with colorectal cancer risk: A meta-analysis. *Pathology & Oncology Research*, *22*(2), 261–267. https://doi.org/10.1007/s12253-014-9843-1

Yang, M., Tang, X., Wang, Z., Wu, X., Tang, D., & Wang, D. (2019). miR-125 inhibits colorectal cancer proliferation and invasion by targeting TAZ. *Bioscience Reports*, *39*(12), Article BSR20190193. https://doi.org/10.1042/BSR20190193

Yue, D., Liu, H., & Huang, Y. (2009). Survey of computational algorithms for microRNA target prediction. *Current Genomics*, *10*(7), 478–492. https://doi.org/10.2174/138920209789208219

Zaha, D. C. (2014). Significance of immunohistochemistry in breast cancer. *World Journal of Clinical Oncology*, *5*(3), 382–392. https://doi.org/10.5306/wjco.v5.i3.382

Zarrabi, A., Alipoor Amro Abadi, M., Khorasani, S., Mohammadabadi, M., Jamshidi, A., Torkaman, S., Taghavi, E., Mozafari, M. R., & Rasti, B. (2020). Nanoliposomes and tocosomes as multifunctional nanocarriers for the encapsulation of nutraceutical and dietary molecules. *Molecules*, *25*(3), Article 638. https://doi.org/10.3390/molecules25030638

مجله بیوتکنولوژی کشاورزی
شاپا چاپی: ۶۷۰۵-۲۲۲۸ شاپا الکترونیکی: ۶۵۰۰-۲۲۲۸

مطالعه ژنتیکی تغییرات انرژی آزاد جایگاه اتصال miRNA و ارتباط آن با ویژگی‌های هیستوپاتولوژیک در سرطان کولورکتال

شیماء ع. العبیدی

* *نویسنده مسئول.* گروه آناتومی و بافت‌شناسی انسان، دانشکده پزشکی، دانشگاه بابل، عراق. ایمیل: shaimaaaloubaidy2010@gmail.com

اسماء محمد مکی

گروه آناتومی و بافت‌شناسی انسان، دانشکده پزشکی، دانشگاه بابل، عراق. ایمیل: med.asmaa.mohm@uobabylon.edu.iq

تاریخ دریافت: ۱۴۰۴/۱۰/۱۳ تاریخ دریافت فایل اصلاح شده نهایی: ۱۴۰۴/۱۲/۰۸ تاریخ پذیرش: ۱۴۰۴/۱۲/۰۹

چکیده

هدف: تغییرات بافت‌شناسی در سرطان کولورکتال یکی از ویژگی‌های اساسی این بیماری است که تحت تأثیر عوامل مختلف قرار دارد و نقش مهمی در طبقه‌بندی و تشخیص ایفا می‌کند. سرطان کولورکتال از شایع‌ترین انواع سرطان بوده که در سال‌های اخیر در جمعیت عراق افزایش یافته است. هدف این مطالعه بررسی ارتباط بین ویژگی‌های هیستوپاتولوژیک سرطان کولورکتال و تغییرات انرژی آزاد اتصال miRNAها به جایگاه‌های هدف آن‌ها بود.

مواد و روش‌ها: این پژوهش به‌صورت مقطعی طراحی شد. در این مطالعه، ویژگی‌های هیستوپاتولوژیک نمونه‌ها بررسی شد و تغییرات انرژی آزاد برخی miRNAها (شامل miR-125a-3p، miR-383-5p، miR-148a، miR-148b، miR-365a، miR-26a، miR-130a و miR-27) در اتصال به توالی‌های هدف، از طریق تعیین توالی PCR و پیش‌بینی هیبریداسیون miRNA-mRNAبه‌صورت in silico ارزیابی گردید.

نتایج: نتایج بررسی‌های بافت‌شناسی نشان داد که آدنوکارسینوم شایع‌ترین نوع تومور در این مطالعه بود (۷۵٪) و حدود ۵۸٪ موارد دارای تمایز خوب بودند. غدد سرطانی به لایه عضلانی نفوذ کرده بودند، اما به چربی اطراف کولون گسترش نیافته بودند. بر اساس آستانه اتصال ΔG >-15 kcal/mol، تغییر معنی‌داری در انرژی آزاد miR-148b مشاهده شد، در حالی که سایر miRNAها تفاوت معنی‌داری بین گروه بیمار و کنترل نشان ندادند. سطح انرژی آزاد بالاتری برای miR-125a-3p، miR-383-5p، miR-148a، miR-365a و miR-26a در آدنوکارسینوم موکوسی مشاهده شد، اما این اختلاف‌ها از نظر آماری معنی‌دار نبودند. تغییرات انرژی آزاد در مراحل مختلف بیماری مشاهده شد، ولی معنی‌دار نبود. در طبقه‌بندی بر اساس درجه تومور، افزایش معنی‌دار انرژی آزاد miR-27 در موارد با تمایز خوب مشاهده شد. تمامی مقادیر انرژی آزاد در ارتباط با متاستاز تفاوت معنی‌داری نداشتند. همچنین در تمامی انواع miRNA، تغییرات انرژی آزاد در دسته‌بندی‌های N (درگیری غدد لنفاوی) معنی‌دار نبود. ارتباط معنی‌داری بین miR-365a و miR-148b مشاهده شد، در حالی که در گروه کنترل بین این دو همبستگی معکوس وجود داشت. همچنین در

گروه کنترل، ارتباط معنی‌داری بین miR-130a و miR-383-5p دیده شد، در حالی که در گروه بیماران این رابطه به صورت معکوس گزارش گردید.

نتیجه‌گیری: یافته‌ها نشان دادند که انرژی آزاد اتصال در تقریباً تمامی نمونه‌ها بیشتر از kcal/mol 15- بود که نشان‌دهنده پایداری نسبی کمپلکس‌های miRNA-mRNA است. miR-27 تأثیر معنی‌داری بر زیرگروه‌های سرطان کولورکتال داشت و می‌تواند در طبقه‌بندی تومور نقش داشته باشد، در حالی که miR-148b اثر مهارکننده تومور قابل توجهی نشان داد. تغییر الگوهای همبستگی miRNAها بین گروه بیمار و کنترل بیانگر اختلال در شبکه‌های تنظیمی است که می‌تواند در تشخیص یا درمان سرطان کولورکتال مورد استفاده قرار گیرد.

کلمات کلیدی: انرژی آزاد، جایگاه‌های اتصال، سرطان کولورکتال، ویژگی‌های بافت‌شناسی، miRNA

نوع مقاله: پژوهشی

استناد: شیماء ع. العبیدی، اسماء محمد مکی (۱۴۰۵) مطالعه ژنتیکی تغییرات انرژی آزاد جایگاه اتصال miRNA و ارتباط آن با ویژگی‌های هیستوپاتولوژیک در سرطان کولورکتال. *مجله بیوتکنولوژی کشاورزی*، ۱۸(۳)، ۱۳۷-۱۵۴.

Publisher: Shahid Bahonar University of Kerman & Iranian Biotechnology Society.