




Identification of OncoMir Biomarkers: Comprehensive Analysis of Fatty Acid Pathway Genes in Colorectal Cancer Progression and Their Prognostic Implications

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Abstract

MicroRNAs play a pivotal role in driving colorectal cancer (CRC) progression through their influence on gene expression. In this study, we conducted a comprehensive analysis of gene expression profiles in CRC cell lines, focusing on the genes involved in the fatty acid pathway and their associated microRNAs. Our analysis unveiled a consistent pattern of decreased expression for miR-16-5p, miR-195-5p, miR-27a-3p, and miR-497-5p across all CRC cell lines studied. Intriguingly, the targeted genes showed a contrasting trend, with increased expression levels. To reinforce our findings, we conducted a comparative analysis with The Cancer Genome Atlas database, which corroborated our results. Notably, we found a strong association between high expression levels of PPARG and VEGFA and a shorter Disease-Free Survival (DFS) in CRC patients. Conversely, elevated expression of PPARGC1A was linked to better overall survival outcomes. These findings underscore the significant impact of microRNAs on the regulation of gene expression in colorectal cancer and highlight potential biomarkers for assessing disease progression and patient outcomes.

Keywords Colorectal cancer · Fatty acid · miRNAs · OncoMir

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Introduction

Colorectal cancer (CRC) stands as one of the most prevalent gastrointestinal malignancies, holding the dubious distinction of being the third most recognized cancer and the second leading cause of cancer-related mortality worldwide at 10.1% and 9.4%, respectively [1]. The current therapeutic approaches primarily hinge on surgery and chemotherapy [2]. However, emerging as a promising avenue is targeted therapy, a novel approach that specifically targets genes and proteins responsible for the growth and development of cancer cells. CRC is now understood as a multifaceted disorder involving genetic, epigenetic, and the activation of oncogenic pathways in its progression [3]. Traditionally, cancer has been viewed as an outcome of accumulating mutations in pivotal genes, including tumor suppressors and oncogenes, leading normal cells to transform into malignant ones through deregulated cell cycle. Nevertheless, genomic sequencing of colon cancer has illuminated that only a small fraction of tumor-specific mutations is involved in CRCs [4]. Recent research shows that cancer reprograms

metabolism and alters metabolites to facilitate tumorigenesis and metastasis, with reviews highlighting abnormal cancer-related metabolites, their potential as noninvasive biomarkers, and the applications and challenges of metabolomics in liquid biopsy [5]. It has been also reported that colorectal cancer (CRC) screening with blood-based biomarkers could improve participation and reduce CRC incidence and mortality, though current blood tests are less effective and costlier compared to established methods like FIT, colonoscopy, and stool DNA testing; higher sensitivity for advanced precancerous lesions (APL) is essential for blood tests to match the effectiveness of traditional methods [6].

In light of this, the influence of epigenetic factors, such as DNA methylation and the regulation of microRNA (miRNA) expression profiles, has gained recognition in cancer risk and its role in tumorigenesis and progression as oncogenes or tumor suppressors [7]. MiRNAs are a group of non-coding RNAs with the length of approximately 22 nucleotides that are highly conserved and single-stranded. Previous research has established the engagement of miRNAs in the development of various types of cancers including CRC [8, 9]. On the other hand, lipid metabolism, as an important pathway of cellular energy metabolism, can affect several cellular processes and linked to the development of the variety of diseases including cardiovascular disease, obesity, diabetes and cancer. Deregulation of lipid metabolism in cancer has been reported previously [10, 11]. For instance, the association between Fatty Acid Synthetic activities in CRC neoplasms had been observed and a higher level of fatty acids in association with higher incidence of CRC was detected [12, 13]. Fatty acid-binding proteins (FABPs), like FABP2, play a role in the transport and metabolism of long-chain fatty acids and other hydrophobic molecules. Overexpression of FABPs has been linked to cell growth, proliferation, and cancer development. FABPs are fatty acid-binding proteins involved in intracellular transport of bioactive fatty acids. FABP2 (I-FABP) is an intestinal fatty acid-binding protein that is in large quantities found in small intestinal epithelial cells. Several lines of evidence suggested that FABPs family is highly over expressed in cell growth, proliferation and cancer development. Over expression of FABP1 has been reported in hepatocellular carcinoma (HCC) which is in correlation to vascular endothelial growth factor (VEGF) expression [14–16]. FABPs act as natural peroxisome proliferator-activated receptor (PPAR γ) receptor ligands and activate PPAR γ directly. This activation results in increased expression of vascular endothelial growth factors (VEGFs) which leads to cancer progression. PPARs family included three isotypes including α , β , and γ , also referred to as NR1C1, NR1C2, and NR1C3, respectively or PPARA, PPARD and PPARG [17]. PPARs family is currently in use as potential therapeutic targets for the variety of disorders and diseases such as obesity, diabetes and cardiac

disease due to their direct impact on energy homeostasis by the activation of fatty acid metabolism as well as stimulation of gluconeogenesis [18–20]. However, the potentiality in the risk of cancer has attracted attention recently and some evidence revealed the role of this gene family as hallmark of cancer progression capability [21]. Fatty Acid Synthesizing (FAS) Enzyme which encoded by the FASN gene is a key metabolic enzyme in the synthesis of fatty acids that has been found to be overexpressed in various tumors. FASN activation is reported as a hallmark in several human malignancies that operate as an oncogene factor [22, 23]. Previous studies show that PGC1 α acts as a co-activator of PPAR γ which is highly adaptable in the metabolism of the cancer. Both high and low expression of this gene have been reported in the association with cancer which indicates its attribution with both oncogenic and tumor suppressive features [24–26]. Vascular endothelial growth factor (VEGF), initially referred to as vascular permeability factor (VPF), is a sub-family which are similar to platelet-derived growth factor family that stimulates blood cells formation with their highly conserved receptor-binding cysteine-knot structure and play a vital role in the signaling pathway of vasculogenesis and angiogenesis [27]. Some members of VEGF family are validated as therapeutic target for cancer treatments for example anti-VEGF-A agent is using as an anti-angiogenesis approach for numerous human cancers. VEGF-B is another member of this family and its over expression has been shown in human cancer with arteriogenesis function [28].

The association between cancer and the alterations in fatty acid (FA) metabolism is increasingly being recognized [29]. Thus, any alteration in fatty acid metabolism pathway genes could be considered as valuable targets to control the cancer improvement which could be achieved through respective miRNAs as effective factors with inducing epigenetic changes. In this present study we aimed to target some important genes involved in the fatty acid pathway with their associated miRNAs using in silico analysis and assess the level of expression of each group in four CRC cell lines. On the other hand, we focused on the intricate relationship between fatty acid metabolism and colorectal cancer (CRC), mediated through the regulatory effects of non-coding RNAs, particularly microRNAs (miRNAs). The genes studied in this article, including members of the FABP, PPAR, and VEGF families, play pivotal roles in lipid transport, metabolism, and angiogenesis. Dysregulation of these genes can contribute to the tumorigenic processes in CRC. By examining the expression profiles of key miRNAs (miR-16-5p, miR-195-5p, miR-27a-3p, and miR-497-5p) and their target genes within the fatty acid pathway, this study aims to provide valuable insights into the molecular mechanisms underlying CRC progression. Understanding how these miRNAs influence the expression of fatty acid-related genes could unveil novel biomarkers and therapeutic targets,

offering potential advancements in CRC diagnosis, prognosis, and treatment. This study bridges the gap between lipid metabolism and cancer biology, highlighting the critical role of non-coding RNAs in CRC.

Materials and Methods

Bioinformatics Studies

In the first step, eight genes were selected based on their association with fatty acid metabolism pathway with CRC. These candidate genes were divided into three main groups included PPARs family, VEGF family and FABP family. We used Human microRNA disease database (HMDD) to detect and extract miRNAs involved in CRC [30–32]. In the

next step, we investigated the possibility of miRNAs and genes interactions using the bioinformatics databases such as miRWalk [33], TargetScan [34], DIANA-TarBase[35], MiRanda[36], Mirbase [37]and MiRTarBase [38]. MiRNAs were selected regarding to the higher score miRNAs-mRNA interactions. The conformation of predicted miRNA:target hybrids were visualized using RNAhybride database [39]. Then, we used miRTarBase database to screen unvalidated miRNA-mRNA interactions. Based on miRBase database, functional arms of miRNAs were selected. Moreover, using Cytoscape 3.6.0, molecular interaction networks integrating with gene expression profiles and other state data were deduced. We also used STRING [40] for predicted protein–protein interactions and their associated signaling. The flowchart of computational study stage is illustrated in Fig. 1A.

Fig. 1 The flowchart of computational analysis procedure: **a** the STRING protein–protein interaction network of the study: **(b)**

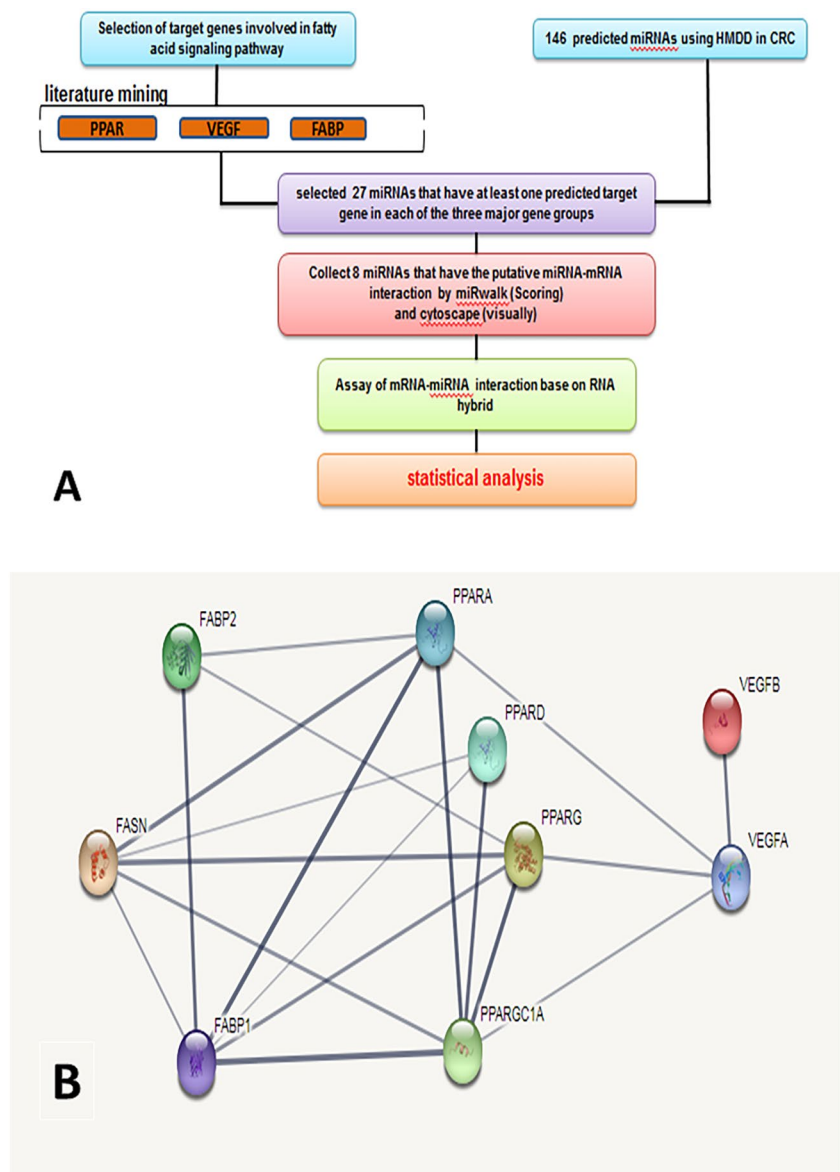


Table 1 Primer pairs used in this study

Forward 5'- TGCAGATTATGCGGATCAAACCTC -3'	<i>VEGFA</i>	151 bp
Reverse 5'- AACAAATGCTTTCTCCGCTCT -3'		
Forward 5'-CCACTCCTCCACCTTTGACG-3'	<i>GAPDH</i>	107 bp
Reverse 5'-CCACCACCCTGTTGCTGTAG-3'		
Forward 5'-TGGAAGAGGAGACTGGGAGG-3'	<i>VEGFB</i>	197 bp
Reverse 5'- TATCAGGGTTGGGGTCACA -3'		
Forward 5'- GAGCTATGGTATGTGGTTC-3'	<i>PPARα</i>	88 bp
Reverse 5'- CATCTGGTCTGTTGGTC-3'		
Forward 5'- AAGTGAGCGATTAGTTGAG -3'	<i>PGC1α</i>	156 bp
Reverse 5'- CATTGCTTTCCCTTATTAGC -3'		
Forward 5'-AGCCTCTTCTCAACGACCAG-3'	<i>PPARβ/δ</i>	102 bp
Reverse 5'-TACCAGCAGCCCGTCCT-3'		
Forward 5'- GTTTGGTGTGTGTGTG-3'	<i>FASN</i>	151 bp
Reverse 5'- ACATGCGGTTTAATTGTGG-3'		
Forward 5'-CAAGGTCATCCATGACAACCTTG-3'	<i>GAPDH</i>	500 bp
Reverse 5'-GTCCACCACCCTGTTGCTGTAG-3'		
Forward 5'- GAAGGAGTAGAAGCCAAAAG-3'	<i>FABP2</i>	148 bp
Reverse 5'-GGACCAATCAATCAGTAACC-3'		
Forward 5'-CTTTACGCCTCGGTGTTAG-3'	<i>PPARγ</i>	217 bp
Reverse 5'-CCTTCCTTACCTACCACTGAC-3'		

Cell Culture

Human dermal fibroblasts (HDF) served as the control cell line, and various human colorectal cancer (CRC) cell lines, namely Caco-2, SW480, HT-29, and HCT116, were acquired from the National Cell Bank of Iran at the Pasteur Institute in Tehran, Iran. SW480, HCT116, and Caco-2 cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS), while HT-29 cells were maintained in Roswell Park Memorial Institute (RPMI) medium supplemented with 15% FBS. All cell lines were incubated at 37°C with 5% CO₂ and 95% air. This study received approval from the Biomedical Ethics Committee of the Royan Institute and adhered to the Ethics Code of IR.ACECR.ROYAN.REC.1397.04.12.

RNA Extraction, cDNA Synthesis and RT-qPCR

Based on the manufacturer's protocol, total RNA was extracted from cells using Trizol. Quantification of the extracted RNA concentration by the NanoDrop was performed. (NanoDrop Technologies, USA). Then cDNA was synthesized using 1000 ng of total RNA (Exiqon, Denmark-TaKaRa, Japan) according to the protocols for miRNAs and mRNAs respectively. RT-qPCR was carried out on thermal cycler (Applied Biosystems, Foster City, CA, USA). The primer pairs were ordered through Pishgam Company (Tehran, Iran) and were used for RT-qPCR. Primers sequences are shown in Table 1. In this study, we used geometric method [41] for normalization of miRNAs expression analysis. Accordingly, U6 and

miR-191-5p were used as internal control transcripts. Meanwhile, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as reference gene for analysis of the mRNA expression. The $2^{-\Delta\Delta C_t}$ method was used for calculating of the relative quantification number.

Statistical analysis

We conducted the statistical analysis using SPSS 23 (SPSS Inc, Chicago, IL, USA) and GraphPad Prism 8 software. To compare the data in this study, we employed either analysis of variance (ANOVA) or a two-tailed Student's t-test. All data were presented as the mean along with the standard deviation (SD), and a significance level of $p < 0.05$ was used to determine statistically significant differences between the parameters.

Results

Identification of Candidate miRNAs and Targeted Genes in CRC

Based on literature review, 8 target genes (FASN, FABP2, PPAR α , PPAR β/δ , PPAR γ , PGC1 α , VEGFA, and VEGFB) which are involved in fatty acid metabolism pathway were selected. These genes were categorized in three groups including FABPs, PPARs and VEGFs. In the next step, each group was assessed in STRING database to visualize the protein-protein interactions and associations in metabolic

Table 2 Target genes and their associated signalings in STRING databased on Kegg and Reactom pathway

Pathway name	Term description	Observed gene count	False discovery rate	Matching proteins in your network
Kegg pathway	1-PPAR signaling pathway	5	4.42E-09	FABP1,FABP2,PPAR α ,PPAR β/δ ,PPAR γ
	2-Pathways in cancer	4	0.00079	PPAR β/δ ,PPAR γ ,VEGFA,VEGFB
	3-Fat digestion and absorption	2	0.0016	FABP1,FABP2
	4-Adipocytokine signaling pathway	2	0.004	PPAR α ,PPARGC1A
Reactome pathway	1-Metabolism of lipids	7	2.26E-07	FABP1,FABP2,FASN,PPAR α ,PPAR β/δ ,PPAR γ ,PPARGC1A
	2-PPARA activates gene expression	4	5.41E-06	FABP1,PPAR α ,PPAR γ ,PPARGC1A
	3-Nuclear Receptor transcription pathway	3	1.34E-05	PPAR α ,PPAR β/δ ,PPAR γ
	4-Transcriptional regulation of white adipocyte	3	7.87E-05	PPAR α ,PPAR γ ,PPARGC1A
	5-differentiation Fatty acid metabolism	2	0.0072	FASN,PPAR β/δ

pathways they are involved (Fig. 1B) (Table 2). According to the data obtained from the HMDD database, a list of 146 miRNAs which are highlighted in colorectal cancer were predicted. In the next step, 27 miRNAs that target at least one of the mentioned genes were selected regarding to their higher interactions and acquired maximal score of *context++score* (CS) in TargetScan online data base including miR-125b-3p, miR-103a-3P, miR-140-5P, miR-497-5P, miR-491-5P, miR-149-5P, miR-185-5P, miR-193a-3P, miR-195-5P, miR-372-5P, miR-181a-5P, miR-181b-5P, miR-30a-5P, miR-328-5P, miR-27a-3P, miR-27b-3P, miR-340-5P, miR-34a-5P, miR-34c-5P, miR-17-5P, miR-186-5P, miR-1915-3P, miR-335-5P, miR-29a-3P, miR-133a-3P, miR-342-3P, miR-16-5P. Finally, among the predicted list 8 miRNAs (miR-140-5p, miR-16-5p, miR-17-5p, miR-195-5p, miR-27a-3p, miR-27b-3p, miR-29a-3p, and miR-497-5p) were selected.

The miRNA-mRNA Interactions

Next, we sought to identify miRNA-mRNA interactions using the RNAhybride database, as illustrated in Supplementary Fig. 1. We have depicted the miRNA-mRNA interaction of hsa-miR-27a-3p as an example. Among the 27 miRNAs examined in this study, eight exhibited a stronger association with their target genes within this network. Additionally, we evaluated the minimum free energy (MFE) for each interaction between miRNAs and their respective mRNA target genes.

Expression Analysis of Candidate miRNAs and their Targets in CRC Cell Lines

Quantitative analysis of the miRNA's expression using RT-qPCR revealed that the expression level of miR-16-5p,

miR-195-5p, miR-27a-3p, and miR-497-5p were significantly reduced ($p < 0.05$) in all CRC cell lines (Caco-2, HCT116, HT-29 and SW480) compared to the control cell line (HDF) (Figs. 2 and 3). However, the expression analysis of other miRNAs did not show any remarkable change (Figs. 4 and 5). Moreover, the highest and lowest expression of miRNAs were analyzed in all cell lines and the result showed that miR-27b-3p, miR-29a-3p, miR-17-5p had highest expression and miR195-5p, miR-497-5p; miR-16-5p had the lowest expression in these cell lines (Table 3). Regarding to the expression of the target genes indicated that all were significantly increased in four cancer cell lines (except FABP2 in SW480) ($p < 0.05$) (Figs. 2, 3, 4 and 5).

Expression Analysis of Targeted Genes Using TCGA Data

For further exploration, we utilized the GEPIA dataset to compare the mRNA expression differences of all eight targeted genes between COAD and READ cancer tissues and their respective normal counterparts. The results revealed significant overexpression of FASN, PPARG, and VEGF-A in both tumor tissues when compared to adjacent normal tissues. In contrast, VEGF-B and PPARGC1A exhibited remarkable overexpression in the normal adjacent tissues, as shown in Fig. 6. It is worth noting that the data for FABP2, PPARA, and PPARD did not show statistical significance, as indicated in Supplementary Fig. 2.

Identification of Prognosis-Related Genes in High and Low Expression Levels

We next used Kaplan–Meier Plotter to explore the prognostic survival rate of the mRNA expression in high and low expression level of all targeted genes using TCGA database obtained from GEPIA dataset. The Kaplan–Meier curve and

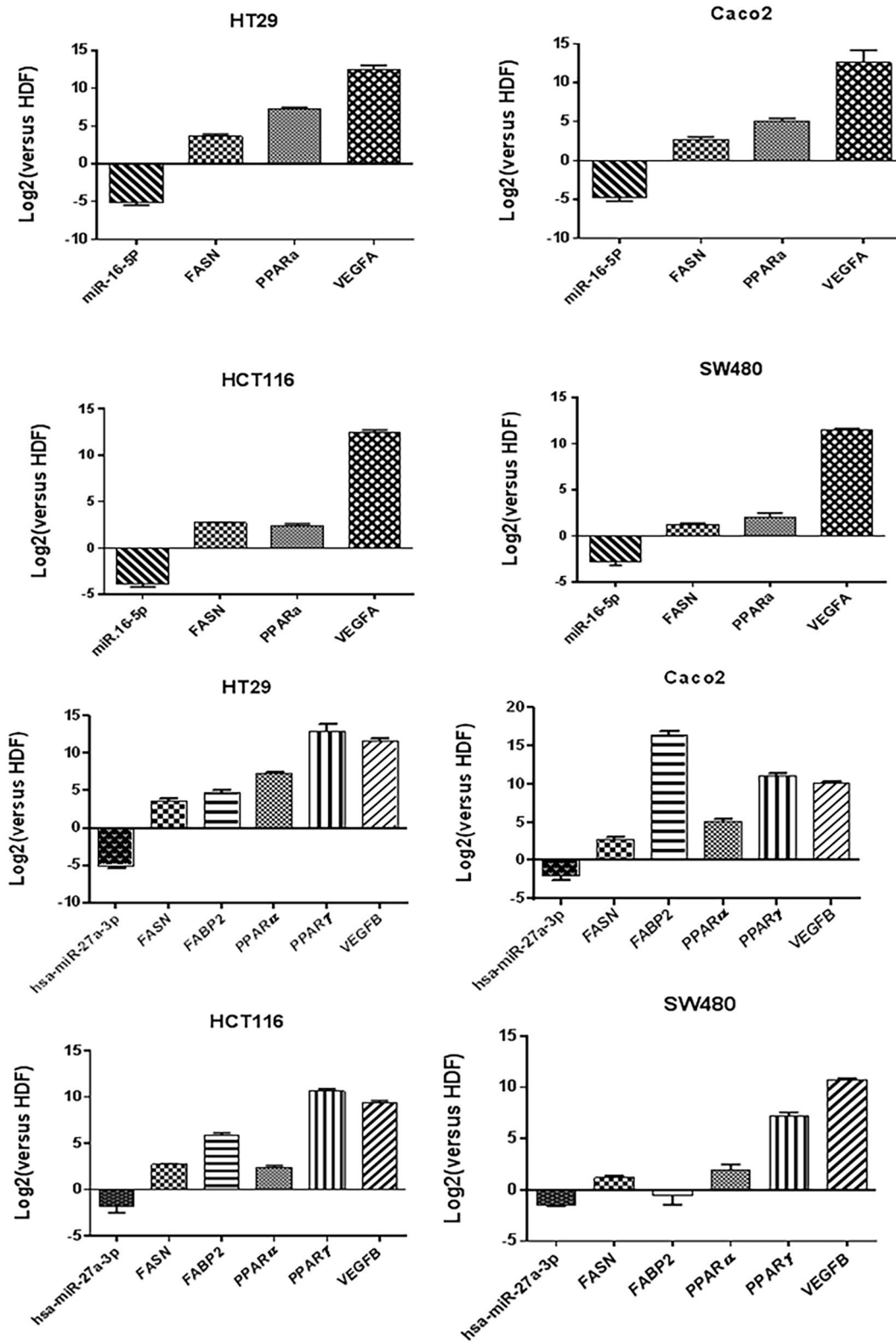


Fig. 2 assessment of the repression of hsa-miR-16-5p and hsa-miR-27a-3p with their targeted genes

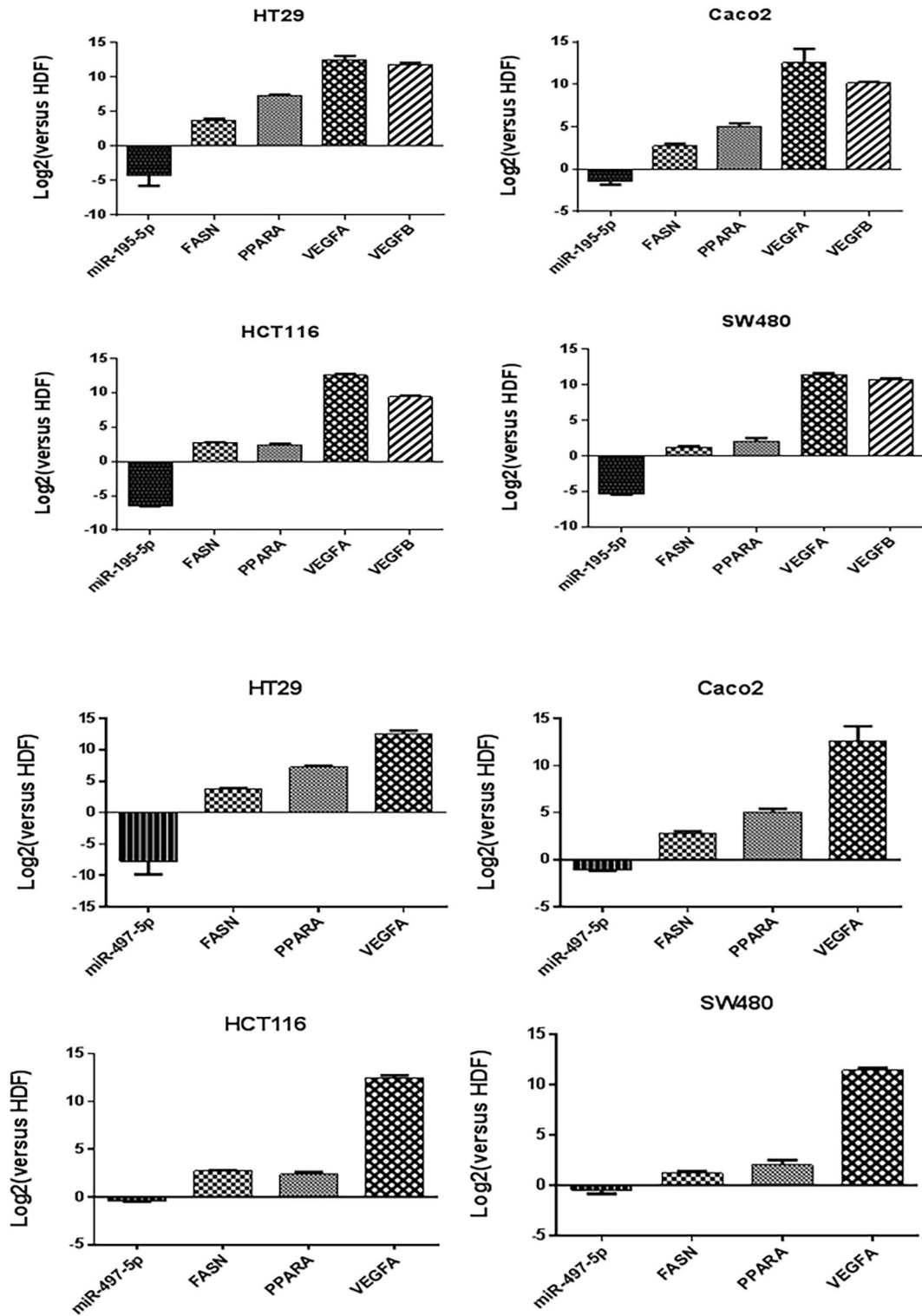


Fig. 3 assessment of the repression of hsa-miR-195-5p and hsa-miR-497-5p with their targeted genes

log rank test were shown in Fig. 7 and as it can be seen, high expression of PPARD and VEGFA were significantly associated with shorter Disease-Free Survival (DFS) rate (Fig. 7A,

B). In contrast, PPARGC1A showed a better overall survival in high expression level (Fig. 7C).

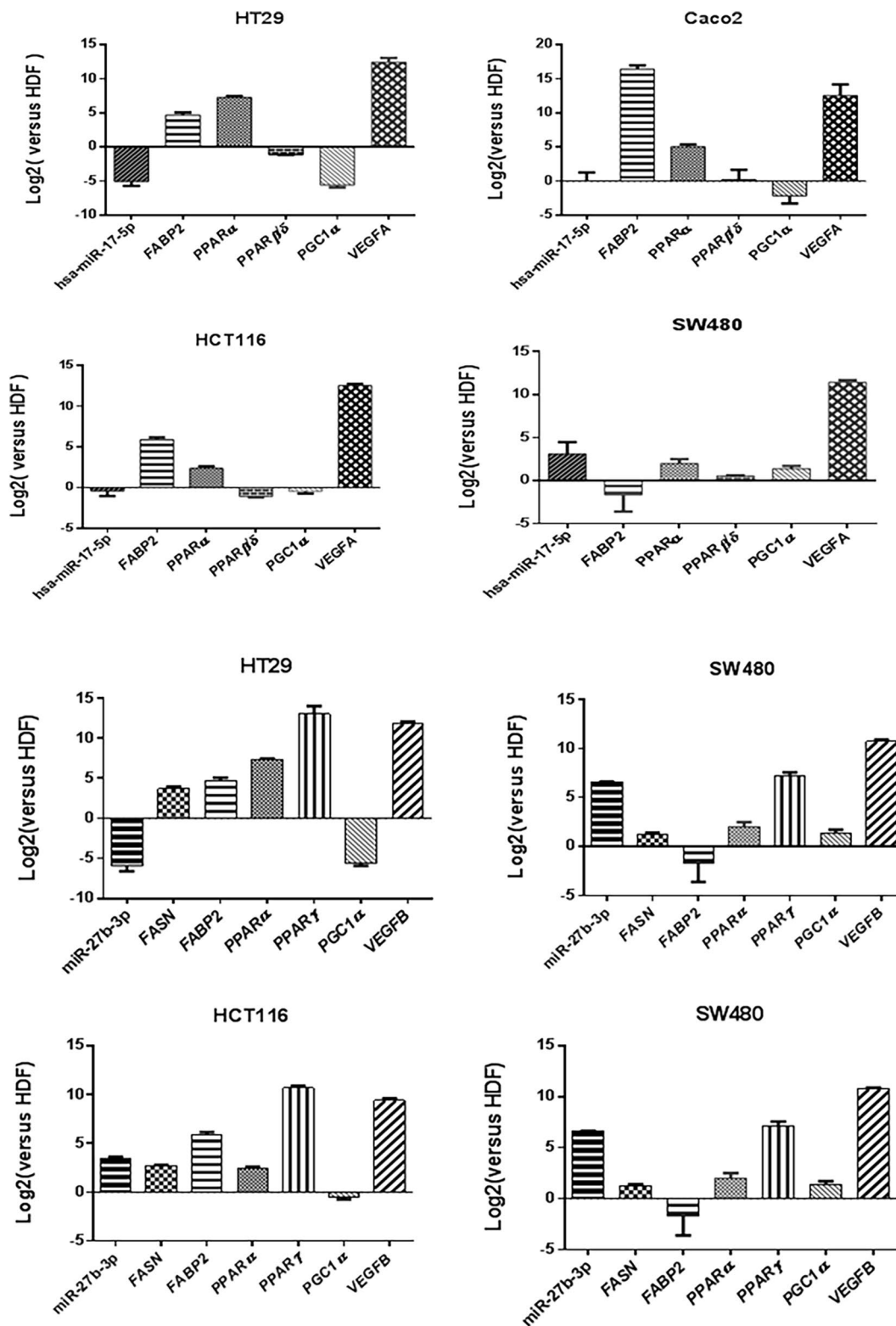


Fig. 4 assessment of the repression of hsa-miR-17-5p and hsa-miR-27b-3p with their targeted genes

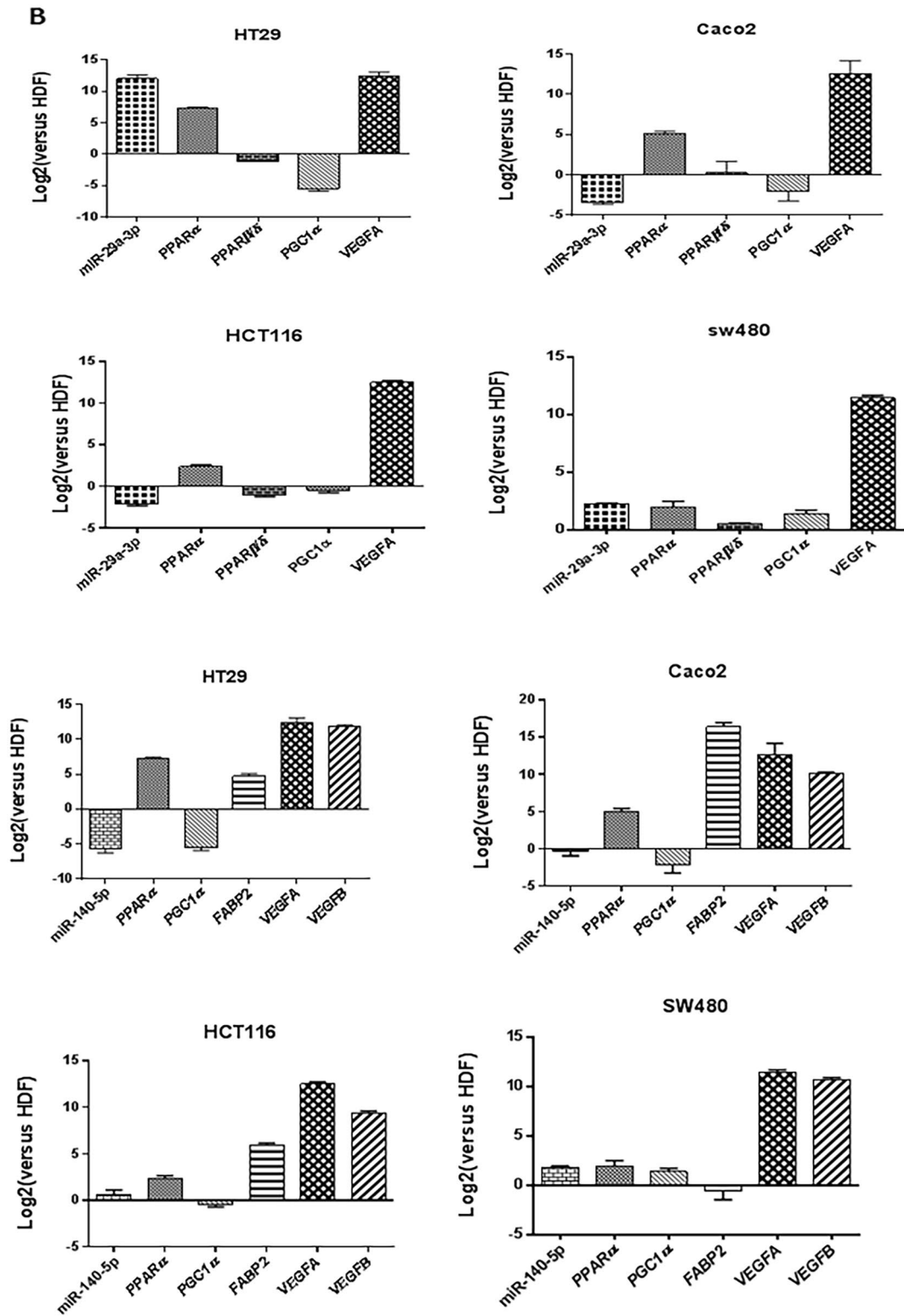


Fig. 5 assessment of the repression of hsa-miR-29a-3p and hsa-miR-140-5p with their targeted genes

Table 3 Expression levels of selected miRNAs in CRC cell lines

Expression	Cell line miR	Caco2	HCT116	HT29	SW480
Highest expression	hsa-27b-3p		@t		@t
	hsa-29a-3p			@t	
	hsa-17-5p	@t			
Lowest expression	hsa-195-5p		@t		@t
	hsa-497-5p			@t	
	hsa-16-5p	@t			

Discussion

Personalized medicine is essential for effectively treating the varied forms of colon cancer, using genomic profiles, biomarkers, and patient history to improve outcomes where surgery, chemotherapy, and radiation may not work. This approach looks at genetic and epigenetic features to find the best treatments and diagnostic tests. Current personalized strategies include immunotherapy, plant-based compounds, and targeted therapies based on specific biomarkers [42]. For CRC, immunotherapy targeting immune checkpoints shows great promise as a novel treatment, although it faces obstacles due to the immune-suppressing function of the tumor microenvironment (TME), resulting in poor patient response. Emphasizing the importance of liquid biopsy, it offers a valuable, noninvasive method for detecting and monitoring these and other therapeutic responses in CRC [43]. Recent research shows that miRNAs, initially known for their role in post-translational gene expression, have emerged as promising biomarkers for CRC detection, offering potential advantages over existing screening tools, though widespread clinical adoption depends on overcoming key challenges [44]. There has been growing attention to the complex role of miRNAs in cancer development, as these powerful RNA molecules regulate gene expression and are involved in both physiological and pathological processes, including carcinogenesis, highlighting current treatment challenges and suggesting future research directions [45].

The important role of lipid metabolism pathway in the development of cancer has been previously observed. The alteration in the regulation of lipid metabolism pathway could provide a tumor microenvironment for cancer cells to thrive [46, 47].

Among different gene families involved in lipid metabolism pathway some are highlighted including VEGF, PPAR, FABP and FASN. VEGF family is studied widely as an angiogenic factor which demonstrated with a tumor overexpression pattern and correlated with poor prognosis in colorectal cancer [48–51]. FABPs are also involved in the coordination of lipid transport and the differential expression level of FABP subtypes in several malignancies had been reported however the

expression of I-FABP is not well determined [52]. PPARs gene family are also express from various tissues and regarding their crucial role in activation of several molecules during cell differentiation and regulation, have been considered as therapeutic targets [53]. The emerging roles of lipid metabolism in the tumor aggressive progression has been reported which leads to a metastatic cancer subsequently [54]. Considering all these aspects, addressing the genes deregulation which is in concert in a same pathway could provide a better big picture of the signaling pathway leads to cancer progression. The aim of present study was to detect some important genes involve in lipid metabolism pathway and compare their expression level in tumor and normal cell lines. On the other hand, as respect the functional interaction of miRNA:mRNA had been described obviously, eight miRNAs were candidate regarding to their strong interaction with their targeted mRNAs of the selected genes. Previous studies had shown that the overexpression of miRNAs can down-regulate tumor suppressor genes which lead to tumor formation or cell proliferation and miRNAs can perform as oncogenes independently. In contrast, it is identified that the down-regulation impact of miRNAs on proto-oncogenes could lead to cell proliferation [55]. Previous study strongly indicated the aberrant expression pattern of miRNAs in CRC which suggest them as prognostic biomarker in cancer diagnosis and treatment [56]. In the current study, comparing the expression level of hsa-miR-497-5p, hsa-miR-16-5p, hsa-miR-27a-3p and hsa-miR-195-5p with their targeted genes showed that the expression level of those miRNAs was significantly decreased while the expression of genes was remarkably over expressed in all cell cultures (Figure Supp 3). This observation may support the hypothesis that the differential expression of miRNAs could play an important role in cancer development therefore their potentiality to be introduced as biomarker (Fig. 8). The observed decrease of hsa-miR-497-5p, hsa-miR-16-5p, hsa-miR-27a-3p and hsa-miR-195-5p might result to the increase expression of the target genes. The results obtained from TCGA is also indicated that FASN, PPARG and VEGFA genes were highly up regulated in COAD and READ tissues while FABP2, PPARA and PPARD genes did not show any remarkable change. Surprisingly, PPARGC1A and VEGFB genes were over expressed in normal tissues compared with COAD and READ (Fig. 6). On the other hand, based on our in-silico investigation, the low expression of PPARGC1A is highly associated with poor survival rate and the high expression of both PPARD and VEGFA genes are associated with the decrease rate of disease-free survival which can be considered with a tumor suppressive effect and angiogenic action respectively (Fig. 7). The identification of miRNAs and their target genes involved in fatty acid metabolism presents several potential clinical applications for CRC. The dysregulated miRNAs, including miR-16-5p, miR-195-5p, miR-27a-3p, and miR-497-5p, and their corresponding target genes, such as FASN, PPARG, and VEGFA, offer promising

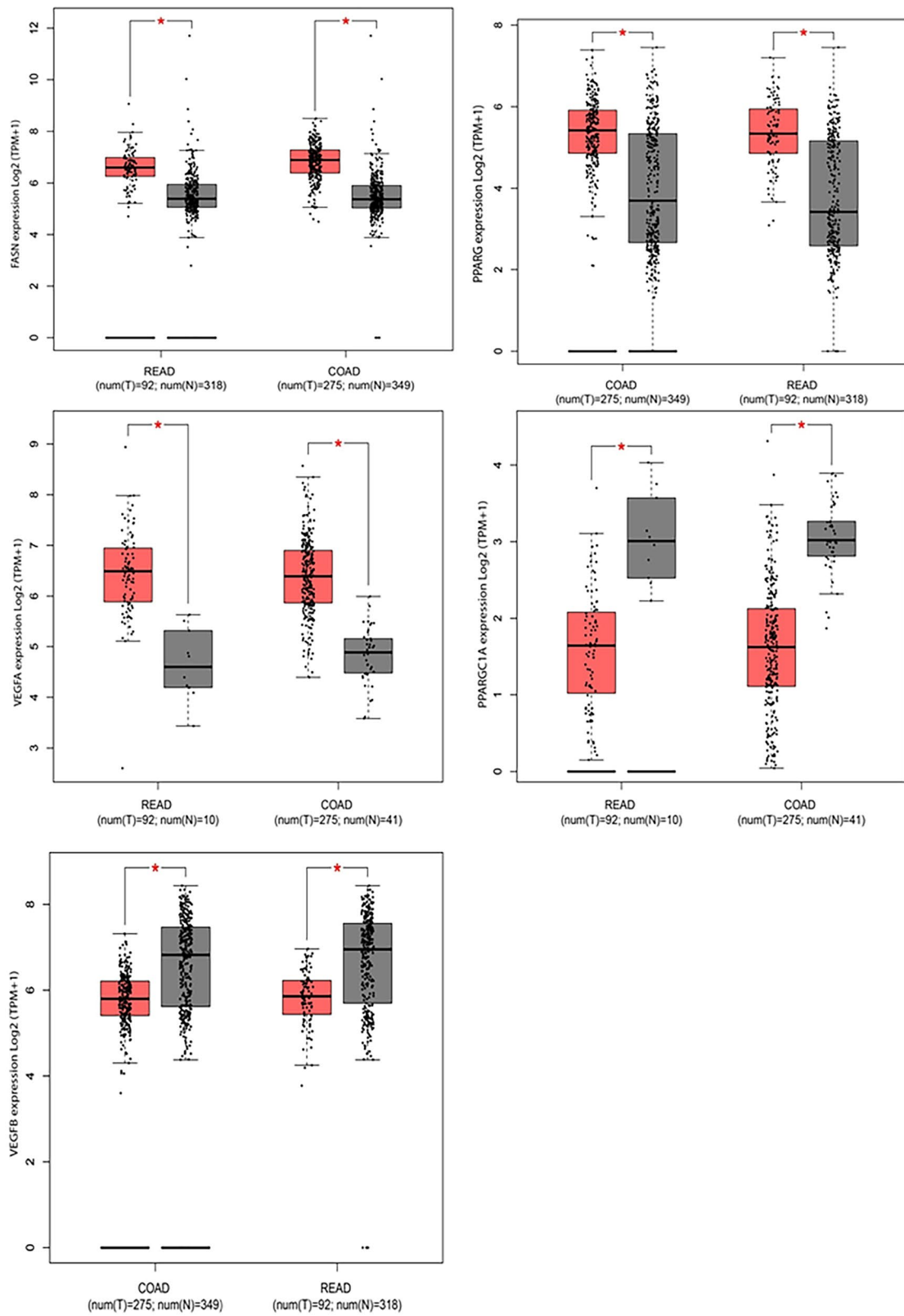


Fig. 6 the expression profile of FASN, PPARG, VEGFA, PPARGC1A and VEGFB genes in COAD and READ tissues

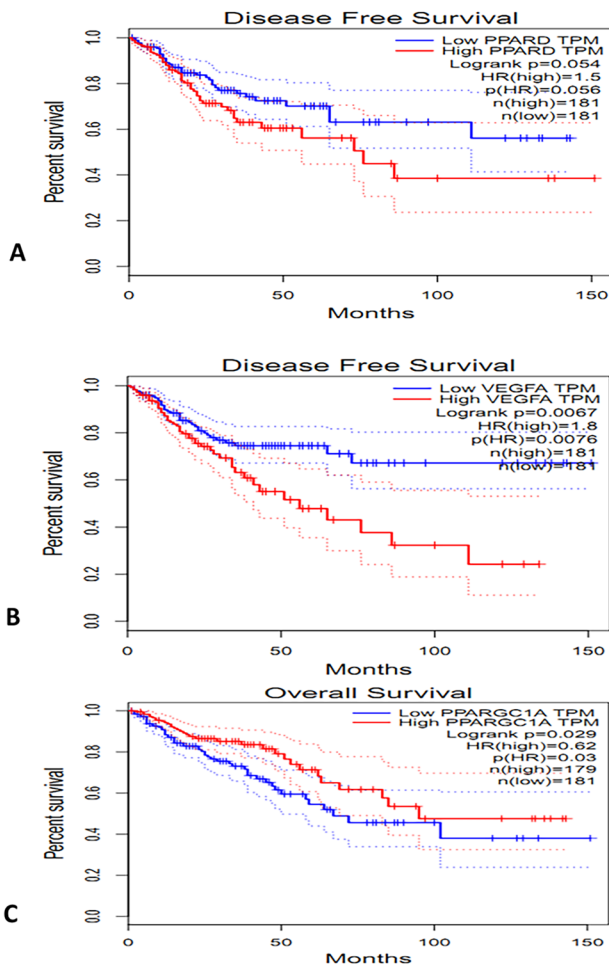
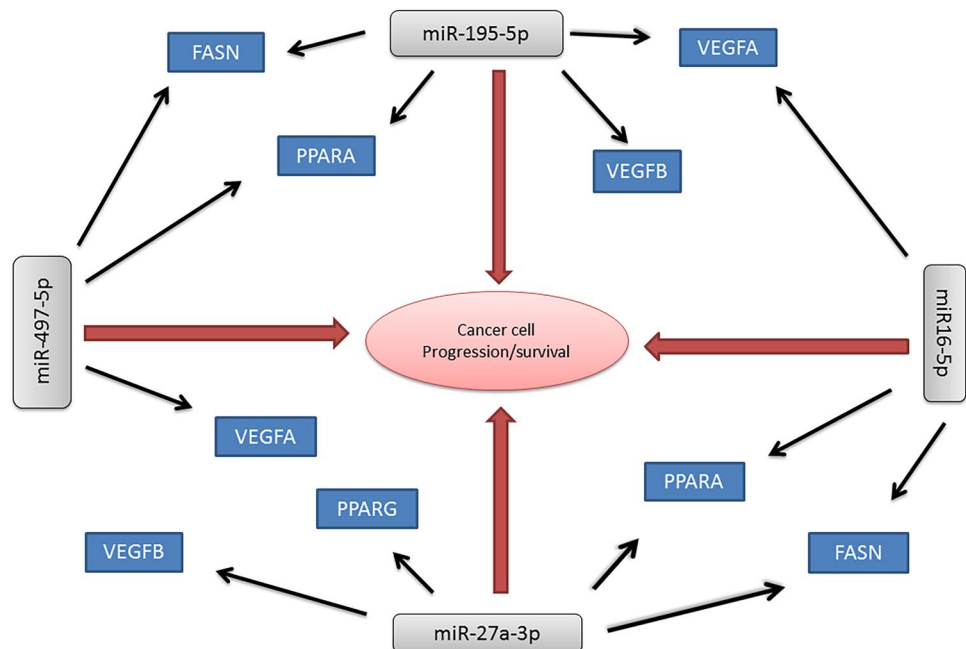


Fig. 7 Kaplan-Meire survival analysis for: **a** PPARD gene in DFS **b** VEGFA gene in DFS and **c** PPARGC1A in OS

Fig. 8 the tumor suppressive effect of hsa-miR-195-5p, hsa-miR-497-5p, hsa-miR27a-3p and hsa-miR-16-5p



avenues for diagnostic and therapeutic strategies. These miRNAs and genes could serve as biomarkers for early detection and as prognostic indicators, aiding in patient stratification and treatment planning. Targeted therapies inhibiting FASN or VEGFA and modulating PPARG activity could suppress tumor growth and angiogenesis, while miRNA-based therapies restoring or inhibiting specific miRNAs could regulate oncogenic targets. Integrating these molecular profiles into clinical practice can enhance personalized treatment plans, with potential for combination therapies achieving synergistic effects. These findings highlight the promise of molecular profiling in CRC management, paving the way for more precise and effective diagnostic and therapeutic approaches.

Conclusion

In overall, our finding indicated that hsa-miR-497-5p, hsa-miR-16-5p, hsa-miR-27a-3p and hsa-miR-195-5p can be suggested as OncoMir therefore the decreased expression of miRNAs is associated with the increase expression of their targeted genes.

Study Limitations and Future Research Directions

Despite the valuable insights provided by our study, several limitations must be acknowledged. Our research primarily focused on a limited number of CRC cell lines and one control cell line, which may not fully capture the genetic and phenotypic diversity of colorectal cancer. Additionally, our in vitro experimental design, while informative, does not replicate the complexity of the tumor microenvironment

in vivo. Future studies should include a broader range of cell lines to provide a more comprehensive understanding of these miRNAs and their target genes in CRC progression.

Exploring the therapeutic potential of targeting these miRNAs or their associated pathways could offer new avenues for CRC treatment. Developing miRNA mimics or inhibitors, as well as small molecules targeting the identified genes, could be a promising strategy for CRC therapy. These future research directions will significantly enhance the impact and applicability of our findings.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s12291-024-01261-5>.

Author's contributions The design and Conceptualization of the study and Methodology were done by Andisheh Oroujalian and Maryam Peymani. Data mining, Formal Analysis, and Investigation were performed by Andisheh Oroujalian, Maryam Keshani, Farnoush Panahi, Mohadeseh Esmaeili, Zahra Koveitypour, Shahrzad Tabrizchi, Hamed Akbari, Mina Amiri, Maryam Esmaeili and Farzad Seyed Forootan. Supervision, Validation, and Visualization were done by Maryam Peymani and Mohammad Hossein Nasr Esfahani. Interpretation of the obtained information was done by Maryam Peymani and Andisheh Oroujalian. The manuscript was written by Andisheh Oroujalian. Review, Editing, and approval by Maryam Peymani. All authors read and approved the final manuscript.

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Data Availability All data of this study are provided with the published article.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical Approval This study was approved by the Biomedical Ethics Committee of the Royan Institute and complies with the Ethics Code of IR.ACECR.ROYAN.REC. 1397.04.12.

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