Gene Reports 24 (2021) 101274

Contents lists available at ScienceDirect

Gene Reports

journal homepage: www.elsevier.com/locate/genrep

Restoration of miR-124 serves as a promising therapeutic approach in CRC by affecting CDK6 which is itself a prognostic and diagnostic factor

Morteza Akbari [a,b,c], Ali Adili [d], Afsaneh Faraji [b], Abbas Pakdel [e], Ramin Aslaminabad [b], Davoud Nasrabadi [b], Shahram Sadeghvand [f], Hossein Saeedi [b,g], Mina Tahavori [g,h], Aliakbar Shabani [a,c,*], Behzad Baradaran [b,**]

[a] Department of Biotechnology, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran
[b] Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[c] Semnan Biotechnology Research Center, Semnan University of Medical Sciences, Semnan, Iran
[d] Department of Oncology, Tabriz University of Medical Sciences, Tabriz, Iran
[e] Department of Biochemistry, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran
[f] Children Hospital, Tabriz University of Medical Sciences, Tabriz, Iran
[g] Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
[h] Department of Pharmaceutics, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

ARTICLE INFO

Keywords:
Colorectal Cancer
5-FU
Oxaliplatin
miR-124
CDK6
Prognosis
Diagnosis
Treatment

ABSTRACT

Background: Colorectal cancer is one of the most common cancers in the world. 5 Fluorouracil and Oxaliplatin are two common chemotherapeutic agents are used in patients with CRC. miR-124 and CDK6 are two notable genes that have been shown to play important roles in various cancers, including colorectal cancer.
Objective: In this study, we investigated the expression of miR-124 and CDK6 in patients with colorectal cancer. Then, the effect of miR-124 and chemotherapeutic drugs on CDK6 expression and cell viability was assessed in the SW480 CRC cell line. Also, the relationship between miR-124 and CDK6 with the clinicopathological features of the patients was evaluated.
Materials and methods: Materials and Methods: Colorectal cancer and its corresponding non-tumor tissues were collected from 50 patients the relative expression of miR-124 and CDK6 was assessed by Real-Time PCR. The relationship between the pathologic features of the patients and the target genes was also evaluated. In addition, the relative expression of CDK6 in response to miR-124 and its combination with 5-FU and Oxaliplatin was evaluated in the SW480 cell line by Real-Time PCR and Western blot analysis. Besides, the effect of miR-124 and chemotherapeutic drugs on cell viability was determined by MTT assay.
Results: miR-124 was down-regulated in CRC patients while CDK6 was up-regulated in CRC tissues compared with adjacent non-tumor healthy controls. In addition, CDK6 was significantly correlated with pathologic features of patients including stage and differentiation. Moreover, CDK6 expression and cell viability were attenuated in response to miR-124, 5-FU, and Oxaliplatin in the SW480 cell line; however, the effect was prominent when miR-124 was transfected with chemotherapeutic drugs.
Conclusion: The results of our study suggest that miR-124 reinforcement may act as a promising therapeutic approach for CRC by affecting CDK6. Nevertheless, it may act as a supreme therapeutic method when combines with chemotherapy drugs. Besides, CDK6 may be used as a diagnostic marker to determine the clinical outcome of patients with colorectal cancer.

Abbreviations: CRC, Colorectal cancer; miR-124, microRNA-124-5p; CDK6, Cyclin-Dependent Kinase 6; 5-FU, Fluorouracil; RB, Retinoblastoma; BRAF, B-Raf proto-oncogene, serine/threonine kinase; KLF4, Kruppel-like factor 4; HMGA2, High-mobility group AT-hook 2; TNM, Tumor-node-metastasis; CAPN2, Calpain 2.

* Correspondence to: A. Shabani, Semnan Biotechnology Research Center, Semnan University of Medical Sciences, Semnan, Iran.
** Corresponding author.
E-mail addresses: shabanialiakbar48@yahoo.com, aashaebani@yahoo.com (A. Shabani), baradaranb@tbzmed.ac.ir (B. Baradaran).

https://doi.org/10.1016/j.genrep.2021.101274
Received 20 February 2021; Received in revised form 14 June 2021; Accepted 28 June 2021
Available online 7 July 2021

1. Introduction

Colorectal cancer (CRC) is one of the most common causes of cancer death in the world. Almost 5% of the world's population suffers from CRC. Despite advances in the diagnosis and treatment of this cancer, it is still the third most common cancer in the world. Early diagnosis of this cancer can have a significant positive effect on the prognosis of CRC and appropriate treatment (Akbari et al., 2019a; Tamjidifar et al., 2021; Azar et al., 2021).

Both environment and genetics play an important role in the progression of colorectal cancer (Varambally et al., 2008; Akbari et al., 2019b). However, environmental protection against colorectal cancer, including the use of non-steroidal anti-inflammatory drugs such as aspirin, daily intake of water, dietary fiber intake, and daily activity should not be overlooked (DeSantis et al., 2016; Shiao et al., 2018; Nan et al., 2015).

The role of environmental factors in the development of colon cancer is more important than hereditary factors. The acquired form is caused by the accumulation of mutations and genetic changes, including deletion and single nucleotide polymorphism and epigenetic alterations such as hypermethylation and hypo mutation of various types of genres such as P53, BRAF, KLF4, and HMGA2 (Fearon, 2011; Shomali et al., 2020).

Current treatment for colorectal cancer is a combination of surgery, radiation therapy, chemotherapy, and targeted treatment. Although surgery is used for patients who have non-metastatic CRC, the patients with metastatic CRC could not be directed to this kind of therapy. Accordingly, it should be noted that surgery, especially in the early stages, is known as the best therapeutic approach for CRC. In chemotherapy, a combination of oxaliplatin and 5-FU is the most common drug which is used in most cancers (Garzon et al., 2010; Gustavsson et al., 2015; Saidi et al., 2017).

Previous studies have shown that genome and protein analysis with a focus on CRC molecular heterogeneity may be a viable way to identify and develop new therapies. In this line, recent studies have revealed microRNAs as effective factors in the further formation of cancers, including CRC (Wu et al., 2010; Iorio and Croce, 2012; Moridikia et al., 2018).

microRNAs are small single-stranded non-coding RNAs that regulate gene expression. They often bind to the 3-UTR (3-untranslated region) region of their target genes and cause gene silencing either by mRNA degradation or translational suppression (Shomali et al., 2019; Mohammadi et al., 2018; Nabipoorashrafi et al., 2020; Alkafaji et al., 2021). miRNAs have a significant role in a wide range of biological processes, such that about 30% of human genes and genetic pathways are adjusted by miRNAs (Azar et al., 2021; Shirafkan et al., 2018; Alizadeh et al., 2020; Hemmatzadeh et al., 2020). There have been several studies in cancer suggesting that miRNAs act as tumor inhibitors or oncogenic microRNAs whose aberrant expression causes metastasis, apoptosis, angiogenesis, and tumor growth (Alizadeh et al., 2019; Vahidian et al., 2019; Azar et al., 2020).

miR-124 was first introduced as a specific brain miRNA, whose function is to support and maintain neural activity. Pre-miR-124 is expressed in the brain, cervix, pancreas, kidney, and muscle and can also help maintain retinal cell properties. Practically several miR-124 target genes have been identified, one of which is CDK6 (Wilting et al., 2010; Sun et al., 2015; Sun et al., 2016; Gebauer et al., 2013; Gennarino et al., 2012; Agarwal et al., 2015). CDK6 (cyclin-dependent kinase 6) is a member of the serine-threonine kinase family that is involved in controlling cell cycle progression (Malumbres, 2014). CDK6 along with cyclin D in the phosphorylation of retinoblastoma protein (RB) participates in the transition of the G1 phase to the S phase of the cell cycle (Santo et al., n.d.; Sherr et al., 2015).

In a newly published study, the researchers found that breast cancer pathogenesis has a relation with CDK6-deficient mice, so it can be concluded that CDK6 plays an important role in tumor pathogenesis (Landis et al., 2006; Malumbres and Barbacid, 2009).

Table 1
Clinicopathological features of patients.

Clinicopathological features	No. of cases (%)
Gender	
Male	29 (58)
Female	21 (62)
Age	
≤55	17 (34)
>55	33 (66)
Tumor location	
Colon	36 (72)
Rectum	14 (28)
Lymph node metastasis	
Negative	39 (78)
Positive	11 (22)
Tumor histology	
Good	25 (50)
Intermediate	19 (38)
Poor	6 (12)
TNM stage	
I	20 (40)
II	15 (30)
III	9 (18)
IV	6 (12)
Lymphatic invasion	
Negative	38 (76)
Positive	12 (24)
Liver metastasis	
Negative	44 (88)
Positive	6 (12)
Venous invasion	
Negative	48 (96)
Positive	2 (4)
Tumor size	
>3	18 (36)
≤3	32 (64)

Since early detection of colorectal cancer improves patients' survival, our aim in this study was to find new molecular markers involved in the diagnosis and treatment of CRC. For this reason, the expression of miR-124 and CDK6 in colorectal cancer, as well as the effect of miR-124 and chemotherapeutic drugs on CDK6 expression and cell viability was investigated in the SW480 cell line.

2. Material and methods

2.1. Colorectal cancer tissue samples

Colorectal cancer tissue samples containing tumor tissues and adjacent non-tumor tissues were gotten from surgical samples of patients undergoing colorectal surgery at Emam Reza Hospital. After surgically removing the tissues from the body they were frozen in liquid nitrogen and maintained in a freezer with a temperature of minus 80. All 50 samples had no radiotherapy and chemotherapy history. The clinicopathologic features of the patients were summarized in Table 1.

2.2. Colorectal cancer cell line

SW480, human adenocarcinoma colorectal cancer cell line was obtained from Pasture Institute (Tehran, Iran). The cell line was cultured in RPMI-1640 medium enriched with 10% fetal bovine serum-containing pen strep (100 IU/ml penicillin and 100 mg/ml streptomycin) (Gibco, USA) and preserved in a humidified incubator containing 5% CO2 at 37 °C.

Table 2
Primer sequences.

Gene	Sequences
U6	F: 5' CTTCGGCAGCACATATACTAAAATTGG 3' R: 5' TCATCCTTGCGCAGGGG 3'
β-actin	F: 5' CAAGATCATCACCAATGCCT 3' R: 5' CCCATCACGCCACAGTTTCC 3'
CDK6	F: TTCAGCCCTGCAGGGAAAGAA R: CTCCTCGAAGCGAAGTCCTCA
miR-124	F: 5′- TAAGGCACGCGGTGAATG -3′ R: 5′- TGGTGTCGTGGAGTCG -3′

2.3. Transfection of miRNA, 5-FU, and oxaliplatin

According to manufacturer protocol, 10 nmol (optimum dose) of miR-124 mimics (Microsyntyh Co, Vienna, Australia) were transfected with a jetPEI reagent (Polyplus, Darmstadt, Germany) to SW480 cells in an approximately 80% confluency. After a while, the medium was replaced with an RPMI-enriched medium (20% FBS) and maintained for 48 h. Then, CRC cells were treated with miR-124 (as described above), Ox (4.25 μmol/l), 5-FU (10 μmol/l), and their combinations and cultured for 48 h.

2.4. Quantitative RT-PCR (qRT-PCR)

CDK6 and Beta-actin primers were purchased from (macrogne, Korea) and miR-124 primer was purchased from Exiqon's company; sequences are shown in Table 2. We used the RiboEx RNA extraction Kit (Geneall, Korea) to extract the total RNA according to the producer's protocol, following this process, a conventional PCR was done to synthesis cDNAs for microRNA and mRNAs by cDNA synthesis kit (Exiqon, Denmark). We used untreated cell lines as our control. Real-time PCR was conducted by light cycler 96 (Roche, Germany) using SYBR Premix Ex Taq. The mRNA level of Beta-actin was used as an internal control for CDK6 and U6 was used for miR-124.

2.5. Western Blot

Briefly, after extraction of total protein by RIPA buffer (Santa Cruz Biotechnology, Santa Cruz, CA), vertical electrophoresis was conducted by the SDS-PAGE. Then, approximately 50 micrograms of total protein were loaded into the gel. In the next step, blots were transferred to polyvinylidene fluoride (PVDF) membranes (Roche, Germany) by a semi-dry western blot transfer system (Bio-Rad). Tween-20 was then used to block the membrane. Then, the membrane was incubated with goat monoclonal antibody against CDK6 and Beta-actin (Santa Cruz, CA). Subsequently, the membrane was incubated with rabbit and mouse anti-goat secondary antibodies conjugated with HRP. Finally, the bands were visualized by an ECL kit (Roche, Germany), and images were taken by a blot imaging instrument (Sabz Co, Iran). The band intensity was normalized to β-actin density.

2.6. MTT assay

Methyl-thiazol tetrazolium was used to determine the effect of miR-124 transfection alone and in combination with 5-FU and Oxaliplatin on cell viability. Briefly, after transfection of miR-124 and chemotherapy drugs into the seeded cells in confluency of almost 80% in 96-well plates, cell viability was measured after 48 h by MTT assay (Takara Bio, Japan) according to the manufacturer's protocol.

2.7. Statistical analysis

Statistical analysis was performed using Graph Pad Prism version 6.00 software to study the expression level of CDK6 and miR-124 in tumor and non-tumor samples based on the Student *t*-test. The effect of miR-124 and chemotherapeutic drugs on CDK6 expression and cell viability was assessed by One-way Anova. To investigate the relationship between the two genes, the Spearman correlation coefficient was used. $P < 0.05$ was measured as statistically meaningful.

3. Results

3.1. miR-124 was downregulated while CDK6 was upregulated in colorectal cancer tissues

As shown in Fig. 1A and B, we found that the mean of miR-124 expression level was significantly down-regulated in CRC tissues compared with adjacent non-tumor tissues (P < ****), while CDK6 was up-regulated in CRC tissues (P < ****). ****$P < 0.0001$

3.2. miR-124 and chemotherapeutic drugs affected CDK6 expression

The results obtained from Real-time PCR and western blot showed a significant modulatory effect of miR-124, Oxaliplatin, and 5-FU on CDK6 expression. Results showed a significant reduction in the expression of CDK6 after transfection of miR-124 and chemotherapeutic drugs; however, the results were more prominent for combination therapy (Figs. 2 and 3, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$)

3.3. miR-124 and chemotherapeutic drugs influenced CRC cell viability

The results obtained from the MTT assay showed that miR-124,

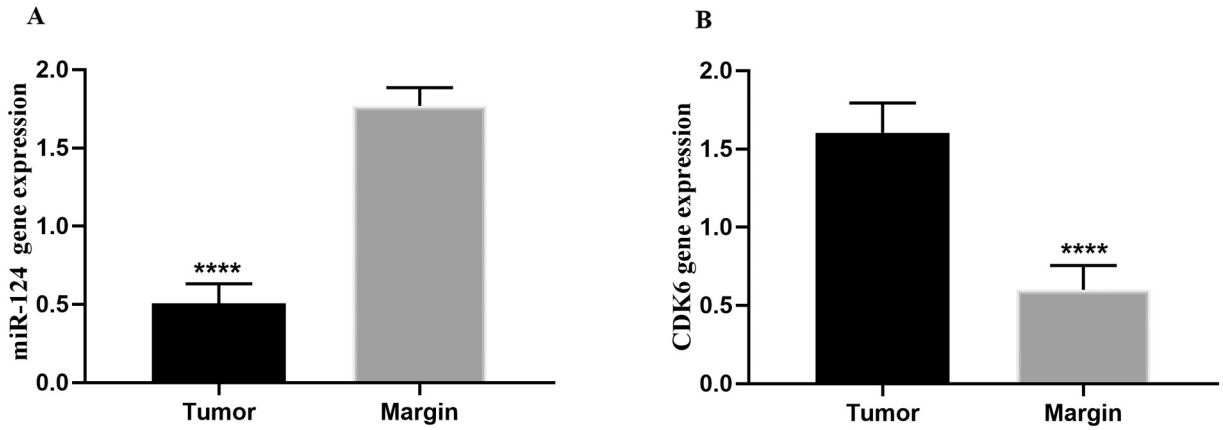
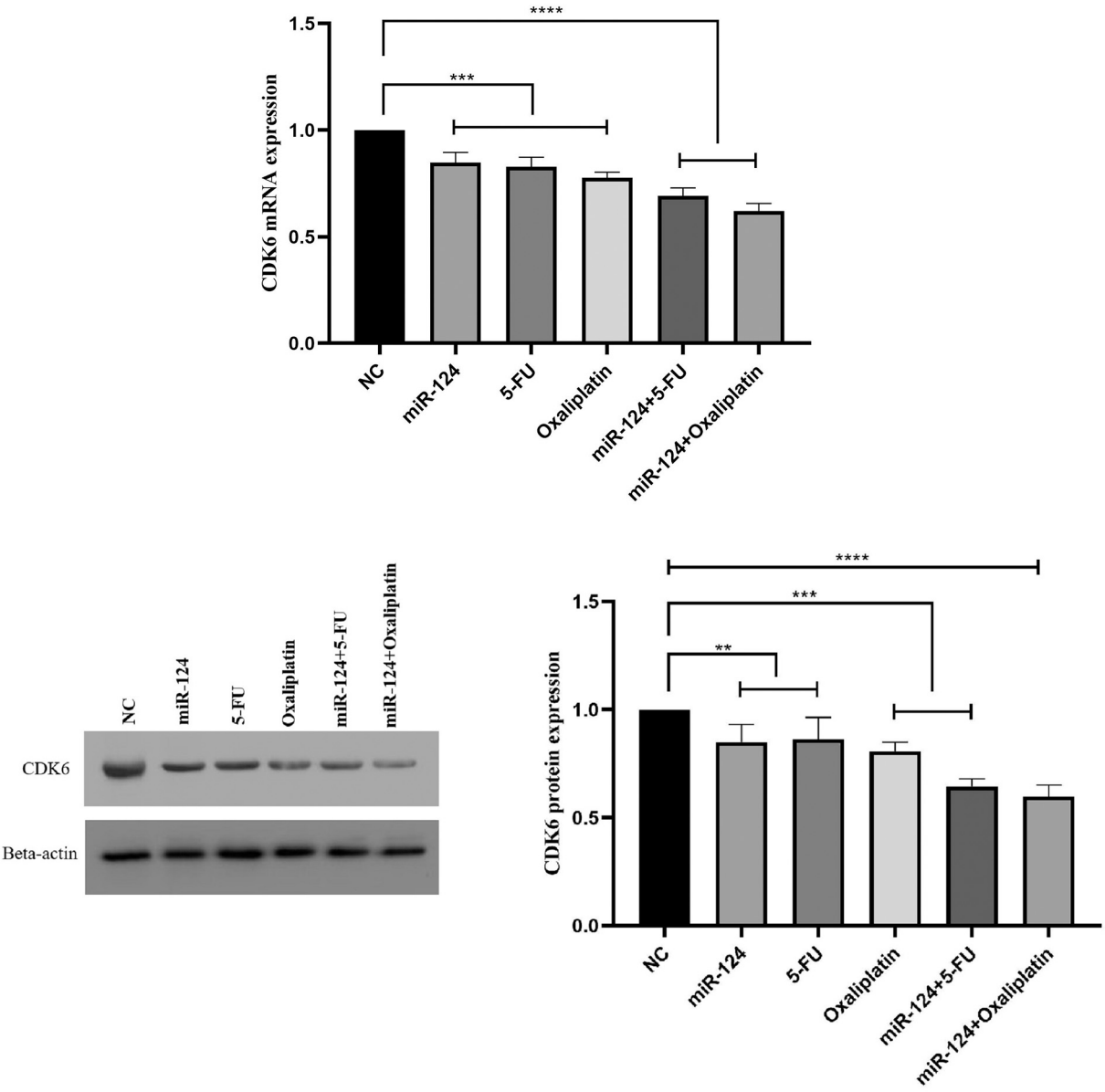


Fig. 1. Expression levels of miR-124 and CDK6 in CRC tissues. (A) miR-124 is downregulated (B) while CXCR4 is upregulated in CRC tissues in comparison with marginal tissues. **** $P < 0.0001$.



Figs. 2 and 3. Relative CDK6 expression levels after transfection of miR-124 and chemotherapeutic drugs. As shown in these figures, the addition of miR-124, 5-FU, and Oxaliplatin reduced mRNA and protein expression of CDK6 in the SW480 cell line; however, the combination of miR-124 with 5-FU and oxaliplatin has a more significant reducing effect on CDK6 expression compared to when one of them was transfected. $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$.

Oxaliplatin, and 5-FU reduced cell viability; however, the results were more prominent for combination therapy such as their effect on CDK6 expression (Fig. 4, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$)

3.4. CDK6 was correlated with clinicopathological features of patients

We found that there was no association between miR-124 and pathologic features of patients; however, CDK6 had a significant correlation with the patients' stage ($P = 0/033$) and differentiation ($P = 0/021$) (Table 3).

4. Discussion

CDK6 is a known oncogenic target of miR-124 involves in cell differentiation and proliferation (Agirre et al., 2009). It can be considered as a suitable candidate to be used as small inhibitory molecules. In a study conducted in 2009, the results showed that reinforcement of miR-124 reduces CDK6 expression, leading to inhibiting the growth of leukemia cells both *in-vitro* and *in-vivo*. Therefore, miR-124 could be considered as a prognostic factor, in which its re-expression may be a therapeutic approach in patients with ALL (Agirre et al., 2009; Wang et al., 2007).

miR 124 is known to suppress tumor microRNA, which exerts its suppressive effects on a variety of human cancers including breast, glioma, colitis, and gastric cancer (Zhou et al., 2016). Previous studies demonstrated that methylation of miR-124 is related to advanced and malignant breast cancer (Gacem et al., 2014; Liang et al., 2012). Additionally, miR 124 has been studied focusing on its potential inhibitory effects on glioma cell invasion and proliferation by blocking the expression of a specific gene (Lu et al., 2014). Furthermore, in progressive uveal melanoma, miR-124 has been epigenetically silenced in which this epigenetic silencing caused the progression of colorectal carcinogenesis (Deng et al., 2011; Chen et al., 2013; Luo et al., 2017).

miR124 was first identified by Sanuki et al. (2011) in the brain tissue and introduced as a brain-specific miRNA, which causes maintaining the neuronal specificity. miR-124 is expressed primarily in the brain,

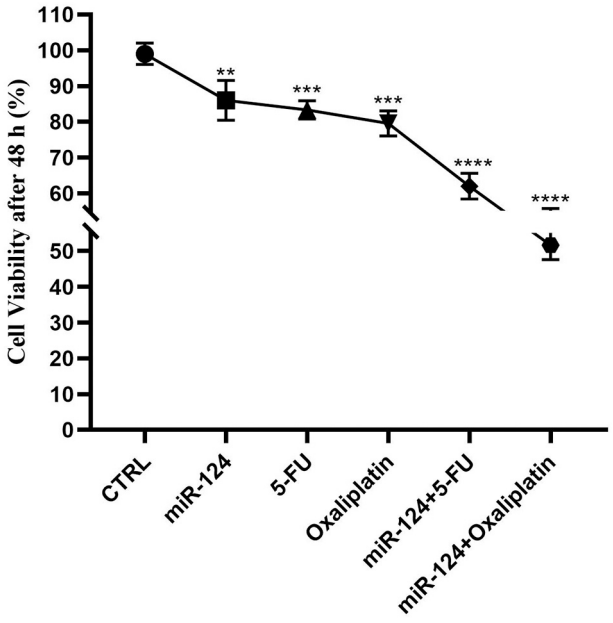


Fig. 4. miR-124 and chemotherapy drugs reduce CRC cell viability. This figure shows that miR-124, 5-FU, and Oxaliplatin decreased cell viability; however, their combination has a potential effect in comparison with when one of them was transfected.**P < 0.01, ***P < 0.00, and ****P < 0.0001.

Table 3
Related correlation between expression of CDK6 with clincopathological features of patients.

Subject	Stage (P-value)	Differentiation (*P*-value)
CDK6	0.039	0.024

pancreas, kidneys, and muscles that play a vital role in preserving the specific properties of retinal cells (Sanuki et al., 2011).

Epigenetic suppression of miR-124 was first reported in CRC cells. This report was conducted by Lujambio et al. (2007) and showed this microRNA was dysregulated in colorectal cancer and proved its reduction in cancer cells (Lujambio et al., 2007).

In another study, Pierson et al. (2008) showed that miR-124 was downregulated in medulloblastoma and reduces the expression of CDK6. They showed that CDK6 is regulated in medulloblastoma by miR-124, which regulates medulloblastoma cell growth. These results were consistent with our study and showed a correlation between miR-124 and CDK6 in colorectal cancer. We also showed the effect of drugs on the expression of miR-124 and CDK6, which was not evaluated by Pierson et al. (2008).

In 2009 Agirre et al. showed that miR-124 exerts its tumor suppressor effects *via* CDK6, thus epigenetic suppression of miR-124 leads to activation of CDK6 and RB phosphorylation, which ultimately leads to cell cycle progression and adverse effects. The results of this study, conducted on acute leukemia, confirm our findings in colorectal cancer (Agirre et al., 2009).

The results of a study revealed that miR-124 inhibited the proliferation of breast cancer by modulating the CDK gene. It was also revealed that miR-124 acts as a tumor-suppressor microRNA in breast cancer (Feng et al., 2016). It has also been revealed that miR-124 re-expression reduced medulloblastoma cells' proliferation (Silber et al., 2013). As we have shown in our study that miR-124 intensifies the effect of chemotherapeutic drugs, it was demonstrated that miR-124 intensified the Oxaliplatin effect by Targeting CAPN2 in CRC (Xie et al., 2020).

5. Conclusion

According to the results of our study, CDK6 was upregulated and miR-124 was downregulated in tumor tissue compared to non-tumor (marginal) healthy tissue. Due association of CDK6 with some clinical features of the patient, it may act as a potential prognostic and diagnostic biomarker in colorectal cancer. Moreover, miR-124 and chemotherapy drugs could reduce CDK6 expression and CRC cell viability, so miR-124 replacement along with chemotherapeutic drugs may act as a therapeutic approach *via* reducing CKD6 expression. However, further studies are needed to be done to determine the exact association of miR-124 and CDK6 in CRC pathogenesis.

CRediT authorship contribution statement

Morteza Akbari: Conceptualization, Investigation; **Ali Adili**: Conceptualization, Investigation; **Afsaneh Faraji**: Writing - review & editing; **Abbas Pakdel**: Writing - review & editing; **Davoud Nasrabadi**: Formal analysis; **Shahram Sadeghvand**: Writing - review & editing; **Ramin Aslaminabad**: Investigation; **Hossein Saeedi**: Investigation; **Mina Tahavori**: Conceptualization; **Aliakbar Shabani**: Conceptualization, Supervision; **Behzad Baradaran**: Conceptualization, Supervision.

Declaration of competing interest

None.

Acknowledgments

This study was carried out and supported at the Immunology Research Center, Tabriz University of Medical Science, Tabriz, Iran.

References

Agarwal, V., Bell, G.W., Nam, J.W., Bartel, D.P., 2015. Predicting effective microRNA target sites in mammalian mRNAs. Elife 4.

Agirre, X., Vilas-Zornoza, A., Jiménez-Velasco, A., Martin-Subero, J.I., Cordeu, L., Gárate, L., San José-Eneriz, E., Abizanda, G., Rodríguez-Otero, P., Fortes, P., 2009. Epigenetic silencing of the tumor suppressor microRNA Hsa-miR-124a regulates CDK6 expression and confers a poor prognosis in acute lymphoblastic leukemia. Cancer Res. 69, 4443–4453.

Akbari, M., Shomali, N., Faraji, A., Shanehbandi, D., Asadi, M., Mokhtarzadeh, A., Shabani, A., Baradaran, B., 2019a. CD133: an emerging prognostic factor and therapeutic target in colorectal cancer. Cell Biol. Int. https://doi.org/10.1002/cbin.11243.

Akbari, M., Shanehbandi, D., Asadi, M., Shomali, N., Faraji, A., Khaze, V., Pakdel, A., Mokhtarzadeh, A., Ebrahimi, A.A., Shabani, A., Bardaran, B., 2019b. Effects of CD133 silencing on survival and migration of HT-29 colorectal cancer cells. Iran. J. Immunol. 16, 246–257.

Alizadeh, N., Asadi, M., Shanehbandi, D., Zafari, V., Shomali, N., Asvadi, T., Sepehri, B., 2019. Evaluation of the methylation of MIR129-2 gene in gastric cancer. J. Gastrointest. Cancer. https://doi.org/10.1007/s12029-019-00239-4.

Alizadeh, N., Asadi, M., Shanehbandi, D., Zafari, V., Shomali, N., Asvadi, T., Sepehri, B., 2020. Evaluation of the methylation of MIR129-2 gene in gastric cancer. J. Gastrointest. Cancer 51, 267–270.

Alkafaji, H.A., Raji, A., Rahman, H.S., Zekiy, A.O., Adili, A., Jalili, M., Hojjatipour, T., Cid-Arregui, A., Shomali, N., Tarzi, S., Tamjidifar, R., Heshmati, R., Marofi, F., Akbari, M., Hasanzadeh, A., Deljavanghodrati, M., Jarahian, M., Sandoghchian Shotorbani, S., 2021. Up-regulation of KISS1 as a novel target of Let-7i in melanoma serves as a potential suppressor of migration and proliferation in vitro. J. Cell. Mol. Med. https://doi.org/10.1111/jcmm.16695.

Azar, M.R.M.H., Akbari, M., Mohammed, H.N., Asadi, M., Shanehbandi, D., Rezai, M., Zafari, V., Niknam, S., Tamjidifar, R., Tarzi, S., 2020. Dysregulation of miR-27a and SMAD2 can be a reliable indicator in the prognosis and diagnosis of CRC as well as in response to chemotherapy drugs. Gene Rep. 21, 100844.

Azar, M., Aghazadeh, H., Mohammed, H.N., Sara, M.R.S., Hosseini, A., Shomali, N., Tamjidifar, R., Tarzi, S., Mansouri, M., Sarand, S.P., Marofi, F., Akbari, M., Xu, H., Shotorbani, S.S., 2021. miR-193a-5p as a promising therapeutic candidate in colorectal cancer by reducing 5-FU and Oxaliplatin chemoresistance by targeting CXCR4. Int. Immunopharmacol. 92, 107355.

Chen, X., He, D., Da Dong, X., Dong, F., Wang, J., Wang, L., Tang, J., Hu, D.-N., Yan, D., Tu, L., 2013. MicroRNA-124a is epigenetically regulated and acts as a tumor suppressor by controlling multiple targets in uveal melanoma. Invest. Ophthalmol. Vis. Sci. 54, 2248–2256.

Deng, G., Kakar, S., Kim, Y.S., 2011. MicroRNA-124a and microRNA-34b/c are frequently methylated in all histological types of colorectal cancer and polyps, and in the adjacent normal mucosa. Oncol. Lett. 2, 175–180.
DeSantis, C.E., Siegel, R.L., Sauer, A.G., Miller, K.D., Fedewa, S.A., Alcaraz, K.I., Jemal, A., 2016. Cancer statistics for African Americans, 2016: progress and opportunities in reducing racial disparities. CA Cancer J. Clin. 66, 290–308.
Fearon, E.R., 2011. Molecular genetics of colorectal cancer. Annu. Rev. Pathol.: Mech. Dis. 6, 479–507.
Feng, T., Shao, F., Wu, Q., Zhang, X., Xu, D., Qian, K., Xie, Y., Wang, S., Xu, N., Wang, Y., Qi, C., 2016. miR-124 downregulation leads to breast cancer progression via LncRNA-MALAT1 regulation and CDK4/E2F1 signal activation. Oncotarget 7, 16205–16216.
Gacem, R.B., Abdelkrim, O.B., Ziadi, S., Dhiab, M.B., Trimeche, M., 2014. Methylation of miR-124a-1, miR-124a-2, and miR-124a-3 genes correlates with aggressive and advanced breast cancer disease. Tumor Biol. 35, 4047–4056.
Garzon, R., Marcucci, G., Croce, C.M., 2010. Targeting microRNAs in cancer: rationale, strategies and challenges. Nat. Rev. Drug Discov. 9, 775.
Gebauer, K., Peters, I., Dubrowinskaja, N., Hennenlotter, J., Abbas, M., Scherer, R., Tezval, H., Merseburger, A., Stenzl, A., Kuczyk, M., 2013. Hsa-mir-124-3 CpG island methylation is associated with advanced tumours and disease recurrence of patients with clear cell renal cell carcinoma. Br. J. Cancer 108, 131.
Gennarino, V.A., D'Angelo, G., Dharmalingam, G., Fernandez, S., Russolillo, G., Sanges, R., Mutarelli, M., Belcastro, V., Ballabio, A., Verde, P., 2012. Identification of microRNA-regulated Gene Networks by Expression Analysis of Target Genes, Genome Research.
Gustavsson, B., Carlsson, G., Machover, D., Petrelli, N., Roth, A., Schmoll, H.-J., Tveit, K.-M., Gibson, F., 2015. A review of the evolution of systemic chemotherapy in the management of colorectal cancer. Clin. Colorectal Cancer 14, 1–10.
Hemmatzadeh, M., Shomali, N., Yousefzadeh, Y., Mohammadi, H., Ghasemzadeh, A., Yousefi, M., 2020. MicroRNAs: small molecules with a large impact on pre-eclampsia. J. Cell. Physiol. 235, 3235–3248.
Iorio, M.V., Croce, C.M., 2012. MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. EMBO Mol. Med. 4, 143–159.
Landis, M.W., Pawlyk, B.S., Li, T., Sicinski, P., Hinds, P.W., 2006. Cyclin D1-dependent kinase activity in murine development and mammary tumorigenesis. Cancer Cell 9, 13–22.
Liang, Y.-J., Wang, Q.-Y., Zhou, C.-X., Yin, Q.-Q., He, M., Yu, X.-T., Cao, D.-X., Chen, G.-Q., He, J.-R., Zhao, Q., 2012. MiR-124 targets slug to regulate epithelial–mesenchymal transition and metastasis of breast cancer. Carcinogenesis. 34, 713–722.
Lu, S.-H., Jiang, X.-J., Xiao, G.-L., Liu, D.-Y., Yuan, X.-R., 2014. miR-124a restoration inhibits glioma cell proliferation and invasion by suppressing IQGAP1 and β-catenin. Oncol. Rep. 32, 2104–2110.
Lujambio, A., Ropero, S., Ballestar, E., Fraga, M.F., Cerrato, C., Setién, F., Casado, S., Suarez-Gauthier, A., Sanchez-Cespedes, M., Gitt, A., 2007. Genetic unmasking of an epigenetically silenced microRNA in human cancer cells. Cancer Res. 67, 1424–1429.
Luo, P., Yang, Q., Cong, L.L., Wang, X.F., Li, Y.S., Zhong, X.M., Xie, R.T., Jia, C.Y., Yang, H.Q., Li, W.P., 2017. Identification of miR-124a as a novel diagnostic and prognostic biomarker in non-small cell lung cancer for chemotherapy. Mol. Med. Rep. 16, 238–246.
Malumbres, M., 2014. Cyclin-dependent kinases. Genome Biol. 15, 122.
Malumbres, M., Barbacid, M., 2009. Cell cycle, CDKs and cancer: a changing paradigm. Nat. Rev. Cancer 9, 153.
Mohammadi, H., Hemmatzadeh, M., Babaie, F., Gowhari Shabgah, A., Azizi, G., Hosseini, F., Majidi, J., Baradaran, B., 2018. MicroRNA implications in the etiopathogenesis of ankylosing spondylitis. J. Cell. Physiol. 233, 5564–5573.
Moridikia, A., Mirzaei, H., Sahebkar, A., Salimian, J., 2018. MicroRNAs: potential candidates for diagnosis and treatment of colorectal cancer. J. Cell. Physiol. 233, 901–913.
Nabipoorashrafi, S.A., Shomali, N., Sadat-Hatamnezhad, L., Mahami-Oskouei, M., Mahmoudi, J., Sandoghchian Shotorbani, B., Akbari, M., Xu, H., Sandoghchian Shotorbani, S., 2020. miR-143 acts as an inhibitor of migration and proliferation as well as an inducer of apoptosis in melanoma cancer cells in vitro. IUBMB Life 72, 2034–2044.
Nan, H., Hutter, C.M., Lin, Y., Jacobs, E.J., Ulrich, C.M., White, E., Baron, J.A., Berndt, S. I., Brenner, H., Butterbach, K., 2015. Association of aspirin and NSAID use with risk of colorectal cancer according to genetic variants. Jama. 313, 1133–1142.
Pierson, J., Hostager, B., Fan, R., Vibhakar, R., 2008. Regulation of cyclin dependent kinase 6 by microRNA 124 in medulloblastoma. J. Neuro-Oncol. 90, 1–7.
Saidi, N., Ghalavand, M., Hashemzadeh, M.S., Dorostkar, R., Mohammadi, H., Mahdian-Shakib, A., 2017. Dynamic changes of epigenetic signatures during chondrogenic and adipogenic differentiation of mesenchymal stem cells. Biomed. Pharmacother. 89, 719–731.
L. Santo, K.T. Siu, N. Raje, Elsevier, pp. 788–800.
Sanuki, R., Onishi, A., Koike, C., Muramatsu, R., Watanabe, S., Muranishi, Y., Irie, S., Uneo, S., Koyasu, T., Matsui, R., 2011. miR-124a is required for hippocampal axogenesis and retinal cone survival through Lhx2 suppression. Nat. Neurosci. 14, 1125.
Sherr, C.J., Beach, D., Shapiro, G.I., 2015. Targeting CDK4 and CDK6: From Discovery to Therapy, Cancer Discovery.
Shiao, S.P.K., Grayson, J., Yu, C.H., Wasek, B., Bottiglieri, T., 2018. Gene environment interactions and predictors of colorectal cancer in family-based, multi-ethnic groups. J. Personalized Med. 8, 10.
Shirafkan, N., Mansoori, B., Mohammadi, A., Shomali, N., Ghasbi, M., Baradaran, B., 2018. MicroRNAs as novel biomarkers for colorectal cancer: new outlooks. Biomed. Pharmacother. 97, 1319–1330.
Shomali, N., Shirafkan, N., Duijf, P.H.G., Ghasabi, M., Babaloo, Z., Yousefi, M., Mansoori, B., Asadi, M., Shanehbandi, D., Baghbani, E., Mohammadi, A., Baradaran, B., 2019. Downregulation of miR-146a promotes cell migration in helicobacter pylori-negative gastric cancer. J. Cell. Biochem. 120, 9495–9505.
Shomali, N., Gharibi, T., Vahedi, G., Mohammed, R.N., Mohammadi, H., Salimifard, S., Marofi, F., 2020. Mesenchymal stem cells as carrier of the therapeutic agent in the gene therapy of blood disorders. J. Cell. Physiol. 235, 4120–4134.
Silber, J., Hashizume, R., Felix, T., Hariono, S., Yu, M., Berger, M.S., Huse, J.T., VandenBerg, S.R., James, C.D., Hodgson, J.G., Gupta, N., 2013. Expression of miR-124 inhibits growth of medulloblastoma cells. Neuro-Oncology 15, 83–90.
Sun, Y., Luo, Z.-M., Guo, X.-M., Su, D.-F., Liu, X., 2015. An updated role of microRNA-124 in central nervous system disorders: a review. Front. Cell. Neurosci. 9, 193.
Sun, B., Liu, X., Gao, Y., Li, L., Dong, Z., 2016. Downregulation of miR-124 predicts poor prognosis in pancreatic ductal adenocarcinoma patients. Br. J. Biomed. Sci. 73, 152–157.
Tamjidifar, R., Akbari, M., Tarzi, S., Sadeghzadeh, M., Abolghasemi, M., Poursaei, E., Shomali, N., Mahdavi, F., 2021. Prognostic and diagnostic values of miR-506 and SPON 1 in colorectal cancer with clinicopathological considerations. J. Gastrointest. Cancer 52, 125–129.
Vahidian, F., Mohammadi, H., Ali-Hasanzadeh, M., Derakhshani, A., Mostaan, M., Hemmatzadeh, M., Baradaran, B., 2019. MicroRNAs and breast cancer stem cells: potential role in breast cancer therapy. J. Cell. Physiol. 234, 3294–3306.
Varambally, S., Cao, Q., Mani, R.S., Shankar, S., Wang, X., Ateeq, B., Laxman, B., Cao, X., Jing, X., Ramnarayanan, K., Brenner, J.C., Yu, J., Kim, J.H., Han, B., Tan, P., Kumar-Sinha, C., Lonigro, R.J., Palanisamy, N., Maher, C.A., Chinnaiyan, A.M., 2008. Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. Science. 322, 1695–1699.
Wang, L., Wang, J., Blaser, B.W., Duchemin, A.-M., Kusewitt, D.F., Liu, T., Caligiuri, M. A., Briesewitz, R., 2007. Pharmacologic inhibition of CDK4/6: mechanistic evidence for selective activity or acquired resistance in acute myeloid leukemia. Blood. 110, 2075–2083.
Wilting, S.M., van Boerdonk, R.A., Henken, F.E., Meijer, C.J., Diosdado, B., Meijer, G.A., le Sage, C., Agami, R., Snijders, P.J., Steenbergen, R.D., 2010. Methylation-mediated silencing and tumour suppressive function of hsa-miR-124 in cervical cancer. Mol. Cancer 9, 167.
Wu, W.K.K., Cho, C.H., Lee, C.W., Wu, K., Fan, D., Yu, J., Sung, J.J.Y., 2010. Proteasome inhibition: a new therapeutic strategy to cancer treatment. Cancer Lett. 293, 15–22.
Xie, X.Q., Wang, M.J., Li, Y., Lei, L.P., Wang, N., Lv, Z.Y., Chen, K.L., Zhou, B., Ping, J., Zhou, Z.G., Sun, X.F., 2020. miR-124 intensified oxaliplatin-based chemotherapy by targeting CAPN2 in colorectal cancer. Mol. Ther. Oncolytics. 17, 320–331.
Zhou, Q., Long, L., Zhou, T., Tian, J., Zhou, B., 2016. Demethylation of microRNA-124a genes attenuated proliferation of rheumatoid arthritis derived fibroblast-like synoviocytes and synthesis of tumor necrosis factor-α. PLoS One 11, e0164207.