Original Article: Determining the diagnostic value of miRNA-221 in colorectal cancer surgery

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ABSTRACT

Introduction: Our study examined the predictive value of six miRNAs of interest for evaluating treatment with FOLFOX and FOLFIRI when used as a first-line therapeutic option in managing patients with metastatic colorectal cancer in this context. **Material and Methods:** We select a combination of endogenous and exogenous control miRNAs to improve the accuracy of the exosomal miRNA expression because there is currently no widely accepted best practice for exosomal miRNA normalization. Recent evidence suggests that using the same type of RNA species (miRNAs) as normalizers may be a more accurate approach than using other RNA species, such as RNU6B small nuclear RNA (U6). Due to this, we include cel-miR-39 as a spike-in (2 108 transcripts) as an exogenous normalizer and miR-16-5p as an endogen normalizer based on prior research on exosomal miRNA normalization. **Results:** We examined the expression of these microRNAs in 17 patients under the FOLFIRI (FIRI) protocol and 14 patients under the FOLFOX (FOX) protocol at 2 different time points (baseline and post-chemotherapy) to determine the associations between the miRNAs of interest and the response to first-line chemotherapy. **Conclusion:** Our findings demonstrated the discriminatory power of exosomal miR-92a-3p, miR-146a-5p, miR-221-3p, and miR-484 for non-responder patients regardless of the treatment employed. High baseline levels of these genes were associated with a lack of response to FOLFOX chemotherapy. Increased exosomal levels of miR-143-3p and miR-221-3p at the start of therapy have a predictive value for shorter OS, whereas increased exosomal levels of miR-92a-3p.

Introduction

In the world today, colorectal cancer (CRC) ranks as the third most lethal neoplasia and the fourth most common cause of cancer-related morbidity and mortality. With a 13.1% 5-year survival rate in a metastatic setting [1-3], the prognosis is still poor. Biomarkers play a crucial role in the diagnosis of cancer, both at the time of the initial diagnosis and at recurrence, as well as in the prognosis and prediction of responses to various therapies, enabling the choice of a personalized therapeutic strategy [4-6].

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First-line chemotherapy doublets/triplets based on fluoropyrimidines, oxaliplatin, and/or irinotecan, frequently supplemented with a molecular targeted therapy, are used to treat metastatic colorectal cancer (mCRC) in patients with a good performance status. Monoclonal anti-EGFR or anti-VEGF antibodies are selected based on the location of the primary tumor in the colon and the level of RAS gene mutation [7].

Predicting treatment outcomes in metastatic CRC is still very difficult, though. Despite the fact that a number of genetic changes, including the KRAS and BRAF gene mutation status and microsatellite instability (MSI), serve as predictors of CRC progression, they are insufficient to forecast treatment outcomes in metastatic CRC. The prognosis and metastasis of CRC could both benefit from microbiota research [8].

However, given the difficulty of studying microbiomes as a component of personalized medicine, it is not practical to use microbiota as a predictive factor for treatment responses in metastatic CRC. There are currently no other verified clinical, biological, or molecular factors for predicting the response to treatment and/or survival of these patients aside from the previously mentioned predictive factors for the response to therapy [9].

Finding new molecular biomarkers, like miRNAs, may herald a paradigm shift in how mCRC is treated. The post-transcriptional regulation of gene expression is greatly influenced by miRNAs, which are small RNA molecules of about 18 to 24 nucleotides in size. Despite not being involved in genetic information coding, miRNAs are extremely important [10].

They have been demonstrated to be involved in the majority of the stages of carcinogenesis, acting either as an oncomiR by targeting tumor-suppressing mRNA or as a tumor-suppressor miRNA by targeting oncogenes. Along with their roles as tumor-suppressors and oncogenes, miRNAs also play a role in promoting inflammation and promoting the development of cancer [11].

Some miRNAs mediate inflammation, even though inflammatory stimuli can change the expression of particular miRNAs. Pro- or anti-inflammatory stimuli are produced when miRNA activity is induced or suppressed, which has an impact on a variety of biological processes. The benefit of plasma miRNAs is that they can provide complex molecular information while only requiring a small amount of blood and being incredibly stable [12].

Previous research suggests that certain serum miRNA panels may serve as prognostic markers for CRC, comparable to conventional markers like carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9), or for the identification of CRC patients with distant metastasis. Other methods, such as examining the role of mast cells or the interaction of miRNAs with other biomarkers like KRAS, are useful in predicting treatment responses to conventional chemotherapy [13].

The majority of miRNAs that are found in serum or plasma are mainly found in exosomes. The first team to demonstrate the transfer of miRNA through exosomes to transport genetic information was Valadi and colleagues. Transmembrane transport of biological material is a crucial component of homeostasis.

Exosomes are secreted by a large number of healthy cells as well as tumors, and their main function is to promote intercellular communication. Exosomes support cancer cell growth, invasion, and metastasis as well as immune system escape, angiogenesis, and chemoresistance. Extracellular miRNA is not rendered inactive by exosomes, allowing them to safely enter their recipient cells [14].

They have been found in the blood, saliva, malignant ascites, breast milk, urine, cerebrospinal fluid, and other bodily fluids. According to some studies, neoplastic patients

have more circulating exosomes than healthy people do, and malignant cells secrete more exosomes than normal cells. Their application as therapeutic biomarkers for diagnosis, prognosis, and prediction, as well as their manipulation to treat various human pathologies, are the main topics of current research. Through better patient selection for pricey therapies, the costs associated with their potential widespread use could be decreased [15].

Numerous preclinical and clinical studies have been carried out to date to pinpoint and validate particular miRNAs as predictive biomarkers in metastatic colorectal cancer, but none have been put into widespread use in clinical practice. Our study examined the predictive value of six miRNAs of interest for evaluating treatment with FOLFOX and FOLFIRI when used as a first-line therapeutic option in managing patients with metastatic colorectal cancer in this context and considering the importance of this first-line treatment option in managing patients with metastatic CRC [16-18].

Material and Methods

Patients and Study Design

Our study was a cohort study, comparing the metastatic subgroups to one another according to how well they responded to treatment, The informed consent form was signed by every patient who agreed to participate in the study. The Institutional Cancer Registry was used to collect all the data. The Institutional Ethics Committee gave the study their blessing. Patients who met the inclusion criteria were adults older than 18 with a histopathological diagnosis of colorectal cancer and either synchronous or metachronous metastases, as well as those who had sufficient information about their demographics, clinical-pathological characteristics, and treatment response. Patients who had multiple synchronous neoplasia were not included, and patients whose blood samples weren't suitable for further processing (i.e. e.), hemoglobin under the microscope).

Application of Treatments and Assessment of Patient Response

First-line chemotherapy, consisting of a fluoropyrimidine (5-Fluorouracil for the majority of patients, or capecitabine), irinotecan, or oxaliplatin, was administered to all patients with metastatic colorectal cancer. Depending on the tumor's RAS mutational status and other factors, chemotherapy was frequently combined with a targeted therapy (anti-EGFR or anti-VEGF). Every 2 to 3 months, CT scans and, in a select few cases, MRIs were used to assess the effectiveness of the treatments. Patients were classified as responders if they had either a complete or partial response and non-responders if their disease was stable or progressing, in accordance with the description of the CT scans and the RECIST 1.1 criteria.

Plasma gathering

Each patient's whole blood was drawn from a vein and placed in an EDTA blood collection tube at the same time (8–12 a.m). The samples were taken at baseline, before the first cycle of chemotherapy for metastatic disease was administered, and at post-chemotherapy, 4-6 months after the start of chemotherapy in patients still receiving first-line chemotherapy, or before switching to second-line chemotherapy in patients who had progressed. All samples were taken before the chemotherapy was given. Each whole-blood sample was registered with a special code and treated similarly right away. Double successive centrifugation at 4000 and 12,000 rpm for 10 min at 4 °C was used to separate the plasma from the whole-blood samples. Before being processed further, the supplied plasma was aliquoted (400 L/tube) and kept at 80 °C.

Extraction of RNA from Plasma-Derived Exosome Isolation

Total Exosome Isolation Kit from Plasma, a specialized commercial conditioning solution from Thermo Fisher, Waltham, Massachusetts, USA, was used to isolate plasma-derived vesicles. To improve the quality of vesicles specific to the exosome domain, additional plasma filtration using a 0.8 m filter was added prior to the precipitation step. Following that, 0.05 v/v Proteinase K was added to the filtered plasma, and it was then incubated at 37 °C for 10 min. After incubation, 120 L of the conditioning solution was added to the plasma, which was then centrifuged at 10,000 g for 30 min at 4 °C.

Using the Total Exosome RNA and Protein Isolation Kit (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's instructions, the precipitate (plasma-derived vesicles/exosomes) was resuspended in 200 L PBS solution and used for RNA extraction. We significantly increased the exosome purity by adding a second step of 0.8 m filtration, which preserved the small EVs while removing the large EVs, as previously discussed when evaluating by TEM and nanoparticle tracking analysis (NTA). While RNA was isolated using the traditional method using an equivalent volume of acid-phenol:chloroform, exosome denaturation was carried out by adding a 2 denaturing solution. After recovery, in the aqueous phase, the RNA was precipitated by 1 point 25 volumes of 100% ethanol and then purified by running it through a gel-silicate column.

For the extraction procedure, a volume of 3.4 L of an exogenous control of miR-39 (2 108 transcripts/extraction) was used for each sample in addition to the protocol. Each sample received a final volume of 100 L of exosome-derived RNA, which was kept at 80 °C until further use. A NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) was used to assess the quality control of the extracted RNAs.

miRNAs that are chosen for study

The available data regarding the predictive role of circulating exosomal miRNA for treatment responses in metastatic CRC are inadequate. As a result, we conducted a literature review to find fresh potential targets that might be looked into as mCRC predictive biomarkers. We started by investigating the precise tissue miRNAs involved in the growth and metastasis of CRCs. The common exosomal and tissue miRNAs for CRC that we discovered can be used as mCRC predictive biomarkers are also known. We also concentrated on the CRC circulating miRNAs linked to treatment prediction and tumor progression. Five miRNAs, miR-92a-3p, miR-146a-5p, miR-221-3p, miR-484, miR-486-5p, and miR-143-3p, were identified in our analysis as candidates for further study in order to make treatment predictions for mCRC.

We select a combination of endogenous and exogenous control miRNAs to improve the accuracy of the exosomal miRNA expression because there is currently no widely accepted best practice for exosomal miRNA normalization. Recent evidence suggests that using the same type of RNA species (miRNAs) as normalizers may be a more accurate approach than using other RNA species, such as RNU6B small nuclear RNA (U6). Due to this, we include cel-miR-39 as a spike-in (2 108 transcripts) as an exogenous normalizer and miR-16-5p as an endogen normalizer based on prior research on exosomal miRNA normalization.

Evaluation of Interest miRNA Expression

In order to conduct the case-control study, we created four RNA aliquot pools, one for each group (FOLFIRI baseline (B), FOLFIRI post-chemotherapy (PC), FOLFOX baseline (B), and FOLFOX post-chemotherapy (PC). We selected the one-step advanced miRNA system because it

has the benefit of allowing multiple miRNAs to be assessed at once using the same pre-amplified cDNA. For the TaqMan® Advanced miRNA cDNA Synthesis Kit, the manufacturer (Thermo Fisher) recommended using a volume of 4 L of exosome-derived RNA for each sample.

The advantage of the advanced miRNA system over the classical system is that it incorporates a pre-amplification stage for the entire spectrum of miRNA contained in the sample, proportionally for all mature miRNA species already present in the samples. Prior to evaluation by polymerization chain reaction (PCR), each cDNA was diluted by 1/10 with RNase-free water after the pre-amplification. Furthermore, 2.5 µL of diluted cDNA was used to investigate the expression of miRNAs of interest with a TaqMan® Fast Advanced Master Mix (2×) and specific miRNA advanced assays (Thermo Fisher Scientific) in a 10 µL reaction volume using a LightCycler (Roche Basel, Switzerland) 480 device under specific miR-advanced PCR conditions: activation of the UNG enzyme at 55 °C for 2 min and Taq polymerase activation at 95 °C for 20 s, followed by 40 PCR amplification cycles based on 2 amplification cycles at 3 s to 95 °C and 30 s to 60 °C, respectively.

With the normalization of CT values at the difference between the two normalization controls, the endogenous one (miR-16-5p) and the exogenous one (cel-miR-39), the expression level of the target miRNA was assessed by CT analysis of NR relative to R groups.

Analysis using statistics

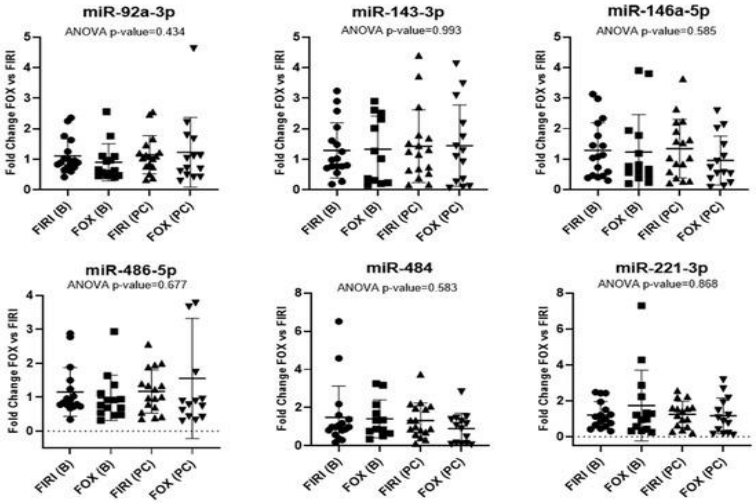
In accordance with the distribution of the data, the qPCR data were analyzed using the Mann-Whitney U test, the unpaired sample t-test for two categorical variables, or the Kruskall-Wallis test, followed by Dunn's multiple comparison post hoc analysis for three categorical variables. The ability of the microRNAs to distinguish between responders and non-responders was assessed using the area under the ROC curve (AUC).

When there was no further progression of the patient's condition, the PFS1 was calculated from the start of the first round of chemotherapy to the start of the second round. The length of survival (OS) was calculated from the beginning of chemotherapy until the date of death or the final evaluation. To examine the relationship between PFS1, the relevant OS, and miRNAs, we used Cox proportional hazards regression models. In order to achieve this, the expression levels of each microRNA were dichotomized based on their median values, dividing the patients into two groups with low and high expression.

Results

31 mCRC patients who had not received treatment for metastatic disease between January 2019 and December 2021 were included in the study. Every patient had access to baseline (B) and post-chemotherapy (PC) plasma samples, which were taken 4-6 months after the beginning of first-line chemotherapy. The patients' clinical follow-up continued until 31 December 2021 [19-21]. Thirty-one of the patients were given chemotherapy, with fourteen receiving oxaliplatin and a fluoropyrimidine (FOLFOX), and seventeen receiving first-line chemotherapy with irinotecan and fluoropyrimidine (FOLFIRI). Depending on the RAS mutational status, 16 patients had targeted therapy added to the first-line chemotherapy protocol between the two timepoints (baseline and postchemotherapy) (bevacizumab in 12 patients, cetuximab in 4 patients) [22-24]. Nine patients had stable disease, and the other five had progressive disease. Only two patients had a complete response, and fifteen had a partial response [25]. We examined the expression of these microRNAs in 17 patients under the FOLFIRI (FIRI) protocol and 14 patients under the

FOLFOX (FOX) protocol at 2 different time points (baseline and post-chemotherapy) to determine the associations between the miRNAs of interest and the response to first-line chemotherapy. At baseline (B) and post-chemotherapy (PC), there were no statistically significant differences in microRNA expression between the two chemotherapy protocols. According to our findings (Figure 1), there is no danger of connecting the degree of false-positive expression with the therapeutic response [26].



Figuring 1. Fold change of the six target miRNAs (miR-92a-3p, miR-143-3p, miR-146a-5p, miR-484, miR-486-5p, and miR-221-3p) in samples taken at baseline (B) and post-chemotherapy (PC) for the two treatment regimens used: FOLFIRI (FIRI) and FOLFOX (FOX).

In addition, we investigated whether the target miRNAs were associated with the therapeutic response regardless of the chemotherapy regimen (FOLFOX or FOLFIRI), but rather with the particular medications used. Regardless of the chemotherapy regimen used, we discovered higher baseline levels of exosomal miR-92a-3p and miR-484 in non-responders compared to responders (Figure 2A), but no significant differences were found in the post-chemotherapy samples (Figure 2B). Additionally, we observed elevated levels of miR-221-3p (FR = 2.7) [27] at baseline in the plasma exosomes of NR patients treated with FOLFOX or FOLFIRI, with a marginal p-value of 0.054 (Figure 2A) [28].

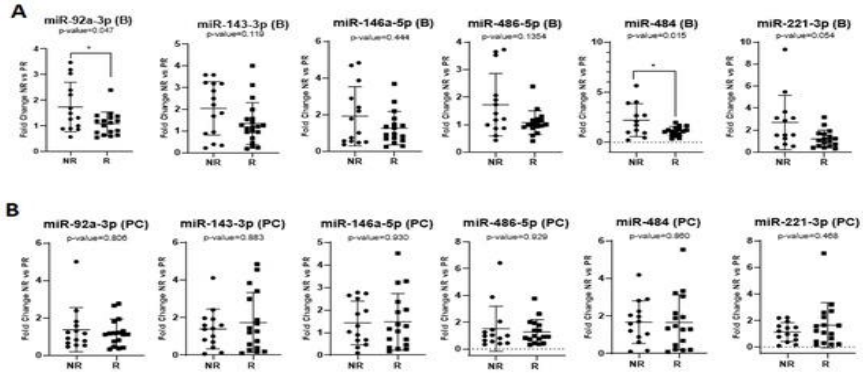


Figure 2. No matter the therapy regimen used in non-responder (NR) vs. responder (R) patients, microRNA expression at baseline (B) and post-chemotherapy (PC) is shown in (A) and (B), respectively. patients who responded (R). In comparison to the PR group, the fold change for each sample was computed. * p = 0 point 05

Our findings showed a significant upregulation of miR-92a-3p, miR-146a-5p, miR-221-3p, and miR-484 expression at baseline in non-responders vs patients who responded to the FOLFOX protocol (FR = 2.17, p = 0.007**, 3.32, p = 0.036*, and 2.62, p = 0.009**, respectively). responding parties (Figure 3A). The expression of exosomal miRNA and the treatment response were not significantly correlated post-chemotherapy (Figure 3B). In the FOLFIRI group, neither pre-chemotherapy nor post-chemotherapy samples from non-responders or responders showed any discernible difference in the expression of the relevant miRNAs.

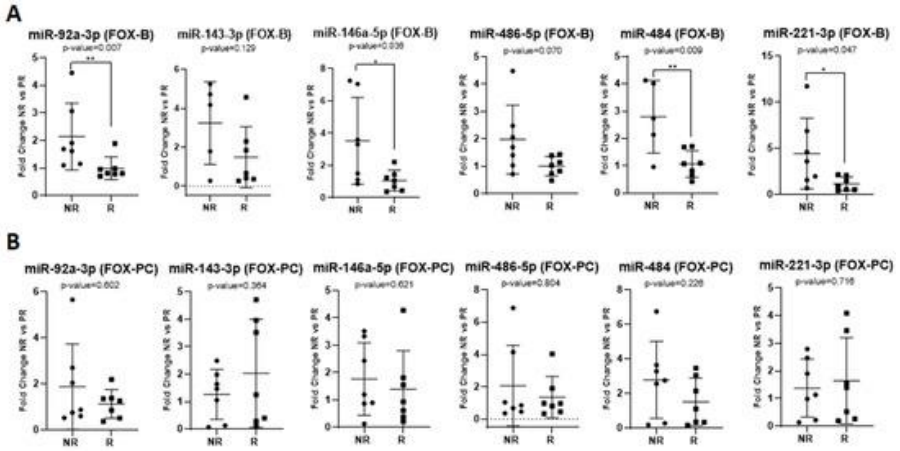


Figure 3. MicroRNA expression in the FOLFOX (FOX) therapy regimen at baseline (B) is shown in (A), and post-chemotherapy (PC) is shown in (B). Each sample's fold change was calculated in relation to the PR group. P is greater than zero in both cases.

We used ROC analysis to evaluate the candidate microRNAs' ability to distinguish between responses and non-responses at baseline and after chemotherapy. The findings suggest that miR-92a-3p, miR-484, and miR-221-3p had the ability to predict non-responders from patients who showed a response to therapy at baseline (AUC = 0.735, 95 percent CI: 0.552-0.919, p-value = 0.026, 0.725, 0.527-0.924, p-value = 0.042, and 0.011, respectively), whereas the post

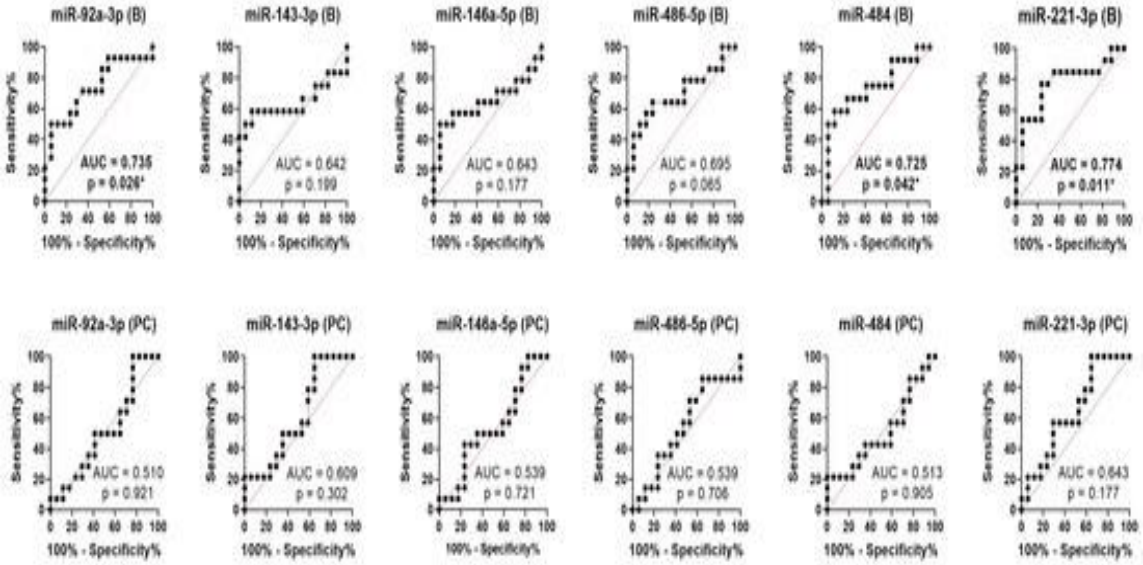


Figure 4. ROC curves for the examined microRNAs, regardless of the type of therapy, at baseline (B) and post-chemotherapy (PC). * P 0.05

Further analysis was done on these miRNAs' capacity to forecast both overall survival (OS) and progression-free survival during first-line chemotherapy (PFS1). The median PFS1 and median OS at the time of evaluation were 17 and 22 months, respectively [29-31]. The univariate COX regression analysis (UA) revealed the statistically significant association of PFS1 with baseline values of miR-486-5p (p = 0.003 **) and miR-92a-3p (p = 0.003 **), showing that the mCRC patients with high levels of baseline exosomal expression of miR-486-5p or miR-92a-3p had significantly shorter PFS than those with low baseline exosomal levels of these miRNAs. Despite having low p-values in UA, miR-484, miR-146a-5p, and miR-143-3p 5p did not pass the significance test [32-34]. We discovered that increased exosomal expression of miR-486-5p (p = 0.019*) and miR-92a-3p (p = 0) at baseline when the OS parameter was taken into account. Among patients with mCRC, miR-221-3p (p = 0.008 **), miR-143-3p (p = 0.009 **), and miR-143-3p (p = 0.009 **) are associated with a worse OS [35-37].

Discussion

While DNA methylation and miRNA changes are epigenetic changes that also contribute to the validation of the cancer phenotype, genetic changes still play a significant role in the development and progression of CRC. It is well known how miRNAs contribute to the growth, invasion, and metastasis of CRCs. However, more research is needed to determine how to identify biomarkers in a liquid biopsy, particularly exosomal miRNAs. Although there have been many publications in the field, there is still work to be done to validate these findings and put them into clinical practice [38-40].

In light of the findings of our study, we were more eager to learn about the findings of other research groups who had studied predictive miRNAs in plasma exosomes in mCRC. We expanded our literature search to include free-circulating miRNAs as potential biomarkers, but due to the scant number of articles found, we did so. For its part in the development, metastasis, and therapeutic responses of CRC tumors, the miR-17-92p cluster has been thoroughly investigated [41-43].

MiR-92a-3p is a miRNA that belongs to the precursor cluster for miR-17-92 and is known to promote CRC tumorigenesis. No matter the chemotherapy regimen used, we demonstrated that a higher baseline plasma exosome miR-92a-3p level was associated with a lack of a therapeutic response, as well as in the subgroup of patients who received FOLFOX chemotherapy . Our data demonstrated that high levels of miR-92a-3p at baseline are associated with low OS; additionally, patients with increased expression had lower PFS1 levels [44-46].

These findings are supported by the research. Hu's team examined the levels of miR-92a-3p in the serum exosomes of patients with chemo-responsive CRC (n = 18) and chemo-resistant CRC (n = 18) who were receiving FOLFOX in 2019. They demonstrated that the level of miR-92a-3p was significantly higher in non-responders than in responders and came to the conclusion that miR-92a-3p in serum exosomes may be a reliable indicator of metastasis and chemoresistance in CRC [47-49].

They also demonstrated that patients with metastatic CRC had the highest serum exosome miR-92a-3p levels in comparison to other patient groups. healthy subjects, highlighting the diagnostic components and its prognostic role. In the same vein, Fu et al Studying the prognostic role of miR-92a-3p and miR-17-5p in mCRC revealed that miR-92a-3p serum exosome levels were significantly higher in patients with metastatic disease than in those with localized disease, making it both a diagnostic and a prognostic biomarker [50].

In their study, Matsumura and colleagues examined serum samples from 124 patients who had been diagnosed with mCRC, whether or not there had been a recurrence of the tumor. The miR-17-92a cluster's gene expression level in the exosomes was linked to the CCR's recurrence. Poel and associates studied miRNAs to find out if they could be used as biomarkers for mCRC patients' sensitivity to palliative chemotherapy [50].

A total of 132 patients receiving 5-FU + Oxaliplatin Bevacizumab were included in the study prospectively, and their response to treatment was assessed using the RECIST 1.1 criteria. MiR-17-5p, miR-20a-5p, miR-30a-5p, miR-92a-3p, miR-92b-3p, and miR-98-5p serum expression levels and baseline tissue levels were examined. The serum levels of miR-92a-3p and miR-98-5p significantly increased the clinical parameters under study's ability to predict the outcome of chemotherapy [5].

The Conev et al. miR-17, miR-21, miR-29a, and miR-92 were examined for their ability to predict relapse in CRC patients who had undergone surgery and adjuvant chemotherapy. Patients who had a relapse of their illness had significantly higher serum expression of miR-17, miR-21, and miR92. Therefore, in this study, the three miRNAs' serum expression levels could identify cancer relapse in stage III CRC patients.

It was discovered that miR-92a may contribute to CRC metastasis through the PTEN-mediated PI3K/AKT pathway. Additionally, by specifically targeting FBXW7 and MOAP1 and mediating 5-FU/L-OHP resistance in CRC, miR-92a can activate the Wnt/-catenin pathway and inhibit mitochondrial apoptosis. MiR-143-3p, which has been identified as a tumor suppressor in CRC, inhibits the growth and metastasis of cancer cells [9].

Its expression is downregulated in CRC, according to previous research. Sahami-Fard and colleagues MiR-143-3p, miR-424-5p, miR-212-3p, and miR-34a-3p serum expression levels were examined in CRC patients. Based on their analysis, miR-143-3p expression in CRC patients was low, which was associated with more aggressive tumor characteristics. MiR-424-5p expression was increased and miR-143-3p expression was decreased. In their study, Romero-Lorca and colleagues examined 76 patients with mCRC and found that overexpression of miR-143-3p in paraffin-embedded biopsies was associated with a significantly longer PFS.

However, our findings demonstrate that elevated miR-143-3p gene expression levels at baseline were related to low OS. In agreement with our findings, a subsequent clinical study found that capecitabine-treated mCRC patients with low miR-143 expression in their primary tumor had a better PFS than those with high expression. The authors speculate that FXYD3, a putative target of miR-143, may be involved in this finding because it can affect some transporters involved in the uptake of fluoropyrimidines and, consequently, the response to treatment.

We were unable to locate any studies that evaluated the role of circulating miR-146a-5p in patients with CRC; however, our data suggest that an elevated baseline level of plasma exosomal miR-146a-5p was related to a lack of a therapeutic response in the subgroup of patients who received FOLFOX chemotherapy. However, Lu et al did show that high levels of miR-146a-5p in CRC are linked to cell invasion and migration through the carboxypeptidase M/src-FAK pathway.

The findings of our study demonstrate that in the subgroup of patients who received FOLFOX chemotherapy, an increased baseline expression of the oncogenic miR-221-3p in plasma exosomes was associated with poor OS and a lack of a therapeutic response ($p = 0.047$). Furthermore, epigenetic reprogramming was used in trials with NP-encapsulated anti-miR-221 to show an anti-metastatic effect. The high

expression of miR-221 in CRC tissues was closely associated with a shorter survival time and a high level of risk for CRC prognosis.

In the ITACa study, patients with mCRC were treated with FOLFOX/FOLFIRI in combination with an antiangiogenic therapy (Bevacizumab), and Ulivi and colleagues examined the predictive role of free circulating miRNAs in these patients. There were 52 patients in total who received treatment in accordance with the protocol. MiR-21-5p and miR-221-3pb were significantly correlated with the RAS mutational status among the miRNAs taken into account, and as a result, they played a predictive role in the response to anti-EGFR agents.

Previously published research by Dokhanci et al revealed that exosomal miR-221-3p controls the STAT3/VEGFR-2 signaling axis by targeting SOCS3 in endothelial cells to induce angiogenesis in vitro. Furthermore, by inhibiting SPINT1 expression and activating the liver growth factor (HGF), which promotes the development of a favorable premetastatic niche and CRC metastasis, miR-221-3p upregulation forecasts a poor overall survival rate. To the best of our knowledge, no studies have examined the relationships between exosomal miR-221-3p and treatment predictions for metastatic CRC. Regarding the modulation of the expression induced by chemotherapy, there was an increased expression level from baseline to post-chemotherapy of 1 point68 x in responders vs.

We also noted that an increased baseline level of miR-484 in plasma exosomes was associated with lack of a therapeutic response, regardless of the chemotherapy protocol administered. non-responders. Kjersem et al investigated the prognostic value of specific plasma-free miRNAs in predicting responses to FOLFOX chemotherapy. In the NORDIC ACT clinical trial, which included 24 patients with CRC (12 responders and 12 non-responders), the gene expression levels of a subset of miRNAs were examined in plasma at baseline and after four cycles of chemotherapy. Following that, 150 patients validated the miRNA that had shown a difference in expression between the two groups. With a notable differential expression at baseline, miR-106a, miR-484, and miR-130b were overexpressed in non-responders. MiR-484, a tumor-suppressor miRNA, targets the DLK1 gene and contributes to the EMT by promoting the process.

Conclusion

Given the small number of samples used in this study and their low statistical power for clinical applicability, the data from this study should be viewed as a hypothesis generator for subsequent research. Our findings demonstrated the discriminatory power of exosomal miR-92a-3p, miR-146a-5p, miR-221-3p, and miR-484 for non-responder patients regardless of the treatment employed. High baseline levels of these genes were associated with a lack of response to FOLFOX chemotherapy. Increased exosomal levels of miR-143-3p and miR-221-3p at the start of therapy have a predictive value for shorter OS, whereas increased exosomal levels of miR-92a-3p and miR-486-5p are associated with lower OS and PFS1. Our findings imply that higher exosomal miR-92a-3p and miR-221-3p basal expression predisposes to chemotherapy resistance and poorer OS. However, the validation of these data on larger cohorts of advanced CRC patients will be able to confirm the function of these miRNAs in predicting a lack of response to FOX chemotherapy in clinical practice.

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