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REVIEW ARTICLE

Exosomal miRNAs in colorectal cancer: the carriers of useful news

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Summary

Purpose: *In this review, we focused on presenting an up-to-date overview of exosomal miRNAs as biomarkers for diagnosis, prognosis, and their therapeutically perspectives in colorectal cancer (CRC).*

Methods: *A comprehensive literature search was conducted using the PUBMED database through February 2019 to identify all studies concerning the role of EXOSOMAL miRNAs in CRC.*

Results: *Among the 77 studies identified, 43 articles were relevant for the collaboration of miRNAs and exosomes as therapeutic and diagnostic opportunities in CRC.*

Conclusions: *This review reveals the role of exosomal miRNAs in CRC management and discusses the promises and challenges associated with the introduction of this APPROACH into clinical practice.*

Key words: *miRNA, exosomes, colorectal cancer, biomarker*

Introduction

The global cancer burden predicted by the GLOBOCAN 2018 is estimated to increase to 18.1 million new cases and 9.6 million deaths in 2018. With an estimation of 1.8 million new cases and about 881,000 deaths annually, colorectal cancer (CRC) ranks third in terms of cancer incidence and second in cancer mortality [1]. Despite early screening protocols, new chemotherapeutic agents and the development of targeted therapy, only 39% of the cases are diagnosed with localized disease for which, with adequate treatment, the 5-year survival rate is 90%. Survival rates decline to 71% and 14% respectively for patients diagnosed with regional disease or metastases [2]. Hence, the development of novel sensitive and specific biomarkers for early diagnosis and personalized therapeutic approach is necessary for the management of CRC.

MiRNAs are small non-coding RNA sequences of 19-25 nucleotides that play a crucial role in almost every cellular process, including differentia-

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tion, proliferation, progression and apoptosis [3]. Numerous studies have shown abnormal expression patterns of miRNAs in both solid and hematopoietic tumors, including CRC, where miRNAs are linked to the diagnosis, prognosis and therapeutic response, making them promising biomarkers [4]. Exosomes are small endocytic membrane vesicles (50-90 nm) released in the extracellular microenvironment after the fusion of multivesicular bodies with the plasmatic membrane. Exosomes can load and transport proteins, lipids, mRNA, and miRNA depending on the origin of the secreting cell [5]. Previous studies have already confirmed that tumor cells release specific miRNAs-containing exosomes in the blood, saliva, urine, ascites and bronchoalveolar lavage fluid of cancer patients. Considering their specificity, circulating exosomal miRNAs have a great potential as noninvasive biomarkers for the management of CRC patients [6].

MiRNAs overview

Only about 2% of the human genome include protein-coding DNAs, known as Protein Coding Genes (PCG), while the rest 98% represents non-coding DNAs including regulatory sequences defining non-coding RNAs (ncRNAs), introns as well as other DNA sequences with unknown functions. Non-coding RNAs represent RNA structures that are not further translated into proteins, but participate actively in the regulation of PCG [7]. The ncRNAs consist of a series of classes of transcripts that can be described by their length or by their function. According to their length, there are small (18 to 200 nucleotides) and long (>200 nucleotides) ncRNAs. As for their function, there are regulatory (e.g., microRNA, lncRNA) and also housekeeping (transfer RNA, ribosomal RNA) RNAs [8]. Because of their capacity to modulate both mRNA transcription and translation, ncRNAs are involved in physiological processes but also in pathological statuses, like cancer. MicroRNAs (miRNAs) have lately become the most studied species of nc-RNAs. A specific feature of miRNAs is that of a single miRNA is capable of targeting tens or hundreds of genes, such as one miRNAs can modulate an entire molecular mechanism or even different molecular pathways [9]. New insights into miRNA properties revealed that they could target DNA promoter regions, RNA 5' untranslated region, proteins, and other ncRNAs. Moreover, miRNAs are directly involved in upregulating of protein translation [10].

If under normal conditions miRNAs are involved in maintaining cell physiology, in cancer, due to their altered expression, miRNAs are involved in the activation and development of the tumor phenotype [11,12]. Abnormal expressions of miRNAs appear to have a specific signature with role in cancer diagnosis, prognosis, and therapeutics [13]. For this perspective, miRNAs are regarded as encouraging tools for clinical use, if further multicentre clinical trials validate them as reliable biomarkers.

MiRNAs role in CRC is sustained by their involvement in most of the CRC processes from cell proliferation to cancer-related inflammation [14].

Exosome overview

First described in 1981 by Trams et al [15], exosomes were considered extracellular debris for a long time, but in recent years, exosome function has generated increasing interest, especially in the field of cancer research. Exosomes may contain a broad array of proteins (oncoproteins, tumor suppressor proteins, transcriptional regulators), lipids (phosphatidylcholines, phosphatidylethanolamines and phosphatidylserines), DNAs (single-strand DNA, genomic DNA, XX DNA, and retrotransposon elements) and RNAs (mRNA, lncRNA, miRNA and other non-coding RNAs) [16]. Circulating exosomes create a communication network between cancer cells and tumor microenvironment and facilitate tumor hallmark processes such as escaping of tumor cells from apoptosis, inducing tumor angiogenesis, reprogramming tumor metabolism, modulating the immune response and remodeling the extracellular matrix that leads to migration and invasion of tumor cells [17]. These biomolecules allow the transfer of oncogenic properties from the primary tumor to the recipient cells in distal organs, contributing to cancer metastasis through the phenomenon of genometastasis [18].

Increasing evidence has demonstrated the utility of using the content of tumor-derived exosomes as biomarkers in the diagnosis, as well as for monitoring the efficacy of cancer treatment. Tumor-released exosomes include the following characteristics: exosomes contain cancer-related protein, lipids, RNA and DNA; have a small volume and can be found in various body fluids making them accessible for clinical detection; the lipidic bilayer membrane protects their contents from degradation. In each ml of blood, there are over 109 exosomes, making the *in vivo* detection of cancer-specific exosome feasible [19].

Exosomes are also emerging as valuable potential therapeutic targets. Besides their role as biomarkers, exosomes could be used as drug delivery vehicles due to nanometric size, protective lipid bilayer-membrane, lower immunogenicity and toxicity than other drug delivery strategies,

and the surface expression of integrins that can direct them to specific organs [20].

Exosomal miRNAs in colorectal cancer

1.Diagnostic features of exosomal miRNAs in CRC

Aiming to investigate the exosome-mediated crosstalk between cancer-associated fibroblasts (CAF) and CRC cells, Bhome et al identified an exosomal cancer-associated fibroblast panel containing six microRNAs: miR-329, miR-miR-181a, miR -199b, miR -382, miR -215 and miR -21 which could serve as a potential CRC stromal biomarkers. Furthermore, they demonstrated the oncogenic role of exosomal miR-21, transferred from cancer-associated fibroblasts (CAF) in CRC progression using an orthotopic model [21].

The diagnostic value of miR-125a-3p and miR-320c was evaluated in 50 early-stage CRC patients and matched healthy controls [22]. Their data highlighted that miR-125a-3p and miR-320c are significantly higher in plasma exosomes of the patients with early-stage colon cancer than in healthy persons. ROC curve analysis demonstrated that miR-125a-3p expression might predict colon cancer with an area of under the curve (AUC) of 68.5%. Moreover, when CEA (carcinoembryonic antigen) was associated with miR-125a-3p, a significant improvement of AUC to 85.5% ($p<0.0001$) was provided.. Additionally, plasmatic exosomal miR-125a-3p and miR-320c level displayed significant correlation with perineural invasion ($p<0.01$), but not with tumor size, infiltration depth, and grade of differentiation ($p>0.05$). On the contrary, plasma CEA was associated to tumor size, infiltration depth, and differentiation grade ($p<0.05$, $r=0.3009$-0.7270), but not to nerve infiltration ($p=0.744$). When searching for a correlation between tumor sidedness and exosomal miRNA, miR-125a-3p was up-regulated in the left colon compared to the right colon [22].

MiRNA-486-5p has been demonstrated to function as oncogene or tumor suppressor in NSCLC [23], prostate cancer [24], esophageal squamous cell carcinoma [25] or papillary thyroid carcinoma [26]. Liu et al., demonstrated that miRNA-486-5p is downregulated in CRC due to higher DNA methylation of its promoter region. An analysis of 50 paired CRC tissues and adjacent normal tissue proved that lower miRNA-486-5p was significantly correlated with advanced TNM stage, nodal metastasis, and larger tumor size. Kaplan-Meier survival analysis showed that high miR-486-5p expression was correlated with better 5-year survival. To evaluate the diagnostic value of exosomal plasmatic miR-486-5p, ROC curve analysis was carried out. The AUC of miR-486-5p to distinguish CRC patients from healthy subjects was 0.713 with 67.5% sensitivity and 77.3% specificity and the AUC of the same miR to differentiate CRC stage I patients from healthy subjects was 0.737 with 63.1% sensitivity and 77.3% specificity [27].

Differential expression of various miRNAs may serve as a potential biomarker for CRC diagnosis allowing 0for earlier diagnosis and a more personalized approach. A panel of seven overexpressed miRNAs (miR-103a-3p, miR-127-3p, miR-151a-5p, miR-17-5p, miR-181a-5p, miR-18a-5p and miR-18b-5p) was identified in the plasma of CRC patients, being further validated in tissue samples and exosomal miRNA in a four-stage manner (screening, training, testing and external validation stage). When testing for sensitivity and specificity of the seven-miRNA panel, the AUC of the ROC was 0.967 and 0.533 in the training stage, 0.853 and 0.351 for the training stage and 0.769 and 0.867 for the validation stage. No significant expression difference was observed between stages I, II vs. III, IV, neither between left vs. right-sided CRC. The analysis of the exosomal expression levels of the selected miRNAs showed high expression in CRC plasma exosomes compared with normal controls, but only four miRNAs (miR-17-5p, miR-181a-5p, miR-18a-5p and miR-18b-5p) were up-regulated with statistical significance [28].

An evaluation of extracellular vesicle (EV) miRNA (shed vesicle miRNA, and exosome encapsulated miRNA) as a potential diagnostic marker for CRC found 345 different dysregulated miRNAs in CRC cell lines. When comparing metastatic cell lines (SW620) to primary carcinoma cells (SW480), 61 and 73 EV miRNAs were upregulated and respectively downregulated in SW620 compared to SW480. Pearson's correlation analysis proved that EV miRNA panels could be used to predict, with high sensitivity (94.9%) and specificity (100%) CRC tumors of different stages listed in the TCGA dataset [29].

Another group of researchers [30] have proposed a three-panel miRNA signature for diagnostic and screening of CRC. Their study was divided into four phases, to elucidate if miR-19a-p, miR-21-5p and miR-425-5p are significantly higher in serum, tissue and exosomal serum of CRC patients than in normal controls. The analysis indicated serious proof for these exosomal miRNAs to predict CRC. They also attempted to screen these three miRNAs in arterial blood, but the data was not consistent because of the small number of patients.

It is also to be mentioned a mathematical model proposed in a recent paper [31] that correlates

exosomal miR-21, miR-23a, miR-92a and miR-1246 with the growth of the colorectal tumor. The model was adapted for early-stage CRC cells taking into consideration the growth of the tumor diameter and the exosomal concentration of these miRNAs. This model also took into account the most frequent mutations that occur in CRC, like KRAS, PI3K, APC, p53, and SMAD. The authors found a specific relationship of these exosomal miRNAs serving as reliable biomarkers for early detection of CRC and future therapeutic measures.

In another study, Kawata et al [32] investigated the miRNAs profiles of serum exosomes and identified a set of seven serum exosomal miRNAs (let-7a, miR-1229, miR-1246, miR-150, miR-21, miR-223, and miR-23a) with higher levels in CRC patients than in healthy controls. Moreover, they observed that after surgical resection of the tumor, these exosomal miRNAs were significantly downregulated. Even in colon cancer cell lines, the expression of this set of miRNAs was significantly higher than in normal cells. The specific signature of these seven exosomal miRNAs based on ROC analysis proposed them as CRC diagnosis biomarkers. Following the normal-adenoma-carcinoma sequence, a study evaluated and compared four miRNAs (miR-21, miR-29a, miR-92a, and miR-135b) in serum and exosomes. These miRNAs in serum were found to be superior non-invasive biomarkers compared with exosomal miRNAs in the early detection of CRC. This method could be useful in the clinic for the detection of high-risk adenomas with an increased chance of developing CRC [33]. In Table 1 is presented a short overview of exosomal miRNAs related to CRC diagnosis.

2. Prognostic features of exosomal miRNAs in CRC

Hypoxia negatively impacts therapy in locally advanced rectal cancer. In the search for potential biomarkers of tumor hypoxia and adverse prognosis, Bjørnetrø et al investigated relevant exosomal miRNAs from hypoxic cell lines and the serum of locally advanced rectal cancer patients. Exosomal miR-486-5p, miR-181a-5p, and miR-30d-5p were selected, based on the frequency or magnitude of variance in hypoxic versus normoxic cell line and prevalence in patient serum. Low plasmatic levels of exosomal miR-486-5p and miR-181a-5p were linked to T4 (p=0.029) and N-positive (p=0.024) disease, while high miR-30d-5p was associated with metastatic progression (p=0.036). No link was observed between exosomal miRNAs selected based on cell line hypoxia and tumor response to neoadjuvant treatment [34].

Exosome encapsulated miR-548c-5p has been associated with CRC prognosis. Low exosomal miR-548c-5p levels are linked to shorter overall survival in CRC adjusted by age, sex, tumor grade, vascular infiltration, TNM stage, and metastasis. Exosomal miR-548c-5p is more significantly reduced in CRC

Table 1. Exosomal miRNAs associated with the diagnosis of colorectal cancer

Exosomal miRNA	*Role*	*Ref*
miR-329, miR-181a, miR-199b, miR-382, miR-215, miR-21	Diagnostic miR21 - progression	[21]
miR-125a-3p, miR-320c	Diagnostic in early stage Predictive	[22]
miRNA-486-5p	Diagnostic / Predictive Differentiate CRC patients from the healthy subject in early stage	[27]
miR-103a-3p, miR-127-3p, miR-151a-5p, , miR-17-5p, miR-181a-5p, miR-18a-5p, miR-18b-5p	Diagnostic Prognostic	[28]
miR-19a-3p, miR-21-5p, miR-425-5p	Diagnostic Predictive Screening	[30]
miR-21, miR-23a, miR-92a, miR-1246	Diagnostic Prognostic in early stage Tumor growth	[31]
Let-7a, miR-1229, miR-1246, miR-150, miR-223, miR-23a	Diagnostic	[32]
miR-21, miR-29a, miR-92a, miR-135b	Diagnostic Predictive risk for high risk adenomas to transform in CRC	[33]

patients with liver metastasis and advanced TNM stage. An analysis of potential miR-548c-5p target genes in TargetScan (miRNA.org, and miRDBA databases) revealed that miR-548c-5p might be involved in CRC by targeting STAT3, PTPRO, IRF, interleukins, and their receptors. When comparing HCT116 cells exposed to high levels of exosomal miR-548c-5p with those incubated with low exosomal miR-548c-5p, a significant inhibition of cellular proliferation could be observed in the high miR group, indicating exosomal miR-548c-5p as a potential tumor suppressor in CRC [35].

MiR-200 family comprises 5 members (miR-200a, miR-200b, miR-200c, miR-429, miR-141) and has raised interest in identifying new miRNA biomarkers. An evaluation of plasmatic exosomes from the mesenteric vein (MV) and peripheral vein (PV) of 50 resected stage I-III CRC patients revealed superior expression in MV. Low miR-200c and miR-141 in MV plasma were linked to longer overall survival (OS), while CEA expression was associated with miR-141 and miR-429 levels in MV. Tumor location was linked to miR-141 and miR-429 expression in the MV. In PV, a correlation was detected between tumor size and miR-200b and miR-141; relapse and miR- 200b, and between K-RAS mutations, histological type and miR-429 [36].

Another study revealed that exosomal cargo of miR-328 is higher in patients with a risk of developing liver metastases. Starting from the idea that taking blood from the place near the tumor can develop a higher concentration of biomarkers the authors proved that taking blood from mesenteric vein had a higher predictive impact than taking it from a peripheral vein [37].

A previous study [38] focused on the predictive value of lncRNA GAS5 and miR-221 in the prognosis of CRC and their role in CRC cell proliferation, migration and invasion was conducted by sampling plasma, tissue and exosomes of 158 CRC patients, 173 healthy subjects and SW480 cell lines. Exosomal miR-221 expression was positively correlated with tumor size, TNM stage, Dukes stage, lymph node metastasis, local recurrence rate, and distant metastasis rate. Exosomal miR-221 was found as an independent prognostic factor for OS, local recurrence rate, and distant metastasis rate. ROC curve analysis showed that exosomal miR-221 had diagnostic value in CRC. SW480 cell line assay revealed that lncRNA GAS5 inhibits CRC cell proliferation, migration, and invasion by down-regulation of miR-221 [38].

A panel of exosomal microRNAs sequencing conducted by Fu et al on SW480 and SW620 cell lines and validated in the serum of CRC patients found miR-17-5p, miR-92a-3p to be upregulated in CRC patients. Higher expression of circulating exosomal miR-17-5p and miR-92a-3p were significantly associated with pathologic stages and grades of the CRC patients. ROC curve analysis for distinguishing between CRC patients and normal controls showed AUC value of 0.897 (95% CI, 0.800-0.994) for exosomal miR-17-5p and 0.845 (95%CI, 0.724-0.966) for miR-92a-3p. When testing for metastatic vs non-metastatic patients AUC values were 0.841 (95% CI, 0.720-0.962) for miR-17-5p and 0.854 (95%CI, 0.735-0.973) for miR-92a-3p. No association was found between exosomal miR-17-5p, miR-92a-3p and KRAS, BRAF, TP53 mutations [39].

In a previous study, Li et al [40] aimed to investigate the clinical significance of the circulating glypican-1 positive (GPC1) plasma exosomes, including miR-96-5p and miR-149 in 85 patients with stage III colon cancer. The authors found that that high levels of circulating GPC1 plasma exosomes before and after surgery alongside low circulating miR-96-5p and miR-149 before surgery indicated poor prognosis in stage III colon cancer patients. Furthermore, miR-96-5p and miR-149 were found to influence GPC1 positive plasma exosomes levels by targeting GPC1 gene. High GPC1 plasma exosome expression stimulated CRC cell invasion and migration by activating endothelial-mesenchymal transition.

Serum exosomal miR-6803-5p was previously associated with advanced disease, lymph node and liver metastasis, while high levels of plasmatic exosomal miR-6803-5p indicated inferior OS and DFS as well as poor prognosis in CRC independent of age, sex, TNM stage, lymph node metastasis and liver metastasis [41].

Substantially decreased levels of miR-6869-5p have been detected in serum exosome and tissue samples of CRC patients. Serum exo-miR-6869-5p values were more reduced in patients with advanced stage or with liver or lymph node metastasis. Additionally, the 3-year survival rate was more inferior in patients expressing low exo-miR-6869-5p levels. TargetScan, (microRNA.ORG) and miRDBA databases revealed toll-like receptor 4 (TLR4) as a potential target for this miRNA. MiR-6869-5p may downregulate cellular proliferation and production of inflammatory cytokines (TNF-α and IL-6) in CRC cells by targeting the TLR4/NF-κB signaling pathway [42].

Aiming to find specific miRNAs in serum exosomes of CRC patients which may serve as diagnostic, prognostic or therapeutic markers Yan et al [43] confirmed a panel of 7 miRNAs (miR-638, miR-5787, miR-8075, miR-6869-5p and miR-548c-5p were downregulated, while miR-486-5p

and miR-3180-5p were significantly upregulated) in the serum of CRC patients. Their data revealed that low levels of exosomal miR-638 were associated with a higher risk of liver metastasis and a more advanced stage. Using network analysis, this group identified the glucose metabolism of CRC cells as a potential target for miR-638, miR-5787, miR-8075, miR-6869-5p, and miR-548c-5p.

Recent studies suggest that exosomal microRNA-21 has been shown to play a specific role in each TNM stage of CRC. The endpoint correlated the level of exosomal miR-21 with DFS and OS. It appears that this exosomal miRNA has a dual role, as patient stratification for chemotherapy of stage II patients and as predictive for recurrence and as a prognostic biomarker for stage III-IV CRC patients [44].

Liu et al [45] provided data about miR-4772-3p and if it could be a biomarker for recurrence and response to adjuvant chemotherapy after resection. Reduced levels of serum exosomal miR-4772-3p were correlated with time to recurrence and OS in stage II-III colon cancer patients. The strength of the study consisted in being the first study on stage II-III patients and another strong point represents the minimum selection and treatment bias.

By drawing on the concept of previous studies that suggested miR-375 as being involved in several human cancers, a team of researchers [46] tried to prove that miR-375 is also downregulated in metastatic CRC and could be used as a prognostic biomarker. By blocking the Bcl-2 pathway, miR-375 functions as a tumor suppressor and inhibits cell progression and invasion. It also interferes with the metastatic cascade controlling the metastatic capacity of CRC cells. This was the first study to correlate exosomal miR-375 and Bcl-2 pathway blockage in colon cancer.

Serum exosomal miR-19a seems to be upregulated independently of CRC stages. In a recent study [47] it was presented that miR-19a expression could serve as a biomarker for early detection of recur-

Table 2. Exosomal miRNAs associated with prognosis of colorectal cancer

Exosomal miRNA	*Role*	*Ref*
miR-486-5p, miR-181a-5p, miR-30d-5p	Prognostic for T4, nodal involvement and metastatic progression	[34]
miR-548c-5p	Prognostic for advanced TNM stage, liver metastasis	[35]
miR-200a, miR-200b, miR-200c, miR-429, miR-141	Prognostic for early stage	[36]
miR-328	Prognostic for liver metastasis	[37]
miR-221	Prognostic Predictive for advanced stage, nodal involvement, relapse and metastasis	[38]
miR-17-5p, miR-92a-3p	Prognostic Predictive for pathologic stage and grade	[39]
miR-96-5p, miR-149	Prognostic for stage III CRC	[40]
miR-6803-5p	Prognostic Diagnostic	[41]
miR-6869-5p	Prognostic for advanced stage, lymph node metastasis and liver metastasis	[42]
mir-638, miR-5787, miR-8075, miR-6869-5p, miR-548c-5p, miR-486-5p, miR-3180-5p	Prognostic for advanced stage and liver metastasis Diagnostic	[43]
miR-21	Prognostic Predictive for recurrence Diagnostic Stratification for chemotherapy	[44]
miR-4772-3p	Prognostic Predictive for adjuvant treatment and recurrence	[45]
miR-19a, miR-17-92a	Prognostic Predictive for recurrence	[47]
miR-200c	Prognostic Predictive for aggressiveness, metastases	[48]

rence. Also, exosomal miR-17-92a cluster expression in serum was correlated with early detection of recurrence in CRC patients. The shortcomings of the study consisted of the lack of explanations of the mechanism between serum exosomal miR-19a and intercellular signalling.

In a spheroid experimental model, the loss of miR-200c in chemoresistant 5-FU CRC cells established an aggressive migratory phenotype in endothelial cells. From a clinical point of view, this could serve as a tool for the prevention of metastases in stage I-II CRC [48]. In Table 2 presented is a brief overview of the exosomal-miRNAs associated with CRC prognosis.

3. Exosomal miRNAs therapeutic perspective

To study the role of exosomes on CRC cells Lucchetti et al used sodium butyrate-induced differentiation of HT29 colon cancer cells. Their findings suggested that the differentiation status of the CRC affects exosome release both quantitatively and qualitatively. Exosomes released by differentiating cells carried increased levels of miRNA and increased proliferation, motility and colony-forming capacity of CRC cells. They evaluated the expression of exosomal miR-24, miR-27a and miR-26a. Upon differentiation the exosomal export of miR-24, miR-27a, and miR-26a, increased along with the decrease of their cellular level; additionally, they noticed an increment of miR-24 and miR-27a in HCT116 exposed to exosomes released from differentiated cells, suggesting that the expression of a subgroup of miRNAs might decline in differentiated cells whereas increasing in released exosomes, which can transfer these miRNAs to recipient cells [49].

Tumor-associated macrophages play an essential role in the tumor microenvironment. They promote proliferation, invasion, and metastasis, stimulate angiogenesis and escape immune surveillance [50]. To evaluate the macrophage-derived factors that control CRC Lan et al assessed the role of macrophage-derived exosomes (MDE) in CRC cells migration and invasion. Their data proved that MDE displayed a high expression level of miR-21-5p and miR-155-5p. M2 tumor-associated macrophages transfer miR-21-5p and miR-155-5p to the CRC cells, via MDE, promoting cell migration and invasion by downregulating BRG1 [51]. Mutation status of BRG1 is reported in several cancers and it is a core motor of SWItch/Sucrose Non-Fermentable (SWI/SNF) complex involved in the regulation of gene transcription [52].

TP53 gene has been reposted in the pathogenesis of most cancers including CRC. Cooks et al [53] demonstrated that p53 mutations reprogram macrophages to a tumor-supportive and anti-inflammatory state. Mutp53 CRC cells selectively shed exosomal miR-1246 biding it via heterogeneous nuclear ribonucleoproteins A2/B1 (hnRNPa2b1), an RNA binding protein. Exosomes containing miR-1246 are internalized by surrounding macrophages which undergo reprogramming and produce anti-inflammatory and tumor supportive factors including IL-10, TGF-β, and matrix metallopeptidase.

Cancer-associated fibroblasts (CAF) promote CRC cell proliferation. A study examining the development of CAF in the tumor microenvironment demonstrated that CRC cell export increased exosomal miR-10b levels compared to normal colonic epithelial cells. Exosomal miR-10b is transferred to fibroblasts. MiR-10b uptake leads to reduction of fibroblast proliferation by targeting PIK3CA expression and diminishing PI3K/Akt/mTOR pathway activity. Additionally, miR-10b stimulated the expression of TGF-b and SM α-actin, implying that exosomal miR-10b may activate fibroblasts to become CAFs that express myofibroblast markers. These activated fibroblasts were able to promote CRC development *in vitro* and *in vivo* [54].

A screening of all intracellular and extracellular microRNAs was performed by Mizoguchi et al to find new tumor suppressors. Exosomal export of miR-8073 was 20% higher in CRC cells. To examine the role of miR-8073 on cellular proliferation, miR-8073 was transfected to HCT116 cells. MiR-8073 mimic reduced cancer cell viability to 4% of the level in the control cells. Similar inhibitory effect was observed in other cell lines. To evaluate whether the reduced viability of the transfected cells was due to apoptosis, the activity of caspase-3 and -7 proteins were measured. A 200% increase in activity was observed in CRC cells compared to control suggesting that miR-8073 can reduce cancer cells viability via apoptosis. Five potential targets for miR-8073 were identified: FOXM1, MBD3, CCND1, KLK10, and CASP2, all known to control cell proliferation, DNA methylation, cell cycle, carcinogenesis, and apoptosis. The binding of miR-8073 to mRNA targets negatively regulated gene expression in all five target genes, making miR-8073 a potential therapeutic goal [55].

To assess whether exosomal loading with miRNA is efficient in targeting cancer stem cell markers claudin7 (cld7) and EpCAM(EpC) in the adjuvant therapy of CRC and pancreatic cancer, Kyuno et al collected non-transformed mouse and rat lung fibroblasts exosomes which were transfected with Tspan8cDNA (NIH3T3-Tspan8, rFb-Tspan8). Exosomes were loaded by electroporation with miRNA. MiR-3541 and -615 target rat cld7 in rat

pancreatic cell lines while miR-342-5p and MiR-498 target rat Cld7 and EpC in human colon cancer cell lines. Exosomal miRNA transfer induced Cld7, respectively, EpC downregulation by 33-60%. Additionally, the Cld7 silencing was associated with reduced expression of NOTCH, reduced vimentin, N-cadherin, and Nanog expression and the transfer of exo-miRNAs presented above may influence growth, motility, and invasion of colon cancer cells [56].

Zeng et al analyzed the role of exosomal miR-25-3p in CRC metastasis. They demonstrated that miR-25-3p could be transferred from CRC cells to endothelial cells via exosomes, inducing vascular permeability and angiogenesis by targeting the Krüppel-like factor (KLF) family, KLF 2 and KLF4. Additionally, the exosomal transfer of miR-25-3p from CRC cells to endothelial cells decreased the levels of KLF2, KLF4, ZO-1, Occludin, Claudin5, and increased the level of VEGFR2 in the lung and liver of mice. Therefore, it can be stated that exosomal miR-25-3p contributes to the formation of the premetastatic niche. Serum of healthy subjects and CRC patients with or without metastasis was analyzed for miR-25-3p detection. Gene expression profiling demonstrated that miR-25-3p from circulating exosomes was higher in CRC patients than in controls, but also miR-25-3p expression was higher in CRC metastatic patients than in CRC patients without metastasis. Significantly, 85% (17/20) of patients presented a decrease of miR-25-5p exosomal expression after surgery. Moreover, a positive correlation of miR-25-3p exosomal expression with CRC tissues was identified, such as the miR-25-3p expression in tissue could anticipate the miR-25-3p exosomal expression. Furthermore, they demonstrated that the blockade of miR-25-3p reduced hepatic and pulmonary vascular permeability and consequent CRC metastasis, proving exosomal miR-25-3p not only as a potential biomarker for CRC progression but also as a promising therapeutic target [57].

Exosomal miR-200b is involved in CRC cell proliferation. TGF-β1 exposure of CRC cell lines increases exosomal levels of miR-200b which targets the 3'-UTR of p27. P27/kip1 represents, an essential regulator of cell cycle progression, involved in G1 arrest. Upon exposure to high exosomal miR-200b, p27/kip1 becomes downregulated, facilitating the proliferation of cancer cells [58]. In another study, Chira et al evaluated the role of exosomal miRNA profiling in assessing the response of DLD-1 CRC model to deuterium depleted water (DDW) modulator of adjuvant therapy using 5-FU and oxaliplatin. Their analysis demonstrated that cell culture DDW-modified condition altered the release of exosomal miRNAs compared with cell culture standard conditions, making exosome released miRNA pattern an efficient method to monitor DDW response [59].

Cancer stem cells are the main culprits of the chemoresistance process. The mechanism of chemoresistance is not clarified. Using human CRC cell lines, Dong Ren et al [60] proved that miR-196b-5p has been involved in 5-FU chemoresistance of CRC cells by targeting SOCS1, SOCS3, and STAT3. They also found that miR-196b-5p is upregulated in CRC tissue and high expression correlates with a low OS. The exosomal serum levels of miR-196b-5p are increased in CRC patients compared to healthy subjects. Having both therapeutic and diagnostic applications, miR-196b-5p represents a valuable candidate for future therapeutic perspectives.

As stated in previous articles, the role of GPC1 in CRC is yet insufficient elucidated. Jian Li et al [61] studied the role of GPC1 plasma exosomes in CRC cancer. The study was conducted on early stage I-II colon cancer patients and found that miR-96-5p and miR-149 were decreased in plasma, tissue and also exosomal GPC1. Interestingly, the levels of these three biomarkers became normalized after surgery. The increased level of exosomal GPC1 and the decreased expression of miR-96-5p and miR-149 are markers for diagnosis, therapeutic efficacy, and furthermore targeted therapies. In their seminal paper, Yun Teng et al [62] showed that advanced CRC has higher levels of exosomal miR-193a. The interesting fact about the results of the study is that MVP (major vault protein) can sort the tumor suppressor exosomal miR-193a into exosomes selectively. This property confers the tumor suppressor miRNA the capacity to promote CRC progression by targeting Caprin1. By suppressing MVP, exosomal miRNA-193a can act as an inhibitor of tumor progression and is a candidate for therapeutic experimentation.

Using CRC LIM1863 cell line, and based on an RNA-Seq approach, Chen et al [63] identified and investigated three different extracellular vesicle subtypes, including exosomes and microvesicles. This particular CRC cell line secrets two types of exosomes, an apical one (EpCAM-Exos) and a basolateral, type (A33-Exos) and also shed microvesicles with specific proteins and miRNA characteristics. Six of the identified miRNAs were specific only for exosomes (miR-320a/b/c/d, miR-3p, and miR-200c-3p), other miRNAs including miR-451-a, miR-374-5p, miR-4454,miR-7641 and Let-7/a/f were common to all vesicles, while miR-98-5p was enriched only in microvesicles. Clancy and Khan described in detail the examination of the entire set of miRNAs from HCT-116 and HT-29 CRC cell secreted exosomes. This examination was the first study to

Table 3. Exosomal miRNAs associated with the presumptive therapeutic role that could become useful for colorectal cancer management

Exosomal miRNA	*Role*	*Ref*
MiR-24, MiR-27a, MiR-26a	Therapeutic	[49]
MiR-21-5p, MiR-155-5p	Therapeutic	[51]
MiR-1246	Therapeutic	[53]
MiR-10b	Therapeutic	[54]
MiR-8073	Therapeutic	[55]
MiR-3541, MiR-615, MiR-342-5p, MiR-498	Therapeutic	[56]
MiR-25-3p	Therapeutic, Diagnostic, Predictive	[57]
MiR-200b	Therapeutic	[58]
MiR-196b-5p	Therapeutic, Diagnostic, Chemoresistance	[60]
MiR-149, MiR-96-5p, GPC1	Therapeutic, Diagnostic	[61]
MiR-193a	Therapeutic, Diagnostic	[62]
MiR-379	Therapeutic	[64]
MiR-200	Therapeutic, Chemotherapy and radiotherapy resistance	[65]
MiR-200c, MiR-141	Therapeutic	[66]

encompass all the miRNAs present in two different CRC cell lines and all their implications in cancer pathogenesis. From all miRNAs examined, miR-379 was found to act as tumor suppressor in CRC cell lines and could become of interest for modulating it through targeted therapies [64].

In an interesting study in a 3D model using spheroids, Holzner et al [65] explained the microenvironmental mechanism behind chemotherapy and radiotherapy resistance. Exosomal miR-200 family including miR-200c, miR-141, and miR-429 was implicated in the modulation of BEC (blood endothelial cells), that represents possible gates for tumor transmigration, by downregulating the expression of ZEB2, SNAI, and TWIST transcription factors underline epithelial to mesenchymal transition in BEC cells.

In 2015, Tanaka et al [66] demonstrated on CRC cells resistant to oxaliplatin, that decitabine, a DNA methyltransferase (DNMT) inhibitor, can suppress the invasion ability. Exosomal miR-200c and miR-141 were upregulated in two of the three cell lines. These miRNAs appeared to be upregulated by decitabine in SW620 and SW620/OxR cell lines, but not in SW480. These cells are very suggestive for CRC because of the resistance to oxaliplatin (SW620/OxR) and also because they show invasive characteristics as mesenchymal-epithelial transition. Oxaliplatin remains one of the most useful drugs in day-to-day care, making this study more valuable for therapeutic clinical biomarkers.

As KRAS status is essential for day-to-day care of metastatic CRC, a study of Cha et al [67] sorted exosomal miRNAs according to their mutant or wild-type characteristics. The results showed that mutant KRAS could modify the expression of secreted miRNAs. MiR-10b was increased in wild-type exosomes, and miR-100 was found to be increased in the exosomes released by CRC mutant KRAS cells. This fact shows that exosomal profiles differ from cellular profiles unveiling potential treatments for CRC.

The metastatic capacity of CRC cells based on intercellular transfer mediated by exosomes was also highlighted in a previous study [68]. In this research, the Asari's group proved that exosomes derived from three different CRC cell lines contained mRNAs, miRNAs, and natural antisense RNA can shuttle between CRC cells and recipient hepatic and lung cells, interfering with the regulation of gene expression in the recipient cells. Table 3 presents a brief overview of the exosomal-miRNAs associated with the presumptive therapeutic role that could become useful for CRC management.

Conclusion

High incidence and mortality characterize CRC despite recent advances in diagnosis and therapeutics. Although many efforts have been made to improve the chances for a better result in the metastatic CRC, the prognosis is still dismal. Therefore, there is an urgent need for noninvasive diagnosis, prediction markers for aggressiveness of the tumor and response to treatment, as well as new therapeutic options. CRC biology has revealed miRNAs as solid players in CRC initiation, progression and chemoresistance. Combining the effect of exosomes carrying miRNAs cargo opens new doors for developing promising tumor bio-

markers into daily clinical management. These findings can considerably improve the outcome of CRC patients. Preliminary data is encouraging although future research is required to confirm their sustainability.

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Conflict of interests

The authors declare no conflict of interests.

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