The Impact of miRNA in Colorectal Cancer Progression and Its Liver Metastases

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Abstract:

Colorectal cancer (CRC) is one of the most commonly diagnosed malignancies with a high incidence and mortality. An essential challenge in colorectal cancer management is to identify new prognostic factors that could better estimate the evolution and treatment responses of this disease. Considering their role in cancer development, progression and metastasis, miRNAs have become an important class of molecules suitable for cancer biomarkers discovery. We performed a systematic search of studies investigating the role of miRNAs in colorectal progression and liver metastasis, published until October 2018. In this review, we present up-to-date information regarding the specific microRNAs involved in CRC development, considering their roles in alteration of Wnt/βcatenin, EGFR, TGFβ and TP53 signaling pathways. We also emphasize the role of miRNAs in controlling the epithelial-mesenchymal transition of CRC cells, a process responsible for liver metastasis in a circulating tumor cell-dependent manner. Furthermore, we discuss the role of miRNAs transported by CRC-derived exosomes in mediating liver metastases, by preparing the secondary pre-metastatic niche and in inducing liver carcinogenesis in a Dicer-dependent manner.

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Keywords: miRNAs, colorectal cancer, exosomes, liver, metastases

I. Introduction

Colorectal cancer (CRC) is the third most frequently diagnosed cancer worldwide, with an annual incidence of 1,4 million new cases and 694.000 deaths [1]. About 15% of CRCs are diagnosed in metastatic stages (stage IV), with an average survival rate of 2.5 years. Moreover, about 50% of patients that have a low TNM stage at diagnosis will eventually develop metastasis, mostly localized in the liver [2]. During the last decade the clinical outcome of patients with metastatic CRC has improved due to the development of new chemotherapeutic drugs and targeted therapies. Today, the median overall survival for patients with metastatic disease is about 30 months, time that has doubled over the past 20 years [3].

Despite the current screening methods and prognosis factors (TNM stage, age, tumor differentiation grade, vessel invasion, performance status and tumoral markers) according to National Comprehensive Cancer Network (NCCN) guidelines, there are still a great number of patients that are experiencing therapeutic failure and metastasis [4]. Therefore, an important task in CRC management is to identify new prognostic factors that could help in selecting patients who could benefit from adjuvant therapies or from intensive screening for disease recurrence and metastasis.

After their discovery, micro-RNAs (miRNAs) have been shown to have important implications in cancer biology. Increasing evidence highlights that deregulated miRNAs expression has a functional role in the progression and metastasis of CRC, acting either as tumor suppressors or oncogenes to regulate expression of their specific mRNA targets. Due to their high stability, miRNAs were considered and investigated as a new class of valuable biomarkers [5].

We will present herein up-to-date information about the specific miRNAs involved in CRC progression and liver metastasis and how these miRNAs control the epithelial-mesenchymal transition of CRC cells, a process responsible for liver metastasis in a circulating tumor cell-dependent manner. We also emphasize the role of miRNAs transported by CRC-derived exosomes in mediating liver metastases, by preparing the secondary pre-metastatic niche and in inducing liver carcinogenesis in a Dicer-dependent manner.

2. Selection of the studies to review

We performed a systematic search of studies profiling the miRNAs involved in colorectal progression and liver metastasis, published until October 2018. Four PubMed string searches (Figure 1) were used for this aim; in the first search we identified the miRNAs involved in CRC progression and metastasis, considering their roles in alteration of Wnt/βcatenin, EGFR, TGFβ and TP53 signaling pathways; in the second search we selected the miRNAs involved in the epithelial-mesenchymal transition (EMT), an important step in CRC metastasis; in the third, we identified the specific miRNAs transported by tumor-delivered exosomes (miRNA-TEX) released by CRC, which support the liver metastasis by preparing the secondary pre-metastatic niche, inducing pro-inflammation and immunosuppression; in the fourth, we found out the overlapping between miRNAs-TEX and miRNAs involved in promoting the hepatocellular carcinoma, in a Dicer-dependent manner.
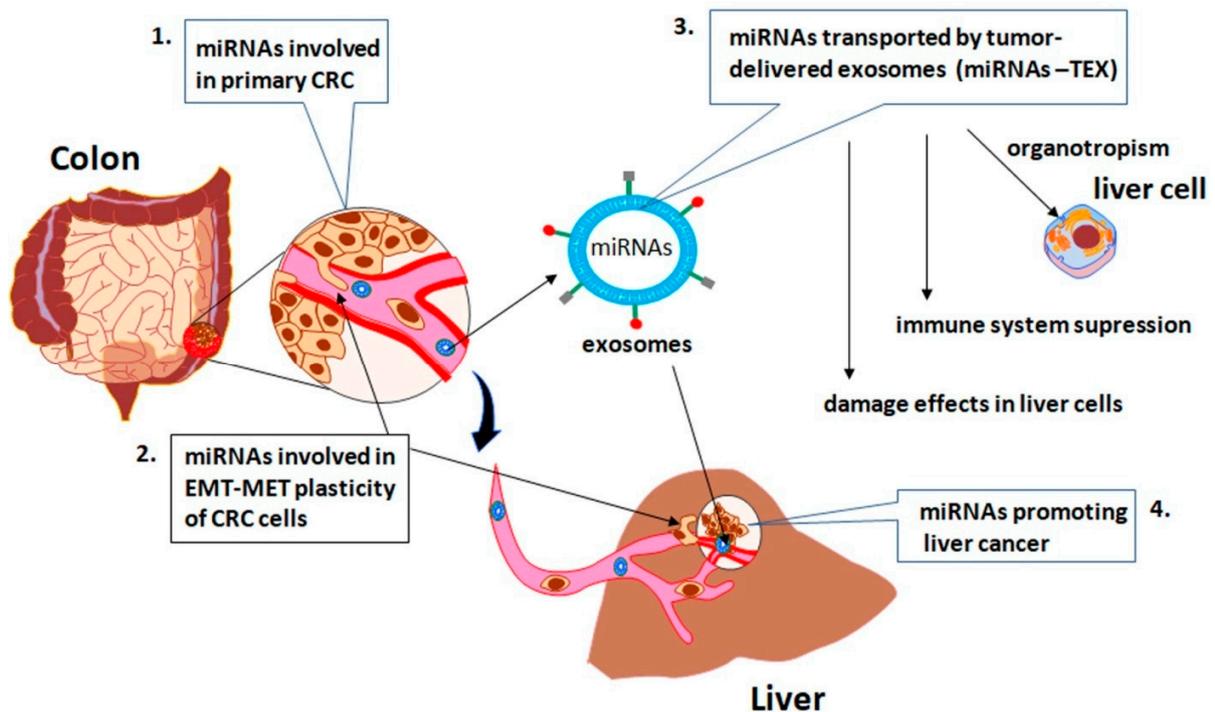
Figure 1. The PubMed string searches used to identify the specific miRNAs involved in CRC development and progression as well as in liver metastasis.

3. The role of the tumor microenvironment in CRC development and progression

In 2000, Hanahan and Weinberg [6] stated that there are "six essential alterations that collectively dictate malignant growth: self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis". A decade later, when tumors were recognized as organs, compared to what was previously accepted as solitary masses of proliferating cells, an enhancement of these cancer hallmarks was provided. Thus, reprogramming energy metabolism and evading immune response became the newest hallmarks of cancers while tumor microenvironment (TME) has been proved to play a substantial role in tumor progression and metastasis [7]. TME represents a very complex network between tumor cells and stromal, endothelial and immune cells, which substantially contributes to the validation of an aggressive tumor phenotype [8]. Moreover, the presence of inflammatory cells and inflammatory mediators such as chemokines and cytokines related to TME facilitates tumor progression, including CRC [9]. Besides that, TME facilitates CRC progression by maintaining a paracrine cross-talk signaling between tumor resident adipocytes that represent a fuel rich source of energy and cancer cells demanding energy for their rapid proliferation [10].

Nowadays it is largely accepted that CRC occurs from an adenoma-carcinoma transition and implies genetic and epigenetic events, supported by TME [11]. The genetic and epigenetic studies in CRC have provided a lot of data about the mutational status and alteration of gene expression [12]. So far, a plethora of papers have broadly presented all these alterations that lead to "oncogene addiction" by activating several key oncogenes (eg. *KRAS, EGFR, MYC, ERBB2,*

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etc) or inactivating tumor suppressor genes (eg. T*P53, PTEN, WNT, SMAD2, SMAD4*, etc) and DNA repair genes (eg. *bMSH2, bMLH1, bPMS1, bPMS2* and *MSH6*) [11, 13, 14].

Colorectal carcinogenesis is also mediated by epigenetic alterations, including changes in the DNA methylation status, both by global hypo-methylation and promoter hyper-methylation. Global hypomethylation is decreased by 10-40% in CRC compared to normal colonic tissue, being associated with genomic instability, while promoter hyper-methylation leads to repression of several tumor suppressor genes and their specific pathways (e.g. *TP53, PI3K/PTEN/AKT/mTOR, TGFb/SMAD*) [15]. Furthermore, non-coding RNAs, especially miRNAs, play important roles in maintaining the physiology of normal colonic cells, while alteration of their levels contributes to CRC development, progression and metastasis, as well as drug resistance and tumor relapse [16]. Moreover, recent evidence indicates that miRNAs are involved in both direct cell-to-cell signaling and paracrine signaling between tumor cells and TME components as secreted molecules in microvesicles or exosomes [17].

4. The role of miRNAs in CRC progression and metastasis

The first association of miRNAs with colon cancer was described by Michael et al [18], fifteen years ago, when they reported decreased miR-143 and miR-145 expression in colon cancer when compared to normal colon tissue. Since then, many studies have been conducted to investigate the miRNAs involvement in colorectal carcinogenesis. Considering the evolutionary progression of CRC from adenomas, in-depth understanding of the miRNAs-modulated molecular mechanisms in CRC has become a major challenge [19, 20]. It has been shown that alterations in Wnt/βcatenin, EGFR, TGFβ and TP53 signaling pathways result in survival, proliferation, invasion and metastasis of CRC [21, 22]. Therefore, many studies have focused on these pathways, to establish the miRNAs:mRNA interaction networks, in order to complete the molecular puzzle that underlines the CRC development, progression and metastasis. Adenomatous polyposis coli (*APC*) is an important tumor suppressor gene with an inhibitory function on Wnt/ß-catenin signaling. When mutated, as happens in 90% of CRC, APC loses its inhibitory function, leading to tumorigenesis, invasion and metastasis. An increasing number of studies have identified specific miRNAs that lead to alteration of APC/Wnt/ß-catenin signaling, either by direct suppression of *APC* (eg miR135a/b, miR-494, miR-19a) and aberrant activation of Wnt pathway (eg miR-21, miR-155, miR-103a, miR-1827, miR-145, miR-34a), or indirectly, targeting other members of these pathways (miR-150, miR-224, miR-146a, miR-574-5p). An up-to-date list of oncomiRs and tumor suppressor miRNAs (TS-miRNAs), and their targets involved in the alteration of Wnt/ß-catenin signaling in CRC is presented in Table 1.

Table 1. MiRNAs and their mRNA targets associated with the development, progression, and metastasis of CRC.

Key signaling pathways	miRNAs	Targets	Targeting effects	Ref
Activation of Wnt/ß-catenin pathway	**oncomiR (high expression) – gain of function**			
	miR-21	*CTNNB1, TGFBR2, PIK3CA, BRAF, ZFHX3, SFRP1*	Tumor development, proliferation, progression	[23, 24]

	miR-135a/b	*APC*	proliferation	[25]
	miR-494	*APC*	Proliferation, tumorigenesis	[26]
	miR-155	*CTNNB1*	Invasion; metastasis	[27]
	miR-146a	*NUMB*	Progression, stemness	[28]
	miR-522	*DACH1*	Proliferation, migration	[29]
	miR-19a	*APC, CTNNB1, c-Myc, PTEN, TIA1*	Proliferation, tumorigenesis, proliferation, invasion, progression, angiogenesis	[30, 31]
	miR-224	*GSK3β, sFRP-2*	Proliferation, metastasis	[32]
	miR-103a miR-1827	*APC, APC2, CTNNB1, WNT3a*	Cell cycle progression, reduced apoptosis	[33]
	miR-574-5p	*Qki 6/7*	Proliferation, tumorigenesis differentiation, angiogenesis	[34]
	TS-miR (low/reduced expression) - loss of function			
	miR-137	*CTNNB1, WNT3a*	Cell cycle progression	[33]
	miR-23b	*FzD-7*	Proliferation, progression, invasion, metastasis	[35]
	miR-7	*YY-1*	Proliferation, reduced apoptosis, cell cycle progression,	[36]
	miR-93	*SMAD-7, CTNNB1*	Proliferation, progression,	[37]
	miR-185	*MYC, CCND1*	Proliferation, progression,	[38]
Activation of EGFR signaling pathway	**oncomiR (high expression) – gain of function**			
	miR-20, miR-21, miR-130b	*PTEN*	Progression , invasion, metastasis	[39]
	miR-26b	*PTEN, WNT5A*	Proliferation, EMT, metastasis	[40]
	miR-182, miR-135b	*ST6GALNAC2, PI3K/AKT*	Proliferation, invasion	[41]
	TS-miR (low/reduced expression) - loss of function			
	miR-43, miR-145	*CD44, KLF5, KRAS, BRAF*	Proliferation, migration	[42]
	Let-7	*KRAS*	tumorigenesis	[43]
	miR-19a	*KRAS, VEGFA*	Proliferation, angiogenesis	[44]
	miR-181d	*PEAK1*	Proliferation, invasion, migration, metastasis	[45]
	miR-30a	*KRAS, ME1,*	CRC developmnet	[46]
	miR-217	*MAPK1, KRAS, Raf-1*	Tumor growth, apoptosis	[47]
	miR-487b	*LRP6, KRAS*	Metastasis	[48]
	miR-16	*KRAS*	Proliferation, invasion,apoptosis	[49]
	miR-384	*KRAS, CDC42*	Invasion, migratiuon, metastasis	[50]
	mirR-125a-3p	*FUT5-FUT6*	Proliferation, migration, invasion, angiogenesis	[51]

Inactivation of TGF-ß signaling pathway	**oncomiR (high expression) – gain of function**			
	miR-135b	*TGFBR2*	Progression, inhibiting of apoptosis	[52]
	miR-301a	*TGFBR2*	Migration, invasion, metastasis	[53]
	miR-20-5p	*SMAD4*	EMT, Invasion, metastasis	[54]
	miR-224	*SMAD4*	Invasion, metastasis	[55]
Suppressing of TP53 function	**oncomiR (high expression) – gain of function**			
	miR-125b	*TP53*	CRC progression	[56]
	miR-504	*TP53*	CRC progression	[57]
	miR-29b, miR-30	*TP53*	CRC progression	[58]
	miR-24	*TP53*	CRC pogressin	[59]
	miR-518c, miR-638	*TP53, PTEN*	CRC progression, invasion, metastasis	[60]
	TS-miR (low/reduced expression) - loss of function			
	miR-638	*SOX2, TSPAN1*	EMT, invasion, migration, proliferation	[61, 62]
	miR-34a	*SIRT1*	Proliferation, reducing apoptosis	[63]

EGFR signaling pathway has a critical role in normal embryogenesis, mediating cell proliferation, differentiation, migration and apoptosis. The gain of function of *EGFR* by genetic alterations leads to cancer development. About 50% of CRC present *EGFR* gene amplification and mutational activation of *KRAS* and *BRAF* downstream mediators [64]. Recent studies have reported that aberrant activation of oncogenic EGFR pathway can be due to TS-miRNAs loss of function. Two of the most important TS-miRNAs involved in the EGFR pathway are miR-143 and miR-145, whose combined action decreases proliferation and migration by targeting several members of the EGFR pathway, including *KRAS* and *BRAF* [42]. Loss of function of let-7a was associated with high levels of *KRAS* and *c-MYC* and colon tumorigenesis [65] while the let-7 *KRAS* rs712 polymorphism was correlated with increased colorectal cancer risk [43]. Recent evidence has confirmed that miR-19a can inhibit CRC angiogenesis by targeting *KRAS* and *VEGFA* [44] and that miR-181d reduces cell proliferation, migration and invasion by triggering *PEAK1*, a downstream regulator in EGFR/KRAS pathway [45]. On the other hand, activation of the KRAS pathway can trigger and up-regulate some oncomiRs, such as miR-210 and miR-181a with a role in CRC development and progression [66] or miR200c that has a pivotal role in CRC aggressiveness and metastasis [67].

AKT-PI3K-mTOR pathway represents the second signaling hub of the EGFR pathway, being amplified in about 15-20% CRC [68]. Amplification of AKT-PIK3K-PTEN pathway is mediated both by the loss of function of TS-miRs, such as miR-126, miR-497 and miR-1, or by upregulation of several oncomiRs including miR-21, miR-19 and miR-96 [69]. More data about miRNAs involved in the EGFR signaling pathway, in CRC developing and progression are presented in Table 1, section EGFR signaling pathway.

TGF-ß signaling has a dual function in colorectal carcinogenesis. It is a tumor suppressor in early stages of CRC development, but acts as a tumor promoter in advanced stages of CRC.

The tumorigenic activity of TGF-ß signaling is due to several up- and down-stream defects that enhance multiple oncogenic pathways leading to increased angiogenesis, evasion of the immune system, evading apoptosis, EMT and metastasis [70]. Mutational alterations of *TGFBR2*, the key component that initiates signaling in the TGF-β pathway, were identified in about 30% of CRC. However, recent evidence indicates that many miRNAs are involved in *TGFBR2* regulation (Table 1). Several of them, including miR-301a and miR-135b, induce proliferation, migration and invasion in CRC cells, through negative regulation of *TGFBR2* expression [52, 53]. Also by negative regulation of *SMAD4*, a mediator of the TGF-β pathway, miR-20-5p and mir-224 induce EMT, invasion and metastasis of CRC cells [54, 55].

TP53, "the guardian of the genome", is a tumor suppressor gene with multiple roles in maintaining the cellular physiology under many stress conditions, including cancer [71]. Almost 50% of CRCs present inactivation of *TP53* by deletions or point mutations [72]. Albeit TP53 can affect transcription and maturation of many miRNAs, both by transactivation of tumor-suppressor miRNAs and by repression of oncogenic miRNAs, there is increasing evidence that TP53 expression is also under the tight control of miRNAs [73]. Translational repression of TP53 in CRC is controlled by several miRNAs including miR-125b, miR-504, miR-25, miR-30d, miR-638. Previous data have demonstrated that miR-125b is an independent prognostic factor in CRC, its high expression being associated with poor prognosis [56], while mir-504 was shown to have a role in the negative regulation of TP53 in several cancers, including CRC [57]. Moreover, miR-25b and miR-30 were found to reduce apoptosis by negative regulation of both gene expression and protein level of TP53 [58]. Interestingly, miR-518c and miR-638 can target and inactivate both *TP53* and *PTEN* genes [60]. However, several studies have reported that miR-638 can also function as tumor suppressor miRNAs, its loss of function leading to proliferation, EMT, migration and invasion of CRC by up-regulating SOX2 and TSPAN1 proteins [61, 62]. Another study has reported that TP53 can indirectly be repressed by *SIRT1*, a target of tumor suppressor miR-34a [63].

However, miRNAs-mRNAs networks in CRC are more complex in the more specific case of CRC metastasis. MiRNAs can control the EMT underlying the movement of the colorectal circulating tumor cells (CTCs). By their encapsulation in exosomes, miRNAs provide support in preparing the liver metastatic niche, suppressing of the immune system and modulating the liver phenotype in a Dicer-dependent manner.

5. The role of miRNAs in CRC metastasis, by modulation of EMT

Understanding the molecular mechanisms underlying CRC metastasis is crucial for improving the treatment strategies for this pathology and consequently, to increase the patients survival rate. Although the miRNAs-mediated mechanisms in colorectal carcinogenesis are quite well understood, those responsible for metastasis are not yet elucidated.

An important feature of cancer cells that lead to invasion and metastasis consists in changing their epithelial characteristics to mesenchymal ones, a process known as the epithelial to mesenchymal transition (EMT). During EMT, cancer cells undergo several processes that modify their phenotype, leading to cell motility, the acquisition of stemness properties, inhibition of apoptosis and immunosuppression. EMT is characterized by dissolution of epithelial tight cell junctions by downregulation of several specific genes that encode proteins like E-cadherin *(CDH1)* and claudins, and by promoting the mesenchymal adhesion by activating several protein-coding genes, as the ones for vimentin, N-cadherin and fibronectin. Stimuli from TME such as inflammatory cytokines and growth factors have a significant role in tumor evolution, by

abnormally regulating the EMT of cancer cells [74]. The EMT modulation requires a complex network of cooperation including many regulators and inducer molecules [75].

The main regulators of EMT are represented by three families of activating transcription factors: *SNAIL, ZEB*, and *TWIST*. However, accumulating evidence has shown that other two families of transcription factors, *PROX1* and *FOX*, are involved in EMT of CRC. Nevertheless, the induction of EMT by members of these families of transcription factors involves multiple pathways, tightly coordinated by miRNAs (Table 2).

MiR-34 family (miR-34a/b/c), directly regulated by TP53, is involved in preventing TGF-ß-induced EMT by suppressing *SNAIL, SLUG* and *ZEB1* transcription factors, as well as the stemness factors *BMI1, CD44, CD133* or *c-MYC*. Conversely, *SNAIL* and *ZEB1* repress miR-34a/b/c expression, promoting EMT. However, the loss of function of TP53 and/or miR-34a/b/c, found in many cancers, represents an important molecular alteration facilitating cancer metastasis [76]. EMT activation by *SNAIL* in CRC directly induces *ZNF281* transcription, leading to repression of miR-34a/b/c that contributes to CRC metastasis [77].

In a recent study, miR-375 was proved to regulate *MMP2* and several EMT-associated genes, including *SNAIL* [78]. When it is down-regulated, miR-375 leads to proliferation, invasion and migration of colorectal cancer cells. MiR-374 represents another tumor suppressor, whose loss of function in CRC induces the activation of the PIK3/AKT pathway, promoting proliferation, invasion, migration and intrahepatic metastasis. Transcription factors *SNAIL, SLUG* and *ZEB1* as well as N-cadherin and vimentin are among the targets of miR-374, all of which being significantly up-regulated by miR-374 inhibition [79]. MiR-200c and miR-429, two members of the miR-200 family, are predominately involved in the regulation of *ZEB* transcription factors in CRC cells. Induction of miR-200c leads to inactivation of EMT by suppressing *ZEB1* expression which results in reduced invasion and migration of CRC cells [80]. MiR-429 could reverse TGFβ-induced EMT by targeting *ONECUT2* and thus inhibiting cell migration and invasion. However, miR-429 is significantly down-regulated in CRC [81]. On the other hand, activation of miR-200 cluster by loss of function of *ASCL2*, a downstream target of WNT signaling, leads to inhibition of *ZEB* and *SNAIL* families of transcription factors and consequently to the regulation of the plasticity from EMT to mesenchymal-epithelial transition (MET) [82]. Down-regulation of other tumor suppressors, mainly miR-335, miR-132 and miR-192, was related to invasion and metastasis of CRC by increasing expression of their *ZEB*2 target gene [83-85]. In hypoxic conditions, miR-675-5p overexpression can regulate hypoxia-induced EMT driven by *HIF1α*, through increasing *SNAIL* transcription by both inhibiting the SNAIL's repressor *DDB2* and stabilizing the activity of the transcription factor *HIF1α* [86].

Although a few papers have described several miRNAs targeting *PROX*1, none of them has focused on CRC. However, the role of *PROX1* inducing EMT in CRC was previously pointed out. *PROX1* can promote EMT by inhibiting *CDH1* expression via binding to the promoter of pre-miR-9-2 and triggering its expression [87]. On the other hand, two members of the FOX family of transcription factors, *FOXQ1* and *FOXM1*, have been recently identified as targets of miR-320c, as part of the EMT induction process. Vishnubalaji R et al reported low levels of miR-320 in CRC compared to normal tissues and an inverse correlation with *SOX4*, *FOXM1* and *FOXQ1* expression , leading to decreased CDH1expression [88].

The main inducers of EMT in CRC are TGF-ß and Wnt/ß-catenin pathways but *TMPRSS4, FMNL2, GDF15, NRP2* or *TUSC3* were also linked to EMT in CRC [75]. TGF-ß-induced EMT in CRC is related to both SMAD-dependent and -independent mechanisms and partly influenced by alteration of miRNAs expression. The role of *SMAD4* in EMT has been

extensively studied. Previous studies have demonstrated that normal levels of *SMAD4* maintain the epithelial phenotype while inactivation of *SMAD4* leads to invasion and metastasis. Down-regulation of mir-187, a downstream effector of the TGF-β pathway with a role in EMT prevention, leads to increased expression of *SOX4, NT5E* and *PTK6*, known as important upstream regulators of SMAD pathway. Loss of function of miR-187 was tightly correlated with CRC metastasis and a reserved prognosis [89]. On the other hand, over-expression of miR-20a induces EMT, promoting metastasis of CRC via suppression of *SMAD4* expression [90]. Moreover, the TGF-ß-induced EMT can be initiated by suppressing the SMAD inhibitors, such as *SMAD7*. Thereby, miR-4775, miR-1269 and miR-21 promote CRC metastasis in a SMAD7/TGFβ signaling-dependent manner [91-93]. MiR-150, the most expressed miRNA induced by Wnt/ß-catenin pathway, enhances the EMT in CRC by targeting CREB signaling pathway [94].

Although Wnt-induced EMT in CRC is mostly activated by canonical Wnt/ß-catenin signaling, there is evidence that also non-canonical Wnt signaling is involved in EMT. The Wnt-induced EMT could be activated by suppressing the negative regulators of several transcription factors. Accordingly, loss of function of the tumor-suppressors miR-34a, miR-145 and miR-29b leads to the induction of EMT. Mir-34 is a suppressor of the ß-catenin pathway and EMT [77, 95] but its inhibition by methylation leads to cancer progression and metastasis [96]. Likewise, the loss of function of miR-145 was inverse correlated with the overexpression of several genes involved in EMT, such as *CTNNB1, VIM, SNAI*, and the downregulation of *CDH1* expression in SW480 cells [97]. The regulation of EMT in colon cancer cells via miR-29b involves blocking of various β-catenin target genes, by suppressing the coactivators of β-catenin such as *TCF7L2, SNAIL*, and *BCL9L* [98]. Moreover, recent data has shown that miR-29b-3p modulated by lncRNAH19 can promote the EMT in CRC by directly binding and targeting the progranulin (*PGRN*) [99].

Recent evidence has shown that circulating tumor cells (CTCs) from CRC are responsible for liver metastasis [100] while the EMT-MET plasticity of CTCs is considered an important hallmark of metastasis [101]. Both the detachment of CTCs from the primary CRC and their attachment in the liver are closely mediated by miRNA. CTCs detaching is facilitated by increasing the activity of the matrix metalloproteinases (MMPs) or by inhibiting the tissue inhibitors of metalloproteinase (TIMPs). On this regard, miR-194 can promote EMT-mediated metastasis of CRC by *MMP2* activation [102], while miR-20a promotes CRC metastasis by inhibiting TIMP2, that leads to increasing of *MMP2* and *MMP9* enzymatic activity [103]. Moreover, by destabilizing *MMP11* mRNA, let-7c plays a role as a metastasis suppressor, in addition to its tumor growth suppressor activity [104]. While CTCs are responsible for tumor metastasis, miRNAs-TEX are responsible for preparing the secondary metastatic niche.

Table 2. MiRNAs and their mRNA targets associated with regulation and induction of EMT, supporting CRC invasion and metastasis.

Key signaling	miRNAs	Targets	Function	Ref
Regulation of EMT	**oncomiR (high expression) – gain of function**			
	miR-675-5p	*SNAIL*	EMT, invasion, metastasis	[86]
	TS-miR (low/reduced expression) - loss of function			
	miR-34a/b/c	*SNAIL, SLYG, ZEB1, BIM1, CD44, CD133, c-MYC,*	EMT, invasion, metastasis, MET,	[76, 77]

	miR-375	*SP1, MMP2, SNAIL, CDH1, VIM, CDH2, CTNNB1*	EMT, invasion, metastasis	[78]
	miR-374	*CCND1, ZEB1, CDH2 VIM, SLUG, SNAIL*	EMT, proliferation, invasion, migration, liver metastasis.	[79]
	miR-200c	*ZEB1, ETS1, FLT1, ASCL2*	EMT-MET plasticity, invasion, migration, liver metastasis	[80, 82]
	miR-429	*ONECUT2, ZEB1, ZEB2*	EMT, invasion, migration	[81]
	miR-335, miR-132, miR-192	*ZEB2*	EMT, Invasion, metastasis	[83-85]
Inducing of EMT	**oncomiR (high expression) – gain of function**			
	miR-20a	*SMAD4, MMP2, MMP9*	EMT, migration, metastasis	[90, 103]
	miR-4775	*SMAD7/ TGF-ß*	EMT, invasion, metastasis	[91]
	miR-1269	*SMAD7, HOXD10*	EMT, invasion, metastasis	[92]
	miR-21	*NRF2, SMAD7*	EMT, invasion, metastasis	[93]
	miR-150	*CREB*	EMT, invasion, migration,	[94]
	miR-194	*MMP2*	EMT, invasion, migration,	[102]
	TS-miR (low/reduced expression) - loss of function			
	miR-320c	*SOX4, FOXM1, FOXQ1*	EMT, proliferation, migration, tumorigenesis,	[88]
	miR-187	*SOX4, NT5E, PTK6*	EMT, invasion, metastasis	[89]
	miR-34a	*CTNNB1, c-Met ZEB1, ZEB2, SNAIL*	Progression, EMT, liver metastasis	[95, 96]
	miR-145	*SIP1, CTNNB1, TCF4, VIM, SNAIL*	Proliferation, migration, EMT, invasion, metastasis	[97]
	miR-29b	*BCL9L, TCF7L2, SNAI1, PGRN*	Cell growth, EMT, angiogenesis, invasion, migration	[98, 99]
	Let-7c	*MMP11*	Cel migration and invasion	[104]

6. The role of miRNAs -TEX in sustaining the CRC liver metastasis

The occurrence of liver metastases from CRC represents a multistep process that involves EMT-MET plasticity of colorectal CTCs, local remodeling of the liver microenvironment and immunosuppression. Generally, these processes are strongly mediated by miRNAs-TEX released by CRC.

Exosomes are small extracellular vesicles of about 30-100 nm surrounded by a lipid bilayer, derived from multivesicular bodies (MVBs) generated within the cell's endosomal system. They are secreted by multiple cell types, including tumor cells, and can be detected in most body fluids such as breast milk, urine, saliva, semen and blood as well as in supernatants of cultured cells. Exosomes can modulate the phenotype of the cells they bind to by transferring

them their cargo content, either in a paracrine or endocrine manner [105]. Preparing the secondary pre-metastatic niche or remodeling the liver microenvironment is a mandatory condition to sustain liver metastasis. Exosomes released by CRC can transfer bioactive molecules including nucleic acids (DNA, mRNA, miRNA, lncRNA, circRNA etc), proteins (transcription factors, receptors, enzymes, etc) and lipids to the liver cells, modulating their phenotype [106]. One of the most important features of TEXs is that they can determine the organotropism and predict organ-specific metastasis due to their distinct integrin expression patterns. Integrins bind to specific receptors found on target cells, preparing the secondary pre-metastatic niche by inducing the pro-inflammatory signals through Src phosphorylation and S100 gene expression [107]. Two chemokine receptors, *CXCR4* [108] and *CCR6* [109] have been previously associated with CRC metastasis to the liver (Figure 2). Moreover, previous data exploring the characteristics of the metastatic pattern of CRC has revealed that the liver is the main metastatic site of CRC, while lungs, bones and the brain are less common [110].

According to the existing data, miRNAs are the most studied exosomes-entrapped molecules that are involved in cancer development, progression and metastasis. Since 2010, when the functional role of miRNAs-TEX in gene expression regulation in recipient cells was demonstrated [111], their essential contribution to the hallmarks of cancer has been acknowledged [112]. However, the amounts of miRNAs that are incorporated into the exosomes released by different tumors remain to be elucidated. One hypothesis mentions that the abundance of miRNAs-TEX depends on the presence of their mRNA target transcripts in the cytoplasm of the donor cell. Therefore a presumptive mRNA-miRNA interaction in the donor cell could lead to low levels of that miRNA in the exosomes [105]. On the other hand, miRNAs sorting into exosomes could be conditioned by their posttranscriptional modifications, so that 3'end adenylated miRNAs are preponderantly enriched in cells while 3′ end uridylated miRNAs are enriched in exosomes [113].

Wang et al demonstrated for the first time that exosomes play a pivotal role in colorectal cancer liver metastasis [114]. Using nude mouse models, the authors have demonstrated that exosomes derived from a highly liver metastatic CRC cell line (HT-29) can induce the same aggressive liver metastasis in the Caco-2 xenograft mouse model that usually has poor liver metastatic potential. The mechanisms by which HT-29-derived exosomes influence the liver metastasis of CRC involve increased CXCR4 expression in many types of stromal cells that contribute to the remodeling of the liver microenvironment and thereby to the development of the secondary pre-metastatic niche. Moreover, the treatment of Caco-2 cells line with HT-29-derived exosomes has led to increased migration of Caco-2 cells, highlighting the main role of exosomes in colorectal liver metastasis. Since then, several studies have identified miRNAs-TEX as relevant molecules in CRC liver metastasis.
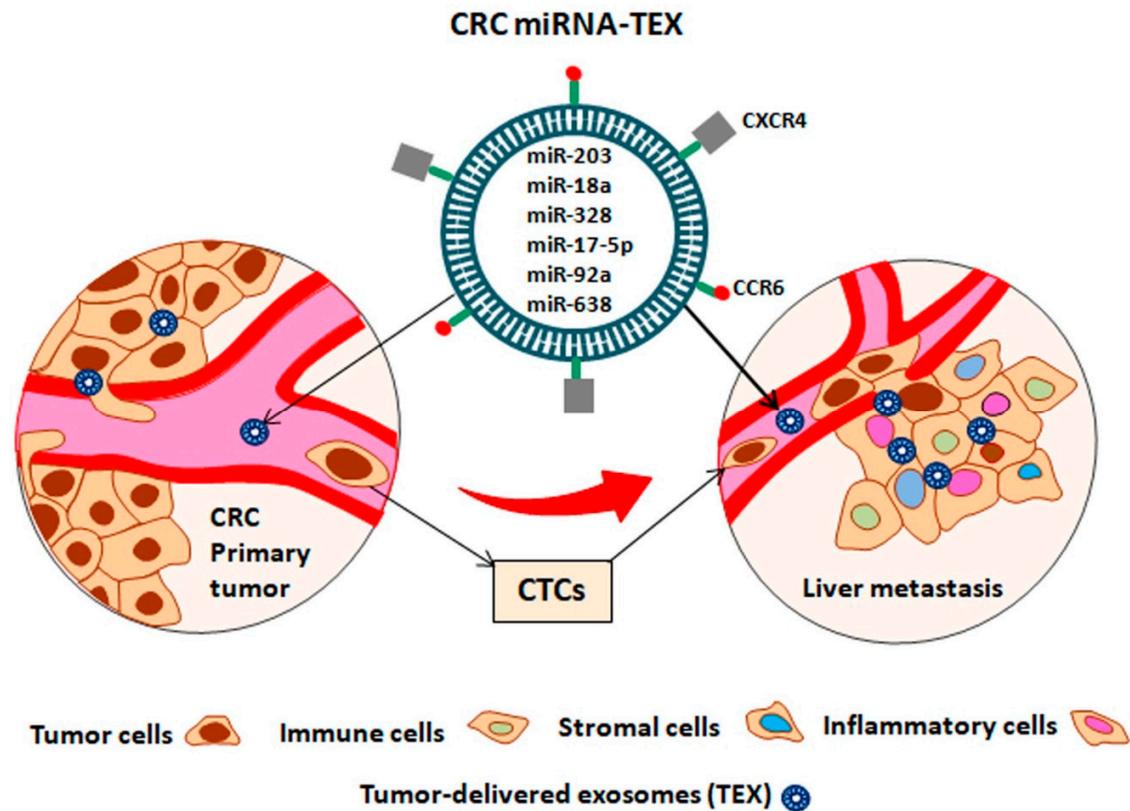
Figure 2. CRC metastasis in the liver through CRC circulating tumor cells (CTCs), mediated by miRNAs transported by CRC-delivered exosomes (miRNA-TEX). CXCR4 and CCR6 chemokine receptors are responsible for liver organotropism of miRNA-TEX, while the miRNAs released in the liver are responsible for preparing the secondary pre-metastatic niche by inducing the pro-inflammatory and pro-tumorigenic signals.

Accordingly, exosomal miR-203 from CRC cells can sustain the formation of the premetastatic niche by promoting the differentiation of monocytes to M2-tumor-associated macrophages (TAMs) involved in liver metastasis of CRC patients [115].

Moreover, recent evidence has suggested that liver pre-metastatic niche could be promoted by exosomal miR-21 of CRC cells, through the miR-21-TLR7-IL6 axis [116]. Significantly increased levels of serum exosomal miR-6803-5p [117] and decreased levels of miR-548c-5p have been recently associated with poor survival and liver metastasis of CRC patients [118]. However, the molecular mechanisms by which both miR-6803-5p and miR-548c-5p influence CRC metastasis remain largely unknown. Likewise, new evidence has revealed that decreased levels of serum exosomal miR-638 are associated with an increased risk of liver metastasis [119]. Previous data demonstrated that miR-638 functions both as an oncomiR by targeting *TP53* and *PTEN* [60] and as a tumor suppressor by inhibiting *TSPAN1*, in CRC [62]. Using a translational research model, including culture cells, mouse models and human samples, Fu et al. [120] have recently demonstrated that miR-17-5p and miR-92a-3p exosomal expression is related to CRC metastasis, but not to the CRC mutational type. The AUC of these serum exosomal miRNAs was about 0.84, while their combination leads to an AUC of 0.91. Based on a

very interesting hypothesis that tumor-draining vein could provide more homogeneous information than blood drawn from the peripheral vein (PV), Monzo et al. demonstrated that CRC primary tumors release higher concentrations of miRNAs through the mesenteric vein (MV) than peripheral vein (PV). Accordingly, they observed that exosomal miR-328 was higher in the MV than in PV plasma of the patients developing liver metastasis, suggesting a possible role of miR-328 in the development of CRC liver metastasis [121]. However, miRNAs loading into exosomes is not a passive process. Teng et al. have recently demonstrated that the sorting of oncomiRs into exosomes is suppressed, while the sorting of TS-miRs into exosomes is increased [122]. Furthermore, they found that exosomal TS-miR including miR-193a and miR-18a are significantly higher in patients with liver metastasis compared to CRC patients having no metastasis. Functional analysis demonstrated that miR-193a modulates CRC progression by *CCDN1* and *c-MYC* expression via *Caprin1*, its direct target.

Quite interestingly, recent evidence has pointed out that miRNA-TEX released by breast cancer are directly involved in changing the normal cell phenotype into a tumor phenotype, promoting CTCs - independent metastasis, in a Dicer-dependent manner [123]. However, no data supporting this approach for CRC metastasis has been presented yet. Besides, previous data have mentioned the role of tumor-derived exosomes in the suppression of the immune system of the host [124]. Nevertheless, identifying miRNAs entrapped in exosomes released by colorectal tumors and involved in the suppression of the immune system during CRC metastasis remains a major challenge.

7. The role of CRC miRNAs-TEX in the promotion of hepatocellular carcinoma

The exosomes delivered by tumor cells contain both miRNAs and proteins/enzymes (Dicer, Argonaute 2 (Ago2) and TRB as well as the RISC Complex) used for miRNAs processing and silencing the mRNA targets of the recipient cells. Considering that miRNAs-TEX could alterate the phenotype of the recipient cells, by modulating the cell transcriptome in a Dicer-dependent manner [123], we further analyzed the association of the CRC miRNAs-TEX mentioned above with the miRNA signature specific for the hepatocellular carcinogenesis (HCC) (Table 3).

MiR-21 is one of the most oncogenic miRNAs and its relationship with HCC has been already proved [125]. Thus, it can be speculated that the transfer of CRC miR-21-TEX into liver cells could contribute to liver metastasis. The tumor suppressor gene *BTG2* represents one of the miR-21 targets, its negative correlation with miR-21 expression being related to hepatocarcinogenesis [126]. Recent data has revealed that miR-18a has an important role in promoting liver cancer, by targeting the tumor suppressor gene *TSC1*, leading to downstream regulation of the mTOR signaling pathway [127]. More data pointed out that miR-18a is involved in liver cell proliferation by targeting *IRF2* and *CBX7* [128], as well as HCC cell migration and invasion, by inhibiting the Dicer I expression [129]. MiR-328 represents another oncomiR found into the exosomes released by CRC which would promote liver metastasis. Previous evidence highlighted the role of miR-328, as a crucial oncogene that targets and suppresses the *PTPRJ* gene, leading to an increased cellular motility in HCC [130]. Likewise, miR-17-5p, found increased in the exosomes of the metastatic CRC has an important role in liver cancer, promoting HCC cell proliferation and migration, by modulating the p38-HSP27 signaling pathway [131]. High levels of exosomal miR-92a were associated with CRC metastasis [120], but high levels of miR-92a in the liver cells have also been associated with HCC development [132]. Recent evidence has pointed out that *FOXA2* represent a direct target of miR-92a, in HCC

development and progression [133]. A very interesting observation is about miR-638, which plays a dual role in colorectal tumorigenesis, modulating invasion and metastasis of CRC. Low levels in exosomal miR-638 were associated with CRC metastasis, but low levels of miR-638 were also associated with invasion and EMT in HCC, by targeting *SOX2* [134]. No data about the role of miR-548, iR-193, miR-6803 in liver carcinogenesis has been reported yet.

Table 3. Common miRNAs identified both in exosomes-delivered by CRC cells and in the early diagnosis of hepatocellular carcinomas.

CRC miRNAs –TEX	HCC targets	Function	Ref
oncomiR			
Mir-21	*BTG2*	Tumorigenesis	[126]
Mir-18a	*TSC1*	Tumorigenesis	[127]
	IRF2, CBX7	Cell proliferation	[128]
	Dicer I	Migration and invasion	[129]
miR-328	*PTPRJ*	Tumor progression, motility	[130]
miR-17-5p	*P38, HSP27*	Proliferation and cell migration	[131]
mir-92a	*FOXA2*	Inhibiting apoptosis, tumorigenesis	[132, 133]
TS-miR			
miR-638	*SOX2*	EMT, development	[134]

Conclusion

Growing evidence consistently shows that miRNAs represent important molecules in the modulation of all hallmarks of cancer. Accordingly, specific miRNAs have been associated with the diagnosis, development and progression of CRC as well as its liver metastasis. Moreover, changes in the circulating miRNAs levels in patients with solid cancers could be considered for developing novel diagnostic and prognostic factors. In this regard, blood miRNAs transported by CRC-delivered exosomes (miRNA-TEX) have become valuble tools that could be used to predict CRC metstasis in the liver. Several CRC oncomiRs, including miR-18a, mir-328, miR-17-5p and miR-92a, delivered into tumor exosomes, are associated with CRC metastasis, but have also been associated with liver carcinogenesis.

However, before their implementing in the clinic as reliable biomarkers, more validation studies are necessary, while standardised methods related to miRNAs processing, expression and normalisation have to be established.

Conflict of interest
The authors declare no conflict of interest

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