Molecular Therapy
Nucleic Acids
Review

Biological Implications and Clinical Potential of Metastasis-Related miRNA in Colorectal Cancer

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Colorectal cancer (CRC), ranking as the third commonest cancer, leads to extremely high rates of mortality. Metastasis is the major cause of poor outcome in CRC. When metastasis occurs, 5-year survival rates of patients decrease sharply, and strategies to enhance a patient's lifetime seem limited. MicroRNAs (miRNAs) are evolutionarily conserved small non-coding RNAs that are significantly involved in manipulation of CRC malignant phenotypes, including proliferation, invasion, and metastasis. To date, accumulating studies have revealed the mechanisms and functions of certain miRNAs in CRC metastasis. However, there is no systematic discussion about the biological implications and clinical potential (diagnostic role, prognostic role, and targeted therapy potential) of metastasis-related miRNAs in CRC. This review mainly summarizes the recent advances of miRNA-mediated metastasis in CRC. We also discuss the clinical values of metastasis-related miRNAs as potential biomarkers or therapeutic targets in CRC. Moreover, we envisage the future orientation and challenges in translating these findings into clinical applications.

Colorectal cancer (CRC) ranks as the third most malignant form of cancer and the fourth leading cause of cancer mortality in the world.[1,2] About 60% patients who diagnosed with CRC are simultaneously observed with localized or distant metastases.[3] Metastasis is significantly associated with poor prognosis in CRC patients.[4] The 5-year survival rates decline with the progression of tumor stage, in which metastasis is one of the principle standards to evaluate the tumor-node-metastasis (TNM) stage, and the 5-year survival rates of patients with stage II are 20% higher than those with stage III.[4,5] Thus, elucidation on the mechanisms of CRC is urgently required for improving clinical outcomes.

Metastasis is a pivotal process, which can not only reveal the malignant transformation of neoplasms, but also partly reflect the clinical stage of malignant tumors.[6] Numerous studies have been conducted on cancer metastasis. The most significant factors affecting cancer metastasis include epithelial-mesenchymal transition (EMT),[7] angiogenesis,[8] hypoxia,[9] and the tumor microenvironment (TME). EMT, a process in which epithelial cells lose their adhesion and then transform to the mesenchymal phenotype, is the early stage of cancer metastasis.[10] Angiogenesis refers to generation of new blood vessels at primary sites or secondary organs.[11] It is a rate-determining step in metastasis because cancer cells require additional nutrition and oxygen.[11] Hypoxia can lead cancer cells to acquire more aggressive phenotypes by regulating genetic programs that can facilitate cells to adapt to hypoxic conditions.[12] The TME is a biological system that contains cancer-associated endothelial cells (CAECs), immune cells, cancer-associated fibroblasts (CAFs), and extracellular matrix (ECM). Transformation of the TME is essential for CRC carcinogenesis.[13,14] Molecules interacting with the microenvironment (MET) may also contribute to metastasis.[13]

MicroRNAs (miRNAs) are short (19–25 nt), endogenous, non-coding, and regulatory RNAs, and they regulate target genes at the post-transcriptional level.[15] miRNAs play crucial roles in biological and pathological processes such as metabolism, apoptosis, differentiation, cell proliferation, cell cycle, as well as invasion and metastasis.[16] Accumulating studies have found that miRNA dysregulation was significantly associated with tumor metastasis. For example, miR-103 promotes metastasis of hepatoma cells by increasing vascular permeability.[17] In breast cancer, miR-182 enhances metastasis by targeting transforming growth factor β (TGF-β)-induced EMT via SMAD family member 7 (SMAD7).[18] Recent studies have also shed light on the role of miRNAs in CRC metastasis. In CRC, miRNAs participate in several cellular processes related to metastasis, including EMT,[19] angiogenesis,[20] and interaction with the MET.[13] miRNAs can act as oncogenes or suppressors via different molecular mechanisms, including the classical signaling pathways,[21] the long non-coding RNA (lncRNA)/miRNA/mRNA axis,[22] and methylation of the DNA promoter.[23] So far, the concrete mechanisms of CRC

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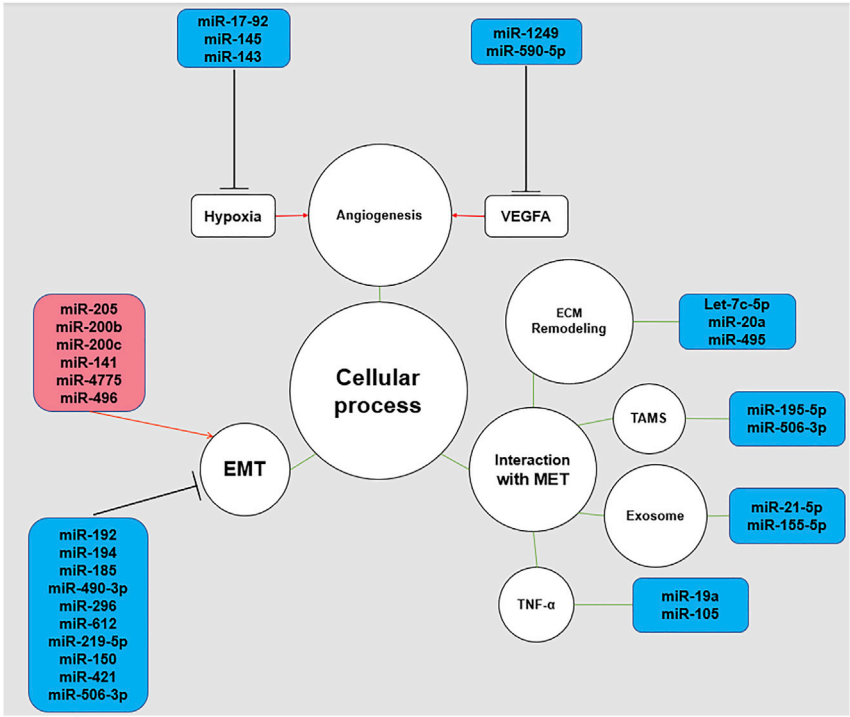

Figure 1. Role of miRNAs in CRC Metastasis
The role of miRNAs in CRC metastasis is shown. The black lines indicate suppression while red lines indicate promotion of downstream targets or processes. The green lines indicate interaction with corresponding processes or molecules. EMT, epithelial-mesenchymal transition; MET, microenvironment.

metastasis are still unknown. Researchers have been devoted to clarifying the specific mechanisms of miRNAs in CRC metastasis.[21] Additionallyt, numerous biomarkers and therapeutic targets associated with CRC metastasis have been reported.[24]

In this review, we searched articles from the PubMed (https://pubmed.ncbi.nlm.nih.gov), Web of Science (http://apps.webofknowledge.com), and EMBASE (https://www.embase.com) databases. We used various combinations and variations of search terms, including "colorectal cancer," "CRC," "colon cancer," "rectal cancer," "metastasis," "miRNA," "microRNA," "diagnosis," "prognosis," "therapy," and their variants. Then, we summarized the recent advances of miRNAs in the metastatic process of CRC and commented on their applications as biomarkers or therapeutic targets in CRC management. Our review may provide new insights into the comprehension and future research on CRC metastasis.

miRNAs and Cellular Process

miRNAs and EMT Process

Emerging evidence has revealed that the dysregulation of miRNAs is associated with cancer metastasis.[13,19] According to the published literature on the role of miRNAs in CRC metastasis, the metastasis-related cellular processes can be divided into four categories: EMT, angiogenesis, hypoxia, and interaction with the MET (Figure 1).[20,23] The dysregulated miRNAs, their confirmed targets, and the corresponding cellular processes are listed in Table 1.

Previously, studies have confirmed that miRNAs directly or indirectly interact with EMT-related molecules such as the E-cadherin/β-catenin complex and N-cadherin and are essential for EMT progression.[47] Some miRNAs can induce the progression of EMT, which is why they function as tumor-promoting genes.[47] For instance, Snail belongs to the family of zinc finger transcription factors and is one of the transcription factors of EMT.[19] miR-205 and let-7i are confirmed to be the downstream molecules of Snail, and they can induce EMT in CRC, indicating their oncogenic role in CRC metastasis.[7,25] Likewise, zinc finger E-box binding homeobox 1 (ZEB1) and E-box binding homeobox 2 (ZEB2), which represent the zinc finger E-box binding homeobox family, are two additional EMT-related transcription factors.[19] A recent study reported that miR-200b, miR-200c, and miR-141 can directly regulate both ZEB1 and ZEB2 in CRC, followed by the activation of EMT and metastasis.[26] Moreover, several miRNA-related signaling pathways can also induce EMT. For example, miR-4775 was reported to promote EMT and metastasis by positively targeting the SMAD7/TGF-β axis.[27] Additionally, hypermethylation of Ras association domain family member 6 (RASSF6) was confirmed as a direct target of miR-496. The miR-496/RASSF6 axis can promote EMT and migration in CRC through Wnt signaling.[28]

Conversely, some miRNAs can inhibit metastasis partly by suppressing the EMT process. Przygodzka et al.[25] reported that miR-192 and miR-194 could suppress the Snail-induced EMT and metastasis. miR-150 can also inhibit metastasis of CRC by directly targeting Snail and Gli1.[7] Moreover, miR-490-3p inhibits Wnt/β-catenin expression via binding frequently rearranged in advanced T cell lymphoma (FRAT) protein and subsequently suppresses EMT.[29] In addition, EGFR signaling is also suppressed by certain miRNAs and finally leads to inhibition of EMT.[19] miR-612 reversely regulates EMT-related process via inhibiting AKT serine/threonine kinase 2 (AKT2), and CRC metastasis is alleviated afterward.[30] Overexpression of miR-219-5p can inhibit the expression of lymphoid enhancer-binding factor 1 (LEF1), resulting in the downregulation of the AKT/extracellular signal-regulated kinase (ERK) pathway, repression of EMT, and lower metastasis rate.[31] Likewise, other oncogenic factors can be targeted by miRNAs, leading to the downregulation of EMT. miR-185 can inhibit EMT and metastasis of CRC cells by inhibiting stromal interaction molecule 1 (STIM1).[32] miR-296 was observed to suppress CRC metastasis via manipulating the S100A4-mediated EMT process.[33] Moreover, miR-421 suppresses

Table 1. miRNA-Related Cellular Processes in CRC Metastasis

Cellular Processes	miRNAs	Alteration	Downstream Targets	References
EMT	miR-205	↑	Snail, SNORD	7,25
	let-7i	↑		
	miR-200b	↑	ZEB1, ZEB2	26
	miR-200c	↑		
	miR-141	↑		
	miR-4775	↑	Smad7	27
	miR-496	↑	RASSF6	28
	miR-192	↓	Snail	7,25
	miR-194	↓		
	miR-150	↓		
	miR-490-3p	↓	FRAT	29
	miR-612	↓	AKT2	30
	miR-219-5p	↓	LEF1	31
	miR-185	↓	STIM1	32
	miR-296	↓	S100A4	33
	miR-421	↓	MTA1	34
Angiogenesis	miR-1249	↓	VEGFA, HMGA	35
	miR-590-5p	↓	NF90, VEGFA	36
	miR-25-3p	↑	KLF2, KLF4, VEGFR2, ZO-1	37
Hypoxia	miR-17~92	↓	HIF-1α, TGFBR2, VEGFA	8
	miR-145	↓	IRS1, HIF-1α	38
	miR-143	↓	IGF-1R	39
ECM remodeling	let-7c-5p	↓	COL1A2	40
	miR-20a	↓	MMP2	41
	miR-495	↓		
	miR-155-5p	↓	MDEBRG1	42
	miR-21-5p	↓		
TAM polarization	miR-195-5p	↑	NOTCH2, GATA3, IL-4	43
	miR-506-3p	↓	IL-6, CCL2	44
Interaction with TME via TNF-α	miR-19a	↓	TNF-α	45
	miR-105	↑	NF-κB, TNF-α, RAP2C	46

SNORD, small nucleolar RNAs with C/D motifs; ZEB1, zinc finger E-box binding homeobox 1; RASSF6, Ras association domain family member 6; STIM1, stromal interaction molecule 1; FRAT, frequently rearranged in advanced T cell lymphoma; S100A4, S100 calcium-binding protein A4; AKT2, AKT serine/threonine kinase 2; LEF1, lymphoid enhancer-binding factor 1; Gli1, GLI family zinc finger 1; MTA1, metastasis-associated protein 1; FoxQ1, forkhead box Q1; CCL2, C-C motif chemokine ligand 2; IL-6, interleukin 6; JAK2, Janus kinase 2; VEGFA, vascular endothelial growth factor A; HMGA, high mobility group A; NF90, IL enhancer binding factor 3; ZO-1, tight junction protein 1; KLF2, Krüpple-like factor 2; HIF-1α, hypoxia inducible factor-1α; IGF-1R, insulin-like growth factor 1 receptor; TGFBR2, transforming growth factor-β receptor type-2; IRS1, insulin receptor substrate 1; TNF-α, tumor necrosis factor alpha; NAT10, *N*-acetyltransferase 10; NF-κB, nuclear factor κB subunit 1; TAM, tumor-associated macrophage; Notch2, notch receptor 2; GATA3, GATA binding protein 3; IL-4, interleukin 4.

the EMT process by repressing metastasis-associated protein 1 (MTA1).[34]

As discussed above, numerous miRNAs are reported to be associated with the EMT process (Figure 2). Therefore, preventing EMT may be a promising method for anti-metastasis.

miRNAs and Angiogenesis

The balance between angiogenic and anti-angiogenic factors is pivotal in the manipulation of angiogenesis.[48] Vascular endothelial growth factor A (VEGFA) and its receptors are two important factors in angiogenesis.[9] Recent researches on tumor metastasis highlighted the importance of miRNAs and angiogenesis.[20] For example, pp53-induced miR-1249 inhibits angiogenesis by negatively targeting VEGFA, which then regulates AKT/mechanistic target of rapamycin kinase (mTOR) signaling pathway.[35] Similarly, VEGFA and interleukin enhancer binding factor 3 (NF90) are downstream targets of miR-590-5p.[36] Meanwhile, miR-25-3p can upregulate VEGF receptor 2 (VEGFR2) by targeting Krüpple-like factor 2 (KLF2) and Krüpple-like factor 4 (KLF4).[37] Additionally, miR-25-3p plays an oncogenic role in stimulating the process of angiogenesis.[37] Therefore, keeping the balance between angiogenesis and anti-angiogenesis may be helpful to restrain cancer cell migration.

miRNAs and Hypoxia

Hypoxia is another hallmark for solid tumors, including CRC.[16] It may result in more aggressive phenotypes by modulation of the genetic programs that facilitate cellular adaption to the hypoxic environment.[12] Hypoxia-inducible factor 1α (HIF-1α) and insulin-like growth factor 1 receptor (IGF-1R) are the major factors that induce a hypoxic environment.[8] To date, increasing studies have found that miRNAs play a significant role in cancer metastasis under a hypoxic environment.[16] For example, the miR-17∼92 cluster can negatively regulate HIF-1α and inhibit CRC metastasis.[9] Yin et al.[38] found that miR-145 blocked CRC metastasis by repressing the expression of HIF-1α. miR-143 inhibits angiogenesis and metastasis through suppressing IGF-1R, another major factor in hypoxia.[39] Given that hypoxia is closely related to angiogenesis and metastasis, targeting hypoxia-related miRNAs may lead to significant consequences for controlling cancer metastasis.

Interaction between miRNAs and TME

TME is vital for the progression of aggressive metastasis.[13] Previous studies have shown that miRNAs are closely related to TME, and that miRNAs could regulate some TME-related genes in cancer metastasis.[49] Moreover, miRNAs can act as modulators among immune cells, CAECs, CAFs, and tumor cells.[41,49–52] For instance, let-7c-5p can interact with TME by negatively regulating the expression of collagen type I alpha 2 chain (COL1A2) in CRC.[40] The balance between matrix metallopeptidases (MMPs) and tissue inhibitor of metalloproteases-2 (TIMP-2) plays a significant role in the degradation of ECM in cancer cells.[53] Makondi et al.[53] demonstrated that miR-20a and miR-495 suppress CRC metastasis by repressing the expression of matrix metalloproteinase, especially MMP2. Likewise, miRNAs

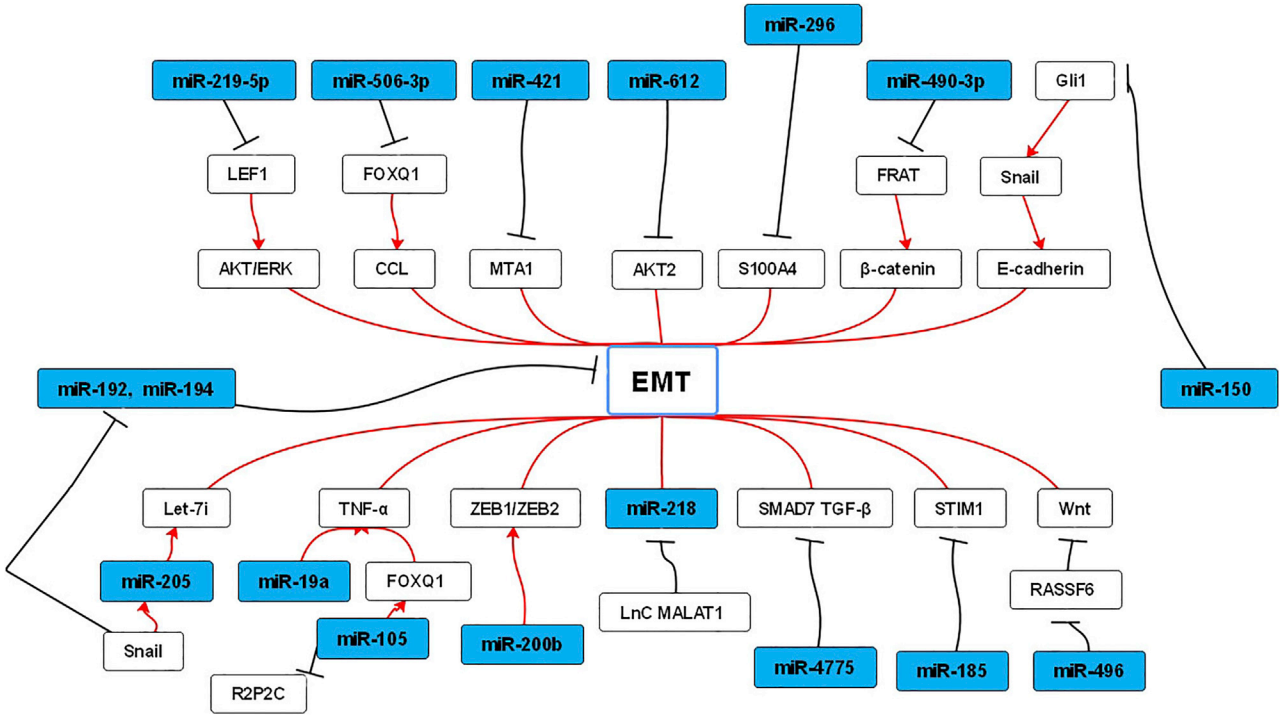

Figure 2. Network of miRNAs and EMT in CRC Metastasis

The interactions between miRNAs and protein-coding genes, lncRNAs, and several signaling pathways are shown. Promotion (red line) or suppression (black line) of EMT is presented.

derived from the exosomes can also interact with the MET.[14] For example, the elevated expression of miR-21-5p and miR-155-5p is induced by M2 macrophage-derived exosomes (MDEs), and then they bind the S-ribonuclease binding protein (SBP) family protein (BRG1), resulting in downregulation of BRG1 and higher rates of metastasis. Importantly, the contents of MDE range with the conversion of the MET.[42]

Tumor-associated macrophages (TAMs) are also pivotal components of TME. During cancer development and progression, TAMs undergo M1-like or M2-like polarization and subsequently manipulate tumor metastasis.[54] Some metastasis-related miRNAs are reported to widely participated in these processes.[13] For example, miR-195-5p directly binds to the 3′ UTR of the notch receptor 2 (Notch2), resulting in the suppression of M2-like TAM polarization and metastasis.[43] Meanwhile, $CD163^{+}$ TAMs participate in the secretion of interleukin 6 (IL-6) and the downregulation of miR-506-3p. miR-506-3p then suppresses metastasis by repressing the production of C-C motif chemokine ligand 2 (CCL2), a molecular marker recruiting macrophages.[44]

Tumor necrosis factor (TNF)-α, a central proinflammatory cytokine, is also involved in TME transformation as the downstream target of specific miRNAs.[55] Current research confirmed that miR-19a promoted TME transformation by enhancing TNF-α, which is required for TNF-α-induced metastasis.[45] Similarly, miR-105 is necessary for the TNF-α-induced TME transformation process, in which the nuclear factor κB (NF-κB) subunit 1 signaling pathway is elevated to activate TNF-α,[46] revealing the role of inflammatory factors in the carcinogenesis of CRC.

Thus, the interaction between miRNAs and TME may be another important point for developing the anti-metastasis strategy.

miRNAs and Molecular Mechanisms

miRNAs in Key CRC Signaling Pathways

Emerging research has revealed that miRNA-mediated CRC metastasis depends on different molecular mechanisms.[21] Table 2 presents these miRNAs, their targets, and the corresponding molecular mechanisms in CRC. Moreover, Figure 3 also illustrates the concrete mechanisms of miRNAs in CRC metastasis, including classical signaling pathways (such as the Notch, Wnt, EGFR, and TGF-β signaling pathways), the lncRNA/miRNA/mRNA axis, and methylation-related pathways.[82]

miRNAs in the EGFR Signaling Pathway

Kirsten Ras (KRAS) is a key molecule involved in EGFR signaling. The mutation of KRAS is the most commonly seen mutated gene (up to 30%–60%) in CRC patients, and it is a main resistant factor to anti-EGFR-targeted therapy.[23,83] As the downstream targets of miR-543, the expression levels of KRAS, MTA1, and high-mobility group AT-hook 2 (HMGA2) are inversely correlated with its

Table 2. miRNA-Related Molecular Mechanisms in CRC Metastasis

Mechanisms	miRNAs	Alteration	Downstream Targets	References
Mutation of KRAS	miR-543	↓	KRAS, MTA1, HMGA2	56
	miR-384	↓	KRAS, CDC42	57
PTEN/AKT/mTOR axis	miR-877	↓	MTDH	58
	miR-495	↓	FAM83D	59
	miR-99b-5p	↓	p-mTOR	60
TGF-β signaling	miR-224	↑	USP3, Smad4	61,62
	miR-27a	↓	SGPP1, Smad2, p-STAT, cleaved caspase-3	63
	miR-320a	↓	ΔNp63α, PKCγ, Rac1	64
	miR-199a-5p	↓	ROCK1	65
Wnt/β-catenin signaling	miR-135	↓	APC	66,67
	miR-494	↓		
	miR-371-5p	↓	SOX17, SOX2	68
	miR-145	↓	PRC2	69
	miR-132	↓		
	miR-212	↓		
	miR-34a	↑	SIRT1	70
	miR-378	↓	SDAD1	71
Notch signaling	miR-1280	↓	JAG2, Zeb1, Suz12, Gata1/3	72
	miR-200b	↓		
lncRNA/miRNA/mRNA axis	miR-218	↑	EZH2, E-cadherin	73
	miR-106-5p	↓	SLAIN2, MT	74
	miR-3679-5p	↑	MACC1	75
	miR-206	↓	NOTCH3, TM4SF1	76
	miR-577	↓	HSP27	77
	miR-215	↑	ZEB2	78
DNA promoter methylation	miR-214	↑	FOXD3, MED19	79
	miR-34c-5p	↑	SATB2	80
	miR-1246	↑	METTL3, SPRED2, MAPK	81

JAG2, jagged canonical Notch ligand 2; Suz12, polycomb repressive complex; SOX17, SRY-box transcription factor 17; NEAT1, nuclear-enriched abundant transcript 1; SRIT1, sirtuin 1; SDAD1, SDA1 domain containing 1P; MTDH, metadherin; FAM83D, family with sequence similarity 83, member D; PTEN, phosphatase and tensin homolog; mTOR, mechanistic target of rapamycin kinase; KRAS, Kirsten Ras; HMGA2, high-mobility group AT-hook 2; USP3, ubiquitin-specific peptidase 3; SGPP1, sphingosine-1-phosphate phosphatase 1; p-STAT3, phosphorylated signal transducer and activator of transcription 3; Rac1, Rac family small GTPase 1; ROCK1, Rho-associated coiled coil-containing protein kinase 1; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; SLAIN2, SLAIN motif family member 2; MT, microtubule; CYTOR, cytoskeleton regulator RNA; MACC1, MET transcriptional regulator; NOTCH3, neurogenic locus notch homolog protein 3; TM4SF1, transmembrane 4 L6 family member 1; HSP27, heat shock protein 27; CCAT2, colon cancer-associated transcript 2; FOXD3, forkhead box 3; MED19, mediator complex subunit 19; SATB2, SATB homeobox 2; METTL3, methyltransferase-like 3; SPRED2, sprout-related EVH1 domain-containing protein 2; MAPK, mitogen-activated protein kinase; ANXA1, annexin A1; DFFA, DNA fragmentation factor subunit alpha; PDCD4, programmed cell death 4; MTSS1, metastasis suppressor 1; FBXW7, F-box and WD repeat domain containing 7; SLC6A8, solute carrier family 6 member 8; CKB, creatine kinase, brain-type; HOXB7, homeobox B7; GALNT5, polypeptide *N*-acetylgalactosaminyltransferase 5; CEMIP, hyaluronidase 1; ADAMTS5, ADAM metallopeptidase with thrombospondin type 1 motif 5; IGFBP5, insulin-like growth factor binding protein 5; SND1, staphylococcal nuclease domain containing-1; EIF5A2, eukaryotic translation initiation factor 5A2; MBD2, methyl-CpG binding domain protein 2; CHD5, chromodomain helicase DNA binding protein 5.

expression in clinical samples, revealing the pivotal tumor suppressive role of miR-543 in CRC.[56] In addition, a recent study showed that overexpression of miR-384 could inhibit KRAS and cell division cycle 42 (CDC42) and act as an inhibitor in CRC metastasis.[57]

The phosphatase and tensin homolog (PTEN)/AKT/mTOR axis is also generally observed to be activated by miRNAs in the EGFR pathway.[58] It is approximately amplified in 15%–20% cases of CRC patients.[84] For example, miR-877 suppresses the malignant phenotype of CRC by directly targeting metadherin (MTDH). As a result, the PTEN/AKT pathway is indirectly suppressed, leading to the repression of metastasis.[58] miR-495 can also repress the proliferation and migration of CRC cells via the PTEN/P13K/AKT/mTOR pathway by repressing the expression of family with sequence similarity 83, member D (FAM83D).[59] Similarly, miR-99b-5p is reported to bind mTOR to suppress the mTOR-activated metastasis.[60]

miRNAs in the TGF-β Signaling Pathway

The TGF-β signaling pathway exerts a dual function on CRC.[85] TGF-β and its receptors are two major factors involved in multiple malignant progression.[85] The TGF-β signaling pathway suppresses the progression of CRC in its early stage while promoting CRC in its advanced stage.[85] As estimated, approximately 30% of CRC cases are associated with TGF-β type II receptor (TGF-βR) mutations.[86,87] SMADs, one of key factors in the TGF-β signaling pathway, are commonly used as the downstream targets of specific miRNAs.[88] Recent studies found that miR-224 could directly target SMAD family member 4 (SMAD4) and suppress the TGF-β signaling-induced metastasis.[61,62] Similarly, miR-27a directly binds sphingosine-1-phosphate phosphatase 1 (SGPP1) and SMAD family member 2 (SMAD2), resulting in the downregulation of TGF-β signaling.[63]

Other molecules in the TGF-β signaling pathway can also interact with miRNAs. For example, ΔNp63α can indirectly impair the expression of Rac family small GTPase 1 (Rac1) (a molecule involved in TGF-β signaling) by regulating miR-320a.[64] miR-199a-5p is identified as a CRC suppressor by inhibiting Rho-associated coiled coil-containing protein kinase 1 (ROCK1), another significant regulator in TGF-β signaling.[65]

miRNAs in Wnt Signaling Pathways

Adenomatous polyposis coli (APC), whose mutation happens in 90% of CRC, plays a suppressive role in Wnt signaling.[21] Several miRNAs, including miR-135 and miR-494, are validated to be associated with

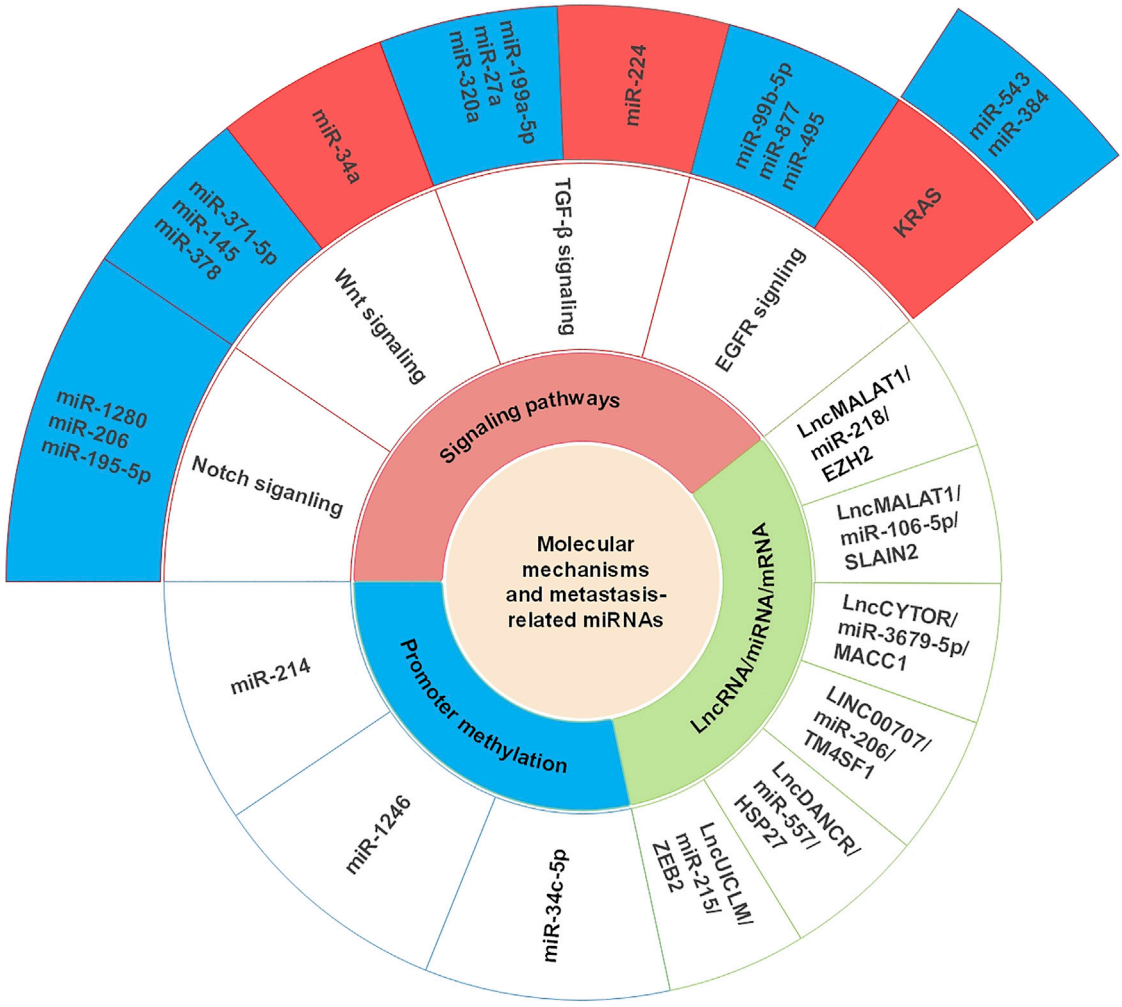

Figure 3. Metastasis-Related miRNAs and Molecular Mechanisms

The interactions between metastasis-related miRNAs and molecular mechanisms in CRC are shown. miRNAs in blue frameworks indicate suppression while miRNAs and KRAS in red frameworks indicate promotion of relevant signaling pathways. Blank frameworks mean interaction with relevant molecular mechanisms.

APC and then result in the dysregulation of Wnt signaling.[66,67] In addition, miRNAs can regulate Wnt signaling by regulating other molecules in CRC metastasis.[68] For instance, miR-371-5p represses the expression of the Wnt/β-catenin signaling pathway and inhibits CRC metastasis via the SOX17/miR-371-5p/SOX2 axis.[68] miR-132, miR-145, and miR-212 can all regulate the Wnt signaling pathway by targeting the TCF4-β-catenin complex and histone trimethylation complex Prc2p (PRC2).[69] Additionally, it is reported that miR-34a can suppress the activation of Wnt signaling via sirtuin 1 (SIRT1) and nuclear enriched abundant transcript 1 (NEAT1).[70] Zeng et al.[71] revealed that miR-378 inhibited metastasis via the suppression of SDA1 domain containing 1P (SDAD1) as well as downregulation of the Wnt/β-catenin pathway.

miRNAs in the Notch Signaling Pathway

Previous studies demonstrated that the Notch signaling pathway was also involved in CRC metastasis and that miRNAs can manipulate it post-transcriptionally.[89] miR-1280 can inhibit metastasis by directly inhibiting jagged canonical notch ligand 2 (JAG2), Zeb1, polycomb repressive complex (Suz12), and Gata1/3, which are activators of the Notch signaling pathway.[72] miR-200b is also validated as an activator of the Notch signaling pathway.[89]

lncRNA/miRNA/mRNA Axis

The lncRNA/miRNA/mRNA axis plays important role in tumor metastasis.[90] lncRNA widely participates in gene regulatory networks at different levels of gene expression, including chromatin modification, transcription, and post-transcription.[91] lncRNA can interact with mRNAs and miRNAs directly or indirectly.[92] According to studies, the regulatory network between lncRNAs, miRNAs, and mRNAs is significant and common in carcinogenesis.[93] lncRNA acts as a positive transcriptional regulator of mRNAs by competitively binding miRNAs, with this process being termed as the sponge-like

effect.[94,95] Research on the novel lncRNA/miRNA/mRNA network is valuable to understand the mechanisms of cancer metastasis.

lncMALAT1 can activate the expression of E-cadherin and induce metastasis by interacting with miR-218.[73] lncMALAT1 is also reported as a metastasis-inducing factor by suppressing miR-106-5p and stimulating the expression of SLAIN2.[74] Moreover, it is reported that lncRNA *CYTOR* could competitively bind to miR-3679-5p and regulate the expression of MET transcriptional regulator (MACC1).[75] Similarly, LINC00707 can promote CRC metastasis by sponging miR-206 and indirectly modulating the expression of neurogenic locus notch homolog protein 3 (NOTCH3) and transmembrane 4 L6 family member 1 (TM4SF1).[76] While lncRNA DANCR can stimulate the expression of HSP27 and CRC metastasis via miR-577 sponging.[77] Additionally, Chen et al.[78] identified that miR-215, as the downstream target of lncRNA UICLM, could negatively regulate ZEB2 and enhance metastasis.

miRNAs and Methylation

Methylation, occurring on the DNA promoter or on miRNA itself, is another significant molecular mechanism related to CRC metastasis.[96] Methylation of the DNA promoter located in or near the CpG island, followed by manipulation of the chromatin structure and gene transcription, is usually observed in tumor development. The specific mechanism is probably transcriptional silencing.[97] Recent studies have revealed that miRNA-related methylation plays a crucial role in cancer metastasis.[79] For example, forkhead box 3 (FOXD3) positively regulates miR-214 via promoter hypermethylation.[79] miR-214 binds mediator complex subunit 19 (MED19) and suppresses metastasis afterward.[79] Similarly, promoter methylation of miR-34c-5p can also impair the process of CRC metastasis.[80] To date, researchers have made great progress in the understanding of the epigenome, and it is generally thought that a small subgroup of the thousands of epigenetic alterations is irreplaceable in driving CRC, among which DNA methylation is the most widely studied.[98] The concrete mechanism is still not clearly verified on how methylation influences the activity of miRNAs.[98] The prospect is broad although further investigation is necessary.

Methylation occurring on the miRNA itself may also serve as a contributor to CRC metastasis.[81] Methyltransferase-like 3 (METTL3), a main methyltransferase involved in the methylation process, assists the maturation of miR-1246 by methylating pri-miR-1246. miR-1246 inhibits the expression of downstream sprout-related EVH1 domain-containing protein 2 (SPRED2) and then upregulates mitogen-activated protein kinases (MAPKs), leading to the malignant phenotype of CRC.[81] We assume that modification of the methylation status of miRNAs may be a novel strategy for controlling cancer metastasis.

Clinical Value of miRNAs Involved in Metastasis in CRC

miRNAs as Biomarkers for CRC Diagnosis and Prognosis

Existing CRC screening methods have their limitations. The fecal occult blood test (FOBT) and fecal immunochemical test, convenient and inexpensive, are widely adopted. However, their sensitivity is relatively low for detecting pre-neoplastic lesions.[99] The current gold standard for CRC diagnosis is colonoscopy. This method can observe the tumor from both macro and micro perspectives. It is not widely applied for its invasive feature.[99] The stool DNA test is characterized by high labor intensity and cost, despite its high sensitivity and specificity. If the concentration of certain miRNAs applied for detection is too low, the result of the stool-based mRNA test varies.[100] Carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) are the most commonly used plasma biomarkers, in which CEA is also widely applied to monitor CRC recurrence, despite their low sensitivity and specificity.[100,101] In conclusion, the diagnostic and prognostic methods need to be upgraded so that early stage CRC can be detected with high accuracy and straightforward approaches.

miRNAs have attracted researchers' attention as biomarkers for several reasons. Primarily, as is revealed from context, accumulating miRNAs have been validated to be significantly associated with CRC progression.[102] Since there are abundant CRC-related miRNAs, it is promising to compare the existing miRNAs to select the most suitable and applicable ones as markers.[103] Additionally, miRNAs are stable and sensitive such that their expression can be detected quantitatively and reliably from tissues, serum, plasm, stool, circulating exosomes, urine, and even saliva and sputum (Table 3).[104,105] Additionally, research on multiple miRNAs and detective technologies is already at the pre-clinical stage. The results indicate that the prospect is extremely broad.[2]

miRNAs as Diagnostic Biomarkers

Ideal biomarkers should be beneficial for early detection and intervention of CRC. Usually, miRNAs are significantly altered in CRC development and progression, and thus they can be used as practical diagnostic biomarkers.[104] Multiple studies have reported that miRNAs in the serum, exosome, and even in stool show potentials for early diagnosis.[56,106] Circulating serum miRNAs are the most commonly explored ones, and even single miRNA can be used to diagnose CRC.[103,106] For example, serum miR-203 has been revealed as a promising diagnostic factor in CRC patients in several studies due to its positive association with early period CRC and its stability for detection.[107,108] miR-122 and the miR-200 family are also associated with early stage CRC. They are quantitatively detectable and could probably be used as one of the multiple markers in the blood test.[106] Similarly, downregulation of miR-126, together with upregulation of miR-21 and miR-210, shows great value for early diagnosis of CRC.[109] Indeed, a series of co-expressed miRNAs or miRNA profiles are more effective in CRC detection.[110]

Experts also revealed that exosomal miRNAs and stool-based miRNA detection are promising for early diagnosis.[111] For example, downregulation of miR-96-5p and miR-149 in $GPC1^+$ exosomes are observed in CRC patients, and they may serve as potential diagnosis biomarkers in CRC.[112] Similarly, early stage CRC patients show increased levels of miR-17-5p and miR-92a-3p in exosomes.[113] In

Table 3. miRNAs as Diagnostic or Prognostic Biomarkers

miRNA	Expression	Clinical Application	References
Circulating Serum miRNAs as Biomarkers for CRC			
miR-203	↑	diagnosis	106,107
miR-122	↑	diagnosis	105
miR-200 family	↑		
miR-126	↓	diagnosis	109
miR-21	↑		
miR-210	↑		
miR-592	↓	prognosis	118
miR-106-5p	↑		
miR-615-3p	↑		
miR-21	↑	prognosis	118
miR-103	↑		
miR-93	↑		
miR-566	↓		
Exosomal miRNAs as Biomarkers for CRC			
miR-96-5p	↑	diagnosis	112
miR-149	↑		
miR-17-5p	↑	diagnosis	113
miR-92a-3p	↑		
miR-21	↑	prognosis	119
miR-548c-5p	↓	prognosis	120
Stool-Based miRNAs as Biomarkers for CRC			
miR-4478	↑	diagnosis	116
miR-1295b-3p	↑		
miR-135b	↑	prognosis	122
miR-92a	↑	prognosis	126

addition, decreased expression levels of miR-4478 and miR-1295-p in stool samples represent non-invasive and effective diagnostic biomarkers for CRC patients.[114,115] Downregulation of fecal miR-4478 and miR-1295b-3p can also be detected in early stage CRC, indicating their potential roles as promising diagnostic biomarkers for CRC.[116]

miRNAs as Prognostic Biomarkers

The expression levels of miRNAs are closely associated with progression of CRC, and they can predict the overall survival (OS), possibility of recurrence, and drug response in CRC.[117] Therefore, detection of miRNAs in CRC patients can help us to make right decisions in cancer management. Several metastasis-related miRNAs have been considered as potential markers for predicting outcome in CRC patients. For instance, miR-106-5p, miR-615-3p, and miR-592 are closely correlated with the prognosis of CRC patients.[118] Similarly, increased miR-21, miR-103, and miR-93, together with downregulated miR-566, are defined as a specific miRNA profile for predicting metastasis and outcome in CRC patients. This profile can be applied to distinguish between primary CRC and CRC with liver metastasis.[118] Recently, a two miRNA-based signature in CRC, including let-7i and miR-10b, has also been proposed for use in distinguishing patients with higher risk of metastasis and hepatic recurrence.[119]

Recent studies also shed light on the roles of exosomal miRNAs in predicting the prognosis of CRC patients.[111] For example, exosomal miR-21 is significantly elevated in higher TNM stage CRC patients, and it can serve as an independent risk factor for CRC.[120] In addition, reduced miR-548c-5p in serum exosomes is independently correlated with shorter OS and higher TNM stage in CRC patients.[121] Researchers have also shown great interest in applying stool-based miRNAs to predict prognosis in CRC patients.[122] Advanced CRC patients with higher miR-135b in stool samples showed a significantly shorter OS than did those with lower miR-135b.[123] miR-92a was reported as a prognostic biomarker in both serum and stool, and a high level of miR-92a is associated with poor progression-free survival in CRC.[124]

Theoretically, miRNAs used as promising diagnostic or prognostic biomarkers for CRC have a broad prospect. However, to date, no miRNA has been applied as a reliable biomarker in clinical practice. The causes are multifarious. First, miRNAs are always involved in various carcinomas, and few specific miRNAs in CRC have been validated.[125] Second, current detection technologies are not sensitive enough. They should be upgraded so that a tiny concentration and alteration of miRNAs can be sensitively observed. Novel tools such as isothermal amplification techniques, near-infrared technology,[126] and energy transfer-based photoelectrochemical[127] and molecular beacons[128] are all promising methods.[102] Additionally, if miRNA detection is to be extensively used in the clinic, convenience and low cost must be taken into consideration.[129]

miRNA-based Targeted Therapy for CRC Metastasis

A single miRNA could regulate the expression of many mRNA genes, and thus targeting miRNAs may be an effective approach for the development of personalized anti-tumor regimens.[130] Moreover, some miRNAs can even target the same mRNA or exert a similar function in CRC. Additionally, manipulating those miRNAs simultaneously may be an available strategy for targeted therapy.[131]

To date, the methods for developing miRNA-based therapy include repression of the oncogenic miRNAs or activation of the suppressors.[122] For the former, we can achieve it by applying anti-miRNA oligonucleotides or blocking related oncogenes via virus-based constructs, miRNA sponges, miRNA-masking, and small molecule inhibitors.[132] To realize the later goal, double-stranded miRNA mimics[133] and viral/liposomal delivery systems[2] can be applicable. However, these methods are inevitably disturbed by toxicity and side effects, including off-target and immune-related effects.[110] Additionally, current studies on the clinical application of miRNAs are limited in the pre-clinical stage.[134] For example, application of MRX34 (one miR-34 mimic encapsulated in lipid nanoparticles) was halted during the phase I clinical trial for various solid tumors (including CRC) due to severe immune-related adverse effects,[135] even though previous evidence proved that it was involved in the

manipulation of multi-oncogenic pathways and it displayed obvious anti-tumor effects in advanced CRC.[134] Fortunately, several recent studies may change the situation. Miravirsen, an inhibitor of miR-122, was also introduced in a phase II clinical trial for HCV infection, which showed lower toxicity and side effects.[136] Moreover, the-poly(amino acid)s/miR-139-5p nanoparticle complex can significantly repress the CRC migration in mice with a much better effect than mere miR-139-5p, and this delivery system is safer and more efficient.[137]

To develop miRNA-based targeted therapy, the most challenging problem is to explore the novel and effective delivery systems.[127] Thus, the use of superior miRNA delivery systems in cancer treatment is expected in the next decade.[137] Moreover, the off-target effects from non-specific miRNAs, the toxic and immune responses from the miRNA mimics and inhibitors, and the reduced efficacy due to the degradation of miRNAs are all beyond our expectation, which are anticipated to be minimized or even eliminated.[127] One solution is to develop lower toxic miRNA mimics and inhibitors, and this relies on studies involving pharmacological, pharmacokinetic, and pharmacodynamic factors.[126] Another approach is to reshape the miRNA expression profiles by self-adjustment of the human body.[126] For example, a study has shown that some miRNAs related to urea synthesis (miR-221-3p, miR-221-5p, and miR-222-3p) could be induced in mice by giving them a low-protein diet, which leads to reduced urea synthesis.[138] Additionally, the combination of miRNA-based therapy with classical chemotherapy may be the effective approach for drug-resistant CRC patients.[137]

Discussion

Metastasis is a malignant phenotype of CRC and it is closely related to poor prognosis. Dysregulation of abundant miRNAs contributes to this malignant process and influences the development of CRC. Therefore, reshaping the expression profiles of these miRNAs can partially control cancer metastasis, and finally improve the OS of CRC patients. In this review, we summarized the recent advances of miRNAs in CRC metastasis and discussed their roles in CRC metastasis. The clinical values (diagnostic role, prognostic role, and therapeutic potential) of certain miRNAs in CRC were also summarized. Our review attempts to provide new clues for future research about miRNAs in metastasis of CRC.

miRNAs are dysregulated and play an important role in metastasis of CRC. miRNA expression patterns in metastatic CRC can be inconsistent among different studies.[139] This phenomenon can be caused by many factors, including the differences in patient selection criteria, collection methods, biological sample processing, and detection approaches.[86] Thus, the normalized criteria and protocol are essential for miRNA research. Moreover, genome-wide searching and bioinformatics analysis can help us to easily identify more miRNAs involved in metastasis, which may improve the accuracy for its clinical application.[117]

Recently, miRNAs in plasm, blood, and stool show potential use as the diagnostic or prognostic biomarkers. However, the efficiency of these markers in the clinical setting still needs further investigation. First, it is necessary to establish miRNA detection methods with high sensitivity, high specificity, and low labor and economic cost. For instance, liquid biopsy, which refers to analysis of DNA, RNA, and proteins in body fluid (blood, urine, and cerebrospinal fluid), presents higher specificity and accuracy than do other approaches.[140] Second, the exosomes containing miRNAs may also be a promising tool for early screening patients. Studies have also indicated that these exosomes can be used as prognostic indicators for cancer metastasis.[85]

The theories of precision medicine and personalized treatment have attracted researchers' attention recently.[21] miRNAs are promising targets for cancer treatments. miRNAs can simultaneously modulate several mRNAs, suggesting that targeting miRNAs can be more effective than targeting one single mRNA.[141] So far, miRNA-based therapy includes miRNA mimics, miRNA inhibitors, and anti-miRNAs.[13] These composites can be used to reshape the expression levels of dysregulated miRNAs in different diseases, including cancers. Moreover, the combination of a miRNA-based strategy with chemotherapy may have a wide application in some drug-resistant cancer patients.[98] Importantly, novel and superior miRNA delivery systems are the bases for developing miRNA-based targeted therapy.

Collectively, studies on metastasis-related miRNAs have made great progress in elucidating their roles in CRC. These miRNAs also show potential in clinical application for CRC management. However, no miRNAs have already been used in clinical practice for multiple reasons. The search for applicable miRNAs and miRNA profiles, the development of detective methods and techniques, the combination of miRNA-based therapy with other chemotherapy, and the construction of delivery systems are all necessary for further research. Additionally, these improvements will finally be beneficial to improve the OS of CRC patients.

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AUTHOR CONTRIBUTIONS

L.N., W.Y., and L.D. conceived this manuscript. X.W., Y.L., Y.Z., and J.L. collected and prepared the related references. L.N., W.Y., and L.D. drafted the manuscript. C.X., C.L., and W.Z. drew the figures. W.Y., Q.Z., L.H., and D.F. supervised and revised the manuscript. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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