



## REVIEW ARTICLE

# MicroRNA-9 as a paradoxical but critical regulator of cancer metastasis: Implications in personalized medicine

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**Abstract** Metastasis, is a development of secondary tumor growths at a distance from the primary site, and closely related to poor prognosis and mortality. However, there is still no effective treatment for metastatic cancer. Therefore, there is an urgent need to find an effective therapy for cancer metastasis. Plenty of evidence indicates that miR-9 can function as a promoter or suppressor in cancer metastasis and coordinate multistep of metastatic process. In this review, we summarize the different roles of miR-9 with the corresponding molecular mechanisms in metastasis of twelve common cancers and the multiple mechanisms underlying miR-9-mediated regulation of metastasis, benefiting the further research of miR-9 and metastasis, and hoping to bridge it with clinical applications.

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## Introduction

Cancer is an uncontrolled growth disease, and significantly increases health and economic burden.<sup>1</sup> Despite surgery and radiation therapy effectively control many cancers at the primary site, metastatic disease is the leading cause of cancer-related death.<sup>2</sup> Therefore, it should be paid more attention to metastasis-targeted therapies in clinical.

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Metastasis is the final product of a multi-step cellular biological process, this process is called the "invasion-metastatic cascade", in which tumor epithelial cells perforate the extracellular matrix, invade into blood vessels, arrest at distant organ sites and penetrate into the tissue parenchyma, eventually proliferating to form new tumor.<sup>3</sup> The process of metastasis is very inefficient, only few cells that metastasize from the primary tumor can succeed in colonizing at a distance.<sup>4</sup> However, metastasis is closely related to the poor prognosis and mortality. Thus, it is very important to elucidate the mechanisms contributing to cancer metastasis or find novel biomarkers for metastasis.

MiRNAs are a class of noncoding single-stranded RNA molecules encoded by endogenous genes with a length of approximately 22 nucleotides.<sup>5</sup> It can bind to the 3'untranslated region (3'UTR) of target mRNA and regulate gene expression at the post-transcriptional level. The biogenesis of miRNAs (microRNAs or miRs) is regulated by multiple steps including DROSHA-regulating nuclear generation of primary (pri-) miRNA, Exportin 5 transferring pri-miRNA to the cytoplasm, and pri-miRNA maturation in the cytoplasm by DICER.<sup>6</sup> More than 60% of human protein-coding genes are regulated by miRNAs.<sup>7</sup> Furthermore, miRNAs have been shown to play crucial roles in tumorigenesis, such as inflammation, stress response, apoptosis, cell cycle regulation, differentiation, metastasis and invasion.<sup>8</sup>

MiR-9 has been shown to be aberrantly expressed in many cancer types and suppress gene expression involved in cell growth, angiogenesis, and metastasis, etc.<sup>9</sup> For instance, ectopic expression of miR-9 inhibits the JAK/STAT3 pathway by targeting interleukin 6 (IL-6), resulting in decreased proliferation and migration of HeLa cells.<sup>10</sup> And MiR-9 has been confirmed to inhibit proliferation of Glioblastoma multiforme cell lines by targeting the cyclic AMP response element-binding protein (CREB) but to promote migration by targeting neurofibromin 1 (NF1).<sup>11</sup> In this review, we focus on the roles of miR-9 in cancer metastasis, expecting that it might be used as a biomarker or potential drug target for metastatic cancers.

## MiRNAs and metastasis

MiRNAs hold a specific characteristic that one miRNA can regulate the expression of numerous genes and one target gene can be targeted by several miRNAs, making a complex regulatory pathway. MiRNAs may influence many aspects of tumor development, the association between miRNAs and cancer metastasis was firstly reported in 2007. Ma et al<sup>12</sup> suggested that miR-10b is induced by the transcription factor Twist and positively regulates invasion and metastasis in breast cancer via targeting homeoboxD10 (HOXD10) directly. Then, lots of studies have shown that miRNAs can function as oncogenes or suppressors in cancer metastasis and coordinate multistep of metastatic process, incorporating migration, invasion, epithelial–mesenchymal transition (EMT), adhesion and motility of cancer cells.<sup>13,14</sup> For example, Chen et al<sup>15</sup> revealed that miR-130a promotes breast cancer cell migration and invasion via targeting FOSL1 and suppressing ZO-1. Lohcharoenkal et al<sup>16</sup> reported that in melanoma, low level of miR-203 is associated with poor overall survival in patients with metastases, and its

expression *in vivo* is shown to inhibit metastasis by regulating Slug. And several members of the miR-200 family were reported that can directly regulate the expression of key EMT-associated genes, such as ZEB1 and SIP1, suppressing EMT and tumor metastasis by downregulating E-cadherin.<sup>17</sup> Furthermore, the important role of miR-9 in cancer metastasis is previously demonstrated by Ma et al<sup>18</sup> and us as well.<sup>19–21</sup> And since then there are many reports showing its role in tumor progression. Here, we reviewed the relationship between miR-9 and metastasis in twelve common metastatic cancers and the underlying mechanisms.

## The promoting role of miR-9 in metastasis of breast cancer

Breast cancer is the most frequently diagnosed cancer in women. It has been confirmed that 25%–50% of breast cancer patients eventually develop fatal metastasis, which can occur even decades after the tumor is diagnosed and removed.<sup>22</sup> Ours and other studies have shown that miR-9 is found to be highly expressed in patients with lymph node metastasis compared to patients without it, and high level of miR-9 is related to poor disease- and distant metastasis-free survival in triple negative breast cancers (TNBCs).<sup>19,23,24</sup> Additionally, a lot of studies provide evidences for the promoting role of miR-9 in breast cancer metastasis. For example, Shi et al<sup>25</sup> described that miR-9 is overexpressed in MDA-MB-231 cells, a model of TNBC, which is more aggressive than MCF-7 cells, a luminal cell line with a weaker invasion ability. And Gravgaard et al,<sup>26</sup> using *in situ* hybridization, found that miR-9 level is also higher in the distant metastases than corresponding primary tumors. These results suggest an explicit involvement of miR-9 in the metastatic process and confirm it as a relevant element in controlling metastatic breast cancer.

C-Myc exerts many physiological functions, such as promoting cell proliferation and apoptosis, contributing to the tumor onset and early growth.<sup>27</sup> In terms of mechanism, several evidences indicated that miR-9 is engaged in c-Myc-mediated regulation on breast cancer. For example, Sun et al<sup>28</sup> demonstrated the increased expression of miR-9 in mammary tumors of MMTV-c-Myc transgenic mice, a c-Myc-induced mouse model; Ma et al<sup>18</sup> confirmed that expression of miR-9 is activated by MYC and MYCN, and miR-9 elevates cell motility and invasiveness via directly targeting the key metastatic-suppressing protein E-cadherin. The down-regulation of E-cadherin sequentially activates  $\beta$ -catenin signaling, which promotes the expression of vascular endothelial growth factor (VEGF), resulting in tumor angiogenesis; on the other hand, the reduction of E-cadherin makes tumor cells sensitive to EMT-inducing signals produced by the tumor microenvironment, both of which in turn leading to metastatic dissemination. Cancer stem cells (CSCs) are the tumor-initiating cell population with ability to self-renew and multi-potential, and induce tumor genesis, expansion, resistance, recurrence, and metastasis process.<sup>29</sup> Zhang et al<sup>30</sup> showed that miR-7 is down-regulated in breast cancer stem cells (BCSCs) and can interfere cell invasion and metastasis, decrease the BCSC population and partially reverse EMT through targeting the oncogene, SETDB1, and then suppressing STAT3 expression,

subsequently down-regulating the expression of c-Myc, twist, and miR-9. And both of twist and miR-9 can inhibit E-cadherin expression, which may explain the mechanisms whereby miR-7 reverses EMT in breast cancer and BCSCs. Moreover, miR-9 was indicated to elevate the generation of CSCs to profit an invasive phenotype.<sup>31</sup> In addition to promote the metastasis of breast cancer through suppressing targeted genes, miR-9 could mediate the competing endogenous RNAs (ceRNAs), which are defined as transcripts that cross-regulate each other by competing for shared miRNAs,<sup>32</sup> and thus engaged in breast cancer metastasis. Like our previous studies suggested that there exists a competition of miR-9 between forkhead box O1 (FOXO1) 3'UTR and E-cadherin 3'UTR, and FOXO1 3'UTR can inhibit the metastases of breast cancer cells via restraining E-cadherin expression through miR-9<sup>21</sup>, and the oncogene CYP4Z1 3'UTR could suppress the migration of breast cancer cells through competitively binding to miR-9 with E-cadherin.<sup>20</sup>

Furthermore, miR-9 could regulate other metastasis-related genes and signaling pathways beyond E-cadherin. Chen et al<sup>33</sup> revealed that leukemia inhibitory factor receptor (LIFR), the downstream effector of the miR-9, suppresses breast cancer metastasis through triggering Hippo pathway by leading to the phosphorylation, cytoplasmic retention and functional inactivation of the transcriptional coactivator YES-associated protein (YAP). Notably, our previous study demonstrated that miR-9 promotes breast cancer EMT and metastasis via post-transcriptionally regulating STARD13 expression.<sup>19</sup> D'Ippolito et al<sup>34</sup> further explored that induction of endogenous miR-9 expression ligand-dependently stimulated by platelet-derived growth factor receptor Beta (PDGFR $\beta$ ) facilitate the vasculogenic ability of TNBC *in vitro* and *vivo*, partially via directly repressing STARD13 expression, this work further confirms our results that miR-9 can target STARD13 in breast cancer.

In recent years, tumor cells-secreted miRNAs, which transferred by exosomes, have become a new mechanism

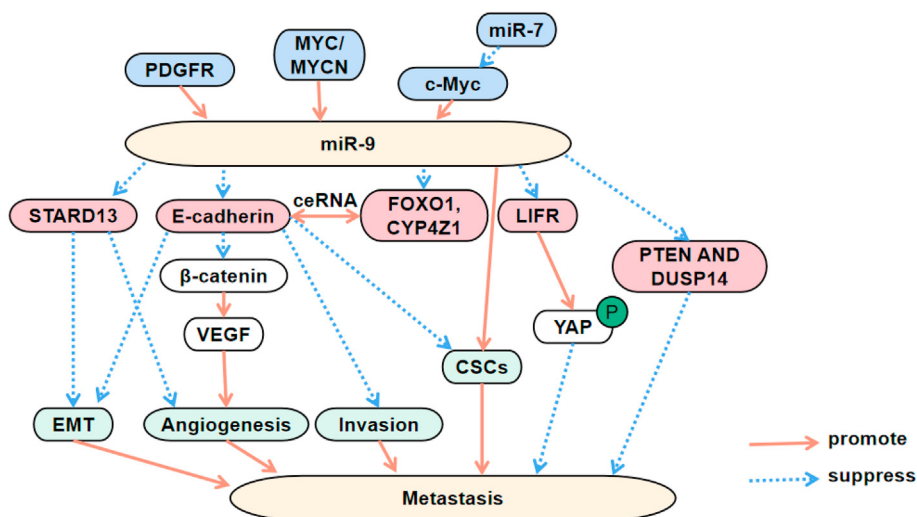
for mediating tumor stroma crosstalk and metastasis. Exosomes are a subset of extracellular vesicles released from cells with diameters <100 nm, have been shown to carry sorts of molecules, including miRNAs.<sup>35</sup> MiR-9 was firstly detected in MDA-MB-231 and MCF-7 exosomes by Vahid Kia et al and it was found that miR-9 is overexpressed in highly metastatic TNBC exosomes. Additionally, treatment of MCF-7 cells with MDA-MB-231 exosomes decreases the expression of miR-9-targeted genes PTEN and DUSP14, two critical tumor suppressors, and then induces metastatic behavior of recipient cells, this study indicates that miR-9 in breast cancer cells-derived exosomes can promote breast cancer metastasis.<sup>36</sup>

Taken together, miR-9 could induce the metastasis of breast cancer via different mechanisms (Fig. 1). And miR-9 is closely correlated with poor prognosis and may serve as a potential therapeutic target for invasive breast cancer patients.

### The promoting role of miR-9 in metastasis of osteosarcoma (OS)

OS is the most familiar primary solid bone malignant tumor. It originates from primitive mesenchymal cells, and 90% of OS patients die from lung metastasis.<sup>37</sup> MiR-9 expression was demonstrated to be elevated in OS tissue and serum. Additionally, the increased miR-9 level in tissue and serum has a strong correlation with the aggressive phenotype of OS, such as advanced tumor-node-metastasis stage, large tumor size and distant metastasis.<sup>38–40</sup> Diao et al<sup>41</sup> screened biomarkers by using gene chips from OS patients with or without metastasis, and identified miR-9 and miR-202 as the potential key factors of OS metastasis.

Mechanistically, Fenger et al<sup>42</sup> validated that miR-9 motivates the invasion and migration in OS through activating the expression of gelsolin (GSN), an actin filament-severing protein involved in cytoskeletal remodeling. Fang



**Figure 1** The underlying mechanisms contributing to miR-9-mediated regulation on breast cancer metastasis. MiR-9 is regulated by PDGFR, MYC/MYCN, miR-7/c-Myc signal and promotes metastasis via targeting STARD13, E-cadherin, FOXO1, CYP4Z1, LIFR, PTEN and DUSP14 signal pathway.

et al<sup>43</sup> showed that high dose of 17 $\beta$ -estradiol (E2) treatment up-regulates miR-9, and metastasis-associated lung adenocarcinoma transcript 1 (MALAT-1) is reduced by miR-9 post-transcriptionally, and then regulates cell proliferation, migration, invasion and EMT processes in an ER-independent ways. Moreover, Gang et al<sup>44</sup> supported the promoting role of miR-9 on OS cell metastasis and certified that its role is correlated with some other signals including cadherin-1 (CDH1), matrix metalloproteinase 13 (MMP-13), FOXO3a, Bcl-2-like protein 11 (BCL2L11), and  $\beta$ -catenin (CTNNB1). Although further experimental studies are needed to confirm these connections, it is still possible to speculate on the important role of miR-9 in OS metastasis.

### The promoting role of miR-9 in metastasis of lung cancer

Lung cancer is the most frequent cancer,<sup>45</sup> and can be divided into small cell lung cancer and non-small cell lung cancer (NSCLC). The metastasis rate of NSCLC is extremely high, more than half of newly-diagnosed NSCLC patients have metastatic disease and become the most lethal cancer in humans.<sup>46</sup> Some recent studies have shown that miR-9 expression is tightly associated with lung cancer metastasis.<sup>47,48</sup> The silence of miR-9 by methylation is related with early-stage and pathologically lymph node-negative case of NSCLC, which suggests that miR-9 is involved in NSCLC metastasis.<sup>49</sup> For example, Xu et al<sup>50</sup> indicated that up-regulation of miR-9 is highly correlated with tumor size and lymph node metastasis, which offers a promising biomarker for poor prognostic in NSCLC patients. Migdalska-Sek et al<sup>51</sup> preliminarily explored the effect of miR-9 on the expression of IL-17A, a pro-inflammatory cytokine, in relation to tumor stage and nodule metastasis in NSCLC. In addition, Li et al<sup>52</sup> suggested that miR-9 promotes cell proliferation and metastasis of NSCLC via decreasing TGFBR2 expression by directly targeting TGFBR2 3'UTR and sequentially restraining the phosphorylation and activation of the downstream effectors smad2/3.

### The promoting roles of miR-9 in metastasis of Prostate cancer and bladder cancer

Prostate cancer is the third commonly diagnosed cancer, accounts for 7.1% of all new cases and caused 3.6 million deaths worldwide.<sup>45</sup> MiR-9 is also found to be involved in prostate cancer progression. Seashols-Williams et al<sup>53</sup> expounded the effect of miR-9 on prostate cancer, and they analyzed and identified miR-9 as an oncogenic miRNA through high-throughput sequencing and RT-qPCR analysis, and that miR-9 is overexpressed in tumor tissues as compared to adjacent benign glandular epithelium. Mechanistically, E-cadherin and suppressor of cytokine signaling 5 (SOCS5) are targeted and suppressed by miR-9, this is responsible for miR-9-mediated effects on tumor progression and metastasis. In addition, the miR-9/STARD13 axis, which is established by us in breast cancer, is further confirmed in prostate cancer metastasis.<sup>54</sup> Furthermore, lncRNA MEG3 can inhibit prostate cancer metastasis through sponging miR-9<sup>55</sup>.

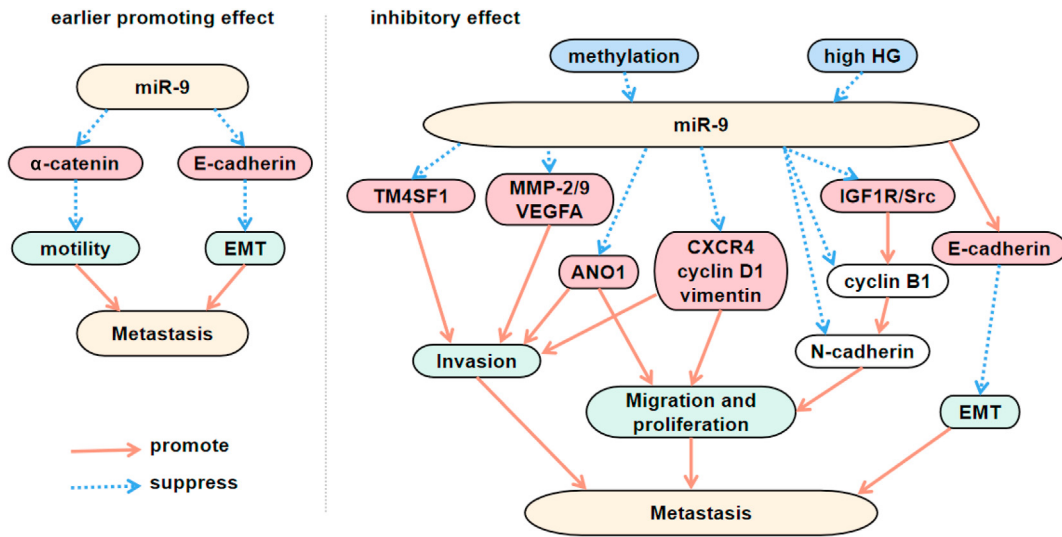
In bladder cancer, Xie et al<sup>56</sup> detected the expression of miR-9 and found that miR-9 expression is increased significantly in bladder transitional cell carcinomas (TCC) samples compared to that in normal bladder transitional cell (NBTC) samples, and the overexpression of miR-9 promotes invasion of bladder transitional cells partly via targeting CBX7, which offers a potential target for TCC therapy.

### The role of miR-9 in metastasis of colorectal cancer (CRC)

CRC is the third most common cancer in the world but second in terms of mortality.<sup>45</sup> Interestingly, earlier studies reported that miR-9 plays a promotive role in colon cancer metastasis. Zhu et al<sup>57</sup> evaluated miR-9 expression and found a significant increase in CRC specimens with distant metastasis comparing with primary CRC specimens without distant metastasis. The ectopic miR-9 expression may be involved in metastasis by enhancing the motility and targeting  $\alpha$ -catenin in CRC cells. Lu et al<sup>58</sup> also suggested that Prospero homeobox 1 (PROX1) increases the invasiveness of colon cancer cells through binding to miR-9-2 promoter, triggering its expression to suppress E-cadherin and thus inducing EMT. However, more inhibitory effects of miR-9 on CRC metastasis have been recently reported. For example, Eva et al<sup>59</sup> showed that miR-9 expression is down-regulated by methylation in patients with CRC, and also related to the presence of lymph node metastasis. Park et al<sup>60</sup> revealed that overexpression of miR-9 suppresses transmembrane-4-L6 family 1 (TM4SF1), and further restrains invasion and metastasis in CRC and miR-9 suppresses MMP-2, MMP-9 and VEGF expression as well. These results suggest that miR-9 acts as a tumor-suppressor for CRC invasion and metastasis. Additionally, the expression of E-cadherin is confirmed to be up-regulated by miR-9 in CRC cells. Xiong et al<sup>61</sup> demonstrated that the ectopic miR-9 expression reduces the level of C-X-C motif chemokine receptor 4 (CXCR4) *in vitro* and *in vivo*, orderly suppressing CRC cell proliferation, migration and invasion. Furthermore, miR-9 overexpression also inhibits Cyclin D1 and Vimentin, whereas it up-regulates the expressions of E-cadherin, and then suppresses cell proliferation and EMT. Chen et al<sup>62</sup> provided evidence for the down-regulation of miR-9 by high glucose (HG) concentration in CRC cells, and miR-9 can decrease the insulin-like growth factor-1 receptor (IGF1R)/Src signal and downstream cyclin B1 and N-cadherin but upregulate E-cadherin, indicating that miR-9 not only modulates EMT protein expression and morphology but also suppresses the cell migration and invasion ability of CRC. Moreover, miR-9 inhibits the proliferation, migration, and invasion of CRC cells via direct targeting anoctamin-1 (ANO1) as also<sup>63</sup> (Fig. 2).

### The suppressive role of miR-9 in metastasis of melanoma

The incidence of malignant melanoma is increasing worldwide. Although most patients are diagnosed with localized disease and can be treated by surgical removal of the primary tumor, metastasis always happens.<sup>64</sup> Shiiyama et al<sup>65</sup>



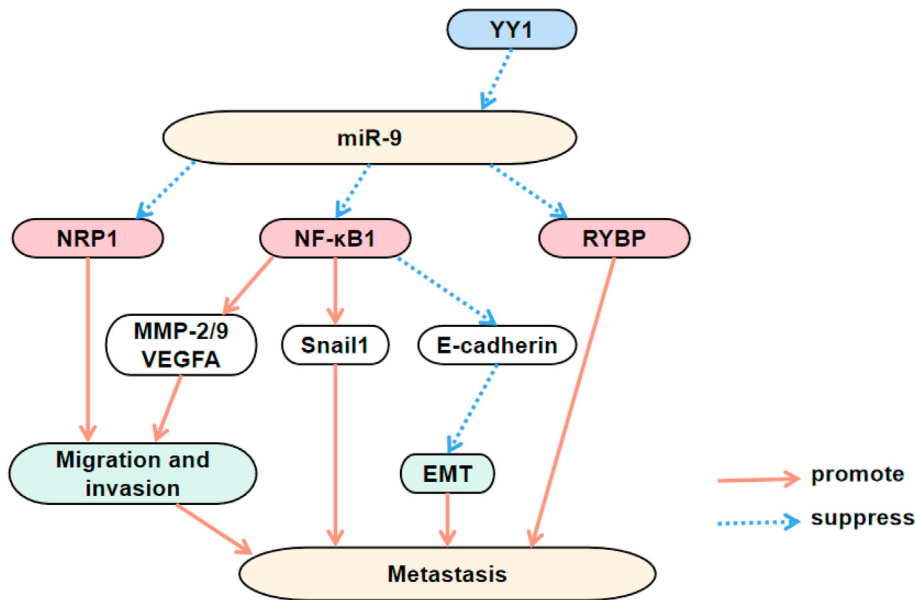
**Figure 2** The mechanisms underlying the roles of miR-9 in CRC metastasis. The promoting effect of miR-9 on metastasis reported by earlier studies is described on the left, and the inhibitory effect is described on the right.

illustrated that miR-9 expression is significantly lower in metastatic patients compared to primary patients, showing the significant correlation between miR-9 and melanoma metastasis. Mechanistically, Liu et al<sup>66</sup> suggested that miR-9 overexpression decreases Snail1 and increases E-cadherin expression via directly targeting 3'UTR of NF-κB1, then inhibiting the proliferation and metastasis. Consistently, Xue Rong et al<sup>66</sup> supported the impact of miR-9 on NF-κB1 and confirmed that the downstream targets of NF-κB1 are regulated by miR-9 in the same pattern, such as MMP-2, MMP-9 and VEGFA, thereby suppressing the migration and invasion of uveal melanoma cell. Besides, miR-9 can also regulate melanoma metastasis through other pathways. For example, Zhao et al<sup>67</sup> determined that the expression of YY1, a transcription factor, is elevated in melanoma and

associated with melanoma metastasis state and tumor stage. YY1 can negatively regulate the transcription of miR-9, which targets RYBP, forming an YY1-miR-9-RYBP axis to promote melanoma growth and progression. Moreover, Dan et al<sup>68</sup> indicated that miR-9 overexpression suppresses the growth, migration and invasion of melanoma cells by targeting neuropilin 1 (NRP1) (Fig. 3).

**The suppressive roles of miR-9 in metastasis of nasopharyngeal carcinoma (NPC), gastric cancer (GC) and brain cancer**

NPC is a highly aggressive and metastatic epithelial carcinoma that occurs in the nasopharyngeal mucosa.<sup>69</sup> MiR-9



**Figure 3** The mechanisms underlying the roles of miR-9 in melanoma metastasis. MiR-9 is regulated by YY1 and inhibits metastasis via targeting NRP1, NF-κB1 and RYBP signaling pathway.

was reported to serve as a biomarker to monitor dynamic change, recurrence and metastasis of NPC. Lu et al<sup>70</sup> evaluated the relationship between the low level of plasma miR-9 and worse lymphatic invasion and advanced tumor lymph nodes metastasis stage via plasma microarray profiling between NPC patients and healthy volunteers. In addition, Lu et al<sup>71</sup> collected patients' blood samples before the treatment initiation, 3 months, 6 months, and 12 months after treatments, and at the time of any recurrence or metastasis and they identified miR-9 level is apparently upregulated after treatment, while downregulated again when recurrence or metastasis happens. Furthermore, Lu et al<sup>71</sup> provided evidence for miR-9 as a potential metastatic target in NPC treatment. The functional analysis supported that miR-9 could suppress the progression of NPC by targeting CXCR4, which is a key metastatic promoter and associated with the clinicopathological features and prognosis.<sup>72,73</sup>

GC is one of the most common malignant tumors in the digestive system.<sup>45,74</sup> Some studies have shown the tumor-suppressing role of miR-9 in GC development and metastasis. For example, Deng et al<sup>75</sup> confirmed that miR-9 downregulates the CUL4A-LATS1-Hippo signaling pathway by directly targeting the 3'UTR of CUL4A and thus suppressing GC cell proliferation, invasion and EMT. In addition, Li duan et al<sup>76</sup> demonstrated that miR-9 inhibits the proliferation, invasion and metastasis of gastric cancer by suppressing the expression of cyclin D1 and Ets1. These results suggest that miR-9 could be a potential therapeutic target for the future treatment of GC.

Glioblastoma is the most common and invasive primary brain tumor in adults, accounting for 45.6% of primary malignant brain tumors.<sup>77</sup> Ben-Hamo et al<sup>78</sup> showed that miR-9 overexpression inhibits MAPKAP signaling through a novel regulation mode. MiR-9 initiates re-organization of actin filaments through regulating a subset of genes, MAPK14/MAPKAP3 complex, of the MAPKAP signal and eventually interfering with cell migration and invasion phenotype of glioblastoma cells. This situates hsa-miR-9 as a therapeutic target of metastasis in glioblastoma multiforme (GBM).

## The role of miR-9 in metastasis of liver cancer

Liver cancer contains primary liver cancer and secondary liver cancer, and hepatocellular carcinoma accounting for approximately 90% of all primary liver cancers. Hepatocarcinogenesis is a multifactorial process, involving multiple signaling pathways during tumor growth and metastasis.<sup>79</sup> Tan HX et al<sup>80</sup> confirmed that miR-9 is overexpressed in highly invasive SK-Hep-1 cells than in other hepatoma cell lines, and inhibition of miR-9 could restrain SK-Hep-1 cell invasion, E-cadherin was up-regulated by miR-9 inhibitor as well. These results suggest that miR-9 could be a booster in HCC metastasis. Sun et al<sup>81</sup> compared primary and recurrence (intrahepatic metastatic) sites of hepatocellular carcinoma, and found that miR-9 is highly expressed in recurrence sites with a higher invasive and migratory potential and miR-9 may promote migration and invasion via regulating KLF17, which directly acts on

the promoter of the EMT-related genes. However, Yi et al<sup>82</sup> provided evidence that miR-9 plays an opposite role by inhibiting the  $\alpha$ -2, 6-linked sialylation via targeting  $\beta$ -galactoside  $\alpha$ -2,6-sialyltransferase 1 (St6gal1). This may be due to the mechanism of miR-9 in liver cancer metastasis has not been thoroughly studied. There may exist some intermediate molecules that have not been discovered, and there may also be mutual influences between pathways. Therefore, although these articles have initially explored the relationship between miR-9 and metastasis of hepatocellular carcinoma, it must be noted that the underlying mechanisms by which miR-9 roles in hepatocellular carcinoma metastasis need to be further studied.

## The roles of miR-9 in metastasis of squamous cell carcinoma (SCC)

Squamous cell carcinoma (SCC)<sup>83</sup> usually occurs in areas covered by scaly epithelium, such as the skin, mouth, lips, esophagus, cervix, vagina, etc, and 95% of cases can be completely removed the primary tumor. Nevertheless, metastasis of some aggressive squamous cell carcinomas leads to metastasis-related death. White et al<sup>84</sup> built a mouse model of SCC through CSCs derived from K15. Kras G12D. Smad4<sup>-/-</sup> cells and showed that miR-9 is related to CSC expansion and metastasis, and miR-9 could be a potential biomarker for metastatic CSCs. They also found that miR-9 is increased in metastatic human primary SCCs and SCC metastases, and is correlated with a low expression of  $\alpha$ -catenin but not E-cadherin. Wu et al<sup>38</sup> claimed that miR-9 expression is increased in laryngeal squamous cell carcinoma (LSCC) and significantly correlated with lymph node metastasis. Song et al<sup>85</sup> demonstrated that miR-9 facilitates the metastasis of esophageal squamous cell carcinoma (ESCC) through down-regulating E-cadherin, followed by activation of  $\beta$ -catenin pathway and induction of EMT. Furthermore, the relationship between miR-9 and head and neck squamous cell carcinoma (HNSCC) was reported by Xue rong et al,<sup>86</sup> and they revealed that miR-9 is up-regulated by insulin receptor substrate 1 (IRS-1) down-regulation and overexpression of miR-9 can promote metastasis via suppressing E-cadherin. These studies support miR-9 acts as a metastasis-promoter in some kinds of SCC. However, it may act a suppressive role in metastasis of other kinds. For example, Sun et al<sup>87</sup> confirmed that serum miR-9 was down-regulated in patients with oral squamous cell carcinoma (OSCC) and low expression is associated with lymph node metastasis, which means that miR-9 might be a tumor suppressor in OSCC.

## Discussion and conclusion

Cancer metastasis, causing 90% of human cancer deaths, consists of a string of extremely complex biological processes that tumor cells migrate from the primary carcinoma to a distant secondary site. It is a daunting and significant challenge, as there are no effective therapies for metastatic cancer so far. MiRNAs represent a critical role in the initiation and development of metastatic process. MiR-9 was reported as an important molecule related to the

**Table 1** MiR-9 roles in different cancer metastasis.

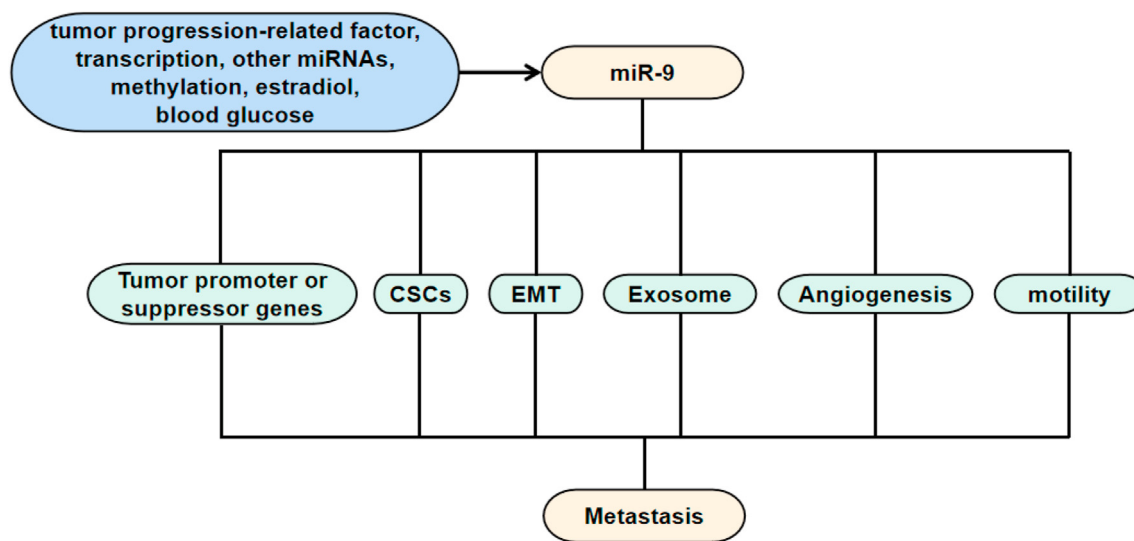
Cancer	Express	Sample	Target
Breast cancer	high	Cell&Tissue	E-cadherin, FOXO1, LIFR, STARD13, PTEN and DUSP14
OS	high	Serum&Tissue	GSN, MALAT-1, CDH1, MMP-13, FOXO3a, BCL2L11, CTNNB1
Lung cancer	high	Cell&Tissue	IL-17A, TGFBR2
Prostate cancer	high	Tissue	E-cadherin, SOCS5
Bladder cancer	high	Tissue	CBX7
CRC	low	Tissue	a-catenin, E-cadherin, TM4SF1, MMP-2/9, VEGF, CXCR4, cyclin D1, Vimentin, IGF1R/Src signal, ANO1
Melanoma	low	Tissue	NF-κB1, NRP1, RYBP
NPC	low	Plasma&Tissue	CXCR4
GC	low	Cell&Tissue	CUL4A, cyclin D1 and Ets1
Brain cancer	—	—	MAPKAP signal
Liver cancer	—	—	KLF17, St6gal1

—, not identified; OS, osteosarcoma; CRC, colorectal cancer; NPC, nasopharyngeal carcinoma; GC, gastric cancer.

metastasis of many cancers through various signaling pathways that modulate tumor development, and may exist same targets in different cancers (Table 1). Such as E-cadherin, directly targeted or indirectly influenced by miR-9 and regulate metastasis of many cancers including breast cancer, prostate cancer, CRC, melanoma, liver cancer, ESCC and HNSCC, but due to tumor specificity, the effects of miR-9 on E-cadherin and subsequent regulation on metastasis are not exactly identical. Moreover, some other miRNAs may also share the same targets and pathways with miR-9, such as miR-210 can also promotes breast CSC metastasis by targeting E-cadherin,<sup>88</sup> and miR-145-5p can hindered the occurrence and metastasis of melanoma cells via inactivating the NF-κB pathway.<sup>89</sup> It is worthy of further exploration and research whether these miRNAs and miR-9 are synergistic or competitive in regulating cancer metastasis. And since the feature of multiple genes-targeting and abundant existences in cells, it is not surprising that miRNAs could serve as a new potential target for metastatic

therapy and have the potential to be a key factor facilitating the therapeutic strategies.

Plenty of studies and research have shown the multiple mechanisms of miR-9-mediated regulation of metastasis as evidenced by this overview. For instance, directly targeting tumor promoter or suppressor genes; modulating EMT, cell motility, angiogenesis and CSC-related properties. Moreover, it also functions in the exosomes secreted by tumor cells (Fig. 4). Meanwhile, miR-9 can exert its effects through the ceRNA mechanism, by which mRNAs, lncRNAs, circular RNAs, and RNAs from pseudogenes, competing with each other through the same miRNA recognition sites. Additionally, the function of miR-9 in metastasis is context and tumor type-specific that miR-9 mainly act as a promoter of metastasis capability in breast cancer, OS, prostate cancer and bladder cancer, while it plays the opposite roles in other cancers such as CRC, NPC, melanoma, GC and brain cancer (Table 2). However, the research on the mechanism contributing to miR-9-induced effects on some



**Figure 4** Multiple mechanisms of miR-9-mediated regulation of cancer metastasis. MiR-9 is regulated by many factors and influence cancer metastasis through multiple mechanisms.

**Table 2** MiR-9 influences metastasis and other properties across cancer types.

Cancer	metastasis	angiogenesis	EMT	invasion	migration	proliferation	CSCs	motility
Breast cancer	↑	↑	↑	↑			↑	↑
OS	↑	↑	↑	↑	↑	↑		
Lung cancer	↑			↑	↑	↑		
Prostate cancer	↑			↑	↑			
Bladder cancer	↑			↑				
CRC	↓		↓	↓	↓	↓		↑
Melanoma	↓			↓	↓	↓		
NPC	↓			↓	↓	↓		
GC	↓		↓	↓	↓	↓		
Brain cancer	↓			↓	↓			

↑, promote; ↓, inhibit; OS, osteosarcoma; CRC, colorectal cancer; NPC, nasopharyngeal carcinoma; GC, gastric cancer.

cancer metastasis is incomplete and needs further exploration.

Selected miRNAs targeting multiple mRNAs to alter disease status can make these molecules as the candidate therapeutic targets and advances in RNA molecules delivery technology have made miRNA-based treatments more feasible. Based on the initial research of systemic or local injection into the target tissue site, the delivery technology has been improved to chemical modifications of miRNAs, including nanoparticle, and some related drugs have entered the clinical stage at present. For example, MesomiR-1 based on miR-16 mimic is in multi-centre phase I clinical trials currently, which deliver miR-16 and targeting cancer disease involving mesothelioma, non-small cell and lung cancer by EnGeneIC Delivery Vehicle (EDV) nanocells coated with epidermal growth factor receptor (EGFR)-specific antibodies (NCT02369198). And miR-34 (MRX34), which delivered by lipid nanoparticles (LNPs), has been used to target and treat multiple solid tumors in phase I trials such as NSCLC, pancreatic cancer and prostate cancer.<sup>90</sup> In other diseases, anti-miR-122 (RG-101) and anti-miR-103 (RG-125), both in clinical trials, act as therapeutic agents in chronic hepatitis C and patients with type 2 diabetes respectively, through N-acetyl-D-galactosamine (GalNAc)-conjugated anti-miR delivery system. As an effective factor governing metastasis and a promising target for novel therapeutic approaches, miR-9 has the capacity to develop into drugs. However, due to its extensive and multiplex effects on various cancers, the technical problems of miR-9 in specific targeting *in vivo* or delivery needs to be further settled.

### Author statement

Yichen Liu, Qiong Zhao, Xiaoman Li conceived and designed the study and also managed the collected data. All authors contributed to data collection and wrote the manuscript. Lufeng Zheng fully revised the manuscript, designed, and presented the figures and supported the work along with Xiaoman Li and Tao Xi.

### Data availability statement

Research data are not shared unless the corresponding authors agree.

### Conflict of Interests

The authors promise no potential conflicts of interest.

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