

# Exosome-derived noncoding RNAs: Function, mechanism, and application in tumor angiogenesis

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**Exosomes are extracellular vesicles released by various cell types that perform various biological functions, mainly mediating communication between different cells, especially those active in cancer. Noncoding RNAs (ncRNAs), of which there are many types, were recently identified as enriched and stable in the exocrine region and play various roles in the occurrence and progression of cancer. Abnormal angiogenesis has been confirmed to be related to human cancer. An increasing number of studies have shown that exosome-derived ncRNAs play an important role in tumor angiogenesis. In this review, we briefly outline the characteristics of exosomes, ncRNAs, and tumor angiogenesis. Then, the mechanism of the impact of exosome-derived ncRNAs on tumor angiogenesis is analyzed from various angles. In addition, we focus on the regulatory role of exosome-derived ncRNAs in angiogenesis in different types of cancer. Furthermore, we emphasize the potential role of exosome-derived ncRNAs as biomarkers in cancer diagnosis and prognosis and therapeutic targets in the treatment of tumors.**

## INTRODUCTION

Tumors are characterized by unlimited replication, nutritional self-sufficiency, and abnormal cellular energy metabolism. This extreme and disordered growth environment depends on angiogenesis.<sup>1</sup> Excessive angiogenesis is a key tumorigenic phenomenon, and it correlates with tumor initiation, malignant progression, and poor prognosis.<sup>2</sup> In recent years, the studies of tumor microenvironments (TME) have provided a new understanding of tumor growth and new ways to treat cancer.<sup>3</sup> Among the TME, exosomes derived from many cells and the substances they carry are an important integral component. They circulate freely *in vivo* and accumulate in TME and have been recognized as a new contributor to angiogenesis.<sup>4</sup>

Some studies have shown that exosomes play an important role in tumorigenesis, proliferation, metastasis, and invasion;<sup>5–8</sup> thus, the extensive and in-depth studies of tumor angiogenesis are inseparable from exosomes and their carriers, especially noncoding RNAs (ncRNAs). ncRNAs constitute a large class of molecules that are not thought to encode proteins and are divided into microRNAs

(miRNAs), long noncoding RNAs (lncRNAs), circular RNAs (circRNAs), small nucleolar RNAs (snRNAs), small nucleolus RNAs (snoRNAs), PIWI-interacting RNAs (piRNAs), ribosomal RNAs (rRNAs), and transfer RNAs (tRNAs) according to their transcriptional length.<sup>9,10</sup> ncRNAs account for up to 98%–99% of the human genome, perform many basic regulatory functions in eukaryotes, and even play roles in abnormal cell functions associated with cancer progression and metastasis.<sup>11</sup> Numerous studies investigating the unique characteristics and vascular endothelial cell function of exosome-derived ncRNAs have shown that they play an important role in tumor angiogenesis.<sup>12</sup> For example, miR-210 released by exosomes secreted by metastatic cancer cells mediated by neutral sphingomyelinase 2 (nSMase2) promoted angiogenesis in the TME by targeting endothelial cells and inhibiting the expression of the specific target gene ephrin-A3.<sup>13</sup> This finding indicates that exosome-derived ncRNAs may potentially become new biomarkers and therapeutic targets for cancer therapy. Therefore, exosome-derived ncRNAs have received increasing attention in exosome research.

In this review, we discuss the basic characteristics of exosomes and exosome-derived ncRNAs and the functional role of exosome-derived ncRNAs in cancers. In particular, we summarize and emphasize the mechanism and clinical application value of exosome-derived ncRNAs in tumor angiogenesis.

## EXOSOME-DERIVED ncRNAs IN CANCERS

Exosomes are 30- to 150-nm extracellular vesicles secreted by mammalian cells<sup>14</sup> resulting from multivesicular endosomes or multivesicular bodies fusing into the plasma membrane and being secreted out of cells under the condition of intercellular communication and regulation between adjacent and distant cells.<sup>15,16</sup> Numerous

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studies have shown that exosomes are excreted from many types of cells (such as tumor cells, B lymphocytes, stem cells, dendritic cells (DCs), platelets, etc.)<sup>17–21</sup> and circulate in bodily fluids (such as blood, urine, saliva, etc.).<sup>22–25</sup> The contents of exosomes are complex and include nucleic acids, proteins, and lipids.<sup>26–28</sup> Exosomes are highly heterogeneous due to the different compositions of their contents, which indicate the source of the cells that produce them.<sup>29</sup> A clinical study has proved the safety of exosomes in the treatment of cancers by inoculating melanoma patients with vaccination based on dendritic cell-derived exosomes.<sup>30</sup> Exosomes activate signal transduction pathways by directly binding their surface membrane molecules to receptor target cells and are internalized into the target cells through the combination of the lipid membrane and the target cell membrane; this process is realized by transmembrane proteins and extracellular matrix proteins.<sup>31,32</sup> For example, exosomes can be located on the extracellular matrix and together with matrix vesicles as the initial location of mineralized nodules.<sup>33</sup> However, the underlying mechanisms remain unclear. The role of exosomal contents internalized into cells has been extensively studied. For example, exosomes can affect tumor angiogenesis through these contents. A study noted that IL-8 and platelet-derived growth factor (PDGF), which are carried by exosomes originating from glioblastoma multiforme cells of hypoxic brain tumors, are considered angiogenic stimulating molecules.<sup>34</sup> Among the contents of exosomes, ncRNAs, such as miR-9, are the most widely studied. Exosomes originating from melanoma cells have been internalized through endothelial cells (ECs), and miR-9 derived from exosomes promotes angiogenesis and metastasis by activating the Janus kinase/signal transducer and activator of transcription (JAK-STAT) pathway.<sup>35,36</sup>

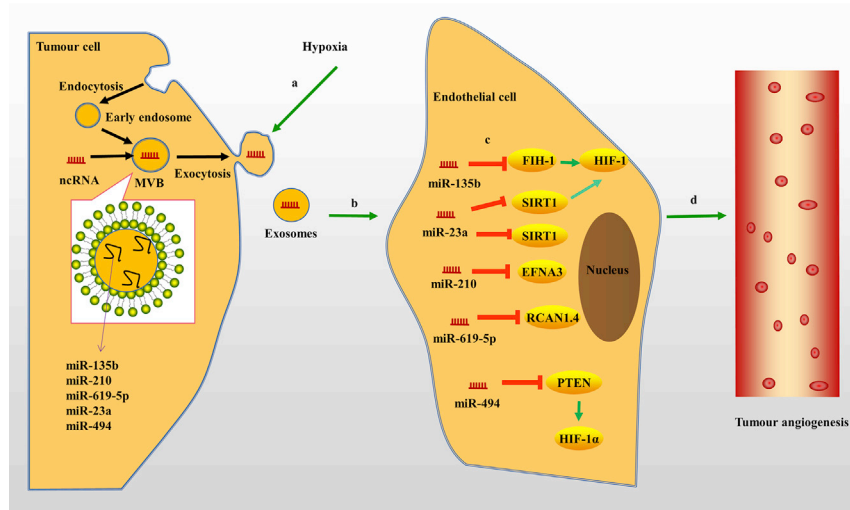
ncRNAs regulate normal cell growth and apoptosis and play an important role in abnormal cells, such as tumor cells.<sup>37</sup> It has been confirmed that ncRNAs can not only act as gene expression and splicing regulators but also serve as epigenetic controllers and provide guidance for chromatin-modification complexes.<sup>38,39</sup> Some ncRNAs are thought to function by being packaged in exosomes and transferred to receptor cells.<sup>26</sup> With the rapid development of RNA sequencing technology and bioinformatics, new information regarding the role of exosome-derived ncRNAs in cancer has gradually emerged. These molecules play a vital role in cancer. Exosome-derived ncRNAs can not only promote cell proliferation and migration, the epithelial-mesenchymal transformation (EMT), tumor proliferation, angiogenesis and metastasis but also participate in tumor immune escape, promote tumorigenesis and even cause tumor cells to acquire chemical resistance, leading to tumor drug resistance.<sup>40–45</sup> For example, exosome-derived miR-210 promotes tumor angiogenesis through endothelial cell uptake and the inhibition of specific target genes highly expressed in breast cancer.<sup>46</sup> Studies have increasingly demonstrated that miRNAs and lncRNAs are regulators of homeostasis and cellular signal transduction and play an important role in tumor progression and metastasis.<sup>47,48</sup> Tumor angiogenesis is also an important part of the TME that can regulate the occurrence and development of tumors.

## TUMOR ANGIOGENESIS

Angiogenesis is the complex process of generating new capillaries from existing blood vessels and usually includes the following steps: vascular endothelial growth factor (VEGF)-stimulating ECs, enzymatic degradation of the vascular basement membrane, proliferation, migration, and germination of vascular endothelial cells, branching, and tube formation.<sup>49</sup> Under physiological conditions in normal adults, the net balance between proangiogenic factors and antiangiogenic factors controls the process of angiogenesis, and blood vessels remain static and rarely form new branches.<sup>50</sup> Tumors secrete various substances to break this balance and induce pathological angiogenesis, which eventually develops into a complex, mature vascular system.<sup>2,51</sup> This system provides the tumor with the necessary nutrients and oxygen to promote tumor growth and causes the tumor cells to leave the original site of the tumor and spread to distant organs through the blood.<sup>52–54</sup> Hypoxia is the main force driving tumor angiogenesis,<sup>55,56</sup> and VEGF plays a key role in tumor angiogenesis by enhancing all the steps of angiogenesis.<sup>57,58</sup>

Hypoxia is the most typical and important feature in the TME and is a marker of malignant tumors.<sup>59</sup> Due to the excessive consumption of oxygen by tumor cell proliferation coupled with tumor-related leakage and a disordered vascular system, tumors cause not only the acute flux of oxygen tension but also the diffusion-limited area of low oxygen levels, leading to hypoxia.<sup>60</sup> Hypoxia triggers proteomic changes in tumor cells and stromal cells, further promoting the aggressiveness and poor survival of tumor cells, while leaky blood vessels cause these cells to metastasize remotely.<sup>61</sup> However, during tumor spread, the oxygen and nutrients necessary for solid tumor growth provided by adjacent capillaries are insufficient; thus, tumor cells must induce new angiogenesis to ensure the supply of oxygen and nutrients.<sup>61</sup> These abnormal blood vessels produce tumor blood vessels but cannot effectively restore the blood supply and further promote temporal and spatial changes in oxygen transport, thereby aggravating the hypoxic phenotype of the tumor. Consequently, the tumor survives and drives tumor growth under these adverse growth conditions.<sup>62</sup>

Hypoxia induces a stress reaction between the tumor and the oxygen-monitoring mechanism, which promotes the hypoxia signaling pathway and changes the transcription of many genes.<sup>63</sup> This pathway is mainly regulated by hypoxia-inducible factors (HIFs; HIF-1 $\alpha$ , HIF-2 $\alpha$ , and HIF-3 $\alpha$ ). HIF-1 $\alpha$  induces the expression of angiogenic proteins, such as VEGF, epidermal growth factor, PDGF, hepatocyte growth factor (HGF), and fibroblast growth factor (FGF).<sup>64</sup> Studies have increasingly shown that in a hypoxic environment the cells in the TME induce ncRNA overexpression, and ncRNAs are released by exosomes and participate in tumor angiogenesis by reacting with ECs and other angiogenic cells, thereby affecting tumor progression<sup>65</sup> (Figure 1). For example, exosome-derived miR-135b from myeloma cells enhances tumor angiogenesis through the HIF-1H (factor hypoxia-inducible factor) signaling pathway.<sup>66</sup> In addition, a study found that in an anoxic environment



**Figure 1. Role of hypoxia-related exosome-derived ncRNAs in tumor angiogenesis**

(A) Hypoxia induces a large release of exosome-derived ncRNAs. (B) Exosome-derived ncRNAs act on ECs. (C) Exosome-derived ncRNAs react with substances in ECs. (D) EC proliferation promotes angiogenesis. The green arrow indicates stimulatory modification; the red "T" indicates inhibitory modification.

nism driving EC reprogramming, which activates ECs to produce VEGF and upregulates VEGFR2 signal transduction.<sup>74</sup> TEXs cause the loss of cadherin and  $\beta$ -catenin on the surface of ECs and promote the movement of ECs.<sup>75</sup> In addition, exosomes carrying tetraspanin Tspan8 induce transcriptional changes in ECs, resulting in the increased expression of angiogenic genes and increased proliferation

tumor cells transfer miR-494 to ECs through exosomes by targeting phosphatase and tensin homolog (PTEN) and then activate the Akt/eNOS pathway, leading to a HIF-1 $\alpha$ -mediated mechanism to enhance EC migration and promote angiogenesis and tumor growth.<sup>67</sup>

## ROLE OF DIFFERENT CELL-DERIVED EXOSOMES IN TUMOR ANGIOGENESIS

Most cells in the body produce exosomes, which play an important role in the process of transmitting biological information to the target by carrying and releasing different types of contents.<sup>68</sup> Studies have shown that the characteristics and functions of cell-derived exosomes are involved in various situations, including diseases of the central nervous system, myocardial ischaemia/circulation injury, liver and kidney injury, the regulation of tumor markers, and the induction of angiogenesis and metastasis.<sup>69</sup> Among these functions, pathological angiogenesis represents a sign of tumor progression, and exosomes derived from various cells in the TME, especially exosome-derived ncRNAs, play an important role in regulating this process.<sup>70</sup> Among these processes, exosomes secreted by tumor cells, stem cells (SCs), and tumor-associated macrophages (TAMs) play a role in regulating the angiogenesis of ECs<sup>17</sup> (Figure 2).

### Tumor-derived exosomes

Tumor-derived exosomes (TEXs) play an important role in tumor growth, angiogenesis, immune regulation, metastasis, and drug resistance.<sup>71</sup> Especially in the early stage of tumor development, TME pressure or hypoxia stimulates tumor cells to increase exosome secretion.<sup>72</sup> TEXs circulate freely in bodily fluids, accumulate in the TME, and interact with ECs and other tissues or immune cells to accelerate tumor angiogenesis. Studies have increasingly confirmed that TEXs play a key role in angiogenesis.<sup>73</sup> Under hypoxia, overexpressed TEXs are absorbed by and internalized into ECs. EGFR transferred to ECs may be involved in the molecular mecha-

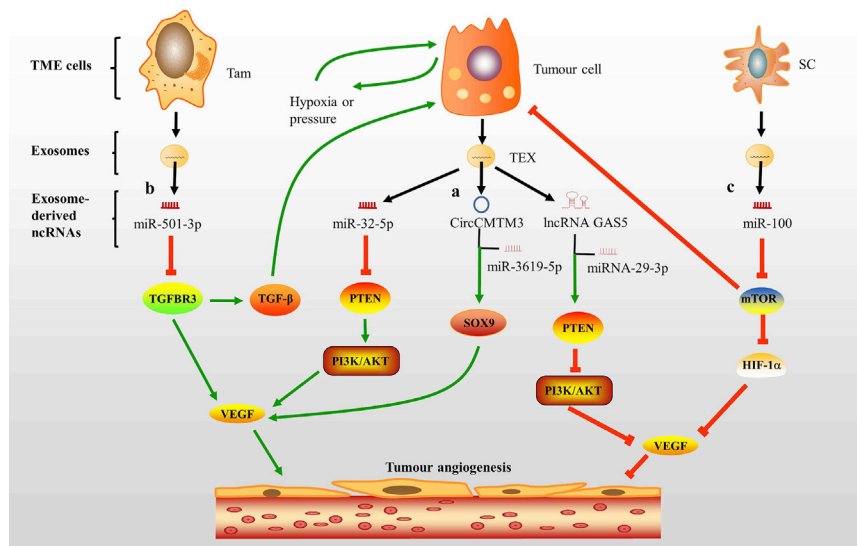
nism driving EC reprogramming, which activates ECs to produce VEGF and upregulates VEGFR2 signal transduction.<sup>74</sup> TEXs cause the loss of cadherin and  $\beta$ -catenin on the surface of ECs and promote the movement of ECs.<sup>75</sup> In addition, exosomes carrying tetraspanin Tspan8 induce transcriptional changes in ECs, resulting in the increased expression of angiogenic genes and increased proliferation of ECs.<sup>76</sup> ECs begin to secrete cytokines and growth factors after TEX uptake, which stimulates pericytes through the PI3K/Akt pathway.<sup>77</sup> In addition, some studies confirmed that TEXs transfer the components of the Notch pathway to ECs to stimulate new angiogenesis and establish a new vascular network.<sup>78</sup> Increasing evidence suggests that TEXs affect tumor angiogenesis mainly through a series of reactions, such as ncRNAs acting on ECs. For example, TEXs secreted by lung cancer cells increase the expression and secretion of VEGF by transferring miR-21 to ECs and stimulating the angiogenesis of ECs *in vitro*.<sup>44</sup>

### SC-derived exosomes

SCs refer to undifferentiated cells. SCs related to tumor progression are usually divided into the following two phenotypes: adult stem cells and cancer stem cells (CSCs).<sup>79</sup> Mesenchymal stem cells (MSCs) are among the most widely studied adult stem cells.<sup>80</sup> Exosomes secreted by MSCs and CSCs, as the main regulators of TME cell signaling, also play an important role in tumor angiogenesis.<sup>81</sup>

How MSC-derived exosomes affect tumor angiogenesis remains controversial. Interestingly, according to current studies, exosomes derived from MSCs have been shown to exert mainly antiangiogenic and antitumor effects. Especially in recent years, numerous experiments investigating the release of ncRNAs confirmed these effects.<sup>82</sup> The overexpression of exosome-derived miR-16 and miR-100 from MSCs downregulates the expression of VEGF in breast cancer cells, thereby inhibiting angiogenesis and tumor growth *in vivo* and *in vitro*.<sup>80,82</sup>

CSCs are regarded as the basic cells in heterogeneous tumor tissue, and their secreted exosomes become effective TME regulators that affect tumor angiogenesis, especially the self-renewing regulatory ncRNAs they carry, which play a key role.<sup>83</sup> Exosomes secreted by CD90+ hepatoma cells, which are defined as CSCs, upregulate



**Figure 2. Different cell-derived exosomes in tumor angiogenesis**

(A) TEX-derived ncRNAs regulate ECs and promote/inhibit angiogenesis. (B) TAM-derived exosomal ncRNAs regulate tumor cells and promote angiogenesis. (C) SC-derived exosomal ncRNAs regulate tumor cells and inhibit tumor angiogenesis. The green arrow indicates stimulatory modification; the red “T” indicates inhibitory modification.

VEGF by passing overexpressed lncRNAH19 to ECs to promote angiogenesis and tumor growth.<sup>84</sup>

#### TAM-derived exosomes

Macrophages have two phenotypes with opposite functions, namely, the proinflammatory, antibacterial, and anti-angiogenic M1-like phenotype and the protumour and proangiogenic M2-like phenotype, and TAMs are considered the latter.<sup>85</sup> In the process of tumor progression, the TME leads to hypoxia, and TAMs release factors related to tumor growth, immunosuppression, and angiogenesis through exosomes.<sup>86</sup> TAM-derived exosomes are important regulators of invasiveness in pancreatic ductal adenocarcinoma (PDAC). TAM-derived exosomes are rich in miR-501-3p, and when cocultured with PDAC cells in animal experiments, the migration and invasive ability of PDAC cells are enhanced, and the expression of VEGFA, VEGFR2, angiopoietin-2 (ANG2), and PIGF is significantly upregulated, promoting tumor angiogenesis.<sup>87</sup>

#### TYPES OF EXOSOME-DERIVED ncRNAs IN TUMOR ANGIOGENESIS

Studies have shown that exosome-derived ncRNAs are involved in many aspects of tumor progression and play an important role in tumor angiogenesis. In recent years, an increasing number of studies confirmed that ncRNAs, especially miRNAs, lncRNAs, and circRNAs, are loaded into exosomes to target the TME or distant cells to directly or indirectly regulate angiogenesis by important angiogenic factors and signaling molecules (Table 1).

#### miRNAs

miRNAs are highly conserved posttranscriptional single-stranded ncRNAs with lengths of 21 to 25 nucleotides that regulate gene expression. miRNAs are abundant ncRNAs in exosomes.<sup>123</sup> In recent years, several exosome-derived miRNAs were shown to affect

different targets in the tumor angiogenesis pathway and regulate tumor angiogenesis by directly or indirectly acting on proangiogenic factors or antiangiogenic factors, such as VEGF, HIF, PDGF, and reactive oxygen species (ROS), to cause tumor progression or stagnation.<sup>124</sup> For example, exosome-derived miR-21 is overexpressed in lung cancer, glioma, and head and neck cancer to promote tumor angiogenesis and induce tumor progression.<sup>44,90–92</sup> Other studies found that miR-210 is highly expressed in HCC cells. SMAD4 is a tumor suppressor that can mediate the TGF- $\beta$  signaling pathway. Interestingly, both *in vitro* and *in vivo* studies have shown that miR-210 is packaged into exosomes secreted by HCC cells and transferred to ECs, and then, these mature miR-210 inhibited the expression of SMAD4 and STAT6 in ECs to achieve HCC angiogenesis.<sup>106</sup>

#### lncRNAs

lncRNAs constitute a large RNA subgroup with a size of more than 200 nucleotides with no or limited protein-coding potential.<sup>125–127</sup> lncRNAs play an important role in the regulation of basic pathological and biological processes. In recent years, lncRNAs have been shown to mediate tumor angiogenesis through exosomes under the regulation of VEGF, matrix metalloproteinase (MMP), hypoxia, stem cells, miRNA, and other conditions and are believed to participate in the “angiogenesis switch” as a key regulatory factor.<sup>128</sup> Among them, lncRNAs and lincRNAs are closely related to the regulation of tumor angiogenesis. Some studies revealed that the lincRNAs CCAT2 and POU3F3 and the lncRNA HOTAIR were overexpressed in exosomes secreted by glioma. By acting on target cells, lncRNAs increase the expression of VEGF, TGF- $\beta$  and FGF, thereby promoting angiogenesis and accelerating tumor progression. Simultaneously, lncRNAs upregulate the expression of Bcl-2 and inhibit Bax and caspase-3, thereby inhibiting hypoxia-induced apoptosis.<sup>92,115,117</sup> Therefore, lncRNAs promote angiogenesis through different mechanisms to create a favourable microenvironment for tumor growth and metastasis.

#### circRNAs

circRNAs are naturally occurring and highly conserved single-stranded closed-loop molecules. Due to the lack of 5' and -3' ends and poly(A) tails, circRNAs resist cleavage by RNA enzymes;

**Table 1. Exosome-derived ncRNAs in tumor angiogenesis**

Exosome-derived ncRNA type	Tumor type	Study cell line	Source cell type	Target cell	Regulatory mechanism	Reference
miR-9	Glioma	A172, U87, and U251	Tumor	HUVEC	Upregulation of VEGF and HIF-1 $\alpha$	Chen et al. <sup>88</sup>
miR-9	Melanoma	SK23	Tumor	EC	JAK-STAT pathway	Zhuang et al. <sup>35</sup>
miR-9	NPC	5-8F and CNE1	Tumor	HUVEC	Upregulation of MDK and the PDK/AKT pathway	Lu et al. <sup>89</sup>
miR-16	BC	MSC	SC	BC cell	Downregulation of VEGF	Lee et al. <sup>82</sup>
miR-21	CML	K562 and LAMA84	Tumor	HUVEC	Downregulation of RhoB	Taverna et al. <sup>90</sup>
miR-21	LC	SV40	Tumor	HUVEC	Upregulation of VEGF	Liu et al. <sup>44</sup>
miR-21	HNSCC	FaDu	Tumor	CD14+ human monocytes	Increase the expression of M2 polarization of TAMs markers	Hsieh et al. <sup>91</sup>
miR-21	Glioblastoma	U-251	SC	HUVEC	Upregulation of VEGF	Sun et al. <sup>92</sup>
miR-23a	NPC	CNE2	Tumor	HUVEC	Inhibition of TSGA10 expression	Bao et al. <sup>93</sup>
miR-23b miR-320b	BC	MCF7	Tumor	EC	Inhibition of PLAU, AMOTL1, NRP1, and ETS2 expression	Hannafon et al. <sup>94</sup>
miR-25-3p	CRC	SW480, NCM460, and HCT116	Tumor	EC	Inhibition of KLF2 and KLF4 expression	Zeng et al. <sup>95</sup>
miR-32-5p	HCC	Bel7402 and Bel/5-FU	Tumor	EC	PI3K/AKT pathway	Fu et al. <sup>77</sup>
miR-92a	CML	K562	Tumor	EC	Targets integrin- $\alpha$ 5	Umezu et al. <sup>96</sup>
miR-100	BC	MDA-MB-231 and MCF-7	SC	BC cell	Downregulation of VEGF and HIF-1 $\alpha$	Pakravan et al. <sup>80</sup>
miR-126	ITAC	MNSC	Tumor	HUVEC	Downregulation of VEGF	Tomasetti et al. <sup>97</sup>
miR-130a	GC	SGC-7901	Tumor	HUVEC	Downregulation of c-MYB	Yang et al. <sup>98</sup>
miR-135b	MM	RPMI8226, KMS-11 and U266	Tumor	EC	Inhibition of FIH-1	Umezu et al. <sup>66</sup>
miR-141-3p	EOC	SKOV-3	Tumor	HUVEC	JAK/STAT3 and NF- $\kappa$ B pathways	Masoumi-Dehghi et al. <sup>99</sup>
miR-141-3p miR-375	LARC	-	Not mentioned	Not mentioned	PI3K/Akt pathway	Meltzer, et al. <sup>100</sup>
miR-142-3p	LAC	H1437 and H2073	Tumor	HMEC	Inhibition of TGF $\beta$ R1	Lawson et al. <sup>101</sup>
miR-148a miR-21-5p	OS	SAOS-2, MG-63, and U-2	Tumor	HUVEC	Upregulation of VEGFA	Raimondi et al. <sup>102</sup>
miR-155	BL	Raji	Tumor	ARPE-19	Upregulation of VEGFA	Yoon et al. <sup>103</sup>
miR-155-5p	Melanoma	NIH/3T3	Tumor	CAF	Downregulation of SOCS1 activates the JAK2/STAT3 pathway	Zhou et al. <sup>104</sup>
miR-205	OC	HO-8910	Tumor	EC	PTEN-Akt pathway	He et al. <sup>105</sup>
miR-210	CML	K562	Tumor	EC	Downregulation of EFNA3	Tadokoro et al. <sup>74</sup>
miR-210	HCC	QGY-7703, HepG2, SK-Hep-1, and Huh-7	Tumor	HUVEC	Inhibition of SMAD4 and STAT6 expression	Lin et al. <sup>106</sup>
miR-210	BC	4T1	Tumor	EC	Upregulation of VEGF	Jung et al. <sup>46</sup>
miR-210	LC	NIH/3T3	Tumor	CAF	JAK2/STAT3 pathway	Fan et al. <sup>107</sup>

(Continued on next page)

Table 1. Continued

Exosome-derived ncRNA type	Tumor type	Study cell line	Source cell type	Target cell	Regulatory mechanism	Reference
miR-221-3p	CSCC	SiHa and C33a	Tumor	EC	Downregulation of THBS2	Wu et al. <sup>108</sup>
miR-340	MM	RPMI8226	BMSC	EC	HGF/c-MET pathway	Umezu et al. <sup>109</sup>
miR-376b-3p	Glioma	U87MG	Serum	Not mentioned	Targets HOXD10	Jiang et al. <sup>110</sup>
miR-451a	HCC	SMMC-7721	Tumor	HUVEC	Inhibition of LPIN1 expression	Zhao et al. <sup>111</sup>
miR-494	NSCLC	A549, H1299, and HCC827	Tumor	EC	Akt/eNOS pathway	Mao et al. <sup>67</sup>
miR-501-3p	PDAC	PANC-1 and BxPC-3	TAM	HMEC	Downregulation of TGF- $\beta$ 3	Yin et al. <sup>87</sup>
miR-619-5p	NSCLC	A549, H460, and BEAS-2B	Tumor	HUVEC	Inhibition of RCAN1.4	Kim et al. <sup>112</sup>
linc-CCAT2	Glioma	U87MG	Tumor	HUVEC	Upregulation of VEGFA and TGF- $\beta$	Lang et al. <sup>113</sup>
lnc-CCAT2	NPC	CNE2 and NP69	Tumor	HUVEC	Not mentioned	Zhou et al. <sup>114</sup>
linc-POU3F3	Glioma	A172	Tumor	HBMEC	Upregulation of bFGF, bFGFR, VEGFA and angiogenin	Lang et al. <sup>115</sup>
lncRNA GAS5	LC	A549, H1299, and 95D	Tumor	HUVEC	PTEN-PI3K/Akt pathway	Cheng et al. <sup>116</sup>
lncRNA H19	HCC	Huh7	SC	HUVEC	Upregulation of VEGF	Conigliaro et al. <sup>84</sup>
lncRNA HOTAIR	Glioma	A172	Tumor	HBMVEC	Upregulation of VEGFA	Ma et al. <sup>117</sup>
lncRNA MALAT1	EOC	SKOV3.ip1 and HO8910.PM	Tumor	HUVEC	Upregulation of certain angiogenesis-related genes	Qiu et al. <sup>118</sup>
lncRNA TUG1	CC	HeLa and CaSki	Tumor	HUVEC	Inhibits caspase-3 activity and impacts apoptosis-related proteins	Lei et al. <sup>119</sup>
circRNA-100338	HCC	Hep3B and MHCC97H	Tumor	HUVEC	May upregulate NOVA2	Yang et al. <sup>120</sup>
circRNA- CMTM3	HCC	Huh7, Hep3B, HCCLM3, SK-HEP-1, MHCC97, and THLE-2	Tumor	HUVEC	Sponges miR-3619-5p and disinhibits SOX9	Hu et al. <sup>121</sup>
HGF siRNA	GC	HEK293T	HEK293T	GC	Inhibition of HGF and VEGF expression	Zhang et al. <sup>122</sup>

HUVEC, human umbilical vein endothelial cell; MM, multiple myeloma; NPC, nasopharyngeal carcinoma; MDK, midkine; BC, breast cancer; MSC, mesenchymal stem cell; CML, chronic myelogenous leukemia; RhoB, ras homolog family member B; LC, lung cancer; HNSCC, human head and neck squamous cell carcinoma; CRC, colorectal cancer; HCC, hepatocellular carcinoma; ITAC, intestinal-type sinonasal adenocarcinoma; GC, gastric cancer; FIH-1, factor-inhibiting HIF-1; EOC, epithelial ovarian cancer; LARC, locally advanced rectal cancer; LAC, lung adenocarcinoma; PTEN, phosphatase and tensin homolog; TGF- $\beta$ 1, transforming growth factor- $\beta$  receptor 1; HMEC, human microvascular endothelial cell; OS, osteosarcoma; BL, Burkitt's lymphoma; ARPE-19, retinal pigment epithelial cells-19; OC, ovarian cancer; EFNA3, ephrin A3; CAF, cancer-associated fibroblast; CSCC, cervical squamous cell carcinoma; THBS2, thrombospondin-2; BMSC, bone marrow stromal cell; NSCLC, non-small-cell lung cancer; PDAC, pancreatic ductal adenocarcinoma; TAM, tumor-associated macrophage; RCAN1.4, calcineurin 14; HBMEC, human brain microvascular endothelial cell; HBMVEC, human brain microvascular endothelial cell; CC, cervical cancer; HEK293T, human embryonic kidney 293T cells; HGF hepatocyte growth factor.

thus, circRNAs are more stable than linear RNA.<sup>10</sup> Research investigating the biological functions of circRNAs in recent years has shown that they play an important role in tumorigenesis, reproduction, metastasis, and invasion, especially in tumor angiogenesis. Some studies noted that abnormal expression of circRNAs is an important regulator of angiogenesis by regulating various cancer markers. This process may be determined by exosomes because they play a key role in regulating the cross talk between normal

cells and cancer cells in the TME.<sup>129,130</sup> Recent studies have shown that circRNA-100338 is overexpressed in exosomes secreted by HCC cells. Exosome-derived circRNA-100338 binds 14 RNA-binding proteins in human umbilical vein endothelial cells (HUVECs), especially NOVA2, and is thought to regulate vascular development and lumen formation, thereby stimulating angiogenesis.<sup>131</sup> Thus, circRNAs play a key role in maintaining and promoting tumor progression.

## DUALITY OF EXOSOME-DERIVED ncRNAs IN TUMOR ANGIOGENESIS

Tumor angiogenesis is a process involving the pathological proliferation of new capillaries in the pre-existing vascular system. This process is controlled by various growth factors and different signal transduction pathways and depends on the balance of proangiogenic and antiangiogenic factors. Exosome-derived ncRNAs play an important role in mediating intercellular communication. Numerous studies have found that ncRNAs play a dually important role in the process of destroying the balance of angiogenesis (Table 2).

In recent years, studies have indicated that exosome-derived ncRNAs directly regulate the expression of the VEGF gene, increase vascular permeability and density, accelerate tumor angiogenesis, and promote tumor growth and metastasis. For example, the lncRNAs HOTAIR and VEGFA have been found to be overexpressed and coexpressed in gliomas. Further studies showed that the lncRNA HOTAIR is transported into ECs through exosomes and then directly targets the VEGFA promoter to promote VEGFA transcription, thereby accelerating the proliferation and migration of ECs to stimulate angiogenesis.<sup>117</sup> Similarly, studies reported that overexpressed miR-155 promotes angiogenesis by transferring exosomes to retinal epithelial pigment cells and upregulating the expression of VEGFA.<sup>103</sup>

In contrast to the angiogenic effect mentioned above, exosome-derived ncRNAs also play an important antiangiogenic role due to their anticancer activity. Some studies showed that miR-23b and miR-320b were overexpressed in exosomes in breast cancer MCF7 cells after treatment with docosahexaenoic acid (DHA). After acting on ECs, the expression of angiogenic target genes (PLAU, AMOTL1, NRP1, and ETS2) is reduced, and endothelial cell tube formation is inhibited.<sup>94</sup> In addition, exosomes secreted by nasopharyngeal carcinoma cells are rich in miR-9, which directly targets the angiogenic gene MDK in ECs and interferes with PDK1/Akt, a proangiogenic pathway. Further studies showed that the expression of exosome-derived miR-9 is negatively correlated with the microvessel density.<sup>89</sup>

Interestingly, the same ncRNAs were found to play dual roles in tumor angiogenesis. In recent years, miR-21 has attracted widespread attention due to its important role in regulating tumor angiogenesis and is enriched in exosomes secreted by various cells. For example, overexpressed exosome-derived miR-21 promotes the formation of the tubular structure of HUVECs by upregulating the level of VEGF in human bronchial ECs.<sup>44</sup> In addition, glioma stem cells promote angiogenesis by overexpressing exosome-derived miR-21 to induce an increase in VEGF in ECs.<sup>92</sup> However, in leukemia, overexpressed exosome-derived miR-21 plays an antiangiogenic role by targeting ECs to inhibit RhoB gene expression and then negatively regulate EC movement.<sup>90</sup>

The regulation of angiogenesis by different exosome-derived ncRNAs in the same tumor also displays duality. For example, in breast cancer, in animal experiments, MSC exosome-derived miR-16 acts directly

on breast cancer cells while reducing the expression of VEGF, showing antitumor angiogenesis and inhibiting tumor growth.<sup>82</sup> In addition, exosomes secreted by breast cancer cells under hypoxia contain a large amount of miR-210. miR-210 is overexpressed in ECs and downregulates the ephrin-A3 and PTP1B genes, which inhibit vascular remodeling. Therefore, exosome-derived miR-210 promotes angiogenesis.<sup>74</sup> These findings reveal the potential role of exosome-derived ncRNAs in breast cancer and provide novel insight into breast cancer prognosis and therapeutic strategies.

## ROLES OF EXOSOME-DERIVED ncRNAs IN THE REGULATION OF ANGIOGENESIS IN DIFFERENT CANCERS

Angiogenesis plays a critical role in the occurrence, development and even prognosis of tumors. Abnormal angiogenesis has been confirmed to be related to human cancer.<sup>132,133</sup> In fact, tumor angiogenesis mostly begins with the release of molecules from tumor cells that signal to promote vascular growth and lead to a local imbalance between stimulating and inhibiting angiogenesis.<sup>134</sup> It has been confirmed that exosomes can participate in the regulation of tumor angiogenesis through ncRNAs to promote tumor progression.<sup>135</sup> Numerous studies have suggested that abnormal ncRNA expression in multiple organs in different human systems activates angiogenic signal pathways in ECs to stimulate angiogenesis, leading to the occurrence and progression of cancer.<sup>134</sup> The function and potential application of exosome-derived ncRNAs in regulating angiogenesis in cancer are worthy of affirmation and may be gradually proven in future studies.

### Digestive system

#### Liver cancer

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver. Previous studies have shown that the expression of miR-451a is downregulated in many human cancers, which is related to tumor suppression. Recent studies showed that exosome-derived miR-451a, as a tumor suppressor, specifically targets LPIN1 to induce apoptosis in HCC cells and HUVECs, thereby inhibiting angiogenesis and, thus, the occurrence of HCC.<sup>111</sup> H19 is closely related to the occurrence of liver cancer, liver metastasis, and angiogenesis. In another study, CD90+ hepatoma cells were shown to be rich in lncRNAH19, which was transferred to ECs through exosome release, promoting an angiogenic phenotype and cell-to-cell adhesion, stimulating angiogenesis, and promoting cell-cell interactions.<sup>84</sup> These studies indicate the important role of exosome-derived ncRNAs in HCC and shed light on the pathogenesis of HCC, providing targets for the development of future therapy.

#### Gastric cancer

C-MYB, a transcription factor, plays an important role in angiogenesis. miR-130a is overexpressed in exosomes from gastric cancer (GC) cells and acts as a driver of angiogenesis by downregulating the C-MYB protein by targeting 3'-UTR C-MYB mRNA in HUVECs, thereby promoting angiogenesis and tumor growth.<sup>98</sup> HGF siRNA packaged in exosomes could be transported to GC cells to

**Table 2. Duality of exosome-derived ncRNAs in tumor angiogenesis**

Function	Exosome-derived ncRNA type	Tumor type	Dysregulation	Reference
Proangiogenesis	miR-9	Melanoma	Up	Zhuang et al. <sup>35</sup>
	miR-21	LC	Up	Liu et al. <sup>44</sup>
	miR-23a	NPC	Up	Bao et al. <sup>93</sup>
	miR-25-3p	CRC	Up	Zeng et al. <sup>95</sup>
	miR-32-5p	HCC	Up	Fu et al. <sup>77</sup>
	miR-92a	CML	Up	Umezu et al. <sup>96</sup>
	miR-130a	GC	Up	Yang et al. <sup>98</sup>
	miR-135b	MM	Up	Umezu et al. <sup>66</sup>
	microR-141-3p	EOC	Up	Massoumi-Dehghi et al. <sup>99</sup>
	miR-142-3p	LAC	Up	Lawson et al. <sup>101</sup>
	miR-148a miR-21-5p	OS	Up	Raimondi et al. <sup>102</sup>
	miR-155	BL	Up	Yoon et al. <sup>103</sup>
	miR-155-5p	Melanoma	Up	Zhou et al. <sup>104</sup>
	miR-205	OC	Up	He et al. <sup>105</sup>
	miR-210	CML	Up	Tadokoro et al. <sup>74</sup>
	miR-221-3p	CSCC	Up	Wu et al. <sup>108</sup>
	miR-494	NSCLC	Up	Mao et al. <sup>67</sup>
	miR-501-3p	PDAC	Up	Yin et al. <sup>87</sup>
	miR-619-5p	NSCLC	Up	Kim et al. <sup>112</sup>
	lnc-CCAT2	NPC	Up	Zhou et al. <sup>114</sup>
	linc-POU3F3	Glioma	Up	Lang et al. <sup>115</sup>
	lncRNA HOTAIR	Glioma	Up	Ma et al. <sup>117</sup>
	lncRNA MALAT1	EOC	Up	Qiu et al. <sup>118</sup>
	lncRNA TUG1	CC	Up	Lei et al. <sup>119</sup>
	circRNA-CMTM3	HCC	Up	Hu et al. <sup>121</sup>
	Antiangiogenesis	miR-9	NPC	Down
miR-16		BC	Down	Lee et al. <sup>82</sup>
miR-21		CML	Down	Taverna et al. <sup>90</sup>
miR-23b miR-320b		BC	Down	Hannafon et al. <sup>94</sup>
miR-126		ITAC	Down	Tomasetti et al. <sup>97</sup>
miR-340		MM	Down	Umezu et al. <sup>109</sup>
miR-376b-3p		Glioma	Down	Jiang et al. <sup>110</sup>
miR-451a		HCC	Down	Zhao et al. <sup>111</sup>
lncRNA GAS5		LC	Down	Cheng et al. <sup>116</sup>

significantly downregulate the expression of HGF (which promotes the growth of tumor cells and vascular cells), thereby inhibiting angiogenesis and cancer cell and vascular cell proliferation and migration.<sup>122</sup> Therefore, antiexosome-derived ncRNAs might be a novel antiangiogenic therapeutic strategy for gastric cancer.

#### Colorectal cancer

In cancer cells from patients with colorectal cancer (CRC) with tumor metastasis, miR-25-3p is abundant and is transferred to ECs and internalized by exosomes. Targeted KLF2 and KLF4 promote vascular

permeability and angiogenesis by upregulating the expression of VEGFR2, ZO-1, occludin and claudin5. Animal experiments confirmed that miR-25-3p enhanced the metastasis of CRC cells to the liver and lung by inducing vascular leakage.<sup>95</sup> Therefore, exosome-derived miR-25-3p is a novel biomarker with promising applications in the clinical diagnosis of CRC.

#### Pancreatic cancer

The most common type of pancreatic cancer is PDAC. Exosome-derived miR-501-3p in M2 macrophages is overexpressed under



hypoxia and inhibits the tumor suppressor TGFBR3 gene to promote PDAC cell migration, invasion, and angiogenesis by activating the TGF- $\beta$  signaling pathway.<sup>87</sup> Therefore, exosome-derived miR-501-3p could be a potential therapeutic pathway in PDAC.

## Respiratory system

### Lung cancer

Lung cancer (LC) is the leading cause of cancer death worldwide. Studies have confirmed that exosomes in lung adenocarcinoma cells accumulate a large amount of miR-142-3p, which is targeted to inhibit TGF- $\beta$  receptor 1 and promote angiogenesis when transferred to ECs. In addition, promoting the phenotype of cancer-related fibroblasts in lung fibroblasts is not related to TGF- $\beta$  signaling.<sup>101</sup> Exosomes of human bronchial epithelial (HBE) cells transformed by cigarette smoke extract (CSE) were rich in miR-21 and transferred to normal HBE cells through exosomes, resulting in STAT3 activation and the upregulation of VEGF expression in HBE cells, which, in turn, led to angiogenesis in HUVECs and the malignant transformation of HBE cells,<sup>44</sup> indicating the critical regulatory role of exosomes in LC angiogenesis.

### Nasopharyngeal carcinoma

Exosome-derived miR-9 from nasopharyngeal carcinoma (NPC) cells plays a special role in the cross talk between tumors and the microenvironment. miR-9 directly inhibits the target gene MDK in ECs, which is positively related to microvessel density and prevents the formation and migration of blood vessels through the PDK/Akt signaling pathway.<sup>89</sup> In another study, researchers established an animal model to confirm that miR-23a in NPC is highly related to the microvessel density, and the overexpression of exosome-derived miR-23a in NPC directly targets testis-specific gene antigens to promote angiogenesis *in vivo* and *in vitro*.<sup>93</sup> These findings suggest that miR-9 or sequence-specific inhibitors targeting miR-23a may serve as novel therapeutic avenues for NPC.

### Sinonasal cancer

A study reported that intestinal-type sinonasal adenocarcinomas (ITACs), which account for approximately 8%–25% of SNCs, are malignant epithelial tumors related to sawdust contact. The ectopic expression of exosome-derived miR-126 from HUVECs inhibits malignant nasal septum carcinoma cell growth and affects cell metabolism, significantly reducing VEGF gene expression associated with reduced colony formation and inhibiting cell migration and proliferation.<sup>97</sup> However, the correlation between miR-126 and HUVECs and the specific mechanism require further experimental studies.

## Blood system

### Multiple myeloma

Some studies have shown that numerous proliferating multiple myeloma (MM) cells lead to hypoxia, and miR-135b in the exosomes of MM cells is significantly upregulated. After being transported to ECs, miR-135b inhibits (FIH-1 and activates the HIF-FIH signaling pathway to enhance angiogenesis under hypoxic conditions.<sup>66</sup> As a

consequence, miR-135b may be a target for controlling MM angiogenesis.

### Burkitt's lymphoma

Exosomes of human EBV-positive Burkitt's lymphoma (BL) cells (Raji) contain a large amount of miR-155, and Raji transfers overexpressed miR-155 into retinal epithelial pigment cells (ARPE-19) and induces angiogenesis by enhancing VEGFA transcription and translation,<sup>103</sup> providing a new method for monitoring the transfer of BL.

### Chronic myeloid leukemia

Some studies have shown that miR-210 in the exosomes of the human leukemia cell line K562 were significantly increased under hypoxia and promoted the angiogenic activity of ECs by downregulating the receptor tyrosine kinase ligand ephrin-A3, thereby promoting the tube formation of HUVECs.<sup>74</sup> In addition, based on fluorescence labeling, the exosomes released by K562 cells contain a large amount of miR-92a, which specifically inhibits the expression of the integrin  $\alpha 5$  gene in HUVECs and promotes angiogenesis.<sup>96</sup> This finding may suggest that exosome-derived ncRNAs have the capacity to predict prognosis and guide the use of drugs in the treatment of chronic myeloid leukemia CML.

## Reproductive system

### Epithelial ovarian cancer

In cancer cells from patients with epithelial ovarian cancer (EOC) with tumor metastasis, the lncRNA MALAT1, which is abundant in exosomes, is transferred to HUVECs through exosomes and internalized to promote angiogenesis. By use of a MALAT1 gene knockout experiment, this proangiogenic activity was greatly weakened.<sup>118</sup> With further understanding of the relationship between ncRNAs and angiogenesis, the lncRNA MALAT1 may provide insight into personalized and accurate treatment for EOC.

### Cervical cancer

A recent study found that the lncRNA TUG1 has a significant upregulation effect in serum exosomes from patients with cervical cancer (CC), and for the first time, it was confirmed that CC cells can transfer the lncRNA TUG1 to HUVECs through exosomes by inhibiting caspase-3 activity and affecting apoptosis-related proteins to promote tumor angiogenesis and HUVEC proliferation.<sup>119</sup> Therefore, the lncRNA TUG1 may be used to evaluate the prognosis of patients with CC.

## Endocrine system

### Breast cancer

Exosomes secreted by hypoxic breast cancer (BC) cells mediate the entry of miR-210 into ECs to inhibit the expression of the ephrin-A3 and PTP1B genes, which increase VEGF and promote VEGF-mediated EC recruitment, thereby promoting the formation of new capillaries and tubular structures.<sup>13</sup> In addition, exosome-derived miR-100 from MSCs induces the decreased expression of VEGF and inhibits angiogenesis and tumor growth by regulating the mammalian target of rapamycin (mTOR)/HIF-1 $\alpha$  signal

transduction axis in BC cells; however, exosome-derived miR-100 from MSCs downregulates the metastasis of BC cells and downregulates VEGF in a time-dependent manner.<sup>80</sup> These studies provide a new perspective regarding intervention strategies to prevent the carcinogenesis of BC.

### Other cancers

#### **Glioma**

Glioma cells are rich in linc-CCAT2, which is absorbed by ECs through exosome release. Its overexpression activates VEGF and TGF- $\beta$ , promotes angiogenesis and the expression of Bcl-2, and inhibits the expression of Bax and caspase-3, thereby reducing apoptosis.<sup>113</sup> Another study confirmed that exosomes derived from glioma stem cells promote the angiogenesis of ECs by carrying miR-21 and VEGF to ECs and then stimulating the miR-21/VEGF/VEGFR2 signaling pathway.<sup>92</sup> These findings suggest that exosome-derived ncRNAs are of great interest in glioma.

#### **Human head and neck squamous cell carcinoma**

Snail is a recruitment transcription factor that promotes TAM activation, which induces the epithelial-mesenchymal transformation and leads to tumor progression and metastasis. The exosomes of human head and neck squamous cell carcinoma (HNSCC) cells are rich in miR-21, which is directly activated and expressed by Snail and captured by CD14+ human monocytes, thereby increasing the polarized expression of M2 labeled by TAMs, promoting angiogenesis, increasing tumor invasion, and accelerating tumor progression.<sup>91</sup> These exosome-derived ncRNAs may serve as candidate biomarkers of tumor progression.

#### **Melanoma**

miR-9 derived from melanoma exosomes effectively promotes tumor angiogenesis by downregulating the level of suppressor of cytokine signal 5 (SOCS-5) and activating JAK-STAT as a carcinogenic signaling pathway.<sup>35</sup> These findings may provide insight into the clinical treatment of melanoma.

### POTENTIAL CLINICAL APPLICATION OF EXOSOME-DERIVED ncRNAs

In recent years, exosome-derived ncRNAs have been shown to have great potential in antitumor clinical applications due to their important role in the key steps of tumor angiogenesis and may be used to identify tumors and even change tumor progression. Exosomes have similar proteins and lipids as their parent cells on the surface, which not only successfully transfer the ncRNAs that they carry to recipient cells but also aid in evading autoimmunity. In addition, exosomes and the ncRNAs they carry are highly stable and exhibit strong biological activity; thus, they play an important role.<sup>136</sup> Therefore, exosome-derived ncRNAs are considered a new tool that could improve cancer diagnosis, treatment, and prognosis.

#### **Tumor biomarkers**

The application of biomarkers is of great value in the diagnosis and prognosis of tumors, and the search for reliable biomarkers is signif-

icant for the early treatment of tumors.<sup>137</sup> Numerous exosome-derived ncRNAs that affect tumor angiogenesis are overexpressed in patients with specific tumors, and their content in bodily fluids is high and stable; thus, they are easily evaluated in clinical practice. Therefore, exosome-derived ncRNAs are used as potential biomarkers for the detection of different malignant tumors (Figure 3).

As a diagnostic indicator, miR-23a is higher in serum exosomes from patients with LC, especially in patients with hypoxic LC, than in normal controls. *In vitro* experiments have shown that the expression level of these exosome-derived miRNAs in hypoxic tumor cell lines is higher than that in normoxic tumor cell lines. A positive correlation exists between the level of exosome-derived miR-23a and angiogenic activity; thus, exosome-derived miR-23a is used as a biomarker for the personalized diagnosis of LC.<sup>65,138</sup> In addition, exosome-derived miR-21 reportedly promotes angiogenesis in HNSCC by participating in the mechanism of EMT-mediated M2 polarization and is listed as a candidate biomarker for the diagnosis of tumor progression.<sup>91</sup>

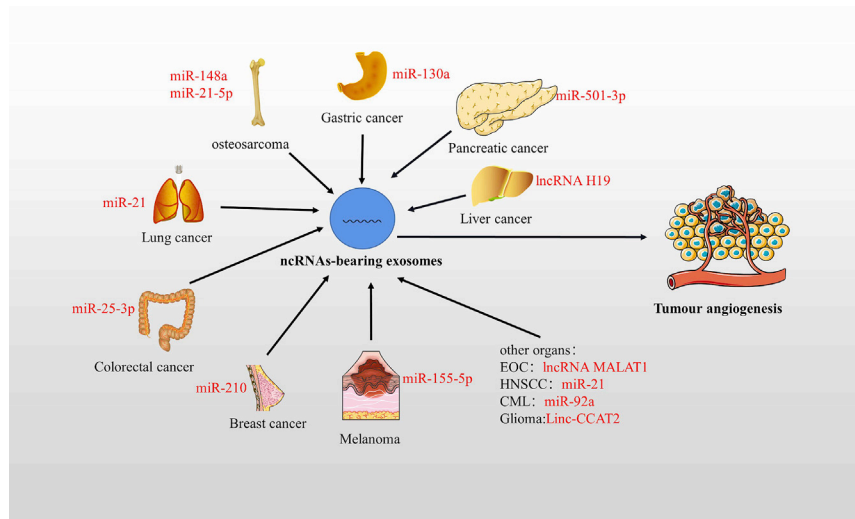
Exosome-derived ncRNAs are also used as prognostic indicators. A study found that circRNA-100338 is significantly increased in serum exosomes from patients with lung metastasis after HCC surgery, and these patients significantly differ from healthy patients, demonstrating that patient prognosis is poor. The sensitivity and specificity of exosome-derived circRNA-100338 are better than those of  $\alpha$ -fetoprotein (AFP), which is a traditional marker; therefore, exosome-derived circRNA-100338 is used as a potential prognostic index.<sup>120</sup>

Generally, exosome-derived ncRNAs are expected to be used as new noninvasive biomarkers in the diagnosis and prognosis of cancer. However, the small size of the experimental sample was not ideal, and the credibility of a single molecule is limited. The potential of exosome-derived ncRNAs as cancer biomarkers must be further verified in more patients with cancer.

#### **Therapeutic targets**

Humans never stop exploring new ways to treat cancer. Aggressive malignant tumors must be powered by pathological angiogenesis. Clinical data have shown that blocking angiogenesis delays tumor progression. Studies have shown that exosome-derived ncRNAs regulate tumor angiogenesis. Therefore, with the in-depth understanding of exosome-derived ncRNAs, the use of their precise targeting to inhibit tumor-angiogenesis-related cells or molecules has become a new means of cancer treatment.

siRNAs are short (21–23 nucleotides in length), double-stranded, noncoding RNAs with an mRNA sequence and an antisense active strand.<sup>139</sup> siRNAs mediate RNA interference (RNAi) to specifically knock out the expression of a target gene. The chemical synthesis of siRNA for clinical use is considered a promising therapeutic tool that acts on sequence-specific endonuclease cleavage of mRNA and leads to specific gene silencing.<sup>140</sup> However, because siRNAs easily decompose *in vivo*, they are generally loaded into exosomes and transported to target cells. Exosomes transmit siRNA to target cells



**Figure 3. Exosome-derived ncRNAs as tumor biomarkers**

Increasing special exosome-derived ncRNAs are stable and abundant in bodily fluids, indicating that they can be potential biomarkers of cancer.

cific exosome-derived ncRNA biomarkers has become a new hot topic in recent years.

In summary, exosome-derived ncRNAs affect tumor progression by regulating tumor angiogenesis. With the in-depth exploration of high-throughput sequencing technology and bioinformatics analyses in the medical field, an increasing number of exosome-derived ncRNAs from different types of cells will be identified and detected, which could further help us understand the mechanism promoting or inhibiting

and interfere with the expression of angiogenesis-related molecules by gene silencing, thereby playing an important role in affecting tumor angiogenesis.<sup>12</sup> Exosome-derived HGF siRNA reportedly inhibits the growth and angiogenesis of gastric cancer. HGF is a tumor marker that upregulates VEGF. Exosome-derived HGF siRNA acts on GC cells and inhibits the proliferation and migration of cancer cells and vascular cells by inducing the HGF/c-Met signal transduction pathway to downregulate VEGF expression.<sup>122</sup> Although the therapeutic effect on exosome-derived siRNA cancer is not yet mature, this method has produced surprising results. Exosome-derived siRNAs may serve as key targets for tumor treatment and open up new directions for future research concerning tumour-targeted therapy.

In addition, some drugs stimulate an increase or decrease in ncRNAs in tumor exosomes to regulate specific genes or pathways to affect tumor angiogenesis.<sup>122</sup> For example, CML cells treated with curcumin overexpress miR-21 in their exosomes, and then, miR-21 is internalized in ECs and directly downregulates the expression of RhoB, which reduces the angiogenesis ability of HUVECs.<sup>90</sup> Therefore, research investigating the targets of exosome-derived ncRNAs as a breakthrough could provide new insight into targeted cancer therapy in the future, and research concerning these topics could become a potential way to prevent and control cancer metastasis.

## CONCLUSION AND FUTURE PERSPECTIVES

Some of the latest research results concerning exosomes and ncRNAs are inspiring. For example, the commonly used exosomal biomarkers differ and do not show universal utility in different cell types.<sup>141</sup> In addition, specific oncogenes promote the release of exosomes and change secreted ncRNAs, such as miRNAs, to reduce the biomass of cancer cells.<sup>142</sup> Recent discoveries related to various ncRNAs that promote the disturbance of a tumor's internal environment have generated discussions regarding the related mechanisms.<sup>143,144</sup> Therefore, the early detection of tumor occurrence and progression by evaluating spe-

ing tumor angiogenesis. Therefore, the development of drugs targeting exosome-derived ncRNAs may represent a new method for the treatment of tumors. However, the role of exosome-derived ncRNAs in angiogenesis is a new field of cancer research, and only a few have a definite function or clinical application. We are currently limited to hypothesizing most functions of exosome-derived ncRNAs, and thus, we still need to explore the detailed mechanism. Exosome-derived ncRNAs show great potential as tumor markers and tumor treatment, but more studies are needed before advanced treatments for cancer are successfully developed and widely used in the clinical field. For example, the challenge of low exosome production needs to be solved in advance, and several strategies, such as serum starvation, have been explored to maximize the harvest. Because of its ability to promote autophagy and apoptosis, serum starvation has become a method that can promote the degree and content of pure exosomes and ensure the biological activity of exosomes, but this method also has the disadvantages of low efficiency and long time consumption. In addition, how to isolate and purify exosomes while retaining only ncRNAs with antitumor angiogenesis properties is a key problem to be solved. We believe that the use of exosome-derived ncRNAs as noninvasive fluid biopsy and noninvasive biomarkers for the early diagnosis of tumors, treatment of cancer, and prognosis prediction is a challenging but interesting task for the future that needs further exploration by researchers and clinicians.

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### AUTHOR CONTRIBUTIONS

K.Y. wrote the manuscript and created the figures. Z.S. and W.Y. provided direction and guidance throughout the preparation of this manuscript. B.Q. and B.S. reviewed and made significant revisions to the manuscript. Q.Z., S.H., and G.W. collected and prepared the related papers. All authors read and approved the final manuscript.

### DECLARATION OF INTERESTS

The authors declare no competing interests.

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