New Insights Into the Regulatory Roles of Extracellular Vesicles in Tumor Angiogenesis and Their Clinical Implications

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Angiogenesis is required for tumor growth and development. Extracellular vesicles (EVs) are important signaling entities that mediate communication between diverse types of cells and regulate various cell biological processes, including angiogenesis. Recently, emerging evidence has suggested that tumor-derived EVs play essential roles in tumor progression by regulating angiogenesis. Thousands of molecules are carried by EVs, and the two major types of biomolecules, noncoding RNAs (ncRNAs) and proteins, are transported between cells and regulate physiological and pathological functions in recipient cells. Understanding the regulation of EVs and their cargoes in tumor angiogenesis has become increasingly important. In this review, we summarize the effects of tumor-derived EVs and their cargoes, especially ncRNAs and proteins, on tumor angiogenesis and their mechanisms, and we highlight the clinical implications of EVs in bodily fluids as biomarkers and as diagnostic, prognostic, and therapeutic targets in cancer patients.

Keywords: extracellular vesicles, tumor angiogenesis, miRNAs, lncRNAs, CircRNAs, proteins

1 INTRODUCTION

Angiogenesis, defined as the establishment of new blood vessels from pre-existing vascular networks, is triggered by proangiogenic factors and depends on the proliferation and migration of endothelial cells (ECs) (Teleanu et al., 2019; Lugano et al., 2020). In normal healthy tissues, angiogenesis is tightly regulated by a balance that is maintained between proangiogenic and antiangiogenic factors. Solid tumors are generally characterized with aberrant angiogenesis, and tumor angiogenesis is critically required for tumor growth and development (Teleanu et al., 2019; Lugano et al., 2020). Many proangiogenic factors are upregulated in tumor cells and tumor-associated stromal cells, including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and delta ligand-like 4 (Dll4). Hypoxia is a key inducer of tumor angiogenesis and promotes the expression of various proangiogenic factors in the tumor microenvironment (Abou Khouzam et al., 2020). Recently, antiangiogenic drugs have been widely applied to the treatment of multiple solid cancers, and cancer patients have gained tremendous survival benefits from antiangiogenic therapy.

Extracellular vesicles (EVs), such as microvesicles and exosomes, are nanosized vesicles with lipid membranes that are secreted by most cells. EVs contain many bioactive molecules, such as microRNAs (miRNAs), long noncoding RNAs (lncRNAs), circular RNAs (circRNAs), and proteins, and these EV cargoes regulate intercellular communication (Mathieu et al., 2019; Liu...
et al., 2021). Donor cell-derived EVs are taken up by recipient cells, and the encapsulated bioactive components are thus delivered to recipient cells, enabling their regulation of recipient cell biological behaviors. An increasing number of studies have demonstrated that EVs play important roles in tumorigenesis, tumor growth, metastasis, immune evasion, drug resistance, and angiogenesis (Todorova et al., 2017; Aslan et al., 2019). Tumor-derived EVs can transfer proangiogenic molecules into ECs to promote their angiogenic activity via various mechanisms such as VEGF/VEGF Receptor (VEGF/VEGFR), Notch, Wingless-type (WNT), and Hypoxia-inducible factor (HIF) signaling pathway (Phng et al., 2009; Horie et al., 2017; Todorova et al., 2017; Aslan et al., 2019). Thus, targeting EVs might be an innovative and promising therapeutic strategy to inhibit tumor angiogenesis.

A wide variety of biomolecules, including ncRNAs and proteins, have been identified as EV cargoes, and these signaling molecules can be transported from donor cells to recipient cells. To date, considerable attention has been directed to the effects of EVs on tumor angiogenesis and the clinical relevance of these effects. A database of exosomes (http://www.exocarta.org/) includes 9,769 proteins, 3,408 miRNAs, and 2,838 miRNAs. The mechanisms triggered by these specific cargos loaded into EVs and delivered from donor cells to acceptor cells are complex (Abels and Breakfield, 2016; Mathieu et al., 2019). This article summarizes the current knowledge on the roles of tumor-derived EVs in angiogenesis, with a particular emphasis on the molecular mechanisms involved. We also discuss the main prospects for their applications in cancer diagnosis, prognosis, and treatment.

2. EXTRACELLULAR VESICLES AND TUMOR ANGIOGENESIS

2.1 EV-Derived ncRNAs and Tumor Angiogenesis

Here, we focus on the effects and mechanisms of EV-derived miRNAs, IncRNAs, and circRNAs on angiogenesis, aiming to elucidate their potential as tumor biomarkers and therapeutic targets for tumor angiogenesis.

2.1.1 miRNAs

Various miRNAs are packaged into tumor-derived EVs and can be transferred into recipient ECs (Muralidharan-Chari et al., 2009). Once internalized by ECs, these miRNAs can initiate an angiogenic switch by modulating EC proliferation and migration and regulating the expression of angiogenesis-related genes (Huang et al., 2020a; Li et al., 2020; Masoumi-Dehghi et al., 2020).

VEGF/VEGFR and HIF signaling pathways are the main targets of miRNAs that regulate angiogenesis. Exosomal miR-130a secreted by gastric cancer (GC) cells targeted c-MYB in ECs and promoted angiogenesis in vitro and in vivo (Yang et al., 2018). Similarly, GC cell-derived exosomal miR-155 downregulated c-MYB but increased the expression of VEGF in ECs, which enhanced EC tube formation and increased microvessel density in xenografted tumors (Deng et al., 2020). Moreover, inhibition of miR-21 in the EVs from colon cancer cells reduced angiogenesis in vivo (Wang et al., 2014). MiR-21 expression levels were upregulated in ECs from tumor tissue compared to normal tissue (Huang et al., 2016a). MiR-21 negatively regulated the expression of VEGF in ECs, suggesting its inhibitory role in angiogenesis (Huang et al., 2016b). MiR-21 levels were significantly increased in EVs from hypoxic ECs compared to normoxic ECs (Liu et al., 2016). MiR-21 targeted VEGFR and inhibited VEGF signaling in ECs (Huang et al., 2016c). MiR-21 inhibition promoted angiogenesis in vitro and in vivo (Huang et al., 2016d).

2.1.2 IncRNAs

Tumor-secreted EV-derived IncRNAs can be transmitted to ECs where they promote the expression of proangiogenic genes and initiate angiogenesis by either binding to endogenous miRNAs or interacting with mRNAs and proteins (Ma et al., 2017; De Los Santos et al., 2019; Zhang et al., 2020). For example, IncRNA–H19 functions as an oncogene and is upregulated in multiple types of cancer (Leempridee, 2017). Exosomes derived from CD90+ liver cancer cells induced angiogenesis in vitro and in vivo (Yang et al., 2018).
TABLE 1 | The effects and mechanisms of miRNAs, lncRNAs, and circRNAs derived from tumor EVs on angiogenesis.

| Cargoes | Cancer types | Recipient cells | Target genes or signaling pathways | Functions | References |
|---------|--------------|----------------|------------------------------------|-----------|------------|
| **miRNAs** | |  | |  | |
| miR-9 | NPC | HUVECs | MDK, PDK/Akt pathway | Inhibition | Lu et al. (2018) |
| miR-17-5p | NPC | HUVECs | COL18A1, THBS2, PTCH1, PHD3, HIF-1α, VEGF | Promotion | Duan et al. (2019) |
| miR-21 | ESCC | HUVECs | SPRY1 | Promotion | Zou et al. (2020) |
| miR-21-5p | NPC | HUVECs | SPRY1 | Promotion | Zou et al. (2020) |
| miR-25-3p | CRC | HUVECs | KLF2, KLF4, VEGFR2, ZO-1, Occludin, Claudin5 | Promotion | Zeng et al. (2018) |
| miR-26a | Gioma stem cells | HBE4Os | PTEN, PI3K/Akt pathway | Promotion | Wang et al. (2019c) |
| miR-27a | PC | HMECs | BeRG2 | Promotion | Li et al. (2019) |
| miR-92a-3p | Retinoblastoma | HUVECs | KLF2 | Promotion | Hou et al. (2021) |
| miR-130a | GC | HUVECs | c-MYB | Promotion | Yang et al. (2018) |
| miR-130b-3p | GC | HUVECs | PTEN | Promotion | Yan et al. (2021) |
| miR-135b | GC | HUVECs | PTEN | Promotion | Bai et al. (2019) |
| miR-135b-5p | CAFs from CRC | HUVECs | TXNIP | Promotion | Yin et al. (2021) |
| miR-141 | EOC | HUVECs | SOCS1, JAK2/STAT axis, VEGFA, FGFR, MMP9 | Promotion | Zhou et al. (2018) |
| miR-148a-3p | GC | HUVECs | c-MYB/VEGFA axis | Promotion | Deng et al. (2020) |
| miR-155 | GC | HUVECs | PTEN/Akt pathway | Promotion | Fan et al. (2020) |
| miR-155-5p | M2 macrophages | MAECs | Targets E2F2 in PDAC | Promotion | Yang et al. (2021) |
| miR-181a | Hypoxic PTC | HUVECs | DACT2, ML13, YAP/VEGFA axis | Promotion | Wang et al. (2021b) |
| miR-182-5p | Hypoxic GBM | HUVECs | KLF2, KLF4, VEGFR, ZO-1, occludin, claudin5 | Promotion | Li et al. (2020) |
| miR-205 | OC | HUVECs | PTEN/Akt pathway | Promotion | He et al. (2019) |
| miR-210 | LC | CAFs | JAK2/STAT3 | Promotion | Lin et al. (2018) |
| miR-210-3p | OSCC | HUVECs | SMAD4, STAT5 | Promotion | Wang et al. (2020a) |
| miR-211-3p | OSCC | HUVECs | SMAD4, STAT5 | Promotion | Wu et al. (2019b) |
| miR-378b | HCC | HUVECs | SMAD4, STAT5 | Promotion | Chang et al. (2019) |
| miR-549a | Tki-resistant ccRCC | HUVECs | HIF-1α, VEGFA | Promotion | Xuan et al. (2021) |
| miR-619-5p | Hypoxic NSCLC | HUVECs | RAC1/4 | Promotion | Kim et al. (2020) |
| miR-944 | Gioma stem cells | HUVECs | VEGF, Akt, Erk1/2 signaling pathway | Inhibition | Jiang et al. (2021) |
| miR-1229 | CRC | HUVECs | HIPK2, VEGF signaling pathway | Promotion | Hu et al. (2019) |
| miR-1266b | NSCLC | HUVECs | HIPK2 | Promotion | Kim et al. (2021) |
| miR-1290 | HCC | HUVECs | SMEK1 | Promotion | Wang et al. (2021a) |
| miR-3157-3p | NSCLC | HUVECs | TIMP2, KLF2, VEGF, MMP2, MMP9, occludin | Promotion | Ma et al. (2021) |
| miR-3682-3p | HCC | HUVECs | ANGPT1, RAS-MEK1/2-ERK1/2 signaling pathway | Inhibition | Dong et al. (2021) |
| **LncRNAs** | |  | |  | |
| LncRNA H19 | Gioma | HBMVECs | miR-29a, VASH2 | Promotion | Jia et al. (2016) |
| LncRNA H19 | CD90+ liver cancer | HUVECs | VEGF, VEGFR, ICAM1 | Promotion | Coriglia et al. (2015) |
| LncRNA HOTAIR | Gioma | HBMVECs | VEGFA | Promotion | Ma et al. (2017) |
| LncRNA CCAT2 | Gioma | HUVECs | VEGFA, TGFβ | Promotion | Lang et al. (2017b) |
| LncRNA POLR3F | Gioma | HBMVECs | bFGF, FGFR, VEGFA, and ANG | Promotion | Lang et al. (2017a) |
| LncRNA MALAT1 | EOC | HUVECs | VEGFA, VEGFD, ENA78, PIGF, IL8, ANG, bFGF, Leptin | Promotion | Chu et al. (2018) |
| LncRNA GAS5 | LC | HUVECs | miR-29-3p, PTEN | Inhibition | Cheng et al. (2019) |
| LncRNA p21 | NSCLC | HUVECs | — | Promotion | Castelletto et al. (2020) |
| LncRNA UCA1 | PC | HUVECs | miR-96-5p/AMOTL2/ERK1/2 axis | Promotion | Guo et al. (2020) |
| LncRNA RAMP2-AS1 | Chondrosarcoma | HUVECs | miR-2355-5p/VEGFR axis | Promotion | Cheng et al. (2020) |
| LncRNA APC1 | CRC | HUVECs | Rab5b, MAPK | Promotion | Wang et al. (2019a) |
| LncRNA TUG1 | CC | HUVECs | — | Promotion | Lei and Mou, (2020) |

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### TABLE 1
(Continued) The effects and mechanisms of miRNAs, lncRNAs, and circRNAs derived from tumor EVs on angiogenesis.

| Cargoes                        | Cancer types | Recipient cells | Target genes or signaling pathways                                      | Functions                  | References        |
|--------------------------------|--------------|-----------------|------------------------------------------------------------------------|---------------------------|-------------------|
| LncRNA X26 nt                  | GC           | HUVECs          | VE-cadherin                                                            | Promotion                 | Chen et al. (2021c) |
| LncRNA OIP5-AS1                | Osteosarcoma | HUVECs          | miR-153, ATG5                                                          | Promotion                 | Li et al. (2021c)  |
| LncRNA AC073352.1              | BC           | HUVECs          | YBX1 stabilization                                                    | Promotion                 | Kong et al. (2021) |
| LncRNA SNHG16                  | HCC          | HUVECs          | miR-4500/GALNT1 axis, PI3K/Akt/mTOR pathway                            | Promotion                 | Li et al. (2021b) |
| LncRNA CCAT1                   | PC           | HUVECs          | miR-1138-5p/HMGAl axis                                                | Promotion                 | Han et al. (2021)  |
| LncRNA LINC00161               | HCC          | HUVECs          | miR-590-3p/ROCK axis                                                  | Promotion                 | You et al. (2021)  |
| LncRNA SNHG11                  | PC           | HUVECs          | miR-324-3p/VEGF axis                                                  | Promotion                 | Fang et al. (2021) |
| **CircRNAs**                   |              |                 |                                                                        |                           |                   |
| Circ-100338                    | HCC          | HUVECs          | MMP9                                                                  | Promotion                 | Huang et al. (2020b) |
| Circ-SHKBP1                    | GC           | —               | miR-582-3p/HUR/VEGF axis                                              | Promotion                 | Xie et al. (2020b) |
| Circ-RanGAP1                   | GC           | HUVECs          | miR-877-3p/VEGF axis                                                  | Promotion                 | Lu et al. (2020)   |
| Circ-CCAC1                     | CCA          | HUVECs          | SH3GL2, EZH2, ZO-1, Occludin                                          | Promotion                 | Xu et al. (2021)  |
| Circ-0044366                   | GC           | HUVECs          | miR-29a/VEGF axis                                                     | Promotion                 | Li et al. (2021a)  |
| Circ-CMTM3                     | HCC          | HUVECs          | miR-3619-5p/SOX9                                                      | Promotion                 | Hu et al. (2021)  |

Abbreviation: Breast cancer, BC; Cervical cancer, CC, Cervical squamous cell carcinoma; CSCC; Clear cell renal cell carcinoma, ccRCC; Cholangiocarcinoma, CCA; Colorectal cancer, CRC; Epithelial ovarian cancer, EOC; Esophageal squamous cell carcinoma, ESCC; Gastric cancer, Gibliostoma, GBM; GC; Hepatocellular carcinoma, HCC; Lung cancer, LC; Mouse aortic endothelial cells, MAECs; Nasopharyngeal carcinoma, NPC; Non-small cell lung cancer, NSCLC; Ovarian cancer, OC; Oral squamous cell carcinoma, OSCC; Pancreatic cancer, PC; Pancreatic ductal adenocarcinoma, PDAC; Papillary thyroid cancer, PTC; Small cell lung cancer, SCLC; Tyrosine kinase inhibitor, TKI.

![FIGURE 1](image-url) The effects and mechanisms of lncRNAs derived from tumor EVs on angiogenesis.
cancer cells were found to be enriched in lncRNA H19 and promoted the angiogenic phenotype of human umbilical vein endothelial cells (HUVECs), probably by regulating VEGF and VEGFR1 expression (Conigliaro et al., 2015). Chondrosarcoma cell-derived exosomes containing lncRNA-RAMP2-AS1 promoted the proliferation, migration and tube formation of ECs by upregulating VEGFR2 by sponging miR-2355-5p (Cheng et al., 2020). LncRNA-UCA1 was highly expressed in exosomes derived from hypoxic pancreatic cancer (PC) cells and promoted angiogenesis and tumor growth by regulating the miR-96-5p/AMOTL2/ERK1/2 axis (Guo et al., 2020). PC-derived exosomal lncRNA SNHG11 promoted the expression of VEGFA by sponging miR-324-3p (Fang et al., 2021). Additionally, glioma-derived exosomal lncRNA-CCAT2 (Lang et al., 2017b) and lncRNA-POU3F3 (Lang et al., 2017a) enhanced angiogenesis by inducing VEGFA expression. LncRNA-APCI, a suppressor of angiogenesis, was significantly downregulated in colorectal cancer cell-derived EVs. It directly bound to and degraded Rab5b mRNA to decrease EV production and block the mitogen-activated protein kinase (MAPK) signaling pathway in HUVECs to suppress angiogenesis (Wang et al., 2019a).

Together, these studies demonstrate that tumor exosomal lncRNAs regulate angiogenesis mainly by modulating VEGFA expression and the VEGF/VEGFR and MAPK pathways. The effects and mechanisms of other EV-derived lncRNAs on tumor angiogenesis are summarized in Figure 1 and Table 1.

2.1.3 CircRNAs
CircRNAs constitute a class of endogenous ncRNAs that form a covalently closed loop without a 5′-cap or 3′-poly-A tail (Gan et al., 2021). They are produced by backsplicing protein-coding precursor mRNAs and regarded as variants of competitive endogenous (ceRNAs) that can sponge and thus inhibit the activity of miRNAs (Hansen et al., 2013). Accumulating evidence has demonstrated that circRNAs are involved in various biological processes by regulating gene expression at the transcriptional or posttranscriptional levels (Du et al., 2016). CircRNAs can also be loaded into EVs and mediate cell-cell communication. Circ-SHKBP1 in GC cell-derived exosomes promoted angiogenesis by sponging miR-582-3p and thus increased the expression of hu-antigen R (HUR), which regulated VEGF mRNA stability (Xie et al., 2020b). Circ-RanGAP1 in secreted exosomes derived from the plasma of GC patients and promoted GC progression by targeting the miR-877-3p/VEGFA axis (Lu et al., 2020). Additionally, circ-0044366/circ29, which is highly expressed in GC cell-derived
exosomes, was delivered into ECs and sponged miR-29a to promote angiogenesis by upregulating VEGF (Li et al., 2021a). In summary, tumor EV-derived circRNAs affect tumor angiogenesis primarily by regulating VEGF expression. The effects and mechanisms of other EV-derived circRNAs on tumor angiogenesis are summarized in Figure 2 and Table 1.

2.2 EV-Derived Proteins and Tumor Angiogenesis

In recent years, researchers have identified thousands of proteins from different types of tumor-derived EVs, and some of these proteins were characterized with proangiogenic properties and can stimulate various steps in the angiogenic cascade. For example, EVs derived from colorectal cancer perivascular cells contained growth arrest specific 6 (Gas6) and promoted the recruitment of endothelial progenitor cells (EPCs) to tumors by activating the Axl pathway, thus leading to tumor revascularization after withdrawal of antiangiogenic drugs (Huang et al., 2021). VEGFα was carried in EVs derived from ex vivo cultured patient-derived glioblastoma stem-like cells and promoted angiogenesis of human brain ECs (Treps et al., 2017). Breast cancer cell-derived EVs contained VEGF<sub>165</sub>, which was generated by VEGF<sub>165</sub> crosslinking and triggered sustained activation of VEGFRs in ECs by interacting with heat shock protein 90 (HSP90) (Feng et al., 2017). Furthermore, EVs secreted by ovarian (ES2), colorectal (HCT116), and renal (786–0) cancer cells, in bodily fluids of tumor-bearing mice, and in ovarian cancer patient ascites could stimulate EC migration and tube formation. These responses were mediated by the 189 amino acid isoform of VEGF (VEGF<sub>189</sub>), which was bound to the surface of these EVs because of its high affinity for heparin (Ko et al., 2019). Collectively, these findings indicate that proangiogenic factors (e.g., Gas6 and VEGFA) and different subtypes of VEGF promote tumor angiogenesis through different mechanisms.

In addition to conventional proangiogenic cytokines, other angiogenesis-related proteins have also been found in EVs. Ephrin type B receptor 2 (EPHB2) in small EVs derived from head and neck squamous cell carcinoma (HNSCC) activated ephrin-B reverse signaling and induced STAT3 phosphorylation in ECs, which promoted angiogenesis both in vitro and in vivo (Sato et al., 2019). Moreover, soluble E-cadherin, which was localized to the surface of exosomes derived from ovarian cancer (OV) cells, activated the β-catenin and nuclear factor-κB (NF-κB) signaling pathways by interacting with VE-cadherin on ECs, leading to angiogenesis in vitro and in vivo (Tang et al., 2018). Exosomal Annexin II secreted by breast cancer cells promoted tPA-dependent angiogenesis in vitro and in vivo (Maji et al., 2017). Wnt5A induced the secretion of exosomes containing proangiogenic proteins (e.g., VEGF and MMP2) and immunomodulatory factors (e.g., IL-8 and IL-6) by melanoma cells (Ekstrom et al., 2014). Additionally, other angiogenic proteins have been found in many cancer cell-secreted EVs, such as yes-associated protein (YAP) (Wang et al., 2019b), angiopoietin 2 (ANGPT2) (Xie et al., 2020a), profilin 2 (PFN2) (Cao et al., 2020), Dll4 (Sheldon et al., 2010), ANG, IL-6, IL-8, tissue inhibitor of metalloproteinases-1 (TIMP-1), TIMP-2, activating transcription factor 2 (ATF2), metastasis associated 1 (MTA1), and Rho associated coiled-coil containing protein kinase 1/2 (ROCK1/2) (Skog et al., 2008; Chan et al., 2015; Yi et al., 2015; Ikeda et al., 2021). More proteins in different types of tumor-derived EVs and their proangiogenic mechanisms are summarized in Figure 3 and Table 2.

3 EXTRACELLULAR VESICLES AND CLINICAL IMPLICATIONS

As ncRNAs or proteins loaded in EVs can be distributed in various biofluids, such as blood, urine, tears, saliva, milk, and ascites (Keller et al., 2011), the ability to analyze their cargoes and levels in bodily fluids makes them promising biomarkers for cancer diagnosis and prognosis (Sun and Liu, 2014). Liquid biopsy is a noninvasive method of detecting precise information about the tumor environment/status, which can provide information prior to treatment (Rekker et al., 2014). Through liquid biopsy, numerous proangiogenic contents in EVs have been identified.

Similar to that on circulating free DNA or cell-free DNA and several oncoproteins, such as prostate-specific antigen (PSA) and alpha-fetoprotein (AFP), emerging evidence has suggested that VEG-associated ncRNAs and proteins can serve as biomarkers and diagnostic, prognostic, and therapeutic targets in cancer patients. The levels of serum miR-210 and serum-derived exosomal miR-210 were much higher in HCC patients than in healthy donors. A high level of miR-210 was associated with higher microvessel density in HCC patients (Lin et al., 2018). Increased expression of exosomal circRNA-100338 in the serum of HCC patients was associated with tumor growth and angiogenesis in primary and metastatic HCC. Exosomal circRNA-100338 can serve as a predictor of poor prognosis and lung metastasis in HCC patients following curative hepatectomy (Huang et al., 2020b). Serum exosomal Annexin II promoted angiogenesis, and a high level of serum exosomal Annexin II was associated with tumor grade, poor overall survival (OS), and poor disease-free survival in African-American women with triple-negative breast cancer (Chaudhary et al., 2020). Increased expression of Inc-UCA1 was positively correlated with microvessel density in PC tissues. Exosomal Inc-UCA1 levels were greatly increased in PC patient serum and were associated with tumor size, lymphatic invasion, late tumor node and metastasis stage, and poor OS (Guo et al., 2020). The elevated expression of metastasis associated lung adenocarcinoma transcript 1 (MALAT1) in exosomes derived from epithelial ovarian cancer (EOC) patient serum was significantly correlated with an advanced and metastatic phenotype and served as an independent predictive factor for the OS of EOC patients (Qiu et al., 2018). NSCLC patients with high levels of IncRNA-p21 in EVs derived from tumor-draining pulmonary veins exhibited shorter relapse-free survival and OS (Castellano et al., 2020). The level of circ-CCAC1 in the EVs in the serum of cholangiocarcinoma patients was significantly
**TABLE 2** | The effects and mechanisms of proteins derived from tumor EVs on angiogenesis.

| Cargoes | Tumor types or donor cells | Recipient cells | Signaling pathways | Functions | References |
|---------|-----------------------------|-----------------|-------------------|-----------|------------|
| Gas6    | Perivascular cells from CRC | EPCs            | Activation the Axl pathway | Revascularization | Huang et al. (2021) |
| VEGF90K | BC                          | HUVECs          | VEGF90K-HSP90 complex | Proangiogenesis | Feng et al. (2017) |
| VEGF189 | OC, CRC, ccRCC, OC patient ascites | HUVECs | Association with the surface of small EVs via heparin-binding | Proangiogenesis | Ko et al. (2019) |
| EPHB2   | HNSCC                       | HUVECs          | Ephrin-B2-STAT3 angiogenic signaling cascade | Proangiogenesis | Sato et al. (2019) |
| Soluble E-cadherin | OC | HUVECs | Activation of the β-catenin and NF-κB signaling pathways in ECs | Proangiogenesis | Tang et al. (2018) |
| Annexin II | BC            | HUVECs          | Activation of the tPA pathway | Proangiogenesis | Maji et al. (2017) |
| YAP     | LC                         | HUVECs          | —                | Proangiogenesis | Wang et al. (2019b) |
| Coagulation factor III, IGFBP3, uPA, TSP-1, endostatin | HNSCC | HUVECs | Functional reprogramming and phenotypic modulation of ECs | Proangiogenesis | Ludwig et al. (2018) |
| ANGPT2  | HCC                        | HUVECs          | —                | Proangiogenesis | Xie et al. (2020a) |
| PFN2    | LC                         | HUVECs          | Activation of the Erk pathway | Proangiogenesis | Cao et al. (2020) |
| ICAM-1, CD44v5 | NPC     | HUVECs          | —                | Proangiogenesis | Chan et al. (2015) |

**Abbreviations:** urokinase type plasminogen activator, uPA; tissue plasminogen activator, tPA.

**FIGURE 3** | The effects and mechanisms of proteins derived from tumor EVs on angiogenesis.
increased compared to that of patients with benign hepatobiliary disease, indicating that circ-CCAC1 in EVs may serve as a biomarker for cholangiocarcinoma (Xu et al., 2021). CRC patients with metastasis showed a higher level of miR-25-3p in exosomes than patients without metastasis (Zeng et al., 2018). The expression of miR-619-5p in exosomes was increased in the serum of NSCLC patients, indicating that miR-619-5p can serve as a diagnostic indicator (Kim et al., 2020). High levels of exosomal miR-1260b were associated with high-grade disease, metastasis, and poor survival in patients with NSCLC (Kim et al., 2021).

Moreover, prostate-specific membrane antigen (PSMA) has emerged as a specific prostate tumor biomarker in prostate tumor-derived exosomes. Ziaei et al. developed a novel biofunctionalized silica nanostructure to capture tumor-derived exosomes through the interaction of PSMA and its ligand TG97, providing a noninvasive approach for prostate cancer diagnosis (Ziaei et al., 2017). The company MiRXES performed a test to analyze the levels of 12 miRNA biomarkers linked to GC and calculated a cancer risk score for each patient (Kapoor et al., 2020). Another study indicated that the level of phosphatidylserine-expressing tumor-derived exosomes in the blood is a reliable biomarker for early-stage cancer diagnosis (Sharma et al., 2017).

4 CONCLUSION AND PERSPECTIVES

Tumor angiogenesis plays a critical role in tumor growth and development, and antiangiogenic therapy has been frequently applied to the clinical treatment of multiple solid tumors. Among the generally known proangiogenic signaling pathways, miRNAs, lncRNAs, circRNAs, and proteins carried by tumor-secreted EVs have recently emerged as important modulators of tumor angiogenesis, acting through a variety of mechanisms, as described in this review.

Antiangiogenic therapy has been widely used for the treatment of various solid tumors and has conferred tremendous survival benefits to cancer patients (Teleau et al., 2019; Lugano et al., 2020). Antiangiogenic drugs, such as bevacizumab, sorafenib, and regorafenib, inhibit tumor growth by suppressing angiogenesis primarily through blocking the VEGF/VEGFR pathway. However, many patients receive only modest survival benefits and develop acquired resistance to antiangiogenic drugs (Huijbers et al., 2016; Gacche and Assaraf, 2018). Drug resistance is one of the most important obstacles to treatment because it limits the clinical applications of antiangiogenic drugs, and the diseases still progress, which results in poor outcomes and unsatisfactory quality of life (Sennino and McDonald, 2012; van Beijnum et al., 2015). Since exosome-derived ncRNAs and proteins play important roles in tumor angiogenesis, targeting ncRNAs and proangiogenic proteins may be a potential therapeutic strategy to inhibit tumor angiogenesis.

Because a single miRNA, lncRNA, and circRNA species has the potential to regulate angiogenesis by modulating multiple targets, these ncRNAs hold great promise for use in therapeutic approaches to the treatment of tumor angiogenesis. However, in addition to tumors, ncRNAs significantly regulate the biological functions of normal cells, and systemic targeting of ncRNAs might affect physiological angiogenesis in normal tissues. Therefore, it is important to develop more specific therapeutic approaches based on angiogenesis-related ncRNAs. Moreover, EVs have turned out to be possible natural carriers of therapeutic agents with long half-time and non-immunogenic properties (Lakhal and Wood, 2011). These EV-based nanocarriers exhibit several advantages such as a high capacity for overcoming various biological barriers and high stability in the blood (Ha et al., 2016). However, the safety, specificity, and proficiency of this promising approach in clinical trials still remain more mysterious. EVs-based nanocarriers still face many challenges in clinical application.

In summary, this review provides deeper insight into the regulatory role of tumor-derived EVs on angiogenesis. Therefore, revealing the mechanisms of tumor-derived EVs on angiogenesis and seeking their potential as biomarkers and diagnostic, prognostic, and therapeutic targets in cancer patients will be popular research directions in the future.

AUTHOR CONTRIBUTIONS

LD, MiH and MaH designed and revised the manuscript. MaH and YL drafted the manuscript. YZ, CC, and MW participated in the procedures. All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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