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Non-coding RNA regulatory networks in post-transcriptional regulation of VEGFA in cancer

Giulia Fontemaggi

Oncogenomic and Epigenetic Unit, IRCCS Regina Elena National Cancer Institute, Rome, Italy

Correspondence
Giulia Fontemaggi, Oncogenomic and Epigenetic Unit, IRCCS Regina Elena National Cancer Institute, Rome, 00144, Italy.
Email: giulia.fontemaggi@ifo.it

Abstract

The switch from the normal quiescent vasculature to angiogenesis in tumors is induced by a variety of growth factors, released from cancer and stromal cells upon oxygen and nutrients deprivation. Vascular endothelial growth factor A (VEGF-A) is a potent-secreted mitogen and the only growth factor specific to endothelial cells that is observed almost ubiquitously at sites of angiogenesis. Expression of VEGF-A in cancer cells is controlled through transcriptional and post-transcriptional mechanisms. Post-transcriptional regulation of VEGF-A occurs at multiple levels, through the control of splicing, mRNA stability and translation rate, enabling a fine-tuned expression and release of VEGF-A. Mounting evidence is highlighting the important role played by microRNAs (miRNAs) in the control of VEGF-A mRNA stability and translation in cancer. Moreover, non-coding RNAs, as long non-coding RNAs and circular RNAs, are emerging as crucial modulators of VEGF-A-targeting miRNAs, with consequent ability to modulate VEGF-A expression. This review discusses the recent progress on the ncRNA-related networks controlling VEGF-A expression in cancer cells and provides insights into the complexity of VEGF-A post-transcriptional regulation.

KEYWORDS

circRNA, microRNA, post-transcriptional regulation, VEGF, VEGFA

1 | INTRODUCTION

Tumor progression includes an initial "avascular" stage when the tumors are small and dormant and a subsequent "vascular" stage, characterized by the development of a unique tumor vasculature necessary for the metabolic demand of tumors that have exceeded a certain size (usually 1–2 mm). The induction of this vasculature, termed "angiogenic switch," is caused by a variety of soluble growth factors, as for example vascular endothelial growth factor (VEGF), tumor necrosis factor alpha

Abbreviations: bFGF, basic Fibroblast Growth Factor; CAFs, Cancer-Associated Fibroblasts; circRNA, circular RNA; CRC, colorectal cancer; GBM, glioblastoma multiforme; GC, gastric cancer; HIF, Hypoxia-Inducible Factor; IL8, interleukin-8; lncRNA, long non-coding RNA; MALAT1, Metastasis-Associated Lung Adenocarcinoma Transcript 1; miRNA, microRNA; MV, microvesicles; OSCC, oral squamous cell carcinoma; TAMs, Tumor-Associated Macrophages; TNF-α, Tumor Necrosis Factor Alpha; TTP, tristetraprolin; VEGF-A, Vascular Endothelial Growth Factor A; VEGF, Vascular Endothelial Growth Factor; VHL, von Hippel–Lindau.

(TNF-α), basic fibroblast growth factor (bFGF) and interleukin-8 (IL8), released by cancer cells and by other cell types infiltrated in the tumor microenvironment, such as cancer-associated fibroblasts (CAFs) and macrophages (TAMs).[1–4] The timing of "angiogenic switch" depends on the tumor type and site, and represents a prerequisite for tumor spreading and metastatic dissemination.

Vascular endothelial growth factor A (VEGF-A) is the key mediator of angiogenesis in cancer.[5] In physiological and pathological conditions, VEGF-A affects both the development of new blood vessels (angiogenesis) and the survival of endothelial cells (vascular maintenance) by binding to the two tyrosine-kinase receptors VEGFR1 and VEGFR2.[6] Moreover, VEGF-A is known as a vascular permeability factor, based on its ability to induce vascular leakage.[7,8] The crucial role played by VEGF-A/VEGFR system in angiogenesis has been evidenced by the study of knock-out mouse models, which showed that loss of a single VEGFA allele leads to developmental deformities in the forming vasculature and embryonic death between days 11 and 12. Mice lacking either VEGFR-1 or VEGFR-2 die even earlier, between embryonic days 8.5 and 9.5.[6] The requirement of a very strict regulation of VEGF-A level during embryonic development has been also highlighted by the analysis of the effect of VEGF-A overexpression. Of note, even two- to three-fold overexpression of VEGF-A from its endogenous locus results in severe abnormalities in heart development and embryonic lethality at E12.5-E14.[9]

Importantly, VEGF-induced tumor vasculature is structurally and functionally different from normal vasculature. Indeed, tumor blood vessels are irregularly shaped, tortuous, leaky, and hemorrhagic. Tumor blood flow is, therefore, suboptimal causing hypoxia and further VEGF production. Major stimuli responsible for VEGF-A up-regulation in cancer cells are low oxygen tension, oncogene activation, hormones, exposure to growth factors, and cytokines.

2 | REGULATION OF VEGFA EXPRESSION

The need for finely tuned VEGF-A expression is highlighted by a complex regulation at multiple levels, including transcriptional regulation, mRNA stabilization, alternative splicing, and translational regulation. Furthermore, even the availability of extracellular VEGF-A is strictly controlled through the intervention of VEGF-releasing proteases and soluble carrier molecules, both regulating VEGF gradient formation.[10] As mentioned above, among stimuli responsible for the transcriptional up-regulation of the VEGFA gene, hypoxia received particular interest because of its role in cancer progression.[11] Specifically, hypoxia-inducible factor (HIF) is stabilized by exposure to low oxygen tension, as a result of von Hippel–Lindau (VHL) protein destabilization, and transcriptionally activates the hypoxia response element (HRE) within VEGFA promoter. However, transcriptional regulation accounts for only a fraction of the VEGFA gene expression, which is strictly controlled by post-transcriptional mechanisms.[12] Among post-transcriptional regulatory layers, alternative splicing is a major mechanism that gives rise, depending on the inclusion/exclusion of exons 6–8, to several distinct isoforms of VEGFA, which differ in their expression patterns, affinity to receptors, biochemical and biological properties. VEGF121 (equivalent to murine VEGF120) and VEGF165 (equivalent to murine VEGF164) are the most abundant isoforms in human tumors. These splice variants differ by the presence or absence of exons 6a, 6b, and 7. Heparin-binding VEGF isoforms (such as VEGF165) produce a branching network with narrow vessels, while non heparin-binding isoforms (such as VEGF120) results in poorly branching, tortuous, and leaky vessels.[10]

Moreover, the use of an alternative 3′ splice site in the last exon of VEGFA (exon 8) leads to the production of the same isoforms but differing in their six C-terminal amino acid sequences, named $VEGF_{XXX}b$, characterized by reduced angiogenic potential, due to their lower affinity to VEGF receptors.[13] $VEGF_{XXX}b$ isoforms are generally down-regulated in human tumors as a result of the activation of splicing factors as SRSF1 (a.k.a. ASF/SF2).[14] Cooperation between SRSF1 and lncRNA MALAT1 (Metastasis-Associated Lung Adenocarcinoma Transcript 1) has been also reported to favor $VEGF_{XXX}$ vs. $VEGF_{XXX}b$ in breast cancer cells.[15] Interestingly, the use of inhibitors of SRPK1, the kinase responsible for SRSF1 activation, has been recently proposed as powerful antiangiogenic strategy to be used for the treatment of various pathological conditions, included cancer. Inhibition of SRPK1, through the blocking of SRSF1 phosphorylation and activity, leads to the induction of $VEGF_{XXX}b$ isoforms and, consequently, significantly reduces the angiogenic potential of targeted cells.[16–18]

mRNAs-encoding VEGF-A isoforms are generally unstable under normal oxygen and nutrient conditions. It has been shown that multiple regions in the VEGF mRNA cooperate both to ensure the rapid degradation of the mRNA under normoxic conditions and to allow stabilization of the mRNA in response to hypoxia.[11] Major factors responsible for destabilization of VEGF-A mRNA are ARE-binding proteins AUF1 and TTP (tristetraprolin), interacting with AU-rich elements

present in VEGF-A 3′-UTR.[12] AU-rich elements within the VEGF-A 3′-UTR may, on the contrary, confer hypoxia-dependent mRNA stability and enhanced translation rate, when involved in interaction with RNA-binding proteins such as HuR (ELAVL1), PTBP1, and NF90 (ILF3).[12,19,20] Expression of VEGF-A is also controlled at translation level by elements present in the long GC-rich 5′-UTR region of the gene, which contains three in-frame alternative CUG start codons, one uORF element and two internal ribosome entry sites (IRES). These last enable efficient translation in the presence of stimuli/stresses (as for example hypoxia) while inhibiting cap-dependent translation (reviewed in Refs 21 and 22).

3 | CONTROL OF VEGF-A EXPRESSION BY MICRORNAS

The long 3′-UTR region of VEGF-A emerged as a major regulator of mRNA stability and translation rate also due to the presence of several binding sites for microRNAs. MicroRNAs (miRNAs) are single-stranded non-coding RNAs of 18–25 nucleotides that predominantly act as translational repressors. Through sequence-specific interaction with the 3′-untranslated region (UTR) of target mRNA, miRNAs suppress gene expression via transcript degradation or inhibition of protein translation.

The first experimental evidence of the relevance of miRNAs in the regulation of VEGFA expression and in angiogenesis was obtained by the analysis of the phenotype of mice lacking miRNA-processing enzyme Dicer.[23] Subsequently, a multitude of miRNAs has been reported to target directly the 3′-UTR of VEGF-A in various cell types; these include miR-20, miR-29b, miR-93, miR-126, miR-150-5p, miR-190, miR-195, miR-200, miR-203, miR-205-5p, miR-206, miR-361, miR-497, miR-503, miR-613, and miR-638 (reviewed in Ref 24 and reported in Refs 25–28).

miRNAs have been shown to control VEGFA expression also in the endothelial cell context.[29–31] An example of this regulation is represented by some miRNAs of the miR-15/107 family,[32] for example, miR-16 and miR-424, sharing the same seed sequence required for target recognition, and inhibiting the expression not only of VEGFA but also of VEGFR2 (reviewed in Ref 33). Interestingly, as VEGF-A has been reported to exert both autocrine and intracrine signaling in the endothelium (reviewed in Ref 34), targeting of VEGFA 3′-UTR by miRNAs might contribute to the lowering of VEGF-A expression in endothelial cells and to the inhibition of these signaling pathways in the absence of angiogenic stimuli.

VEGF-A-targeting miRNAs are generally downregulated in tumors, contributing to the induction of proliferation, invasion, and angiogenesis. Interestingly, miRNAs can compete with VEGF-A stabilizing RBPs by sharing common binding sites in the 3′-UTR. A representative example is the antagonistic effect of miR-200 family miRNAs and the protein HuR.[35] Of note, the evidence that many of the miRNAs that target VEGF-A 3′-UTR are also responsible for the negative regulation of other key players in angiogenesis, as for example HIF-1α, highlighted that inhibition of angiogenesis in physiological conditions is based on a complex network of regulatory layers. The inhibitory activity of miRNAs on the expression of angiogenesis-related genes is almost always lost in cancer cells, through both downregulation of these tumor suppressor miRNAs as well as the inhibition of their activity exerted by additional non-coding RNAs as circular RNAs (circRNAs) and long non-coding RNAs (lncRNAs) which are frequently upregulated and function as sponges to limit microRNAs' function. These circRNA-miRNA and lncRNA-miRNA regulatory networks are discussed below.

4 | CIRCULAR RNAS CONTROLLING VEGF-A EXPRESSION

CircRNAs are covalently closed RNA loops that originate from back-splicing events occurring mainly in protein-coding genes. Owing to the closed continuous loop structure, circRNAs can escape exonuclease-mediated degradation and are more stable than other RNA species.[36] CircRNAs may be overexpressed in cancer cells, contributing to the various features of malignancy, as proliferation, migration, and invasion, and are efficiently released by cancer cells into extracellular vesicles, acting as autocrine and paracrine mediators.[37] CircRNAs may exert at intracellular level various functions, the best known of which is miRNA sponging.[38] Through base-pairing with a miRNA sequence, a circRNA may sequester the miRNA and block its inhibitory activity on target mRNAs. Therefore, the overexpression of a circRNA usually results in the upregulation of the target mRNAs of the sequestered miRNA. Other major activities of circRNAs are the enhancement of protein complexes formation and/or activity, and the regulation of transcription or translation. Intriguingly, a few circRNAs may be also translated into polypeptides, when presenting an epitranscriptomic m6A modification, needed for translation initiation.[37] With regard to VEGF-A, a number of circRNAs have been functionally characterized as inducers of VEGF-A protein expression, mainly through their ability to sequester miRNAs targeting the 3′-UTR region of VEGF-A (summarized in Table 1 and Figure 1). Interestingly, various miRNAs that are sponged by

TABLE 1 CircRNAs involved in the regulation of VEGF-A expression in the indicated cancer types

circRNA (host gene)	Sponged miRNA or other function	Cancer type	Refs
circSMARCA5	Interacts with SRSF1 leading to enhanced VEGFxxx/VEGFxxxb ration	Glioblastoma	47
circRPL15	miR-146b-3p	Glioma	53
circPVT1	miR-195	Papillary thyroid carcinoma	54
circ_0001429 (MANBA)	miR-205-5p	Bladder cancer	55
circ_0056618 (SPOPL)	miR-206	Colorectal cancer	56
circMYLK	miR-29a	Bladder cancer	40
circ_0044366 (a.k.a. circ29) (ATP5G1)	miR-29a	Gastric cancer	42
circ_001971 (alias hsa_circ_0001060) (UXS1)	miR-29c-3p	Colorectal cancer	41
circRhoC	miR-302e sponging and interaction with VEGFA protein	Ovarian cancer	57
circITGA7	miR-34a-5p	Glioma	58
circ_0001178 (USP25)	miR-382-5p	Hepatocellular carcinoma	59
circAP2A2	miR-382-5p	Hemangioma	60
circSCAF11	miR-421 sponging, leading to Sp1 increase and VEGFA transcriptional activation	Glioma	46
circMYOF	miR-4,739	Pancreatic ductal adenocarcinoma	61
circ_0023404 (RNF121)	miR-5,047	Cervical tumors	62
circ_0030998 (LAMP1)	miR-567	Colorectal cancer	63
circSHKBP1 (circ_0000936)	miR-582-3p sponging, leading to HuR protein induction and VEGFA mRNA stabilization	Gastric cancer	45
circCCT3	miR-613	Colorectal cancer and pancreatic ductal adenocarcinoma	64
circASH2L	miR-665	Ovarian cancer	65
circ_0001766 (PDIA4)	miR-877-3p	Oral squanous cell carcinoma	44
circRanGAP1	miR-877-3p	Gastric cancer	43
circPOK (Zbtb7a)	Interaction with ILF2/ILF3 complex and VEGFA mRNA stabilization	Mesenchymal tumors	66
circATXN1	miR-526b-3p	Glioma	67

VEGF-A-inducing circRNAs are also down-regulated in cancer cells, as, for example, members of the miR-29 family, miR-195, miR-205-5p, and miR-613. miR-29 family comprises three mature miRNAs, namely miR-29a, miR-29b, and miR-29c, characterized by identical seed sequence (AGCACCA). The miR-29-3p arm represents the most abundant and functionally relevant arm of all three family members. miR-29 is recognized as one of the critical miRNAs with tumor suppressor activity that play a role in cancer pathogenesis[39]; it is down-regulated in the vast majority of cancer types, where it regulates proliferation, apoptosis, epithelial-mesenchymal transition (EMT), fibrosis and metastasis, by targeting key regulatory players in various oncogenic pathways. Of note, miR-29 may be sponged by three circular RNAs, namely circ-MYLK, circ_001971, and circ_0044366, all leading to the induction of VEGF-A expression in various cancer types (Table 1). Circ-MYLK promotes bladder cancer progression by activating VEGFA/VEGFR2 and downstream Ras/ERK signaling pathway.[40] Circ_001971 expression induces proliferation, invasion, and angiogenesis in colorectal cancer (CRC).[41] Circ_0044366 (termed circ29) is released in exosomes by gastric cancer (GC) cells and reaches endothelial cells, where it modulates VEGF-A expression and VEGF-A/VEGFR signaling by sequestering miR-29a.[42] Also miR-877 is potentially sponged by multiple circRNAs in cancer cells, enabling VEGF-A induction. Specifically, circ-RanGAP1-mediated miR-877–3p/VEGFA axis promotes GC

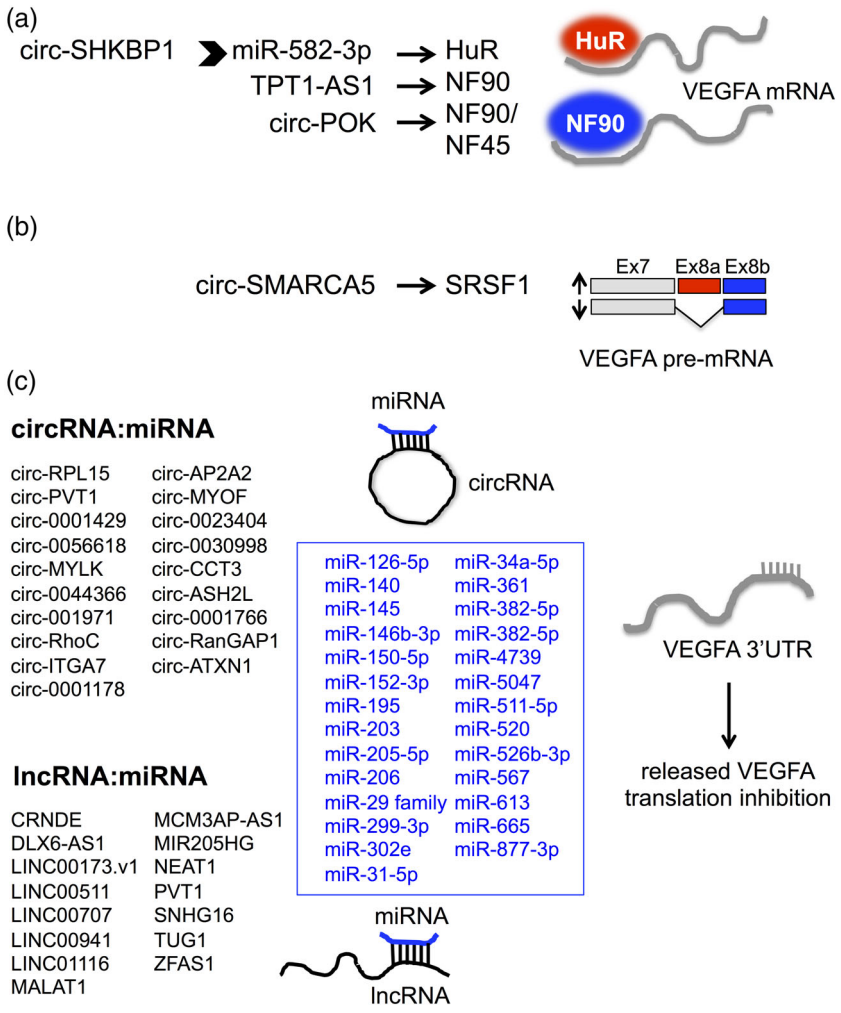

FIGURE 1 Different ncRNA-driven post-transcriptional mechanisms regulating VEGFA expression in cancer cells. (a) ncRNAs may induce the expression of VEGFA by regulating the expression (as in the case of the circ-SHKBP1/miR-582-3p/HuR axis) or the activity (as in the case of TPT-AS1/NF90 and circ-POK/NF90 axis) of key proteins involved in VEGFA mRNA stabilization, such as HuR and NF90. (b) circ-SMARCA5 impinges on the expression level of VEGFA pro- and anti-angiogenic isoforms, favoring the former over the latter, by interacting with splicing factor SRSF1. (c) Summary of the so far reported circRNAs (upper list) and lncRNAs (lower list) able to sponge VEGFA-targeting miRNAs (indicated in blue) in various cancer types

progression. Circ-RanGAP1 is upregulated in the plasma exosomes from GC patients and, interestingly, these exosomes are able to induce the invasive potential of cultured GC cells.[43] miR-877–3p has been shown to be targeted by the sponging activity of circ_0001766 in oral squamous cell carcinoma (OSCC), thus enhancing cell proliferation.[44] A number of additional circRNA-miRNA interactions impinging on the expression of VEGF-A and angiogenesis have been identified in various cancer types and are listed in Table 1.

CircRNA may also affect VEGF-A expression indirectly, for example, by targeting VEGF-A regulatory proteins and impinging on different VEGF-A regulatory layers. Interesting examples of these alternative mechanisms are represented by circ-SHKBP1, circ-SCAF11, circ-SMARCA5, and circPOK. In the first case, circ-SHKBP1 (circ_0000936) is able to sponge miR-582-3p in GC cells, leading to released expression of HuR protein, which in turn enhances VEGF-A mRNA stability and expression. Of note, GC cells release circ-SHKBP1 in the extracellular space and exosomal circ-SHKBP1 contributes to enhance GC cell growth.[45] Circ-SCAF11 is involved in VEGF-A induction during the progression of glioma; circ-SCAF11indeed is able to sponge miR-421 and release the expression of its target Sp1, a key transcription factor responsible for VEGF-A expression.[46] The last example refers to a circRNA, circ-SMARCA5, which acts in advanced brain tumors. Circ-SMARCA5 controls the ratio between pro- and anti-angiogenic VEGF-A isoforms in glioblastoma multiforme (GBM), a highly vascularized tumor, by interacting with SRSF1, a key splicing factor controlling the $VEGF_{xxx}/VEGF_{xxx}b$ ratio. Of note, blood vascular microvessel density negatively correlated with the expression of circ-SMARCA5 in GBM biopsies while positively correlated with that of SRSF1 and the $VEGF_{xxx}/VEGF_{xxx}b$ ratio.[47] The last example is represented by circPOK, a proto-oncogenic RNA in mesenchymal tumor progression, encoded by the *Zbtb7a* gene. CircPOK acts antithetically to its linear transcript counterpart (Pokemon), which acts as a tumor suppressor gene. CircPOK favors proliferation and angiogenesis by interacting with and activating the NF45/NF90 complex (encoded by *ILF2* and *ILF3* genes), leading to the activation of many pro-angiogenic and

growth factors, such as IL6 and VEGF-A, through transcriptional and post-transcriptional regulation, respectively.[66]

5 | LNCRNAS CONTROLLING VEGF-A EXPRESSION

The inhibitory activity of miRNAs on VEGF-A 3′-UTR may be also regulated by long non-coding RNAs (lncRNAs), which may function as sponges for miRNAs. Moreover, lncRNAs may impact on VEGF-A abundance by regulating the activity of transcriptional or post-transcriptional modulators of VEGF-A expression (Table 2 and Figure 1). The most functionally relevant lncRNA:miRNA networks enclose miRNAs with a well-established tumor suppressor activity, such as miR-29 family members (sponged by LINC00511 and PVT1), miR-195 (belonging to miR-15/107 group and sponged by NEAT1 and DLX6-AS1), miR-34a-5p (sponged by TUG1), and miR-145 (sponged by MALAT1).

The example of PVT1 is of particular interest as the *PVT1* (Plasmacytoma Variant Translocation 1) gene locus

TABLE 2 LncRNAs involved in the regulation of VEGF-A expression in the indicated cancer types

LncRNA	Sponged miRNA or other function	Cancer type	Refs
CamK-A	Activation of NF-kB and consequent VEGFA induction	Multiple tumors	68
CRNDE	miR-203	Hepatoblastoma	69
DLX6-AS1	miR-195	Bladder cancer	70
FLANC	Increases the half-life of phosphorylated STAT3 to induce VEGFA expression	Colorectal cancer	71
LINC00173.v1	miR-511-5p	Lung cancer	72
LINC00511	miR-29b-3p	Pancreatic ductal adenocarcinoma	73
LINC00707	miR-382-5p	Cervical cancer	74
LINC00941	miR-877-3p	Non-small cell lung cancer	75
LINC01116	miR-31-5p	Glioma	76
MALAT1	miR-126-5p	Colorectal cancer	51
MALAT1	miR-140	Hepatocellular carcinoma	77
MALAT1	miR-145	Breast cancer	78
MALAT1	miR-150-5p	Osteosarcoma	79
MALAT1	miR-206	Endothelial cells	51
MIR205HG	miR-299-3p	Melanoma	80
NEAT1	miR-126-5p	Thyroid carcinoma	81
NEAT1	miR-195	Sinonasal SCC	82
NEAT1	miR-205-5p	Colorectal cancer	83
NEAT1	miR-361	Hemangioma	84
PVT1	miR-152-3p	Colorectal cancer	85
PVT1	miR-29c	Non-small cell lung cancer	86
SNHG16	miR-520	Lung cancer	87
TNK2-AS1	Interacts with STAT3 and elevate VEGFA expression	Lung cancer	88
TPT1-AS1	Interacts with NF90 and enhances VEGFA mRNA stability	Colorectal cancer	89
TUG1	miR-299-3p	Renal cell carcinoma	90
TUG1	miR-34a-5p	Hepatoblastoma	91
TUG1	Sponges miR-143-5p enabling HIF-1a expression and VEGFA upregulation	Osteosarcoma	92
ZFAS1	miR-150-5p	Colorectal cancer	93

encodes many ncRNA variants, including both linear and circular isoforms, the most abundant being the exon 2-derived circPVT1 (reviewed in Refs 48 and 49) and it has been reported that linear PVT1 and circPVT1, both overexpressed in cancer cells, may positively regulate VEGF-A expression.

Overexpression of PVT1 and circPVT1 in cancer cells may result from gene amplification and/or from promoter activation. Interestingly, different promoters drive expression of PVT1 and circPVT1, as a second promoter region present in the first intron of the gene regulates circPVT1 transcription. Upregulation of PVT1 and circPVT1 in cancer cells may be caused by c-Myc transcription factor and by the TEAD/YAP/mutant p53 complex, respectively (reviewed in Refs 48 and 49). CircPVT1 could promote VEGFA expression by sponging miR-195, thus contributing to the malignant progression of papillary thyroid carcinoma. Interestingly, also linear PVT1 had been previously reported to sequester miR-195, despite its effect on VEGF-A wasn't analyzed in that study.[48] Linear PVT1 induces VEGF-A expression by sponging miR-152-3p and miR-29c in colorectal cancer and non-small cell lung cancer, respectively (Table 2).

Another lncRNA relevant in the cancer context, MALAT1, highly expressed in a variety of tumors following transactivation by oncogenic transcription factors such as HIF-1 and YAP,[50,51] and implicated in various cancer-related activities as proliferation, invasion, and angiogenesis, has been shown to control VEGF-A expression by sponging a variety of miRNAs, as miR-126-5p, miR-140, miR-145, miR-150-5p in various cancer types and miR-206 in endothelial cells. Interestingly, MALAT1 had been previously reported to control VEGF-A splicing, favoring $VEGF_{xxx}$ vs $VEGF_{xxx}b$ isoforms.[15] This lncRNA thus emerges as a regulator of VEGF-A acting at different post-transcriptional levels. Several groups have shown that many lncRNAs contribute to tumor angiogenesis and progression, inducing VEGFA expression by sequestering distinct miRNAs in a wide variety of cancer types (summarized in Table 2). In addition to sponging of VEGFA-targeting miRNAs, various lncRNAs may impact on VEGF-A expression through the modulation of the expression and/or activity of VEGF-A post-transcriptional regulators. One such example is lncRNA TPT1-AS1, a liver-metastasis associated lncRNA in colorectal cancer. TPT1-AS1 is upregulated in CRC and its high expression is associated with poor outcome. Functionally, TPT1-AS1 interacts with NF90 protein thus favoring VEGFA mRNA stability, leading to enhanced angiogenic and metastatic potential of CRC cells.[89] Of note, TPT1-AS1 and VEGFA mRNA are significantly correlated in a CRC cohort.

6 | CONCLUSIONS

Mounting evidence has emerged in the last few years about the relevance of VEGFA post-transcriptional regulation exerted by non-coding RNAs in a wide variety of cancer types. These regulatory networks impinge on the selection of specific VEGFA isoforms, on their stability and translation rate. ncRNA-based networks impinging on the angiogenic potential of cancer cells represent a powerful source of inspiration for the development of novel therapeutic approaches aimed at blocking the spreading of tumors. As the use of anti-VEGF agents for the treatment of cancer has not been encouraging as hoped, mainly due to development of resistance, the possibility of exploiting ncRNA-driven networks to block the angiogenic potential of cancer cells could present some advantages, compared to single-target treatments such as the anti-VEGF therapies. These include (a) the direct impact of some ncRNAs on the expression of VEGFA, therefore, acting upstream, with respect to current antiangiogenic therapies; (b) the ability of some ncRNAs to impinge, simultaneously, on the expression of various growth factors controlling angiogenesis, thus potentially overcoming the development of resistance; (c) the possibility of using cell-derived microvesicles (MV) to deliver ncRNA molecules, efficiently and in targeted manner, to cancer cells. With regard to this last possibility, it has been recently reported that the MV-delivered miR-29a/c significantly suppresses VEGFA expression in GC cells, inhibiting vascular cell growth, metastasis, and tube formation. In vivo data proved that MVs function as a potential carrier of miRNAs for targeted therapy in gastric cancer.[52] Interestingly, the miR-29 mimic MRG-201 is currently being tested in phase I clinical trials via intradermal injection (Clinical-Trials.gov: NCT02603224) for fibrosis-related pathologies and could represent a potential effective therapy for the treatment of cancers presenting miR-29 down-regulation/inactivation. ncRNA-based therapeutics thus represents an attractive approach to target angiogenesis-related networks for the treatment of cancer.

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ORCID

Giulia Fontemaggi https://orcid.org/0000-0001-8332-8842

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