

Review

Role and expression of angiogenesis-related miRNAs in gastric cancer

Martina Giuppi ¹, Anna la Salvia ², Jessica Evangelista ³ and Michele Ghidini ^{4,*}

¹ Faculty of Medicine, CEU San Pablo University, Madrid, Spain; mar.giuppi@ceindo.ceu.es (M.A.G)

² Department of Oncology, University Hospital 12 de Octubre, Madrid, Spain; alasalvi@ucm.es (A.L.S)

³ Thoracic Surgery, Fondazione Policlinico Universitario A. Gemelli IRCCS, 00168 Rome, Italy; evangelistajessica664@gmail.com (J.E.)

⁴ Oncology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; michele.ghidini@policlinico.mi.it (M.G.)

* Correspondence: Michele Ghidini, Operative Unit of Oncology, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy. E-mail: michele.ghidini@policlinico.mi.it; Phone: +39.02.5503.2660; Fax: +39.02.5503.2659.

Simple Summary: The aberrant expression of several microRNAs (miRNAs) has been showed to be involved in neoplastic angiogenesis, which is a crucial mechanism in gastric cancer onset and progression. In this review the possible prognostic and predictive roles of angiogenesis-related miRNAs as novel biomarkers of gastric cancer have been evaluated, but neither tissue nor circulating biomarkers have shown a predictive role for response to antiangiogenic treatment. Nevertheless, we consider that in future studies miRNAs should be evaluated as candidate biomarkers with prognostic and predictive features.

Abstract: Gastric Cancer (GC) is the fifth most frequently diagnosed malignant tumor and the third cause of cancer mortality worldwide. For advanced GC, a large number of novel drugs and combinations have been tested, but results are still disappointing and the disease incurable in the majority of cases. In this regard, it is critical to investigate the molecular mechanisms underlying GC development. Angiogenesis is one of the hallmarks of cancer with a fundamental role in GC growth and progression and ramucirumab, a monoclonal antibody binding to vascular endothelial growth factor-2 (VEGFR-2) is approved in the treatment of advanced and pretreated GC. However, no predictive biomarkers for ramucirumab have been identified so far. MicroRNAs (miRNAs) are a class of evolutionarily conserved single-stranded noncoding RNAs playing an important role, via post-transcriptional regulation, in essentially all biologic processes such as cell proliferation, differentiation, apoptosis, survival, invasion, and migration. Notably, in our review, we focused on miRNAs involved in angiogenic pathways in GC. Moreover, we evaluated the possible prognostic and predictive role of angiogenesis-related miRNAs as novel biomarkers of GC.

Keywords: MicroRNAs (miRNAs), gastric cancer, angiogenesis, VEGF, ramucirumab, biomarkers.

1. Introduction

1.1 Gastric Cancer

Gastric cancer (GC) is one of the most frequent causes of death in cancer patients. Due to its late diagnosis and poor response to treatment, it still represents a major public health problem all over the world. In 2018 the estimated number of GC-related death worldwide was 783,000 (i.e., 1 per 12 patients), affecting men twice more than women [1]. Approximately 95% of GCs are adenocarcinomas which can be mainly classified in intestinal and diffuse types according to Lauren classification [2], cardia and non-cardia GC according to World Health Organization (WHO) classification [3]. Endoscopic approach, surgery, chemotherapy, radiation therapy, chemoradiation, targeted therapy and immunotherapy are standard of treatments employed in the management of GC patients. However, unfortunately in the majority of cases GC is diagnosed at advanced stages and the prognosis remains disappointingly poor in this patient population [4]. Clinically, the sensitivity and specificity of traditional tumour markers such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) are relatively low. Therefore, finding reliable non-invasive tumor biomarkers for gastric cancer remains an urgent clinical need.

1.2 Angiogenesis and Cancer

Angiogenesis is one of the hallmarks of cancer [5]. Tumor-associated neo-vasculature is important for delivering nutrients and oxygen to growing tumors, and also it plays key roles in multi-aspect of tumor biology, including tumor dissemination/metastasis [6], metabolic deregulation [7] and cancer stem cell maintenance [8,9]. For its fundamental role in tumor growth and progression, angiogenesis has become an appealing target in cancer treatment.

Angiogenesis is a complex process by which new blood vessels are formed from pre-existing ones by sprouting, remodeling and expansion of primary vascular networks [10]. In normal conditions, following this morphogenesis, the vasculature becomes largely quiescent. However, within tumors, an “angiogenic switch” is always activated, causing continuous generation of new vessels [11]. The “angiogenic switch” is governed by pro-angiogenic and anti-angiogenic signals elicited by tumor cells or tumor microenvironment [12,13]. When pro-angiogenic signals are activated or anti-angiogenic signals are inhibited, the “angiogenic switch” is turned on. Understanding the regulation of tumor angiogenesis is critical to developing therapeutic strategies against cancer.

1.3 Angiogenesis and Gastric Cancer

Vascular endothelial growth factor (VEGF) family is a crucial mediator of angiogenesis¹⁴. Approximately 50% of GCs express VEGF, and the overexpression of VEGF-A and VEGF-D in GC is associated with poor prognosis [14,15].

Besides these typical activators of angiogenesis in GC, recent studies showed that a new non-classical activator can stimulate angiogenesis in vitro and in vivo. Tryptase is

stimulating proliferation of endothelial cells by activation of the proteinase-activated receptor-2 (PAR-2) [16]. VEGF is produced by this process [17].

Other known pathways such as NOTCH- and WNT-signaling are involved in the process of angiogenesis [18,19]. Other molecules such as integrin influence the interaction between tumor and stroma tissue [20].

Many clinical trials have demonstrated that GC patients can benefit from angiogenesis inhibitors [21-23]. As a consequence, a significant number of clinical trials have been developed to evaluate the activity of different antiangiogenic agents, including monoclonal antibodies directed against VEGFR (as ramucirumab and bevacizumab), small molecules inhibiting tyrosine-kinase activity of receptors such as VEGFR, PDGFR or other related (as apatinib, afatinib, lapatinib, sunitinib and TSU-68 [24]), and other antiangiogenic compounds with different mechanisms of action (as everolimus, cetuximab, panitumumab) [25].

Ramucirumab is a monoclonal antibody that selectively binds to VEGFR-2, blocking the downstream effects of the VEGF pathway in angiogenesis. The survival benefits in the REGARD [26] and RAINBOW [27] studies led to the approval of ramucirumab for the treatment of advanced GC (hazard ratio [HR] for OS 0.776, 95% confidence interval [CI] 0.603–0.998, $p=0.047$, HR for overall survival [OS] 0.807, 95% CI 0.678–0.962, $p<0.0001$, respectively, and HR for progression-free survival [PFS] 0.483, 95% CI 0.376–0.620, $p<0.0001$, HR for PFS 0.635, 95% CI 0.536–0.752, $p<0.0001$, respectively).

Although angiogenesis inhibitors can improve OS and PFS and achieve a better response rate in advanced GC, the clinical effect is quite different in individuals due to heterogeneity of the tumor. Therefore, it is essential to develop biomarkers that allow identifying the subgroup of GC patient who really benefit from this type of therapy. These biomarkers could be used to predict efficacy and choose the most suitable patients to reduce the blindness of clinical medication [24].

1.4 MicroRNAs and Cancer

MicroRNAs (miRNAs) are a class of evolutionally conserved single-stranded noncoding RNAs of 19–22 nucleotides [28]. MiRNA play an important role, via post-transcriptional regulation, in essentially all biologic processes such as cell proliferation, differentiation, apoptosis, survival, invasion, and migration [29]. MiRNAs form the RNA-inducing silencing complex (RISC)-miRNA functional unit, which regulates the expression of nearly 30% of the known human genes [30] (Figure 1). Three basic mechanisms of miRNA-mediated gene regulation are present: translation repression, direct mRNA degradation, and miRNA-mediated mRNA decay [31]. Many studies have demonstrated that mutations in miRNA-encoding genes or deregulated expression of miRNAs are integral to many human diseases including cancers development and metastasis [32]. Thus, they can act as oncogenes or tumor suppressors depending on the function of their target genes. As a consequence, MicroRNAs have shown great promise for use in anti-metastatic cancer therapy.

1.5 Micro RNAs and Angiogenesis

Several MiRNAs have been showed to be involved in neoplastic angiogenesis. In particular, VEGF expression in different types of cancer has been recognized to be regulated by miRNAs as MiR-20 [33], miR-29b [34], miR-93 [35], miR-126 [36], miR-190 [37], miR-195 [38], miR-200 [39], miR-203 [40], miR-497 [41], miR-503 [42], and miR-638 [43]. Some of these, as miR-29, inhibit angiogenesis by downregulating VEGF when overexpressed. Others, as miRNA-195 promote angiogenesis and metastasis via VEGF and the pro-metastatic factors.

Apart from directly targeting VEGF, a handful of miRNAs regulate VEGF-dependent tumor angiogenesis by targeting VEGF inducers, such as Hypoxia-Inducible Factor-1 (HIF-1) pathway (miR-22 [44], miR-107 [45], miR-519c [46], miR-145 [47]).

However, the direct connection between the role of miRNAs in angiogenesis and cancer metastasis remains to be established.

In this review, we will summarize the current evidence, new insights and the main challenges about angiogenesis-related miRNAs in GC. Furthermore, our study will analyze the diagnostic and prognostic role of angiogenesis-related miRNAs as novel biomarkers of GC, and potential novel GC treatments based on miRNAs, resulting from a better molecular knowledge.

2. Materials and Methods

We performed a comprehensive literature review of the PubMed, Scopus and Google Scholar databases regarding angiogenesis-related miRNAs in GC, using terms “MiRNA” AND “angiogenesis” AND (“gastric cancer” OR “gastric carcinoma” or “gastric adenocarcinoma”). The search was limited to articles published in English. We considered all the original studies concerned angiogenesis related MiRNA in GC. A total of 75 publications were identified, from which we selected a final pool of 28 articles based on the relevance in this context.

3. Results

Our search identified 28 studies focused on miRNAs targeting angiogenesis in GC. The details of the selected studies are reported in Table 1. We decided to order and divide the selected miRNAs according to their referral pathway. Therefore, we identified five groups: 1) miRNAs related to VEGF pathway, 2) miRNAs involved in HIF pathway, 3) miRNAs related to Hepatocyte growth factor (HGF)/c-MET signaling, 4) miRNAs involved in phosphatidylinositol 3-kinase (PI3-K) pathway and 5) miRNAs related to signal transducer and activator of transcription 3 (STAT3) signaling (Figure 2). We will briefly describe the importance for the angiogenesis process of these five pathways, and we will detail, for each of them, the significant studies about angiogenesis-related miRNAs that we have found through our literature search. Some of the analyzed studies are focused on different miRNAs, so we have reported the same work in the different paragraphs, where indicated.

3.1 miRNAs involved in VEGF pathway

The VEGF family consists of the seven major subtypes including VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental grow factor 1 and 2 (PIGF-1 and PIGF-2, respectively) [48]. VEGF subtypes stimulate cellular responses by binding to tyrosine kinase receptors (VEGFR-1, -2, -3) on the cell surface. VEGF is produced by several cell types such as fibroblasts, inflammatory cells and many tumoral cells, often in response to increasing tumor hypoxia via HIF-1a pathway. Notably, the activation of VEGFR has a critical role in GC angiogenesis [49]. In this context, we have identified eight relevant published papers about miRNAs targeting VEGF signaling.

Wu et al. aimed at determining the potential relevance of miR-616-3p, located on the chromosome region 12q13.3, in GC tumorigenesis. miR-616-3p has previously been reported to be up-regulated in prostate, lung and hepatocellular carcinoma. In this study, miR-616-3p has demonstrated to facilitate tumor angiogenesis through elevating expression level of VEGFA/VEGFR2 in GC [50]. A second work suggested that the downregulation of another miRNA, miR-1, could favor angiogenesis in GC. In this analysis, miR-1 resulted under-expressed in primary GC tissues when compared with adjacent normal mucosa and several GC cells lines. The restoration of miR-1 significantly inhibited endothelial cell tube formation via decreasing expression of VEGA-A and endothelin 1 (EDN1), the main angiogenic factors contributing to the development and maintenance of blood vessels [51]. MiRNA-126 has been recognized as promising biomarker and therapeutic target in several types of tumors, including GC. It is an endothelial-specific microRNA essential for governing vascular integrity and angiogenesis. Overexpression of this miRNAs leads to direct inhibition of VEGFA expression, reduction of cell proliferation and inhibition of angiogenic process, thereby inhibiting tumor growth both in vitro and in vivo [52-54]. In another work, Zhang et al. reported that the downregulation of miR-29a/c increases VEGF expression and release in GC cells, promoting the growth of endothelial cells. MiR-29a/c significantly suppresses VEGF expression in GC cells, inhibiting vascular cell growth, metastasis, and tube formation. Authors also used a tumor mouse model to show that secreted microvessels (MV) containing overexpressed miR-29a/c significantly reduced the growth rate of the vasculature and tumors in vivo [55]. In addition, VEGF-C was found to be a direct target gene of other three miRNAs, miR-27b, miR-101, and miR-128, respectively [56]. The expression levels of these miRNAs were inversely correlated with higher MV density. Thus, the overexpression of miR-27b, miR-101, or miR-128 was demonstrated to suppress migration, proliferation, and tube formation in human umbilical vascular endothelial cells (HUVECs) by repressing VEGF-C secretion in GC cells. MiR-27b, miR-101 and miR-128 inhibit angiogenesis by down-regulating VEGF-C expression in GC. Mei et al. reported that miR-590 can simultaneously regulate VEGFR1/2 in GC. In this study, miR-590 has also been showed to contribute to the regulation of the expression of Neuropilin 1 (NRP1) in GC. NRP1 is a transmembrane protein that can bind VEGF₁₆₅ isoform and enhance cell migration via VEGFR2, inducing vascular permeability and arteriogenesis as well. Authors reported that miR-590 was downregulated in GC tissues and cell lines, and this was related to the dysregulation of the

transcription factor SNAIL. SNAIL inhibits the expression of miR-590, thereby upregulating the expression levels of NRP1 and VEGFR1/2; this leads to the promotion of epithelial-mesenchymal transition (EMT) process in GC and upregulation of SNAIL. In addition, the overexpression of miR-590 inhibits the migration, invasion, proliferation and digital microvascular (D-MVA) levels of gastric cancer cells *in vivo* and *in vitro* by targeting VEGFR1/2 and NRP1 [57].

3.2 miRNAs involved in HIF pathway

HIF-1 is a dimeric protein (HIF-1 α , HIF-1 β) complex playing an important role in the response to low oxygen concentrations or hypoxia. HIF-1 is a crucial physiological regulator of homeostasis, vascularization and anaerobic metabolism, but in cancer it allows for survival and proliferation of cancerous cells due to its angiogenic properties. HIF-1 is responsible for the migration of mature endothelial cells towards a hypoxic environment via the regulation of VEGF transcription. In our search, we identified two works reporting data about the aberrant expression in GC of HIF-related miRNAs. The first of them, by Zhang et al., has demonstrated how under hypoxic condition (consisting in GC cells cultured under 2% O₂ or in medium containing CoCl₂), HIF-1 elevation leads to the increase of miR-574-5p expression level in GC cells. Authors have suggested that the molecular mechanism involved is that miR-574-5p activates 44/42 Mitogen-Activated Protein Kinases (MAPKs) by suppressing the expression of its target gene, PTPN3 (protein tyrosine phosphatase), promoting angiogenesis by enhancing expression of VEGFA [58]. Furthermore, authors have confirmed the role of miR-574-5p in mice tumor xenografts. In this case, the inhibition of miR-574-5p reduced the expression of CD31, a well-known endothelial cell marker.

In the second study, Seo et al. reported that another miRNA, named miR-210, was found to be progressively upregulated in response to HIFs in hypoxic conditions in GC. In this work, miR-210 was identified as a hypoxia-induced miRNA playing key roles in biological processes such as cell cycle progression, metabolism, apoptosis, angiogenesis and in the metastasis of cancer [59].

3.3 miRNAs involved in HGF/c-MET signaling

A third important pathway for angiogenesis and tumorigenesis is the one regarding HGF/c-MET signaling. HGF is a pleiotropic cytokine that has been reported to prevent and attenuate disease progression by influencing multiple pathophysiological processes and promoting cell proliferation, survival, motility, scattering, differentiation and morphogenesis [60]. c-MET is a receptor tyrosine kinase that binds with its ligand HGF and activates a wide range of different cellular signaling pathways, including those that are involved in proliferation, motility, migration and invasion [61]. When the tyrosines within the multifunctional docking site become phosphorylated, they recruit: 1) signaling effectors such as the adaptor proteins growth factor receptor-bound protein 2 (GRB2), src homology 2 domain-containing (SHC), v-crk sarcoma virus CT10 oncogene homolog (CRK) and CRK-like (CRKL); 2) effector molecules as PI3-K, phospholipase C γ (PLC γ) and

the Proto-oncogene tyrosine-protein kinase SRC, the src homology 2 domain-containing 5' inositol phosphatase (SHIP-2), and 3) signal transducer and activator of transcription STAT3 [62,63]. Therefore, both HGF and c-MET have been recognized as part of a critical pathway with a key role in angiogenesis in cancer, including GC. In this context, we identified one work, carried out by Si et al., in which this signaling has been demonstrated to be involved in GC angiogenesis by miRNA deregulation. In this study, authors focused on miR-26a/b that can potentially target HGF in GC. As a result, HGF was up-regulated in GC in vivo and in vitro, while miR-26a/b was significantly down-regulated. Authors found that the expression of VEGF was induced by HGF, and HGF was up-regulated as a result of downregulation of miR-26a/b. Thus, miR-26a/b appears to promote angiogenesis in GC [64].

3.4 miRNAs involved in PI3-kinase pathway

The phosphatidylinositol 3-kinase (PI3K) signaling pathway regulates growth, survival proliferation and angiogenesis [65-67]. This pathway has two major positive and negative regulators, PI3-kinase and PTEN respectively, which are two of the most frequently mutated proteins in human cancers involved in tumorigenesis. PTEN mainly regulates PI3K signaling by dephosphorylating the lipid signaling intermediate PIP₃ but may have additional phosphate-independent activities and other functions in the nucleus. PTEN is a tumor-suppressor gene that negatively regulates mTORC1 activity, which role is to activate translation of proteins. The restoration of PTEN expression may block angiogenesis in GC by inactivating the PI3K/AKT pathway [51]. Additionally, several miRNAs have been identified to target the Forkhead box O (FOXO) transcription factors and the Tuberous Sclerosis Complex Subunit 1 (TSC1) [51]. FOXO is a key substrate of AKT that is conserved in insulin signaling in invertebrates. AKT-mediated phosphorylation of the transcription factor FOXO can increase proliferation and survival by causing FOXO to be retained in the cytoplasm, preventing it from activating transcription of cell-cycle-regulatory genes such as p27Kip1 and proapoptotic genes such as Fas Ligand (FASL) and B cell lymphoma-2 like 11 (BIM). TSC1 and TSC2, the Tuberous Sclerosis Complex Subunit 2, form a complex that inhibits activity of the small G protein Rheb. AKT-mediated phosphorylation of TSC2 relieves its inhibition of Rheb activity, leading to the activation of the rapamycin-sensitive mTOR complex, mTORC1. The TSC complex is also activated under nutrient-/energy-poor conditions by the action of the serine/threonine kinase LKB1/STK11 (liver kinase B1/serine-threonine protein kinase 11) and AMPK (AMP-activated protein kinase), leading to the attenuation of mTORC1 signaling [65]. Moreover, mTOR is comprised of two complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). The function of mTORC1 is modulated within the PI3K/Akt pathway via phosphorylation alongside inactivation of TSC1/TSC2. The last component of this signalling that we have taken into account is nuclear factor kappa B (NF- κ B). NF- κ B proteins function as dimeric transcription factors that regulate the expression of genes influencing a broad range of biological processes

including innate and adaptive immunity, inflammation, stress responses, B-cell development, and lymphoid organogenesis.

In our literature search we have identified 11 relevant studies about miRNAs belonging to this signaling pathway: four studies describe miRNAs that target PTEN, in three studies the target is the Forkhead box O (FOXO), three studies reported miRNAs targeting mTOR and in 1 study the target is NF- κ B.

3.5 miRNAs targeting PTEN

In the first of these studies, miR-23a resulted in augmented in HGC-27-derived exosomes, and facilitates angiogenesis by targeting PTEN, as verified by elevated expression level of VEGF and reduced expression level of the matricellular protein thrombospondin-1 (TSP-1) [51]. The same study demonstrates that the up-regulation of another miRNA, MiR-616-3p in GC tissues results in the PTEN down-regulation and protein kinase B (AKT)/ mammalian target of rapamycin (mTOR) pathway activation through PTEN, which then contributes to EMT and angiogenesis [51].

A second study confirmed a relevance for miR-23a and a third study for MiR-616-3p's in GC, respectively [68,50]. In the study by Du et al., miR-23a was highly expressed in GC tissues and cells and GC cell-derived exosomes. This miRNA demonstrated in a co-culture system to promote angiogenesis via the repression of PTEN [68]. MiR-616-3p's role as promoter of angiogenesis via the PTEN/AKT/mTOR pathway in GC has also been confirmed in the study by Wu et al [50].

Two other miRNAs, miR-718 and miR-382 have been suggested to target PTEN, thus inhibiting the angiogenesis and progression of GC [69]. In this study by Liu S et al., low expression of PTEN and increased expression of miR-718 demonstrated to be independent unfavorable prognostic factors for GC [69].

3.6 miRNAs targeting FOXO

Two works have reported that miR-155 alone and into GC-derived exosomes could promote angiogenesis in vitro models of GC through inhibiting FOXO3a expression. MiR-155 also seems to have the same role in vivo, where it has been shown to facilitate the angiogenesis in GC [70,71].

In the same direction, miR-135b has been found to suppress the expression of FOXO1 protein and enhanced the growth of blood vessels in GC [72].

3.7 miRNAs targeting mTOR

The overexpression of miR-18a has been shown to inactivate the mTOR pathway and downregulate HIF1 α and VEGF expression in a cell line of GC named SGC-7901. Furthermore, miR-18a has been identified as the cause of the substantial reduction in the number of microvessels in SGC-7901 xenograft model of GC [53].

Emerging data have shown that circulating miR-18a expression level was significantly different between GC individuals and healthy groups, implying that miR-18a may be a

potential biomarker for gastric cancer [73]. In this study, miR-18a levels were associated with the progression of advanced GC and effectiveness of XELOX chemotherapy.

Another work has reported that the exogenous expression of miR-101-2, miR-125b-2 and miR-451a decreased the expression of their putative targets MTOR, PIK3CB and TSC1, respectively [74].

3.8 miRNAs targeting NF-κB

Finally, a study by Zhang et al., demonstrated that miR-532-5p attenuates NF-κB signaling by directly inhibiting NCF2 expression, while miR-532-5p silencing in GC enhances NF-κB activity. miR-532-5p overexpression inhibits GC metastasis and angiogenesis in vitro and in vivo, whereas miR-532-5p silencing had the opposite effect. Furthermore, it was demonstrated that miR-532-5p down-regulation was caused by aberrantly high expression of lncRNA LINC01410 in GC. Mechanistically, overexpression of LINC01410 promoted GC angiogenesis and metastasis by binding to and suppressing miR-532-5p, which resulted in up-regulation of NCF2 and sustained NF-κB pathway activation. Interestingly, NCF2 could in turn increase the promoter activity and expression of LINC01410 via NF-κB, thus forming a positive feedback loop that drives the malignant behavior of GC [75].

3.9 miRNAs involved in STAT3 signaling

STAT3 belongs to a member of a family of seven proteins (STATs 1, 2, 3, 4, 5a, 5b, 6) that relay signals from activated cytokine and growth factor receptors in the plasma membrane to the nucleus, where they regulate gene transcription. STAT-3 activated genes block apoptosis, favor cell proliferation and survival, promote angiogenesis and metastasis, and inhibit antitumor immune response [76].

Among all these seven STAT proteins, STAT3 plays a critical role in angiogenesis [77,78]. In our literature search we have identified a study which analyzed the effect of miR-874 on the VEGFA gene in GC. This study demonstrated that the overexpression of this miRNA determines an inhibition of STAT3 gene expression, leading to the inhibition of VEGFA expression and, in this way, to a reduction of tumor growth and angiogenesis in vitro and in vivo [79]. These results suggest that miR-874 overexpression may inhibit the VEGFA pathway, angiogenesis and tumor growth by acting on the JAK/STAT pathway in GC [52].

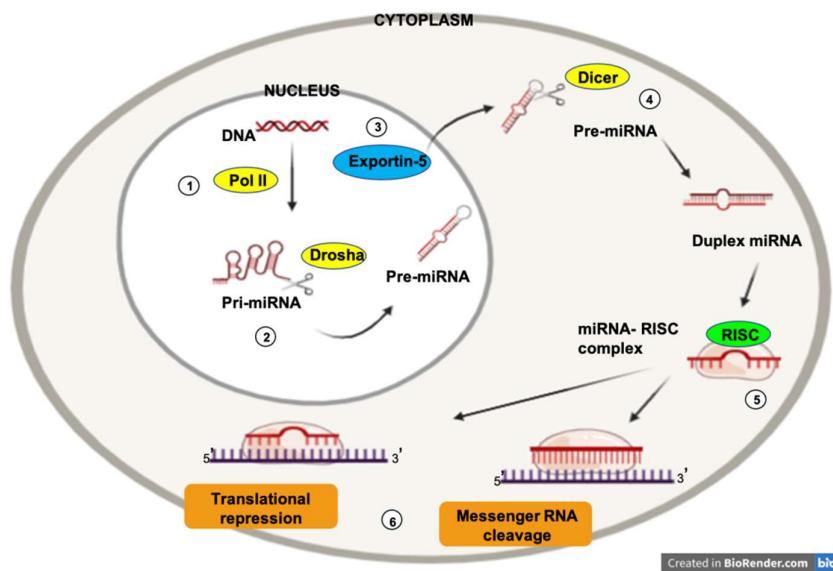


Figure 1. Expression of miRNAs is a complex biological process. RNA polymerase II is responsible for the transcription of a precursor RNA, several thousand nucleotides long and with a loop (hairpin) shape, known as "primary miRNA" (pri-miRNA) (1). The nuclear endonuclease Drosha processes the pri-miRNAs by cleaving the distal portion and making shorter chains (70-100 nucleotides) (2). This yields the pre-miRNA, which is transported into the cytoplasm via the nuclear receptor Exportin-5 (3). Once in the cytoplasm, the Dicer enzyme processes the pre-miRNA to obtain a short (19-25 nucleotides) double chain RNA sequence (4). Subsequently, one of the two chains is rapidly degraded, while the remainder represents the mature miRNA. Once processed, mature miRNAs can interact with Ago2, an enzyme of the Argonaute family of endonucleases, to form so-called RNA-induced silencing complexes (RISCs) (5). This allows the interaction between the mature miRNA and the target mRNA (6). The miRNA can perform its function (degradation or translational repression).

Table 1. MiRNA classification based on referral pathways.

miRNA	Target genes	Classification	Reference
miR-1	VEGF-A EDN1 MET	VEGF pathway	Azarbarzin S, et. al 2020 [51]
miR-29a/c	VEGF	VEGF pathway	Zhang H, et al. 2016 [55]
miR-27b			
miR-101	VEGF-C	VEGF pathway	Liu HT, et al. 2015 [56]
miR-128			
miR-126	VEGF-A	VEGF pathway	Cuzziol CI, et al. 2020 [52] Yang Q, et al. 2015 [53]
miR-590	VEGFR1/2 NRP1	VEGF pathway	Mei B, et al. 2020 [57]
miR-210	HIF	HIF pathway	Seo AN, et al. 2019 [59]
miR-574-5p	PTPN3	HIF pathway	Azarbarzin S, et. Al 2020 [51] Zhang S et al. 2020 [58]
miR-616-3p	PTEN	PI3K pathway VEGF pathway	Wu ZH, et al. 2018 [50]

miR-26a/b	HGF	HGF/c-MET signaling	Si Y, et al. 2017 [64]
miR-18a	mTOR	PI3K pathway	Yang Q, et al. 2015 [53]
miR-23a	PTEN	PI3K pathway	Azarbarzin S, et. Al 2020 [51] Du J, et al. 2020 [68]
miR-101-2		PI3K pathway	
miR-125b-2	MTOR/PIK3CB/TSC1	PI3K pathway	Riquelme I, et al. 2016 [74]
miR-451a		PI3K pathway	
miR-135b	FOXO1	PI3K pathway	Bai M, et al. 2019 [72]
miR-382	PTEN	PI3K pathway	Du J, et al. 2020 [68] Seo AN, et al. 2019 [59]
miR-532-5p	NCF2	PI3K pathway	Zhang JX, et al. 2018 [75]
miR-718	PTEN	PI3K pathway	Du J, et al. 2020 [68]
miR-155	c-MYB/VEGF FOXO3a	Other - c-MYB PI3K pathway	Azarbarzin S, et. Al 2020 [51] Deng T, et al, 2020 [70] Zhou Z, et al. 2019 [71]
miR-874	STAT3/VEGF-A	STAT3 signaling	Cuzziol CI, et al. 2020 [52] Zhang X, et al. 2015 [79]

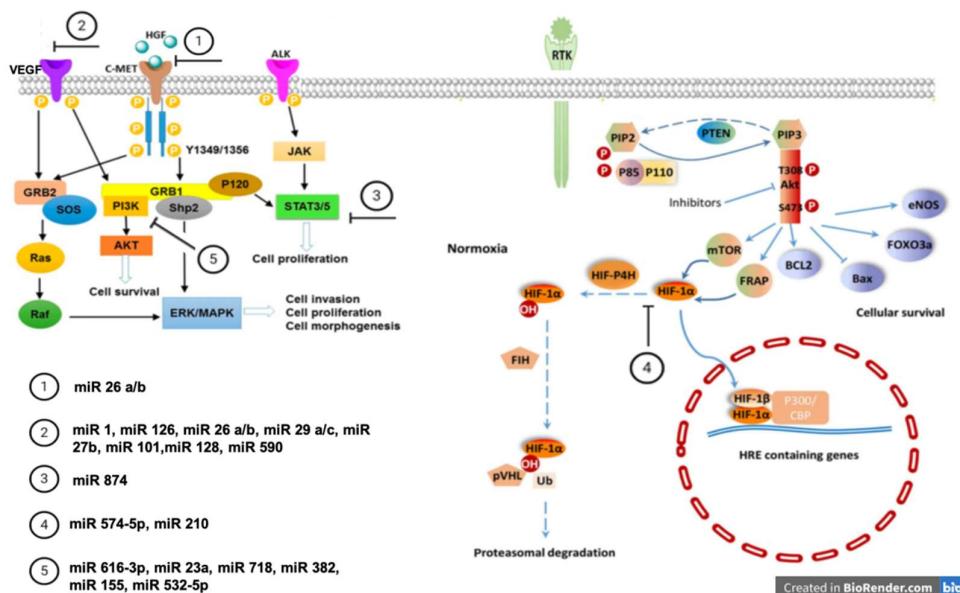


Figure 2. Major groups of microRNAs and related pathways by which they act on endothelial cells.

4. Discussion

Much investigation has been carried out in order to identify predictive biomarkers of response to antiangiogenic agents and mechanisms of resistance to the same drugs in advanced GC. VEGF-A and C high tissue expression was found to be correlated with poor prognosis and higher risk of relapse in patients with resected GC [80-82]. On the other

hand, although ramucirumab acts by blocking the downstream effects of the VEGF pathway, VEGF subtypes expression has never shown to be a predictor of response to antiangiogenic treatment in GC. An exploratory biomarkers analysis of the REGARD trial tested VEGFR expression with immunohistochemistry (IHC), while serum samples were assayed for VEGF-C and D and sVEGFR-1 and 3. Results were not able to identify a strong potentially predictive biomarker of ramucirumab efficacy [83]. Similarly, the biomarker analysis of the RAINBOW study was unsuccessful in identifying circulating predictive factors in plasma samples of GC patients [84]. Ramucirumab is frequently used in combination with paclitaxel, because the combination of both drugs brings to a synergistic inhibitory effect on cell growth [85]. Ramucirumab acts by enhancing the growth inhibition of paclitaxel and the inhibitory effects of chemotherapy on cell migration and actin polymerization. Moreover, the two agents modulate the cell expression of VEGF-A and VEGFR-2 and the signaling of MAPK and PI3K/AKT/mTOR pathways [85]. Promotion of angiogenesis is typical of chromosome instable tumors, accounting for the majority (50%) of GC. These tumors typically harbor overexpression of the gene encoding VEGF-A [86]. As previously shown, many miRNAs act by interfering with these pathways and may play both a prognostic and a predictive role of response to ramucirumab single agent and in combination with paclitaxel. Unfortunately, miRNAs have not been evaluated as predictive biomarkers of response to antiangiogenic treatment so far.

Recently, there has been increasing interest in the development of antiangiogenic options for GC. Apatinib, a tyrosine kinase inhibitor (TKI) targeting VEGFR-2, was tested in a phase III placebo-controlled trial in Chinese patients with advanced pretreated GC. Rivoceranib (apatinib) treatment significantly improved PFS and OS with compared to placebo but with a poor clinical impact on survival outcomes (median PFS 2.6 for apatinib vs 1.8 months for placebo, $p<0.001$; median OS 6.5 for apatinib vs 4.7 months for placebo, $p<0.0149$) [87]. In the randomized phase III placebo-controlled ANGEL trial, including both Eastern and Western patients, rivoceranib treatment significantly improved median OS from forth line of treatment (6.43 for apatinib vs 4.73 months for placebo, $p=0.0195$), while median PFS was improved from third line ahead (2.83 for apatinib vs 1.77 months for placebo, $p<0.0001$) [88]. Among ongoing and recruiting studies, a phase II trial is evaluating the combination of apatinib and the PD-1 inhibitor sintilimab in unresectable GC with oligometastases as conversion therapy (NCT04267549). In another trial, apatinib is being combined with docetaxel and S-1 as first line treatment for advanced GC (NCT03154983). Moreover, the TKI regorafenib is being tested as maintenance treatment after first-line treatment for metastatic GC and absence of progression (NCT03627728). Lastly, a phase I/II trial is testing ramucirumab together with the PARP inhibitor olaparib in advanced and unresectable GC (NCT03008278), while a phase II study is comparing ramucirumab plus FOLFIRI to ramucirumab plus standard paclitaxel in second-line treatment (NCT03081143).

5. Conclusions

MiRNAs are involved in the regulation of neoplastic angiogenesis, which is a crucial mechanism in GC onset and progression. Neither tissue nor circulating biomarkers have shown a predictive role for response to antiangiogenic treatment so far. In future studies,

miRNAs should be evaluated as candidate biomarkers with prognostic and predictive features, especially in the majority of GC harboring a pro-angiogenic molecular signature.

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