# Chapter 17
# miRNA Regulation of VEGF/VEGFR Signaling

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## 1 Introduction

Tumor angiogenesis is a multifaceted molecular mechanism and depends on the release of angiogenic factors/growth factors by neoplastic cells specific for endothelial cells and development of microvasculature. There is a mutational activation of oncogenes and signaling cascades that promote endothelial cell migration and invasion of the surrounding extracellular matrix (ECM) [1].

Vascular endothelial growth factor (VEGF) gene is an extensively investigated regulator reported to be involved in angiogenesis and lymphangiogenesis thus promoting growth and metastasis of neoplasms. VEGF encodes five polypeptide growth factors, VEGF-A, -B, -C, -D, and -E. VEGF signals through VEGFR-1 (Flt-1) and VEGFR-2 (KDR) thus transducing signals intracellularly [2]. Ligands and receptors are shown in Fig. 17.1. In the upcoming sections we will discuss regulation of VEGF and VEGFR by miRNA. However, before discussing intricate network of miRNA regulation of VEGF/VEGFR signaling axis, we provide an overview of miRNA biogenesis.

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F.H. Sarkar (ed.), *MicroRNA Targeted Cancer Therapy*,
DOI 10.1007/978-3-319-05134-5_17, 

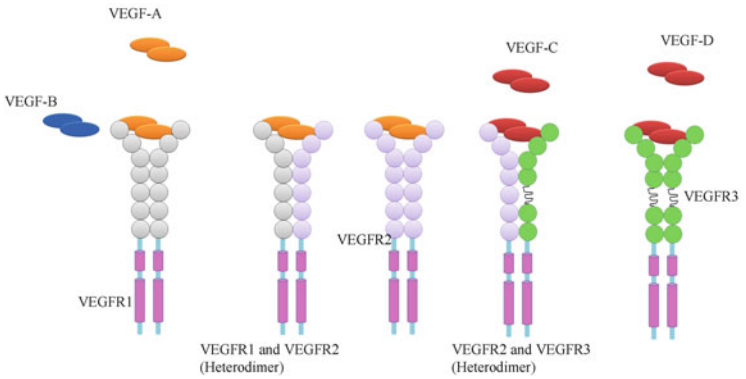


**Fig. 17.1** Shows ligands and receptors. Homodimerization and heterodimerization of receptors is also shown

## 2 miRNA Biogenesis

The miRNA biogenesis is a well orchestrated mechanism and tremendously mounting scientific information is improving our understanding of this biological phenomenon that includes the RNA polymerase II or III mediated generation of the primary miRNA transcript (pri-miRNA). Another important mechanism that occurs in the nucleus is Drosha–DGCR8 complex mediated processing of pri-miRNA into ~70-nucleotide precursor hairpin. The pre-miRNA hairpin is transported from the nucleus to cytoplasm by Exportin-5-Ran-GTP [3]. After its export from nucleus to the cytoplasm, pre-miRNA hairpin is processed by Dicer that yields ~20-bp miRNA/miRNA* duplex. This step is necessary for loading of one strand of the miRNA/miRNA* duplex (the guide strand) into miRNA-induced silencing complex (miRISC) and binding of target mRNAs to miRNAs in RISC is followed by translational inhibition or RISC mediated mRNA degradation. Passenger strand is degraded [4]. In the next section miRNA regulation of VEGFR1 and VEGFR2 will be discussed more extensively. VEGFR3 is insufficiently studied and needs more experimental evidence for a better understanding and information regarding the miRNA subsets which regulate VEGFR3 expression.

## 3 VEGFR1

Extracellular ligand-binding region of full-length VEGFR1 (FLT1) has signal peptide at N terminal followed by 7 immunoglobulin-like domains [5]. FLT1 domains 2 and 3 are necessary and sufficient for binding VEGF [6]. VEGFR1 has been shown to be targeted by different miRNAs particularly, miR-10 and miR-200.

### 3.1 miR-10

There is a recent report suggesting miR-10 mediated quantitative control of VEGFR1 and it was shown that miR-10 depleted HUVECs represented reduced phosphorylation of VEGFR2 upon treatment with low dose VEGF. It was indicated that VEGF/VEGFR1 interaction reduced VEGF mediated activation of VEGFR2 [7].

### 3.2 miR-200

Cancer-associated fibroblasts (CAFs) are isolated from murine lung adenocarcinomas. In-vitro assays have shown that CAFs secrete VEGF thus enhancing tumor cell invasion. Tumor cells are observed to be more invasive in coculture with CAFs as compared to normal fibroblasts (LFs) in the culture. CAFs mediated invasive potential of 344SQ cells was notably reduced upon using neutralizing antibody against VEGF-A. Moreover, enforced expression of miR-200 resulted in downregulation of VEGFR1 thus suppressing CAF mediated invasion promoting effects in 344SQ cells. Role of VEGFR1 in transducing VEGF mediated signals intracellularly was further confirmed by subcutaneously injecting VEGFR1 expressing 344SQ cells into syngeneic mice. Syngeneic mice bearing VEGFR1 silenced 344SQ cells did not show metastasis [8].

## 4 VEGFR2

VEGFR2 is also expressed on the surface of blood endothelial cells and accumulating research is underscoring wide ranging VEGFR2 mediated responses in endothelial cells including proliferation, regulation of survival, migration, and vascular tube formation. VEGFR via phosphotyrosine residues located in the carboxy-terminal region activates downstream effectors, for example pTyr1175 results in activation of PKC and ERK pathway [9]. The pTyr1214 consequently activates tyrosine kinase Fyn which further activates Cdc42 and p38MAPK thus modulating reorganization of the actin cytoskeleton [10]. The following section will provide an overview of regulation of VEGFR2 by miR-200b and miR-200c.

### 4.1 miR-200b and miR-200c

The miR-200b has been shown to negatively regulate GATA2 and VEGFR2 and intriguingly, miR-200b mimic inhibited the angiogenic tube-forming ability of

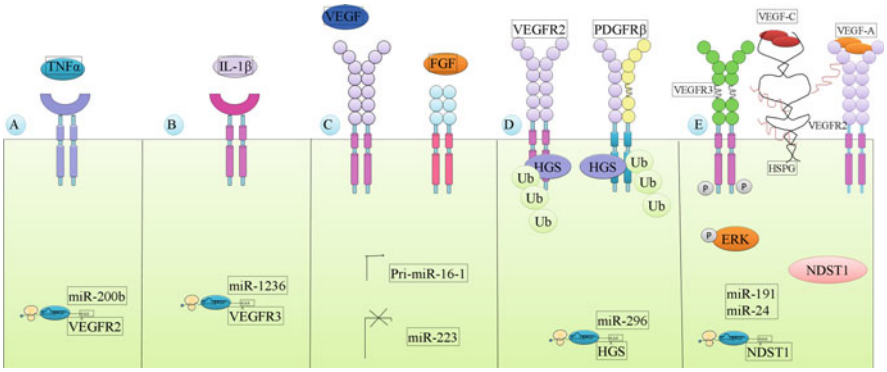


**Fig. 17.2** (*A*) TNFα induced miR-200b negatively regulated VEGFR2. (*B*) IL-1β induced miR-1236 controlled expression of VEGFR3. (*C*) VEGF and FGF induced signals repressed expression of miR-223. (*D*) Ubiquitylated VEGFR2 and PDGFRβ are recognized by the endocytosis machinery and targeted through the endosomes and the multivesicular body for degradation. Hepatocyte growth-factor-regulated tyrosine kinase substrate (HRS) is an adaptor molecule required for the intracellular trafficking. HGS is a target of miR-296. (*E*) Heparan Sulphate Proteoglycan (HSPG) is post-translationally modified by NDST1. HSPG bound VEGF transduces signals through VEGFRs. miRNA mediated targeting of NDST1 resulted in suppression of VEGF induced intracellular signaling through VEGFR

endothelial cells. Experimental data provided convincing evidence that treating human dermal microvascular endothelial cells (HDMECs) with TNF-α considerably reduced angiogenic response. Detailed mechanistic insights suggested TNF-α mediated upregulation of miR-200b, shown in Fig. 17.2. The anti-miR-200b treated HDMECs had substantially enhanced GATA2 and VEGFR2 protein levels [11]. Non-Small-Cell Lung Cancer Cell Line A549 was radiosensitized by enforced expression of miR-200c. It was shown that miR-200c negatively regulated VEGFR2 in A549 cancer cells [12].

## *4.2 miR-15*

VEGFR2 has also been shown to be regulated by miR-15. HUVECs overexpressing miR-15 displayed significant decrease in migration and tubulogenesis however, ginsenoside-Rg1 (Rg1) mediated repression of miR-15 enhanced VEGFR2 expression. Moreover, in-vivo analysis verified that injecting pre-miR-15b precursor into zebrafish embryos substantially inhibited subintestinal vessels formation [13].

Mice bearing Lewis lung carcinoma (LLC) or B16.F10 melanoma were treated with vandetanib (ZD6474). The results revealed that vandetanib significantly suppressed protein expression of VEGFR2 thus retarding the tumor growth in mice. Moreover, miR-296 mediated negative regulation of VEGFR2 was not observed [14].

## 5 VEGFR3

Lymphatic endothelial cells upon treatment with IL-1β revealed considerably reduced protein expression of VEGFR3 however mRNA level was not altered. Mechanistically it was shown that IL-1β exerted its inhibitory effects on VEGFR3 protein expression through miR-1236, shown in Fig. 17.2. Using antagomirs against miR-1236 verified the fact that VEGFR3 protein expression was under direct control of miR-1236 [15].

## 6 VEGF and bFGF Mediated Repression of miRNAs

VEGF- and bFGF have been shown to transcriptionally upregulate the expression of pri-miR-16-1. However, miR-424 was not under transcriptional control of VEGF- and bFGF. However, post-transcriptional processing of miR-424 was noted to be triggered by these cytokines. It has been shown that enforced expression of miR-16 or miR-424 considerably inhibited basal migration, as well as VEGF- or bFGF-induced migration, in bovine aortic ECs. The miR-16 or miR-424 transfected HUVECs upon treatment with VEGF or bFGF demonstrated significant impairment of cord formation as well as under basal conditions. Transfecting HUVECs with VEGFR2 or FGFR1 cDNA lacking respective 3′UTR rescued migration in miR-16 or miR-424 transfected ECs. Akt and ERK1/2 are downstream effectors of growth factor mediated signaling. It was noted that pAkt and pERK1/2 were remarkably reduced in miR-16 transfected ECs upon treatment with VEGF or bFGF [16]. In line with this mechanism, another recent study indicated VEGF- and bFGF mediated repression of miR-223, shown in Fig. 17.2. Interestingly, cells reconstituted with miR-223 displayed dramatically reduced phosphorylation of receptor and Akt upon treatment with VEGF- and bFGF. The β1 integrin was noted to be negatively regulated by miR-223 and miR-223 overexpressing cells reconstructed with β1 integrin rescued growth factor mediated signaling and angiogenesis [17].

## 7 HSPG, NDST-1 and VEGF: Companionship During Signaling

Increasingly it is being realized that heparan sulfate proteoglycans (HSPG) act as co-receptors thus regulating angiogenesis. HS proteoglycan is post-translationally modified by NDST-1 and later binds with VEGF. HS expressed by primary lymphatic endothelium binds to VEGF, thus transducing signals intracellularly via phosphorylation of VEGFR and downstream effectors ERK1/2. Heparinase treated cells displayed dramatically reduced VEGFR3 phosphorylation in response to VEGF-C. RNA interference strategies against N-deacetylase/N-sulfotransferase-

1 (Ndst1) have shown that VEGF-C-mediated Erk1/2 phosphorylation was reduced markedly [18]. Circumstantial evidence substantiates the fact that Ndst1 is negatively regulated by miR-191 and miR-24 [19, 20]. Overexpressing miR-24 in cells remarkably suppressed Ndst1, thus inhibiting VEGF-A mediated activation of VEGFR2 [20], shown in Fig. 17.2. It is concluded that targeting of NDST-1 is an effective strategy to suppress VEGF/VEGFR signaling axis.

## 8 DCLK1 Suppresses miRNA Expression

DCLK1 is an intestinal and pancreatic stem cell marker. It is frequently upregulated in the stroma and epithelium of pancreatic ductal adenocarcinoma. It is appropriate to mention that ablation of Dclk1 expressing cells in $Apc^{min/+}$ mice resulted in regression of polyps. Use of nanoparticle-encapsulated siRNA against DCLK1 (NPsiDCLK1) in tumor xenografted mice has been noted to upregulate expression of miRNA subsets including miR-143/145 cluster, let-7a and miR-200a, b and c. NPsiDCLK1 treated tumors had remarkably reduced mRNA and protein expression of VEGFR1 and VEGFR2 [21].

## 9 miRNAs Enter into HUVECs to Regulate VEGFR

It has recently been persuasively revealed that miRNA produced from epithelial ovarian cancer cells (EOC) is secreted into the local microenvironment and enters HUVEC cells within 24 h. The findings were substantiated by co-culturing HUVECs with control and miR-484–overexpressing SKOV-3 cells. It was found that VEGFR2 protein on endothelial cells was considerably reduced in HUVECs co-cultured with miR-484–overexpressing SKOV-3 cells [22].

## 10 Dicer Expression Is Suppressed in Hypoxic HUVECs

It seems intriguing to note that hypoxia represses mRNA and protein expression of Dicer in HUVECs treated with hypoxia mimetic desferrioxamine. Enforced expression of dicer in HUVECs remarkably reduced the expression of HIF-2α via miR-185. It was noted that there was an accumulation of miR-185 precursors instead of mature miR-185 in hypoxic HUVECs [23].

## 11 miRNA Regulation of VEGF-A

VEGF-A has emerged as a potent mitogen that underlies physiological and pathological angiogenesis. Interestingly, intricacy and multiplicity of regulatory mechanisms involved in VEGF-A expression are deeply studied and significant breakthroughs have been made in identification of miRNAs involved in regulation of VEGF-A.

### *11.1 miR-26a*

Migration and tube forming of HUVECs is remarkably reduced upon co-culturing with HepG2 cells reconstituted with miR-26a. VEGF-A was noted to be targeted by miR-26a in HepG2 cells. The results obtained from in-vivo studies were encouraging as evidenced by miR-26a mediated suppression of ectopic and orthotopic tumor growth and vascularity in nude mice. PIK3C2α belongs to class II PI3Ks and is a known target of miR-26a. pAkt and HIF-α was substantially reduced in miR-26a transfected cells. To verify that PI3K/Akt/HIF-α signaling axis is involved in mediating VEGF-A, cells were treated with PI3K inhibitor and HIF-1α inhibitor that resulted in suppression of VEGF-A expression [24].

### *11.2 miR-29a/b*

DNA methyltransferase is a direct target of miR-29b and it has been shown that transfection of miR-29b in multiple myeloma cells restored expression of SOCS-1 via promoter demethylation. SOCS-1 expression notably suppressed pSTAT3 levels in miR-29b transfected cells. Shown in Fig. 17.3. VEGF-A mRNA was also repressed in miR-29b transfected multiple myeloma cells [25]. VEGF-A is also negatively regulated by miR-29a in gastric cancer cells. Ectopic expression of miR-29a in low expressing gastric cancer cells dramatically reduced VEGF-A expression [26].

### *11.3 miR-125a and miR-126*

MMP11 and VEGF-A are regulated by miR-125a in HCC cells. Proliferation and metastasis of HCC cells was suppressed upon overexpression of miR-125a [27]. The miR-126 gene is embedded in intron7 of EGF-like domain 7 (EGFL7). The miR-126 was notably repressed in breast cancer cells MCF-7 and reconstitution strategies effectively reduced VEGF/PI3K/AKT signaling activity [28]. Enforced

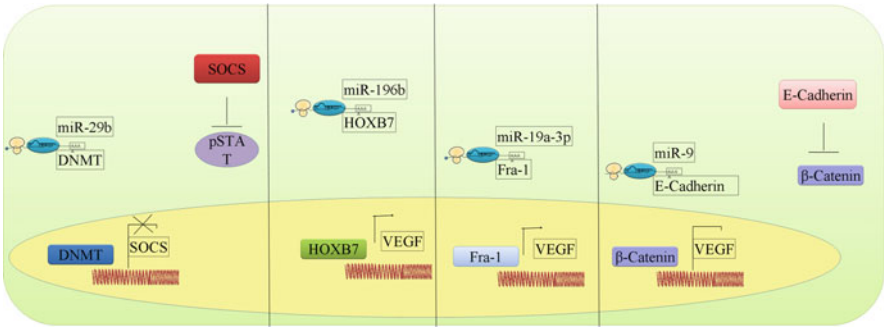


**Fig. 17.3** Shows miRNA regulation of regulators involved in modulation of VEGF. DNMT is targeted by miR-29b. Suppressor of Cytokine Signaling (SOCS) is epigenetically repressed and targeted inhibition of DNMT restored expression of SOCS that inhibited STAT. HOXB7 is also involved in regulation of VEGF and targeted by miR-196b. Fra-1 is involved in regulation of VEGF and targeted by miR-19a-3p. β-Catenin modulates the expression of VEGF. βCatenin is negatively regulated by E-Cadherin. However, E-Cadherin is targeted by miR-9

expression of miR-126 resulted in an improved response of drug resistant NSCLC cancer cells to adriamycin and vincristine. The miR-126 transfected cancer cells remarkably reduced tumor formation in xenografted mice via negative regulation of VEGF-A [29]. VEGF is a known target of miR-126 reported to be epigenetically repressed in colorectal cancer cells. Colorectal cancer cells treated with 5-aza-CdR displayed an increase in miR-126 expression and consequently VEGF was downregulated [30].

## *11.4 miR-185*

Leucine-rich repeat C4 (LRRC4) belongs to LRR protein superfamily and specifically expressed in brain tissue and regulates the expression of miR-185. CDC42 and RhoA are direct targets of miR-185 in glioma cells and downregulation of miR-185 restores their expression. Enforced expression of miR-185 resulted in suppression of CDC42 and RhoA. Additionally, VEGF-A was also found to be indirectly regulated by miR-185 [31].

## *11.5 miR-203 and miR-205*

VEGF-A is negatively regulated by miR-203 and enforced expression of miR-203 resulted in inhibition of tumor growth and angiogenesis in nude mice [32]. miR-205 is suppressed in glioblastoma cells and cells reconstituted with miR-205 resulted in induction of apoptosis and cell cycle arrest [33], shown in Table 17.1.

**Table 17.1** Shows list of miRNAs regulating VEGF-A and VEGF-C

| | |
|---|---|
| VEGF-A | miR-26a, miR-29a, miR-29b, miR-126, miR-203, miR-361-5p, miR-503 |
| VEGF-C | miR-27b, miR-1826 |

### *11.6 miR-361-5p and miR-503*

The miR-361-5p targets VEGF-A [34]. Overexpression of miR-503 in hepatocellular carcinoma cells inhibited VEGF-A [35], shown in Table 17.1.

### *11.7 miR-196b*

The miR-196b suppressed expression of VEGF via targeting of HOXB7 in cervical cancer cells. VEGF was noted to be reduced in HOXB7 silenced cancer cells. Overexpression miR-196b or gene silencing of HOXB7 resulted in suppression of VEGF in cervical cancer cells [36], shown in Fig. 17.3.

### *11.8 miR-378*

5-aza-dC treated gastric cancer cells presented enhanced expression of miR-195 and miR-378 and consequent suppression of VEGF [37]. It is surprising to note that there is a report indicating that stable miR-378 overexpression in NSCLC NCI-H292 cells dramatically enhanced the expression of VEGF. It was concluded that miR-378 promoted non-small cell lung carcinoma growth, vascularization, and metastasis [38].

### *11.9 miR-20b*

The miR-20b negatively regulates HIF-1α and VEGF. HIF-1α transfected normoxic H22 cells showed downregulation of miR-20b [39].

## 12 miRNA Regulation of VEGF-C

There is a recent report that suggests dual targeting of VEGF-A and VEGF-C in gastric cancer cells. In-vitro analysis provided evidence that Lentivirus-mediated RNAi suppressed mRNA and protein expression of VEGF-A and VEGF-C in the SGC7901 cells [40]. Data obtained through 3′UTR luciferase assay has revealed

that VEGF-C mRNA has complimentary binding site with miR-1826 within its 3′UTR. Interestingly, VEGF-C protein expression is suppressed in miR-1826-transfected bladder cancer cells [41]. VEGF-C has also been reported to be targeted by miR-27b. miR-27b is downregulated in colorectal cancer cells because of hypermethylation of CpG islands [42]. Shown in Table 17.1.

## 13 VEGF Regulation

Substantial fraction of information has been added into the modes underlying expression of VEGF. There are various proteins which are involved in regulation of expression of VEGF. In this segment we will discuss regulatory mechanisms of VEGF by β-catenin, N-RAS, IRS, NF-κB1, Fra-1 and p70S6K1.

### *13.1 β-Catenin*

Surprisingly, miR-9 induced activation of β-catenin that consequently triggered expression of VEGF. miR-9 modulated targeting of E-cadherin promoted nuclear translocation of β-catenin thus stimulating expression of VEGF. miR-9 was reported to be triggered by MYC/MYCN in breast cancer cells [43], shown in Fig. 17.3.

### *13.2 N-RAS, IRS1 and NF-κB1*

N-RAS and IRS1 mediated expression of VEGF is also reduced in miR-145 overexpressing colorectal cancer cells. Phosphorylated AKT and ERK1/2 levels were reduced notably in miR-145 overexpressing cells [44]. Similarly, VEGFA, MMP2 and MMP9 are transcriptional targets of NF-κB1. NF-κB1 itself is under direct control of miR-9 in uveal melanoma cells [45].

### *13.3 Fra-1*

It is getting increasingly clear that tissue associated macrophages (TAMs) overexpress Fra-1, Stat3 and c-Jun. It is noteworthy that RAW mouse macrophages displayed enhanced expressions of Stat3 and p-Stat3 which was dependent on cytokines primarily released from tumor cells. TAMs co-cultured with 4T1 tumor cells indicated activated intracellular JAK/Stat3 signaling pathway and an increased expression of VEGF. Certain clues have emerged which point towards

miR-19a-3p mediated negative regulation of Fra-1 and transfecting RAW264.7 macrophages with miR-19a-3p mimic resulted in considerably reduced expression of Fra-1 and its target gene VEGF. Shown in Fig. 17.3. The strategy was found to be effective upon intratumoral injection of miR-19a-3p in tumor bearing mice which inhibited growth of 4T1 breast tumor cells [46].

## *13.4 mTOR/p70S6K1*

The mTOR/p70S6K1 regulates tumor angiogenesis and tumorigenesis and p70S6K1 is a direct target of miR-128. Reintroduction of p70S6K1 cDNA or ectopic expression of p70S6K1 in U87 and U251 cells resulted in upregulation of VEGF [47].

## *13.5 Specificity Proteins Regulate Expression of VEGF and VEGFR*

VEGF and VEGFR are triggered by specificity proteins. It has been experimentally verified that targeting of specificity proteins using natural and synthetic agents effectively reduced expression of VEGF and VEGFR.

Methyl 2-cyano-3,11-dioxo-18beta-olean-1,12-dien-30-oate (CDODA-Me) is isolated from licorice extracts. RKO colon cancer cells treated with CDODA-Me displayed remarkably reduced expression of Sp1, Sp3 and Sp4 mRNA levels. Surprisingly, Sp-target genes including VEGFR1 (Flt-1), and VEGF were also downregulated. CDODA-Me effectively induced regression of tumor load in athymic nude mice inoculated with RKO cells [48], shown in Table 17.2.

GT-094 is a novel nitric oxide (NO) chimera containing an NSAID and NO moieties. RKO and SW480 cancer cells treated with GT-094 demonstrated gradual reduction in Sp1, Sp3, and Sp4 proteins with increasing concentration of the drug. Additionally, protein expression of VEGF and VEGFR reduced significantly in a concentration dependent manner [49], shown in Table 17.2.

ER-negative MDA-MB-231 breast cancer cells displayed an increase in zinc finger ZBTB10 gene upon treatment with antisense miR-27a. Furthermore, ZBTB10 mediated repression of specificity proteins (Sp), Sp1, Sp3, and Sp4 was associated with reduced expression of target genes including VEGF and VEGFR1 as evidenced by RT-PCR and western blot assays [50].

**Table 17.2** Shows CDODA-Me and GT-094 mediated regulation of specificity proteins, VEGF and VEGFR

| Agent | Sp1 | Sp3 | Sp4 | VEGF | VEGFRI |
|---|---|---|---|---|---|
| CDODA-Me | ↓ | ↓ | ↓ | ↓ | ↓ |
| GT-094 | ↓ | ↓ | ↓ | ↓ | ↓ |

### *13.6 HER2 and HER3 Mediated Up-Regulation of VEGF*

Mounting evidence suggested that conditioned medium from miR-199a or miR-125b overexpressing OVCAR-3 and A2780 cells induced substantially reduced tube formation by HUVEC. Furthermore HER2 and HER3 were also noted to be targeted by miR-199a and miR-125b. pAkt and VEGF mRNA were suppressed in miR-199a or miR-125b transfected ovarian cancer cells. However, reintroduction of HER2 or HER3 in miR-199a or miR-125b transfected ovarian cancer cells resulted in restoration of pAkt levels and upregulated VEGF mRNA expression [51], shown in Table 17.3.

## 14 Therapeutic Interventions

The miRNA research has revolutionized molecular oncology and there is a rapidly increasing interest in developing strategies particularly, plasmids containing anti-VEGF miRNA clusters. These plasmids have shown gene silencing effect exerted by miRNA clusters composed of multiple miRNA-mimicked RNA interference effectors. Combinatorial approach using different miRNAs against VEGF revealed that delivery of miR-5,10,7 resulted in a knockdown of VEGF by approximately 75 %. This strategy was further tested in-vivo and noted to be effective as injection of scAAV2/8 vectors expressing miR-5,10,7 into murine hindlimb muscles, resulted in a 44 % suppression of VEGF [52].

Lentivirus-mediated expression of miR-20a precursor has been shown to inhibit endothelial cell migration upon treatment with VEGF. Astonishingly miR-20a reconstituted cells displayed reduced phosphorylated p38 protein levels. Results indicated miR-20a mediated negative regulation of MKK3. MKK3 is a modulator situated upstream to p38 MAPK in protein hierarchy [53]. Monitoring of the expression of VEGFR is of sufficient importance during evaluation of therapeutic strategies in vivo. In accordance with this approach, VEGFR2-luc transgenic mice have been used to monitor the VEGFR2 expression using bioluminescent imaging (BLI). VEGFR2-luc transgenic mice implanted with 4T1 murine breast cancer cells were treated with antagomir-21. Tumor volumes of control group and scramble treatment group were notably larger as compared to antagomir-21 treated group of mice [54].

There is a direct piece of evidence highlighting hepatocyte growth factor-regulated tyrosine kinase substrate (HGS) mediated degradation of VEGFR2 and

**Table 17.3** Shows list of miRNAs positively and negatively regulating VEGF

| | | |
|---|---|---|
| VEGF | Tumor suppressor miRNAs | Oncomir |
| VEGF | miR-199a<br>miR-125b | miR-378 |

PDGFRβ. miR-296 was found to be upregulated in human brain microvascular endothelial cells and targeting of miR-296 restored expression of HGS, shown in Fig. 17.2. Nude mice bearing U87 glioma cells injected with synthetic cholesterol-conjugated antagomir-296 displayed marked regression of tumor growth [55].

Confluence of information suggested Notch signaling mediated expression of truncated intracellular isoform transcribed from intron 21 (i21 Flt1). Breast cancer cells MDA-MB-231 were treated with γ-secretase inhibitor DAPT and i21 Flt1 was notably repressed. Similar results were obtained in Notch-1 and Notch-3 silenced MDA-MB-231 cells. Retinoic acid mediated inhibitory effects on i21 Flt1 expression were achieved via miR-200 upregulation [56].

## *14.1 Natural Agents Mediated Inhibition of VEGFR*

Barbigerone is an isoflavone recently reported to inhibit VEGF induced phosphorylation of VEGFR2 and downstream effectors including ERK, p38, AKT. Moreover, Barbigerone effectively inhibited tumor growth in A549 and SPC-A1 bearing mice [57]. VEGF mediated VEGFR2 phosphorylation was also noted to be inhibited by quercetin-4′-O-β-D-glucopyranoside (QODG), a flavonoid isolated from *Hypericum attenuatum Choisy* [58].

## *14.2 Natural Agents Mediated Regulation of miRNA*

Increasingly it is being realized that hypoxia-induced expression of miR-21 and miR-210 in pancreatic cancer cells. However, synthetic derivative of curcumin (CDF) inhibited miR-21 and miR-210 expression. Moreover, cancer stem cell (CSC) markers CD44 and EpCAM in CSC-like cells derived from pancreatic cancer cells were remarkably reduced in MiaPaCa-2 tumor sphere cells under hypoxic conditions. VEGF production by MiaPaCa-2 tumor sphere cells was suppressed significantly upon CDF treatment [59]. Garcinol is a polyisoprenylated benzophenone derivative isolated from Garcinia indica. Garcinol works synergistically with gemcitabine in pancreatic adenocarcinoma cells thus controlling different miRNAs including miR-21, miR-196a, miR-495, miR-605, miR-638, and miR-453 [60].

## 15 Conclusion

In this chapter we discussed recent advances in miRNA regulation of VEGF/VEGFR signaling axis. Moreover, it is evident that VEGF and VEGFR controlling miRNAs are frequently suppressed, therefore, modulation of miRNA levels via either antagomirs or miRNA mimicry seems to be a promising target for future therapeutics.

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