Angiogenesis and vasculogenesis determine the generation of blood vessels during development and in the adult. In general, angiogenesis involves growth of existing endothelial cells (ECs), whereas vasculogenesis comprises the de novo formation of blood vessels. The analysis of EC biology is of utmost importance to delineate pro- or antiangiogenic signaling cascades triggered by a variety of growth factors in health and disease. Prominent angiogenic factors include the vascular endothelial growth factor (VEGF) and the basic fibroblast growth factor (bFGF), which activate downstream mediators in a highly dynamic process.1,2 Endothelial migration and proliferation enhance the generation of primary capillaries that may undergo remodeling by sprouting, branching, or intussusception.3 In line with this, loss of VEGF in mice is embryonically lethal because of enhanced EC apoptosis and hemorrhage, thus emphasizing the essential role for EC survival.4,5 Enhanced angiogenesis is the major cause of tumor progression defining an angiogenic switch.6 Of note, stimulation of angiogenesis is favorable in ischemic diseases such as myocardial infarction, thus presenting strategies to sustain cardiac vascularization and function.7 Hypoxia-driven angiogenesis mainly relies on VEGF induction by stabilization of the transcription factor hypoxia-inducible factor-1α.8 Besides such classical genomic regulation, post-transcriptional regulation via microRNAs (miRNA, miR) has been reported both in physiological and pathological conditions.9–11 Mechanistically, miRNAs repress a set of target genes (miRNA targetome) at the same time and thus have an impact on whole cellular gene networks.12 Complementary base pairing between nucleotides 2 to 8 (the seed sequence) of the miRNA and the respective sequences in the target gene’s 3′-untranslated region (UTR) mediate either mRNA degradation or translational inhibition.13,14 Indeed, miRNAs are also capable to control angiogenic signaling and especially EC biology.15

See accompanying article on page 2595

In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Chamorro-Jorganes et al16 report that the endothelial miR-16 and miR-424 critically regulate the expression of the proangiogenic factor VEGF, as well as the angiogenic receptors VEGF receptor-2 (VEGFR2) and fibroblast growth factor receptor-1 (FGFR1). The interference of respective signaling cascades inhibits EC migration and proliferation in vitro and in vivo, consequently altering essential EC characteristics. Because of the putative role of miR-15, -16, and -424 in cancer biology,17,18 these miRNAs came into focus to analyze EC-intrinsic functional aspects. Another important hallmark of the chosen miRNAs is their common and identical seed sequence, implicating a similar miRNA targetome. Thus, the set of miRNAs is referred to as miR-16-like miRNAs. Bioinformatic analysis applying different miRNA target prediction databases revealed the presence of target genes with well-known functions in proliferation, cell cycle progression, and apoptosis. Next, further in depth-analysis showed that the majority of relevant effectors are characterized by a proangiogenic function. This initial screening approach pointed to an important regulatory function of miR-16-like miRNAs toward the expression of VEGFR2, FGFR1, and VEGF. Both miR-16 and miR-424 are expressed in ECs but at a negligible level in the fast-proliferating HeLa tumor cell line. To narrow down direct miRNA binding to the 3′-UTR of VEGFR2, FGFR1, and VEGF, luciferase reporter gene assays were performed. The respective miRNAs repressed luciferase activity in wild-type conditions, whereas the effects disappeared in mutated 3′-UTR, clearly validating bona fide miRNA targets. In line with this, protein levels of VEGFR2, FGFR1, and VEGF dropped down in miRNA-overexpressing ECs, whereas endogenous miRNA knockdown induced expression levels. Interestingly, VEGF and bFGF themselves induced expression level of mature miR-16/-424, whereas the effects on precursor pri-miRNA were different. This in turn led to miRNA binding to VEGFR2 and FGFR1 3′-UTR and was absent in miR-16/-424-depleted ECs before growth factor stimulation. Mimicking elevated miRNA expression in vitro impaired EC proliferation, migration, and tube formation. Importantly, these defects were also present under stimulatory conditions (VEGF, bFGF), highlighting miRNA targeting of proangiogenic receptors. At least for EC migration, miRNA-induced defects were rescued via miRNA target overexpression lacking a full miRNA-sensitive 3′-UTR. Mechanistically, cellular survival pathways (extracellular signal regulated kinase and Akt) were involved in the antiangiogenic functions of miR-16/-424, concomitantly reflecting the alteration of VEGFR2 and FGFR1 expression. Translating these findings into an in vivo approach, human ECs were lentivirally transduced with miR-16. Modulated cells were embedded into a matrix and transplanted to monitor engraftment and neovascularization capacity in immunodeficient mice. Capillary density was respectively analyzed after 14 and 21 days. Mimicking the in vitro scenario, elevated miR-16 levels impaired EC function and thus significantly decreased capillary (microvessel) density. Antiangiogenic characteristics of miR-16 are demon-
Angiogenic capacity of ECs is regulated by microRNAs (miRNAs) comprising the same seed sequence (microRNA [miR-16]-like miRNAs, eg, miR-16, miR-424) and is depicted in the plotted mechanistic scheme. The proangiogenic factor VEGF induces expression level of mature miR-16/-424. This in turn leads to repression of receptor tyrosine kinases vascular endothelial growth factor receptor-2 (VEGFR2) and fibroblast growth factor receptor-1 (FGFR1), subsequently interfering with downstream survival signaling cascades. In addition, vascular endothelial growth factor (VEGF) itself is targeted by miR-16/-424. In summary, endothelial cell function is impaired, and angiogenic capacity decreases.

Impaired angiogenic capacity

Transcriptional activation (e.g., via VEGF)

miR-16-like miRNAs (miR-16, -424)

Feedback loop

Enhanced expression level

VEGF, VEGFR2, FGFR1

Reduced expression level

Figure. Angiogenic capacity of ECs is regulated by microRNAs (miRNAs) comprising the same seed sequence (microRNA [miR-16]-like miRNAs, eg, miR-16, miR-424) and is depicted in the plotted mechanistic scheme. The proangiogenic factor VEGF induces expression level of mature miR-16/-424. This in turn leads to repression of receptor tyrosine kinases vascular endothelial growth factor receptor-2 (VEGFR2) and fibroblast growth factor receptor-1 (FGFR1), subsequently interfering with downstream survival signaling cascades. In addition, vascular endothelial growth factor (VEGF) itself is targeted by miR-16/-424. In summary, endothelial cell function is impaired, and angiogenic capacity decreases.

Sources of Funding

This work was supported by the Integrated Research and Treatment Center Transplantation (BMBF 01EO0802 to T.T.) and Deutsche Forschungsgemeinschaft TH 903/10-1 (to T.T.).

Disclosures

Dr. Thum and Dr. Fiedler have filed patents in the field of cardiovascular miRNA diagnostics and therapeutics.

References


Key Words: angiogenesis | molecular biology | signal transduction | microRNAs
MicroRNAs Looping Around Angiogenesis
Jan Fiedler and Thomas Thum

Arterioscler Thromb Vasc Biol. 2011;31:2367-2368
doi: 10.1161/ATVBAHA.111.237602
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2011 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/31/11/2367