

Role of microRNAs in endothelial cell pathophysiology

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ABSTRACT

MicroRNAs (miRNAs) are a family of small, noncoding RNAs that repress gene expression at the post-transcriptional level. Over 700 miRNAs have been identified in the human genome, of which 20% to 30% regulate human protein-coding genes. Functional *in vitro* studies have shown that miRNAs are critical for endothelial cell gene expression and function. miRNAs were found in atherosclerosis, cardiac hypertrophy, arterial hypertension, coronary artery disease, diabetes, and inflammatory diseases. We review the current knowledge about the role of miRNAs in endothelial cells with emphasis on the regulation of cellular senescence, angiogenesis, and vascular inflammation. It has been shown that *miR-34a*, *miR-217*, *miR-200*, *miR-146c*, and *miR-181a* are responsible for the regulation of cell stress and proliferation processes. Proangiogenic factors include *miR-130a*, *miR-210*, *miR-424*, *miR-17-92*, *miR-27-b*, *let-7f*, and *miR-217*, while *miR-221* and *miR-222* have antiangiogenic properties. Other known miRNAs, including *miR-31*, *miR17-3p*, *miR-155*, *miR-221*, *miR-222*, and *miR-126*, are important factors in the regulation of vascular inflammation. Studies show that miRNA expression analysis can be used in the diagnosis and treatment of various diseases; however, additional research is needed before it is used in routine clinical setting.

Introduction MicroRNAs (miRNAs) are a family of highly conserved noncoding single-stranded RNAs (20–26 nucleotides in length) that regulate gene expression in eukaryotes. They were originally described in *C. elegans* and have since been identified in many organisms including humans.¹ miRNAs silence gene expression by repressing protein translation or by accelerating messenger RNA (mRNA) degradation.² They play an important role in various physiological and pathological processes including cell proliferation, differentiation, apoptosis, and metabolism as well as angiogenesis, oncogenesis, and hematopoiesis.^{3,4} Over 700 miRNAs have been identified in the human genome so far, and their sequences are deposited in the miRNA database (<http://www.mirbase.org>).⁵ Computational predictions suggest that about 20% to 30% of known human protein-coding genes are regulated by miRNAs.⁶

Deregulation of miRNA expression has been reported to be associated with several human diseases (atherosclerosis, cardiac hypertrophy, arterial hypertension, inflammatory diseases). Furthermore, miRNAs present in body fluids are highly tissue specific; therefore, they could represent useful clinical biomarkers.^{7,8}

miRNAs are transcribed by RNA polymerase II into a primary molecule (pri-miRNA). Long primary transcripts of miRNA are cleaved in the nucleus by RNA-specific RNase III type endonuclease, Drosha, and its cofactor, DiGeorge syndrome critical region (8DGC8). The 60–70 nt length precursor RNA (pre-miRNA) is actively transported through nuclear pores by the action of Exportin 5 (Exp5) and its partner – Ran GTP-binding protein.^{9,10} Pre-miRNAs are then cleaved into ~22-nt duplexes by Dicer, a cytoplasmic RNase III type endonuclease. Dicer inter-

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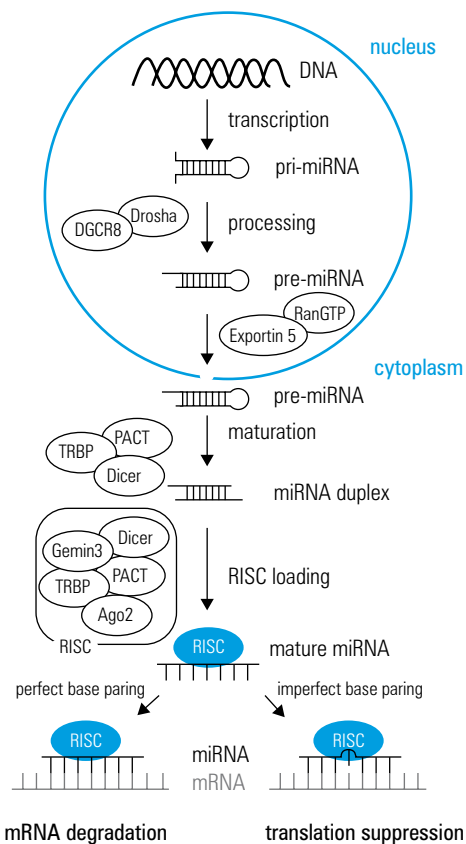
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FIGURE Biogenesis of microRNAs (miRNAs). miRNAs are transcribed by RNA polymerase II into primary miRNAs (pri-miRNA). Maturation of miRNAs is mediated by RNase III endonucleases, Drosha and Dicer, and by numerous partner proteins. Abbreviations: Ago2 – Argonaute 2, DGCR8 – DiGeorge syndrome critical region 8, Gemin3 – DEAD-box RNA helicase, PACT – protein activator of PKR, pre-miRNA – hairpin structures miRNA precursor (~65 nt), pri-miRNA – hairpin stem-loop primary transcript (~70 nt), RanGTP – RanGTP-binding protein, TRBP – trans-activation RNA-binding protein, RISC – RNA-induced silencing complex



acts with double-stranded (ds)-RNA-binding protein partners, such as trans-activation response RNA-binding protein (TRBP) and protein activator of the interferon-induced protein kinase, PKR, (PACT). In the next stage, mature miRNAs are unwound by helicase A and loaded into the RNA-induced silencing complex (RISC).¹¹ Only 1 strand of ds-miRNA is incorporated and the other is released or destroyed. The RISC complex consists of the Dicer enzyme and cofactors – TRBP, PACT, Gemin3, and Argonaute 2 (Ago2). The miRISC complex interacts with mRNA and binds to the complementary sequence in the 3' untranslated region (3'UTR) of target RNA.¹²

There are two ways of silencing gene expression depending on the level of complementarity with target mRNA. The nonperfect complementarity with target mRNA leads to translational repression, while full complementarity is required for mRNA degradation (FIGURE).¹³

Specific classes of miRNAs were demonstrated to affect the function of endothelial cells (ECs). ECs are involved in many aspects of vascular biology, producing different factors that regulate platelet aggregation, inflammatory cell adhesion, smooth muscle cell proliferation, and vascular tone.¹⁴ miRNA molecules participate in the control of EC-mediated homeostasis. Poliseno et al.¹⁵ demonstrated 27 highly expressed miRNAs in human umbilical vein ECs (HUVECs), most of which were predicted to modulate expression of receptors for angiogenesis-regulating factors (Flt-1, Nr1p-2, FGF-R, c-Met, c-Kit). The highly expressed miRNAs in ECs included *miR-126*,

miR-221, *miR-222*, *mir-130a*, *let-7* family, *miR-21*, and *miR-27b*.³

The importance of miRNAs in EC function was demonstrated by the knock down of the Dicer enzyme. Recent data have shown that silencing Dicer results in a significant reduction of the mature miRNA profile. The expression of several significant regulators of angiogenesis, such as endothelial cell-specific receptor kinase, vascular endothelial growth factor receptor 2, endothelial nitric oxide synthase (eNOS), and interleukin 8, was affected. Silencing Dicer resulted in an increased activation of the eNOS pathway and reduced EC proliferation and cord formation.¹⁶

Another study showed that Dicer and Drosha are involved in the regulation of angiogenesis in vitro. Knocking down of both enzymes alters the reduction of capillary development and tubule forming activity in ECs. Drosha and Dicer small interfering RNAs (siRNAs) significantly reduced expression of *let-7a* and *miR-27b*. The knockdown of Dicer also decreased migration of ECs.¹⁷

To examine the importance of Dicer, another group generated Dicer^{ex1/2} knockout mice, which have a deletion at the two first exons of the Dicer gene. The homozygous embryos died between 12.5 and 14.5 days of embryogenesis demonstrating that the presence of Dicer activity is necessary for normal mouse development.¹⁸

The above results show that Dicer and Drosha are the key enzymes in endothelial miRNA formation and maturation and confirm that miRNAs are important in the physiological function of ECs.

MicroRNAs control endothelial senescence Senescence is connected with cellular response to various environmental stressors and damages defined as permanent cell cycle arrest.^{19,20} Senescent cells are important in atherothrombosis and are related to various age-related diseases, including atherosclerosis, and cardiovascular disorders.²¹ Of note, cellular senescence plays a pivotal role in protection against cancer.²²

Several miRNAs are involved in the regulatory mechanisms of cellular senescence of ECs. A recent study indicated that *miR-34a* is already expressed in primary ECs and the degree of expression increases during cell senescence. *miR-34a* regulates proliferation and differentiation of many cell types, for example in ECs it decreases Sirtuin 1 (silent mating type information regulation 2 homolog; SIRT1) levels.²³ *SIRT1* is a longevity gene that protects cells against oxidative and genotoxic stress. Mammalian SIRT1 is a NAD⁺-dependent class III histone deacetylase and functions as a metabolic regulator by deacetylation of histones and large numbers of proteins including protein 53 (p53), Ku70 protein, nuclear factor κβ (NF-κβ), and peroxisome proliferator-activated receptor γ.²⁴ Overexpression of *miR-34a* in ECs decreases SIRT1 and increases acetylation of p53. Additionally, acetylated tumor suppressor p53 promotes *miR-34a* expression, which

TABLE MicroRNAs implicated in endothelial cell function

Endothelial cells	miRNAs	Target	Function		
cellular senescence	<i>miR-34a</i>	SIRT1 – p53	stress resistance		
	<i>miR-217</i>	SIRT1 – FOXO	stress resistance		
	<i>miR-200c</i>	ZEB1	cell proliferation		
	<i>miR-146a</i>	NOX4	cell proliferation		
	<i>miR-181a</i>	NOX4	cell proliferation		
angiogenesis	antiangiogenesis	<i>miR-221</i>	c-Kit	cell proliferation and migration	
			eNOS	vessel permeability	
		<i>miR-222</i>	c-Kit	cell proliferation and migration	
			eNOS	vessel permeability	
		proangiogenesis	<i>miR-130a</i>	GAX, HOXA5	cell proliferation and migration
	<i>miR-210</i>		Ephrin-A3	tube formation, chemotaxis	
	<i>miR-424</i>		CUL2/HIF-1 α	cell proliferation, chemotaxis	
	<i>miR-17-92</i>		<i>miR-17-5p</i>	TSP-1/CTGF	cell proliferation and migration
			<i>miR-18a</i>	TSR/VEGFR-2	cell proliferation and migration
			<i>miR-19a</i>	TSR/VEGFR-2	cell proliferation and migration
	<i>miR-20a</i>		VEGF	cell proliferation and migration	
			<i>miR-92a</i>	ITG- α 5	cell adhesion and cell interactions
	<i>miR-27b</i>		ND	sprout formation	
	<i>let-7f</i>		ND	cell proliferation and migration, sprout formation	
	<i>miR-217</i>	SIRT1 – FOXO/eNOS	vessel formation and maturation		
vascular inflammation	<i>miR-31</i>	E-SELE	leukocyte recruitment to sites of inflammation		
	<i>miR17-3</i>	ICAM-1	cell adhesion and migration		
	<i>miR-155</i>	AT1R/VEGFR-2	cell proliferation and migration		
	<i>miR-221</i>	c-Kit	cell proliferation and migration		
		eNOS	vessel permeability		
	<i>miR-222</i>	c-Kit	cell proliferation and migration		
		eNOS	vessel permeability		
	<i>miR-126</i>	VCAM-1	cell adhesion and cell interactions		
		SPRED, PIK3R2/VEGFR-2	cell proliferation and migration		

Abbreviations: AT1R – angiotensin II type 1 receptor, CTGF – connective tissue growth factor, CUL2 – cullin 2, eNOS – endothelial nitric oxide synthase, FOXO – forkhead box protein O1, GAX – growth arrest homeobox, HIF-1 α – hypoxia-inducible factor 1 α , HOXA5 – homeobox A5, ITG- α 5 – integrin α 5, ND – not determined, NOX4 – nicotinamide adenine dinucleotide phosphate oxidase 4, PIK3R2 – phosphoinositide-3-kinase regulatory subunit 2, SELE – selectin E, SIRT – Sirtuin 1, SPRED – sprouty-related protein with an enabled/VASP homology 1 domain, TSP-1 – thrombospondin 1, TSR – protein containing thrombospondin type 1 repeats, VCAM-1 – vascular cell adhesion molecule 1, VEGF – vascular endothelial growth factor, VEGFR-2 – VEGF receptor 2, ZEB1 – zinc finger E-box-binding homeobox 1

accelerates suppression of SIRT1 and ultimately results in cell senescence.²³ Overexpression of *miR-34a* in the endothelial progenitor cells (EPCs) also reduces SIRT1 and remarkably increases cell senescence. Similarly, silencing *SIRT1* by siRNA resulted in reduced EPC-induced angiogenesis and increased cell senescence.²⁵

Another miRNA, named *miR-217*, is expressed in young HUVECs, human aortic endothelial cells, and human coronary artery endothelial cells. *miR-217* promotes endothelial senescence through inhibition of SIRT1, which affects functions of the SIRT1/forkhead box protein O1 (FOXO1) pathway.²⁶ *miR-217* was absent in young cells but increased during endothelial senescence.²⁶

A recent study has demonstrated that *miR-200c* influenced growth arrest, apoptosis, and senescence of HUVECs in response to reactive oxygen species (ROS). *miR-200c* targets zinc finger

E-box-binding homeobox 1 (ZEB1). Down-modulation of ZEB1 protein by ROS and increased expression of *miR-200c* alters induction of the p53 and retinoblastoma protein tumor suppressor pathways responsible for cellular senescence.²⁷ Microarray analyses have shown that *miR-146a* affects cellular senescence by targeting the expression of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 4 (NOX4) gene. The NOX4 complex catalyzes the reduction of molecular oxygen to ROS in the vessel wall.²⁸

To summarize, decreased expression of the above miRNAs could be considered as potential therapeutic targets for delaying senescence, aging processes, and cancer development affecting EC homeostasis.

The function of microRNAs in angiogenesis Angiogenesis is the process of new blood vessel and

capillary network formation in the body. A large number of miRNAs are responsible for angiogenesis and are expressed in ECs.^{15-17,29-31} Poliseno et al.¹⁵ proved that *miR-221* and *miR-222* are anti-angiogenic factors and that they affect the expression of the human proto-oncogene *c-Kit* receptor in ECs. Therefore, they can modulate the activity of stem cell factor, one of the main growth factors involved in cell fate and angiogenesis. Overexpression of *miR-221* and *-222* in HUVECs significantly reduced cell migration.¹⁵ Dicer silencing has revealed that *miR-221* and *miR-222* also regulate eNOS in the endothelium.¹⁶ The regulatory mechanisms of nitric oxide (NO) are essential for angiogenesis, capillary network maturation, and vascular remodeling.^{32,33}

Other important miRNAs involved in angiogenesis regulation are proangiogenic *miR-130a*, *miR-210*, *miR-424*, *miR27-b*, *let-7f*, and the *miR-17-92* cluster.³ *miR-130a* regulates expression of growth arrest homeobox (GAX) and homeobox A5 (HoxA5) proteins, which inhibit angiogenesis.³⁰ GAX is an inhibitory factor, which affects proliferation, migration, and tubulogenic activity of ECs. Also, HoxA5 protein plays a critical role in inhibiting tubule formation in ECs. Fasano et al.³⁴ showed that *miR-210* is induced by hypoxia in HUVECs and regulates Ephrin-A3. Up-regulated *miR-210* affects cell survival, migration, and differentiation in response to hypoxia.³⁴ Overexpression of *miR-210* stimulates primary capillary network formation and the vascular endothelial growth factor (VEGF)-driven cell migration, while silencing of *miR-210* inhibits the formation of capillaries and decreases cell migration in normoxia.³⁴

A more recent study has provided new evidence of the novel miRNA, *miR-424*, which plays an important role in postischemic vascular remodeling and angiogenesis.³⁵ *miR-424* targets the 3'UTR of cullin 2 (*CUL2*) gene, inhibits *CUL2* expression and finally stabilizes hypoxia-inducible factor 1 α (HIF-1 α) levels. The concentration of *miR-424* is increased in hypoxic ECs and during vascular remodeling in vivo.³⁵ Transfection of ECs with *miR-424* construct increases expression of HIF-1 α and HIF-2 α , which results in an increased proliferation and migration of ECs.³⁵

let-7f and *miR-27b* have been reported to play a significant role in EC-driven angiogenesis.⁴ Inhibition of their expression reduces sprout formation by HUVECs.¹⁷

The polycistronic cluster, *miR-17-92*, consists of several miRNAs: *miR-17*, *miR-18a*, *miR-19a*, *miR-20a*, *miR-19b*, and *miR-92a*.³⁶ *miR-17-92* is the first miRNA with oncogenic activity, which promotes angiogenesis of tumor endothelium through downregulation of thrombospondin 1 (antiangiogenic molecules) and connective tissue growth factor.³⁷ Another study has shown that the *miR-17-92* cluster targets p53-mediated transcriptional repression in hypoxia.³³ Dews et al.³⁸ suggested that *miR-92a* is a negative regulator of angiogenesis in ECs. Overexpression of *miR17*,

miR-18a, *miR-19a*, *miR-20a*, and *miR-19b* down-regulated sprouting in ECs, while silencing enhanced endothelial sprouting.³⁹

MicroRNAs associated with inflammation Recent reports have shown that miRNAs can control vascular inflammation by controlling leukocyte activation and infiltration through the vascular wall.³ Vascular/endothelial effect on inflammation is a process based on white blood cells and microvascular EC interactions. ECs, activated by shear stress, lipopolysaccharides, or cytokines, can modulate various mechanisms including expression of adhesion molecules and chemokines, leukocytes rolling over the endothelium, adhesion to vessels, and transmigration across the EC blood vessel walls.^{20,40,41} Interactions of leukocytes with the affected vessel wall cells are important for the pathophysiology of atherosclerosis.⁴²

Suarez et al.⁴³ used a proinflammatory cytokine, tumor necrosis factor α (TNF- α), to induce the generation of several miRNAs in HUVECs.⁴³ Using the microarray approach, they observed that TNF- α increases the level of *miR-155*, *miR-31*, *miR-17*, *miR-191*, and *miR-125b*. In silico analyses have shown that *miR-31* targets selectin E (SELE), *miR-17-3p* targets intercellular adhesion molecule 1 (ICAM-1), and *miR-155*, *miR-221*, and *miR-222* cotarget Ets-1.^{43,42} To examine the role of *miR-31* and *miR-17-3p* in the expression of SELE and ICAM-1 proteins, Suarez et al.⁴³ transfected HUVECs with sense and antisense miRNA antagonists. The sense *miR-31* and *miR-17-3b* mimic reduced TNF- α -induced SELE and ICAM-1 levels. Overexpression of sense significantly mimicked reduced neutrophil/endothelial binding, whereas the inhibition of *miR-31* and *miR-17-3p* increased neutrophil adherence to TNF- α stimulated ECs. Results of the study indicate that *miR-31* and *miR-17-3p* are essential for the regulation of neutrophil adhesion through the regulation of SELE and ICAM-1 expression.⁴²

miR-126 is involved in vascular dysfunction and inflammation.³ Several studies have shown that *miR-126* regulates inflammatory cell migration, capillary network formation, and cell survival.²⁹ *miR-126* has an intronic location in the epithelial growth factor (EGF)-like domain-containing protein 7 (EGFL7) gene and regulates its transcription in ECs.⁴⁴ Fish et al.²⁹ demonstrated that *miR-126* regulated the response of ECs to VEGF through modification of sprouty-related protein with an enabled/VASP homology 1 domain (SPRED1), phosphoinositide-3-kinase regulatory subunit 2 (β) (PIK3R2), the negative regulators of VEGF signaling.^{29,45} Harris et al.⁴⁶ found that *miR-126* inhibits vascular cell adhesion molecule 1 (VCAM-1) expression, which is involved in leukocyte adhesion to ECs.⁴⁶ Inhibition of *miR-126* increases proinflammatory TNF- α expression, which activates NF- κ B and interferon regulatory factor 1, and finally induces expression of VCAM-1 and adhesion of leukocytes to ECs. The angiogenic activity of *miR-126* was shown in the zebrafish

and mice with the knockdown of *miR-126* causing a loss of vascular integrity.⁴ Thus, *miR-126* can modify vascular inflammation through suppression of leukocyte adhesion to ECs.⁴⁶

miR-155, *miR-221*, and *miR-222* generated by HUVECs regulate Ets-1 transcription factor.⁴² Ets-1 is stimulated by angiotensin II, TNF- α , and thrombin and plays a key role in inflammation and microtubule formation by ECs.⁴³ In addition, *miR-155* targets angiotensin II type 1 receptor (AT1R) activity.⁴⁴ Activation of AT1R by angiotensin II initiates endothelial dysfunction, structural remodeling, and vascular inflammation.⁴⁷ *miR-155*, *miR-221* and *miR-222* have been reported to regulate inflammatory response in ECs mediated by angiotensin II.⁴² *miR-155* also regulates expression of adhesion molecules in inflammatory ECs.⁴² A silent polymorphism +1166 A/C of the 3'UTR of the *AT1R* gene was reported to be a response to vascular inflammation and cardiovascular complications.⁴⁸ The presence of +1166C allele interferes with the ability of *miR-155* to interact with the binding site.⁴⁸

Recent studies have revealed that microvesicles, exosomes, and apoptotic bodies can transfer miRNA.⁴⁹ It is possible that this is a novel genetic exchange between adjacent or distant cells. The tissue-specific miRNAs released into body fluids might serve as paracrine signaling molecules and reflect physiological and/or pathological conditions. For this reason, miRNAs are emerging as interesting, noninvasive early diagnostic biomarkers. Fichtlscherer et al.⁵⁰ suggested that the levels of circulating miRNAs associated with vascular and inflammatory conditions were downregulated in coronary artery disease.⁵⁰ Another group demonstrated that the levels of antiangiogenic *miR-503* is upregulated in diabetic patients with critical limb ischemia.⁵¹ The plasma level of *miR-503* is significantly increased in these patients in comparison with controls and can serve as a potential circulating marker of ongoing ischemia. However, there are some limitations of the use of circulating miRNA as biomarkers. Multiple parameters, such as changes in expression in tissue, secretion of miRNA by cells, and stability of miRNA molecules, may influence its levels in plasma or serum. In addition, determining absolute amounts of miRNA has not been well-established because there is no stable control of miRNA, especially in disease conditions.

In summary, the results described above indicate that several types of miRNAs can target a different aspect of EC/vessel wall/blood homeostasis. These findings also provide possible future therapeutic intervention for diseases connected with endothelial dysfunction. Circulating miRNAs could be measured in body fluids as early biomarkers for disease diagnosis, prognosis, and response to treatment, but large-scale studies are required to confirm potential usefulness as predictive markers. Knowledge about miRNAs provides an opportunity to use antagomirs (oligonucleotide inhibitors to silence endogenous

miRNAs) and miRNA mimics to modulate biological function in the pathology of diseases. Moreover, exogenous miRNAs could be useful in the treatment of many diseases, because they are upstream regulators of gene expression involved in modification of EC activity. Further research on the roles of miRNAs in vascular disease is required for the future development of miRNA therapeutics.

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Rola mikroRNA w patofizjologii komórek śródbłonna

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SŁOWA KLUCZOWE

angiogeneza,
ekspresja genów,
miRNA, stan zapalny
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STRESZCZENIE

MikroRNA (miRNA) to grupa małych, niekodujących RNA, które regulują ekspresję genów na poziomie potranskrypcyjnym. Dotychczas w ludzkim genomie zidentyfikowano około 700 miRNA, spośród których 20–30% reguluje geny kodujące białka. Badania funkcjonalne *in vitro* wykazały, że miRNA są ważnym regulatorem ekspresji genów oraz funkcji komórek śródbłonna. Cząsteczki miRNA zidentyfikowano w takich stanach chorobowych, jak miażdżycza, przerost mięśnia sercowego, nadciśnienie tętnicze, choroba wieńcowa, cukrzyca oraz choroby zapalne. Niniejszy artykuł stanowi przegląd aktualnej wiedzy na temat roli miRNA w komórkach śródbłonna i koncentruje się głównie na regulacji procesu angiogenezy i starzenia komórek oraz stanu zapalnego naczyń. Wykazano, że *miR-34a*, *miR-217*, *miR-200*, *miR-146c* oraz *miR-181a* wpływają na regulację procesów stresu komórkowego oraz proliferację. Do czynników proangiogennych należą *miR-130a*, *miR-210*, *miR-424*, *miR-17-92*, *miR-27-b*, *let-7f* i *miR-217*, podczas gdy *miR-221* i *miR-222* wykazują działanie antyangiogenne. Inne znane miRNA, takie jak *miR-31*, *miR17-3*, *miR-155*, *miR-221*, *miR-222* oraz *miR-126*, są ważnymi czynnikami regulującymi stany zapalne naczyń. Z badań wynika, że analiza poziomu ekspresji miRNA może być wykorzystana w diagnostyce i leczeniu różnych chorób, jednak wprowadzenie jej do rutynowej diagnostyki klinicznej wymaga dalszych badań.

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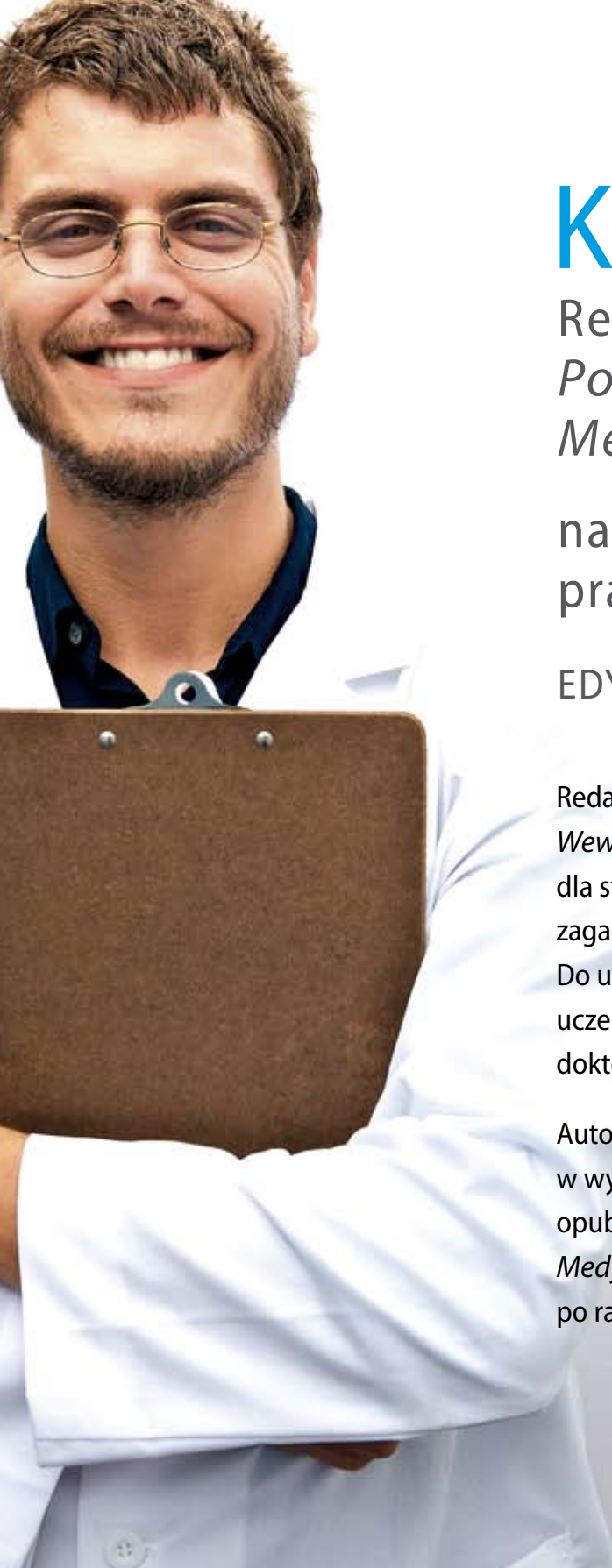
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