Chapter 4

p53 and Vascular Dysfunction: MicroRNA in Endothelial Cells

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Additional information is available at the end of the chapter

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Abstract

In many cancer cells, p53 gene is mutated and accumulated, which is considered as a mechanistical target of tumorigenesis. The role of p53 in non-cancerous cells has been focused on, since p53 activation diversely affects as human diseases, including vascular dysfunctions. p53 regulates vascular events, including vascular inflammation and senescence as well as cardiac dysfunction. Many researchers also have paid attention to the role of noncoding RNAs (ncRNAs), especially small-sized microRNAs (miRNAs) for the last decade and their noble biological cellular functions have been discovered. miRNAs expressed in endothelial cells (endothelial miRNAs) have been shown to control vascular events. Firstly, the importance of p53 in a variety of vascular events, such as vascular inflammation and senescence, are summarized. Secondly, the way to regulate miRNAs by p53 and the involvement of miRNAs on p53 function are demonstrated. Finally, several endothelial miRNAs that have important roles are focused on. The aim of this chapter is to understand the role of p53 in vascular diseases in the view of endothelial cell biology and the contribution of miRNAs related to p53.

Keywords: endothelial cells, miRNAs, p53, Dicer, Drosha

1. Introduction

1.1. p53 overview

p53 is one of well-known tumor suppressor protein and plays crucial roles in inhibiting tumor progression [1]. Tumor suppression by p53 might be carried out mostly through genotoxic stress, however, recent studies revealed that p53 is activated by oncogene activation, oxidized

lipoproteins, and hypoxic condition [2]. In general, p53 is rapidly degraded by the interaction with MDM2 and these stimuli increase p53 levels and activate antiproliferative or proapoptotic responses via downstream signaling molecules [3]. The structure of p53 consists of amino terminal transactivation domain linked to the DNA-binding domain by proline-rich region (**Figure 1**) [4]. The DNA-binding domain on the other end is bound to the tetramerization domain by another proline-rich residue and this tetramerization domain is linked to carboxyl terminus [5]. The core domain (residue 94–312) is naturally unstable and is prone to have mutation [6, 7]. Once bound to the DNA, the whole structure closes around the DNA double helix. The whole process is facilitated by flexible proline-rich region between the core and the tetramerization domain [5]. Although the expression of p53 is in lower level during normal condition, upon activation, p53 increases its level along with the increase of its half-life [8] and gets translocated to the nucleus [9]. p53 is activated mainly by any signals that could damage the DNA [10]. Further p53 undergoes phosphorylation, acetylation, methylation, ubiquitination or SUMOylation to exert its respective activity [11]. p53 interacts with p300/CBP to get acetylated which stimulates the binding of p53 to the DNA, however, p53 requires only p300 but not CBP for the well-known G1 arrest [12]. Two members of p53 family, p73 and p63, are also involved in this p53 world [13], which are not mentioned in this chapter. Regulation and function of p53 in cancer really become complex.

1.2. p53 and endothelial cells

In the complex network of cellular signaling, p53 is a transcription factor that plays an important role in controlling angiogenesis and it is a hub for cellular signaling [14]. p53 itself controls angiogenesis by taking cells under apoptosis or by downregulating mediators of angiogenesis [15]. The role of p53 in vasculature is the same as the other tissues, including cell cycle, apoptosis, senescence, and angiogenesis.

Mice genetically deleted p53 can develop normally, however, these p53 knockout mice had spontaneous tumors [16]. Conditional knockout of p53 in endothelial cells improves angiogenesis of hindlimb ischemia mice model [17]. When mice were fed diet with high calorie, p53 expression increased in endothelial cells [18]. High calorie diet impaired the activation of endothelial nitric oxide synthase (eNOS), which was restored in endothelial p53 disruption. Knockdown of p53 in endothelial cells increased the expression of eNOS and thrombomodulin in vitro [19]. Therefore, accumulating evidence suggested that p53 is one of the key transcriptional factors to regulate endothelial cell function.

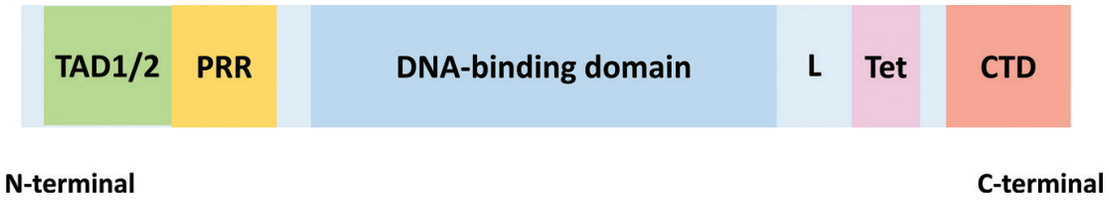

Figure 1. Linear structure of p53. p53 consists of 393 amino acid sequence. The protein is divided into the following domains. The transcriptional activation domain 1/2 (TAD1/2), DNA-binding domain, and the tetramerization domains (Tet) are lysine-rich basic C-terminal domain (CTD). PRR, proline-rich region; L, the linker region; Tet, tetramerization domains.

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2. p53 and vascular function

Normal cells including endothelial cells keep p53 levels quite low. Low grade upregulation of p53 is not apoptotic but it is engaged in other functions like inhibition of endothelial cell migration through downregulation of beta-3 integrin [20] and inhibition of cell survival by causing reversible cell cycle arrest [21]. The modulation of p53 varies vascular function, such as vascular inflammation, senescence, and remodeling.

2.1. Vascular inflammation

Vascular inflammation leads to form atherosclerotic lesions, in which many cells are orchestrating [22]. Role of p53 in atherosclerosis has been investigated by many researchers. Guevara et al. performed the experiments using double knockout mice with apolipoprotein E (apoE) and p53. This apoE$^{-/-}$, p53$^{-/-}$ double knockout mice fed with high fat diet showed significant increase of bulky, hypercellular lesion in aorta, suggesting that p53 is involved in atherosclerotic change [23]. van Vlijmen et al. demonstrated that the role of p53 in subendothelial macrophages is one of the major components of atherosclerosis [24]. This study indicated that deficiency of p53 in macrophage increased atherosclerotic lesions. Oxidative stress induces p53 accumulation in human macrophage, which is prevented by nitric oxide (NO) [25]. NO blocked the secretion of von Willebrand factor in endothelial cells and inhibited vascular inflammation [26]. The molecular mechanism by which p53 regulates atherosclerosis has been aggressively investigated.

2.2. Senescence

Aging is an independent risk factor for atherosclerosis-related diseases and impairment of vascular function is involved in systematic senescence. The molecular difference between senescence and cell death is not an easy question. Disturbed blood flow (d-flow) causes atherosclerosis. Heo KS et al. identified protein kinase zeta (PKC zeta) as a d-flow-activated protein in endothelial cells [27]. d-Flow promotes endothelial cells apoptosis through p53 SUMOylation. Apoptosis in aortic endothelial cells by d-flow decreased in p53$^{-/-}$ mice compared to wild type mice. Endothelial cells constitutively express Nox2 and Nox4, two important isoforms of catalytic subunit of NADPH, which are a major source of reactive oxygen species. Nox2 especially affects endothelial cell cycle arrest and cell death by modulating p53 and p21cip1 [28]. In turn, cellular senescence is a stress-induced phenomenon as well. Senescent cells delay or lose the ability to proliferate. In endothelial cells, hydrogen peroxide or frequent passage induces cellular senescence via p53 and NAD-dependent deacetylase sirtuin-1 (SIRT1) [29]. The expression of endothelial SIRT-1 is reduced during aging process [30, 31]. Reduced SIRT-1 in endothelial cells accumulates genomic instability, resulting in p53 activation and promoting more senescence [32, 33]. AMPK and mTOR signaling is thought to be important for endothelial aging [34, 35]. These molecules are also connected to p53 signals, suggesting p53 as a key regulator of senescence of endothelial cells.

2.3. Vascular and heart remodeling

Vascular remodeling is a process of structural change of vascular walls, involving changes of cellular function, including growth and death. In this process, p53 is an important player.

Chronic hypoxia promotes pulmonary vascular remodeling, causing pulmonary hypertension. Mizuno S et al. demonstrated that p53 suppress hypoxia-induced pulmonary arterial remodeling and pulmonary arterial smooth muscle cell proliferation [36]. Kruppel-like factor 4 (KLF4) controls vascular smooth muscle cell proliferation through p53 induction [37]. Cardiac remodeling and development occur during embryogenesis but stop in postnatal life due to the reduction of the genes responsible for cell cycle progression and growth factors [38, 39]. For remodeling, new cardiomyocytes are derived for pre-existing cardiomyocytes. The rate of the pre-existing cells differentiation is very low (less than 1% per year) and it decreases with age [40] and lesser then 50% of the cells are replaced during a lifespan [41]. One important molecule for cardiomyocyte division is survivin [42]. Downregulation of survivin contributes to cardiac development in spinal muscular atrophy mice model [43]. Survivin is negatively regulated by p53. Survivin expression was downregulated at mRNA and protein level by p53 through histone acetylation. While overexpression of survivin inhibited p53-induced apoptosis [44]. One of MAPK, p38, has been shown to be an important molecule that negatively regulates cell cycle in cardiomyocyte cell [45]. Treatment with FGF1 and p38 inhibitor enhanced heart regeneration by increasing cardiomyocyte proliferation and angiogenesis [46]. Repression of cyclin D1 result into downregulation of cardiac cell proliferation [47] and C reactive protein, besides downregulating cyclin D1 has been shown to accumulate and phosphorylate p53 which leads to cell cycle arrest [48]. Since p53 controls actin cytoskeleton through mechanoresponsive molecules, remodeling may be processing via p53 in mechanical environment-dependent manner.

3. p53 and miRNA in endothelial cells

3.1. miRNA overview: general information

MicroRNAs (miRNAs) are small noncoding RNAs (about 20–24 nucleotides in length) that controls gene expressions mainly by binding to 3′ untranslated region (3′ UTR) of their messenger RNAs (mRNAs). The biogenesis of miRNAs in animals is very unique (**Figure 2**). Primary miRNAs (pri-miRNAs) are transcribed from miRNA-encoding genes. miRNAs are encoded in any place; some are located on protein-coding region, and some are in noncoding region or intron [49]. The pri-miRNAs are cleaved into hairpin-structured small size RNAs (precursor miRNAs; pre-miRNAs) by microprocessor complex containing RNase III, Drosha and DiGeorge critical region 8 (DGCR8) [50]. Exportin 5 (XPO5) and Ran-GTP transported pre-miRNAs into the cytoplasm from the nucleus, then pre-miRNAs are cleaved in double-stranded smaller RNAs (miRNA duplexes) by another RNase III, Dicer [51]. One of the strand (mature miRNAs) are incorporated into miRNA-induced silencing complex containing Argonaute 2 (Ago2) and transactivation response RNA-binding protein (TRBP) in human and these miRNAs are ready to bind to target mRNAs [52] (**Figure 2**).

How do miRNAs inhibit the expression of target protein? In general, miRNAs use two ways of silencing: repression of translation and mRNA decay [53]. The seed sequence of miRNA (2–7 position from 5′ end) can bind to 3′UTR of target mRNA with incomplete match in animals. This miRNA-mRNA binding leads to repress the translation or destroy miRNA [54]. More than 60% of protein is regulated by miRNAs in human [55, 56]. Therefore, miRNAs are involved in modifying ubiquitous cellular functions.

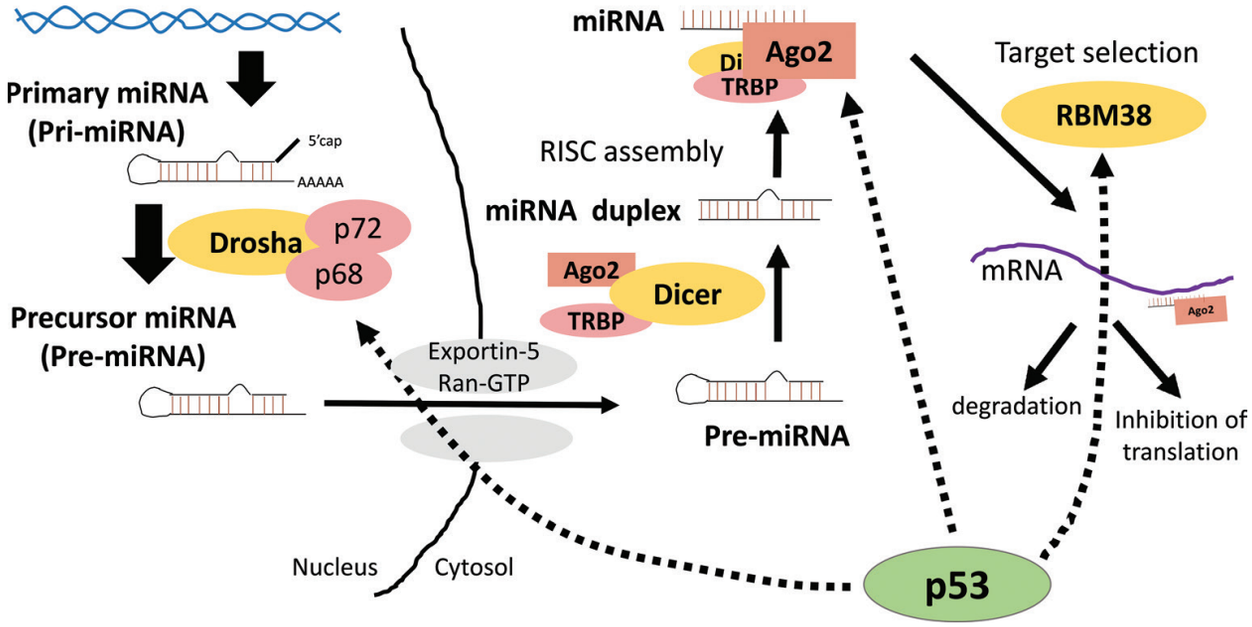

Figure 2. p53 regulation of miRNA biogenesis.

3.2. miRNAs and p53

The relationship between miRNA and cancer was first described by Calin GA et al. in 2002. They described downregulation of miR-15a and miR-16-1 in B cell chronic lymphocytic leukemia patients [57]. miRNAs associated with cancer are called 'oncomiR', which have been identified in many types of cancer [58]. Some oncomiRs decrease in cancerous tissue. In contrast, increased oncomiRs are also found in cancer, which in case inhibit tumor suppressor genes, following the proliferation of cancer cells. The proto-oncogene c-Myc is a transcriptional factor and dysregulation of c-Myc was found in many cancers. The studies for regulation of transcriptional factors by miRNAs have been started since O'Donnell et al. identified mir-17-92, a polycistronic miRNA transcript that yields six individual miRNA components, as c-Myc-regulated miRNAs in human B cell line [59]. It was not hard to assume that the next target of 'transcription factors' regulating miRNAs in cancer was p53.

3.3. Direct regulation of miRNAs by p53

In 2007, several articles about p53 regulation of miRNAs have been published independently from different research groups. All these studies revealed that p53 upregulated the expression of miR-34 family in different cells [60–64]. The miR-34 family comprises three members (**Figure 4C**). miR-34a is generated from the large transcript on chromosome 1p36 and miR-34b and miR-34c are generated from bicistronic transcript on chromosome 11q23 [65]. Though the expression levels of miR-34a, -34b, and -34c were not consistent in non-small cell lung cancers (NSCLCs) compared to the adjacent normal tissue, lower expressions of three miR-34 family members are lower in many cancer cell lines; H1299 (lung cancer), MCF-7 (breast cancer), U-2OS (osteosarcoma), HCT116 (colon cancer), and many pancreatic cancer cell lines such as PANC1 [60, 64]. In addition to cancer, p53 regulates miR-34 family in non-cancerous cells, such as mouse embryonic fibroblasts (MEFs) and human fetal lung fibroblasts (IMR-90 cells) [63].

Forced expression of miR-34 family induce growth arrest and apoptosis in a variety of cell lines, whatever cancers or non-cancerous cells [62]. A lot of target genes of miR-34 family have been identified, including cyclin E2 (CCNE2), cyclin-dependent kinase 4 (CDK4) and the hepatocyte growth factor receptor (MET), B cell CLL/lymphoma 2 (BCL2), baculoviral IAP repeat-containing 3 (BIRC3), and decoy receptor 3 (DcR3 also known as TNFRSF6B). Many miRNAs directly induced by p53 have been identified in cancer cell lines. As described above, miR-34a might be the most fascinating one. Among these p53-induced miRNAs, several miRNAs that affect endothelial function are demonstrated in **Figure 5A**.

3.3.1. miR-34 family

The expression of miR-34 family, which consists of miR-34a, -34b, and -34c are induced by p53 activation [60, 64]. In many cancers, miR-34a-promoted apoptosis as described in the previous section. In primary normal human cells, miR-34 family can change cellular senescence. A series of miRNAs, including miR-34a, were upregulated in hydrogen peroxide-induced premature senescence in human fibroblasts [66]. There are two human p53 isoforms, p53 beta which lacks C-terminal oligomerization domain and delta133 p53 which lacks N-terminal transactivation and proline-rich domains. Human fibroblasts (MBC-5 and WI-38) at early passage had many delta133 p53 but not p53 beta. In contrast, p53 beta expressed well in fibroblasts at late passage. In fibroblasts, miR-34a control replicative cellular senescence and delta133 p53 repressed miR-34a expression, extending cellular replicative lifespan [67].

Aging of endothelial cells is one of the factors for cardiovascular diseases. miR-34a, expressed relatively higher in late-passage endothelial cells, modulated endothelial cell survival and senescence [30]. Overexpression of miR-34a triggers endothelial senescence mainly by blocking SIRT1. In mice, miR-34a expression also increases in heart and spleen from older ones. Endothelial progenitor cells (EPCs) are essential for many physiological processes such as wound healing. miR-34a impairs EPC-mediated angiogenesis by increasing the number of senescent EPC probably through SIRT1 inhibition [68]. SIRT1, the major target of miR-34a, was known to deacetylate p53. Activation of p53 increased miR-34a expression, which inhibit SIRT1 expression, causing accumulation of acetylated p53. Acetylated p53 induces cell cycle arrest and apoptosis, and this increase of p53 activity induced more miR-34a expression. This suggests that p53 – miR-34a – SIRT1 works as a positive feedback loop (**Figure 3B**) [69].

Notch signaling has crucial role in artery-vein differentiation, blood vessel sprouting, and branching. Dysregulation of Notch signaling causes cardiovascular diseases [70]. miR-34a could regulate Notch signaling pathway in vascular inflammation. In the case of placental dysfunction, miR-34a exacerbated vascular endothelial inflammation via suppression of regulator of calcineurin 1 (RCAN1) [71]. Shear stress is one of the central regulators of endothelial inflammatory responses. The expression of miR-34a decreased by anti-inflammatory physiological high shear stress, in turn, inflammatory oscillatory shear stress-induced miR-34a expression in endothelial cells [72]. Increased miR-34a promoted acetylation of NF-kB p65 subunit (Lys310) by downregulating SIRT1, which lead to upregulate vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) protein expression. miR-34a also contributed to shear stress-induced EPC differentiation through a novel target Forkhead box j2 (Foxj2) [73]. The important molecules targeting by endothelial miR-34a are listed in **Figure 3A**.

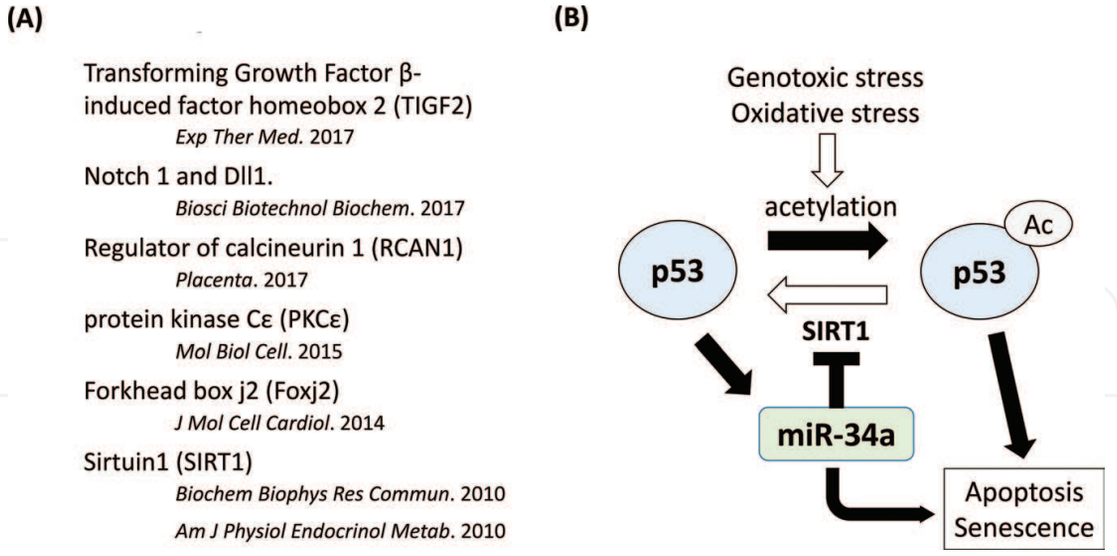

Figure 3. The role of miR-34a in regulating endothelial functions. (A) miR-34a target in endothelial cells. (B) miR-34a – p53 feedback loop.

3.3.2. miR-103 and miR-107

P53 positively regulates expressions of miR-103 and miR-107 in colorectal cancer cell lines [74]. miR-107 is encoded within an intron of the gene for pantothenate kinase enzyme 1, PANK1, while miR-103 is produced from primary miRNAs which are on miR-103-1 and miR-103-2 locus (within introns of PANK2 and PANK3, respectively) (**Figure 4A**). The seed sequences of miR-103 and miR-107 are the same, therefore, these miRNAs should have similar function [75]. miR-103 and miR-107 (miR-103/107) were originally recognized as a key regulator of metabolism and a hypoxia responsible miRNA [76]. The levels of miR-103/107 increased in liver of obese mice, ob/ob mice and diet-induced obese (DIO) mice, and knockdown of miR-103/107 improved insulin sensitivity [77]. Caveolin-1 was one of miR-103/107 targets that altered the level of insulin receptor on lipid rafts.

miR-103/107 also affected angiogenesis as members of hypoxia-responsive microRNAs (HRMs) induced by HIF1α under hypoxia in endothelial cells [78, 79]. The crucial proteins for miRNA biogenesis, Dicer-1 and Ago-1, were identified as miR-107 and miR-103/107 targets, respectively. In both cases, miR-107 provided translational de-suppression of vascular endothelial growth factor (VEGF) mRNA and increased VEGF expression. AGO1 levels regulated by miR-103/107 were associated with higher survival rate in human hepatocellular carcinoma [78]. Antagomir-107 decreased the number of capillaries in ischemic boundary zone after permanent middle cerebral artery occlusion (pMCAO) in rats, which was caused by miR-107 – Dicer-1 – VEGF axis [79].

In sepsis, miR-107 plays an important role in endothelial cells. One of the major complications of sepsis is the development of acute kidney injury (AKI) [80]. Septic AKI activates renal endothelial cells and leads to inflammation and breakdown of endothelial barrier in kidney [81]. Wang et al. isolated circulating endothelial cells (CECs) from septic AKI patients and prepared CEC-conditioned media. Human tubule epithelial cells (HK2 cells) treated with this CEC-conditioned media became more apoptotic, which was regulated by miR-107 [82].

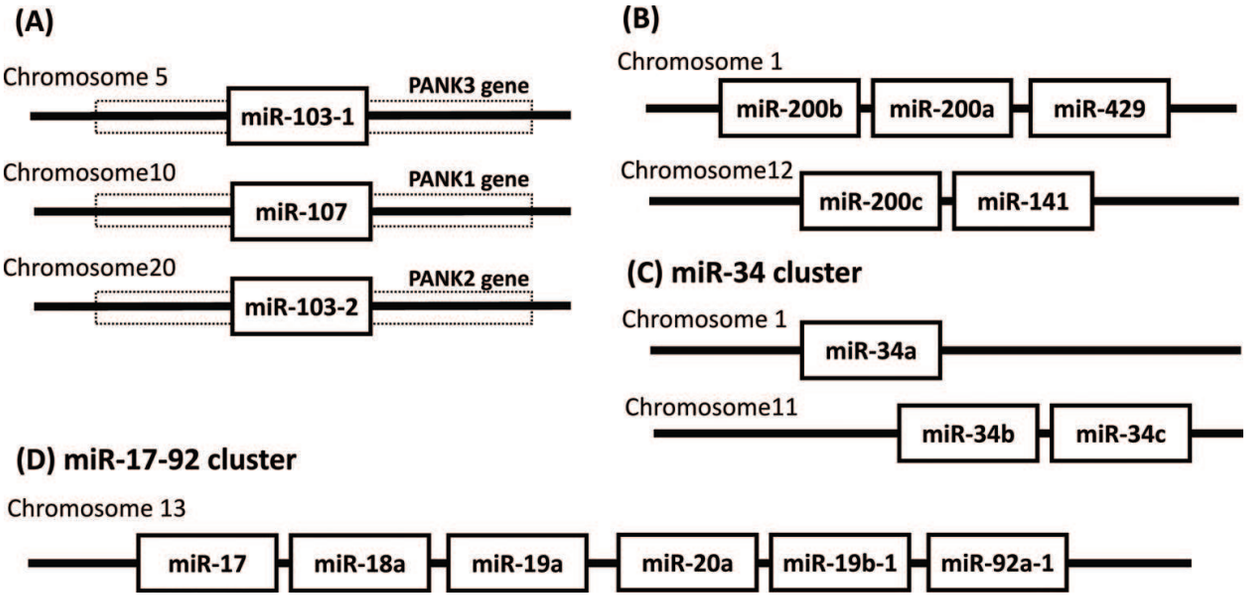

Figure 4. Scheme of miRNA cluster (miR-103/107, miR-200/141, miR-34, and miR-17-92). (A) miR-103/107 cluster. (B) miR-200/141 cluster. (C) miR-34 cluster. (D) miR-17-92 cluster.

In brain, miR-107 is enriched in neuron and the expression of miR-107 decreased in cerebral cortical gray matter of patients with Alzheimer's disease (AD) [83]. The authors demonstrated that beta-site amyloid precursor protein-cleaving enzyme 1 (BACE1) was identified as a miR-107 target. The cerebrovascular deposition of the amyloid beta protein, the key molecule in Alzheimer's disease, causes the disruption of blood-brain barrier (BBB) and brain microvascular endothelial cell dysfunction [84]. Another studies showed that miR-107 prevented amyloid beta-induced endothelial cells dysfunction by targeting endophilin-1 [85]. In a transgenic mouse model of AD, miR-107 expression in brain was lower compared to that in wild type mice [86]. Cofilin, which maintains the structure and function of cytoskeleton, was proved to be regulated by miR-107 in this mouse model. These data from AD patients and mice model suggest that relative high level of miR-107 in neurons and endothelial cells might negatively control the onset and progression of AD.

3.3.3. miR-143/145

miR-143 and miR-145 forms a bicistronic cluster (miR-143/145 cluster) in 5q33.1. The miR-143/145 cluster has been recognized as a tumor suppressor [87]. In cervical cancer, overexpression of Musashi RNA-binding protein 2 (MSI-2) correlated with poor survival. MSI-2 was repressed by p53 regulated miRNAs, miR-143 and miR-107, resulting in the prevention from proliferation and invasion of cervical cancer cells [88]. miR-143 and miR-145 have also potential roles in differentiation of vascular smooth muscle cells [89, 90]. The expression of miR-143/145 cluster decreased in aortic aneurysms and coronary artery diseases [91, 92]. miR-145-5p controls vascular neointimal lesion formation in balloon-injured rat carotid arteries [93]. The expression of miR-145 was upregulated in the lung of bone morphogenetic protein receptor type 2 (BMPR2)-deficient mice and puomonary arterial hypertension (PAH) patients [94]. Deng L. et al. identified transcriptional factors that regulate miR-143 and miR-145 expression in the promoter of miR-143/145 cluster [95]. Each miRNA in this cluster has

each function; however, how this cluster or miR-143 and miR145 independently regulated has not been fully understood yet.

Shear stress suppressed angiotensin-converting enzyme (ACE) expression and increased miR-143/145 levels in HUVEC [96]. The authors have shown that shear stress elicited the AMP-activated protein kinase alpha2 (AMPKα2)-dependent phosphorylation of p53 (Serine 15), and that p53 downregulation prevented the shear stress induced decrease in ACE expression. Since overexpression of miR-143/145 decreased ACE expression, AMPKα2 suppresses ACE expression through p53 activation and upregulation of miR-143/145 in EC. AMPKα2 knockout mice showed higher ACE levels and impaired bradykinin-induced vasodilation compared to wild type mice. In streptozotocin-induced diabetes mellitus (DM) mice model, phosphorylation of p53 and miR-143/145 expression increased, leading to the decrease of ACE expression. Therefore, miR-143 and miR-145 may contribute to the vascular events in atherosclerosis and DM.

miR-143 itself has been studied for the role of VSMCs as well as miR-145. The expression of miR-143 decreased in proliferating hemangiomas and miR-143 overexpression suppressed cell viability and proliferation of hemangioma-derived endothelial cells [97]. Bai Y et al. showed that miR-143 is upregulated in the brain microvessels of methamphetamine-treated mice [98]. Knockdown of miR-143 protected brain-blood barrier (BBB) damage-related vascular dysfunction by methamphetamine exposure. They identified an apoptosis inducing molecule, p53 upregulated modulator of apoptosis (PUMA), as a target of miR-143. Since the expression of miR-143 was regulated by p53 and miR-143 decreased PUMA, miR-143 might act for negative feedback of p53 signaling.

3.3.4. Others: miR-192, miR-200 family, and miR-194

Dysregulation of redox balance affects vascular homeostasis. Hydrogen peroxide treatment significantly increased miR-192 levels, which were prevented by p53 knockdown in endothelial cells [99]. Overexpression of miR-192 inhibited endothelial cell growth. Another study has shown that miR-200 family and miR-141 were upregulated in HUVEC exposed to hydrogen peroxide and in skeletal muscle in acute hindlimb ischemia mice model [100]. miRNA-200 family consists of two clusters, one encodes miR-200b, miR-200a, and miR-429 from chromosome 1 (1p33.36) and the other has miR-200c and miR-141 from chromosome 2 (12p13.31) (**Figure 4B**). These miRNAs share the similar seed sequence and mostly target the same genes. miR-200 family targets ZEB1 and ZEB2, affecting endothelial cell proliferation and senescence as well as epithelial-mesenchymal transition (EMT). Astrocytes are involved in controlling central nerve system (CNS) damage. During repair process of CNS, astrocytes undergo phenotypic changes into endothelial cells. This astrocyte-endothelial cell transition was modulated by a p53 inducible miRNA, miR-194. Therefore, miR-194 could promote angiogenesis in CNS.

3.4. Regulation of miRNA biogenesis by p53

There are two mechanisms by which p53 regulates miRNA production - control of miRNA transcription and modulation of miRNA maturation. These representative miRNAs regu-

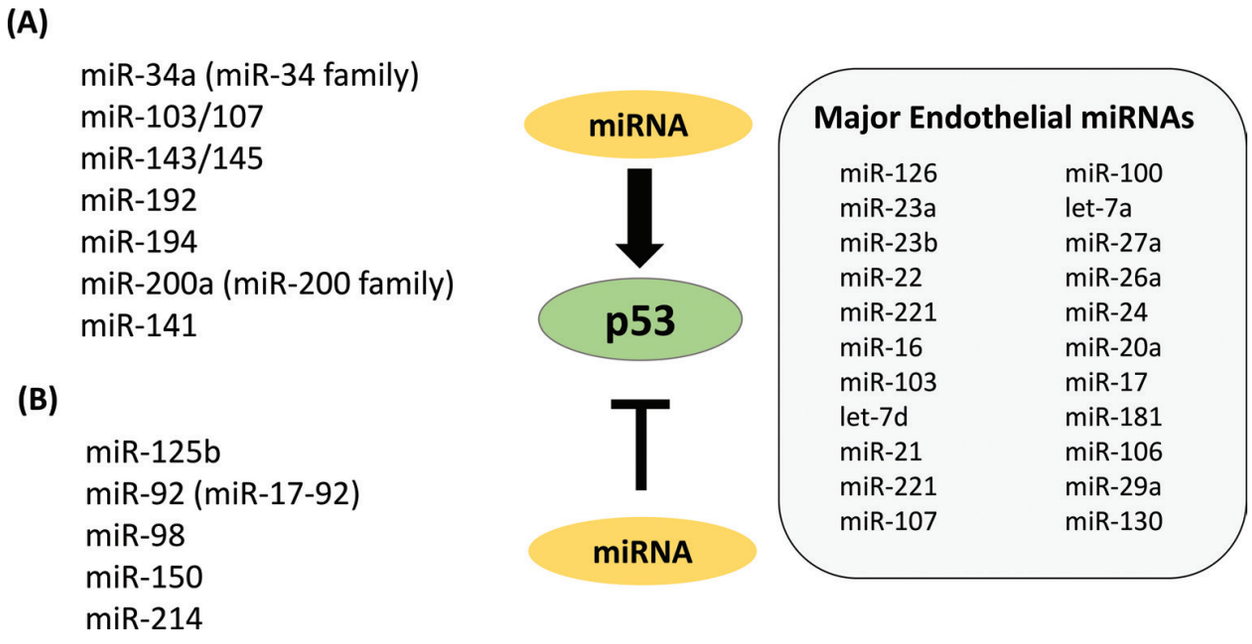

Figure 5. Endothelial miRNA and p53. (A) Endothelial miRNAs regulated by p53. (B) Endothelial miRNAs directly target p53.

lated by p53 are summarized in Section 4.3. Several miRNAs can modulate the process of miRNA biogenesis. The impact of miRNA biogenesis by transcriptional factors has first reported about transforming growth factor beta (TGF-beta) signaling in 2008 [101]. TGF-beta family orchestrates biological processes in vascular development [102]. Davis et al. demonstrated that smads, downstream transcriptional factors of TGF-beta signaling, play a critical role in processing miRNAs by the RNase III-type protein Drosha in nucleus [101]. Similarly, p53 affects the maturation process of miRNAs. The nuclear RNase III Drosha complex contains Drosha, DiGeorge syndrome critical region gene 8 (DGCR8), and the DEAD box RNA helicases, such as p68 and p72 (DDX5 and DDX17, respectively) [103]. P53 interacts with Drosha through p68, facilitating the process of primary miRNAs into precursor miRNAs [104]. Maturation of some precursor miRNAs from primary miRNAs, such as miR-16-1, miR-143, miR-145, and miR-206, is promoted by Doxorubicin stimulated wild type p53 in colon cancer cell lines, HCT-116. Association between a set of miRNAs and Ago2 protein was controlled by p53 [105]. Activated p53 interacts with AGO2 to affect incorporation of let-7 family members. Moreover, p53 induced RNA-binding-motif protein 38 (RBM38) that determined target mRNA selection with miRNAs [106]. Interestingly, Rbm38 deficient mice were likely to accelerate senescence and prone to spontaneous tumors [107]. All these studies had no data using endothelial cells; however, basic insights would be connected to the future study about p53 and miRNAs in the cardiovascular research.

3.5. Regulation of p53 by miRNAs

Many miRNAs regulate p53 directly and indirectly. Endothelial miRNAs can target p53. More than 20 miRNAs that modulated p53 are reported. Among them, miR-92, miR-25, miR-214, and miR-638 play important roles in endothelial cell (**Figure 5B**).

3.5.1. miR-125b

TGF-beta2 induces endothelial-to-mesenchymal transition (EndMT) [108]. The expression of miR-125b in EndMT-derived fibroblast-like cells is significantly higher compared to that in the original mice endothelial cells [109]. In this experiment, miR-125b elevation was negatively associated with p53 expression after EndMT change. Since p53 is a direct target of miR-125b in several human cells, such as neuroblastoma cells and lung fibroblast cells, downregulated p53 by miR-125b possibly modulate TGF-beta-induced profibrotic signaling in endothelial cells [110]. The expression of miR-125b was altered by cell-matrix adhesion in human mesenchymal stem cells (hMSCs) [111]. miR-125b targeted p53, which regulate survival of hMSCs and endogenous miR-125b increased during reprogramming of mouse embryo fibroblasts (MEFs) to induced pluripotent cells. Indeed, miR-125b was not increased by loss of cell adhesion in HUVEC. Sepsis damages endothelial cells, causing multiple organ failure [81]. Transfection of endothelial cells with miR-125b mimics attenuate LPS-induced ICAM-1 and VCAM-1 expression by inhibiting TRAF6 and NF-κB activation [112]. Induction of miR-125 in mice heart attenuated cecal ligation (CLP)-induced sepsis as well and improved survival. These studies suggest that miR-125b regulates angiogenesis and vascular inflammation.

3.5.2. miR-17-92 cluster

Seven individual mature miRNAs (miR-17-5p, miR-17-3p, miR-18a, miR-19a, miR-19b, miR-20a, and miR-92a) are produced from primary miR-17-92, located in the open reading frame 25 (C13orf25) on chromosome 13 in human (**Figure 4D**). Mice knockout or overexpressing of miR-17-92 cluster died shortly after birth, suggested that the balance of miR-17-92 expression are involved in normal development [113, 114]. Originally, miR-17-92 has shown to be a highly conserved cluster, called oncomir-1, and extensively studied the molecular mechanism of tumorgenesis [115]. The roles of miR-17-92 for cardiovascular diseases have been investigated.

One miRNA of this cluster, miR-92a, blocked angiogenetic function in endothelial cells and inhibition of miR-92a by systemic administration of an antagomir-enhanced neovascularization and functional recovery from damaged tissue in hindlimb ischemic mice model [116]. Inhibition of miR-92a-enhanced endothelial cell proliferation and migration, probably through an increased phosphorylation of ERK1/2, JNK, and eNOS. miR-92a promotes pro-atherogenic changes in endothelial cells [117]. Disturbed flow increased miR-92a level in endothelial cells and miR-92a suppressed KLF2 and phosphatidic acid phosphatase type 2B (PPAP2B) that is involved in coronary artery disease (CAD) by genome-wide association studies (GWAS), driving inflammatory and adhesive endothelial phenotype [117]. Although no reports about miR-17-92 regulation of endothelial p53, according to accumulating data above, miR-17-92 may be involved in vascular events and p53 took some parts in them.

3.5.3. Others: miR-98, miR-150, and miR-214 and beyond

There are many miRNAs that directly regulate p53 in cancer; however, a few in endothelial cells. A variety of miRNAs, including miR-98, miR-150, and miR-214 has been shown to decrease p53 expression in cancer [118]. Hypoxia and reoxygenation conditions promote apoptosis and oxidized low-density lipoprotein (ox-LDL)-induced dysfunction of endothelial cells.

miR-98 rescues these phenomenon by targeting caspase-3 and lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1), respectively [119, 120]. Stromal cell-derived factor 1α (SDF-1α) and its receptor CXCR4 control mobilization and migration of EPC. miR-150 decreased CXCR4 expression, leading to impaired EPC migration [121]. In mice studies, decreased miR-150 in EPC helped to revascularize the ischemic heart. miR-150 affected blood-brain barrier (BBB) permeability. Antagomir-150 treatment protected BBB, reduced infarct volume in post-stroke rat via angiopoietin receptor Tie-2 [122].

Targets of miRNAs are recognized by pairing between the seed sequence of miRNA and complementary sites in target mRNAs. There are many useful tools to search for miRNA target genes. Among them, Targetscan is one of the reliable resources many researchers are widely taking. Targetscan predicts four miRNAs, let-7, miR-22, miR122, and miR-150, which are broadly conserved among vertebrates to bind onto 3′UTR of human p53 mRNA (http://www.targetscan.org/vert_71/). Future studies could reveal the function of these miRNAs and their relationship to p53.

4. Conclusion

miRNAs are crucial regulators of gene expression for diverse physiological and pathological processes. Endothelial miRNAs have been intensively studied since Kuehbacher A et al. released that genetic knockout of Dicer and Drosha, miRNA-processing enzymes, inhibited capillary sprouting of endothelial cells and tube formation [123]. Recently Hratmann P et al. demonstrated that Dicer in endothelial cells promoted atherosclerosis and endothelial inflammation [124]. In contrast, p53 is involved in a variety of diseases, such as vascular remodeling, atherosclerosis, hypertension, and hypoxic pulmonary artery remodeling as well as cancer biology.

The importance of p53 and miRNAs in endothelial cells has been shown here. We demonstrated the regulation of endothelial miRNAs by p53 and the modulation of p53 by miRNAs in endothelial cells. These miRNAs play pivotal roles in vascular development and the onset of cardiovascular diseases. The ubiquitin E3 ligase Mdm2 stimulates p53 degradation, in turn, p53 promotes Mdm2 gene expression. Therefore, there is a negative feedback loop between p53 and Mdm2. miR-192, miR-194, and miR-215 targeted Mdm2 protein, which could disrupt this p53-Mdm2 feedback loop [125]. Future studies will unveil the complex and fascinating pathway and loop composed by p53 and miRNAs and develop therapeutic machinery of vascular diseases.

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