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REVIEW

Molecular therapies delaying cardiovascular aging: disease- or health-oriented approaches

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Abstract

Regenerative medicine is a new therapeutic modality that aims to mend tissue damage by encouraging the reconstitution of physiological integrity. It represents an advancement over conventional therapies that allow reducing the damage but result in disease chronicization. Age-related decline in spontaneous capacity of repair, especially in organs like the heart that have very limited proliferative capacity, contributes in reducing the benefit of conventional therapy. ncRNAs are emerging as key epigenetic regulators of cardiovascular regeneration. Inhibition or replacement of miRNAs may offer reparative solutions to cardiovascular disease. The first part of this review article is devoted to illustrating novel therapies emerging from research on miRNAs. In the second part, we develop new therapeutic concepts emerging from genetics of longevity. Prolonged survival, as in supercentenarians, denotes an exceptional capacity to repair and cope with risk factors and diseases. These characteristics are shared with offspring, suggesting that the regenerative phenotype is heritable. New evidence indicates that genetic traits responsible for prolongation of health span in humans can be passed to and benefit the outcomes of animal models of cardiovascular disease. Genetic studies have also focused on determinants of accelerated senescence and related druggable targets. Evolutionary genetics assessing the genetic basis of adaptation and comparing successful and unsuccessful genetic changes in response to selection within populations represent a powerful basis to develop novel therapies aiming to prolong cardiovascular and whole organism health.

Key Words

- ▶ vascular regeneration
- ▶ genetics
- ▶ epigenetics

Introduction

The number and proportion of older people is growing in every country in the world. According to data from World Population Prospects (2019 Revision), by 2050, 16% of the people in the world will be over age 65, almost doubling

the figure in 2019; and the number of persons aged 80 years or over is projected to triple. In Europe and Northern America, the proportion of 65 or over is forecasted to be 25% by 2050. This demographic transformation has remarkable social implications, including work, markets, request, and availability of services such as housing, health,

and mobility. Prolongation of lifespan correlates with an increased number of individuals with chronic pathologies often requiring hospitalization. Many countries are already facing problems related to the impact of the aging population on the national health system, pension schemes, and social protections. Specific actions on health and nutrition, protecting elderly people, housing and environment, family, and social/medical welfare are in the agenda of policy makers and governmental agencies.

All around the world, scientists are trying to beat age-related diseases, such as heart attacks, cancer, and dementia; stop people getting ill is an obvious goal to aid the individual wellbeing and reduce pressure on society. At the whole organism level, aging has been defined as the time-related deterioration of the physiological functions necessary for survival and fertility. This definition applies to all the individuals of a species and overlaps with disease-related aging. Aging of the vasculature plays a key role in morbidity and mortality of older people. It is often assimilated with endothelial dysfunction, that is, the failure of vascular endothelial cells to respond to vasoactive stimuli and mount reparative transformation upon tissue damage. Zooming into the molecular level, aging of the vasculature consists in small, incremental amounts of damage that spreads to all vascular cells, including vascular smooth muscle cells and pericytes, and, owing to the system dependency on vascular homeostasis, to tissues and organs; eventually, the whole organism will suffer from this accumulation of damage.

In this article, we focus on molecular therapies that can aid an organism to correct deviations from or maintain physiological homeostasis. Two specific fields are considered. A review of recent research on ncRNAs and new genetic approaches inspired by extreme longevity aims to prolong health-span.

Mechanisms of vascular aging

The development of novel treatments targeting vascular aging and prevention of age-related vascular pathologies requires a better knowledge of the cellular and functional changes that occur in the vasculature during aging. These include oxidative stress, mitochondrial dysfunction, susceptibility to molecular stressors, chronic low-grade inflammation, genomic instability, cellular senescence, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, and stem cell dysfunction. Senescent cells secrete autocrine or paracrine factors, including cytokines, growth factors, proteases, and soluble receptors

called senescence-associated secretory phenotype (SASP) (1). One dominant SASP molecule is the monocyte chemoattractant protein-1 (MCP-1/CCL2) which acts through binding to cognate receptor CCR2. Hence, MCP-1 could be exploited as a marker of cell senescence both *in vitro* and *in vivo*. Review articles and commentaries have been published on this topic in recent years (2, 3, 4, 5, 6).

The capacity to repair and regenerate empowers living organisms with resilience to natural fluctuations and events that cause disturbance or damage. Aging and regeneration are two sides of the same coin and this has been confirmed through the examination of species with extreme regenerative capabilities, such as planarians and salamanders, which show no signs of aging or quantifiable age-associated functional decline (7). In contrast, in complex organisms like humans, aging is characterized by a decay in the regenerative capacity and reparative activities (8). Tissue-specific stem cells and progenitor cells incur in age-related defects, such as the loss of self-renewal capacities and proliferative activity and the deterioration in functionality and potency (9). Likewise, differentiated cells become progressively incapable of regulating protein synthesis and metabolism, especially under stress conditions, eventually undergoing irreversible proteotoxic damage (10). Exhaustion of compensatory mechanisms increases the susceptibility to risk factors and diseases and results in excess morbidity and mortality.

miRNAs involved in aging and regeneration

The first part of this review article is dedicated to the emerging role of miRNA in aging and regeneration. ncRNAs and among these several miRNAs genes can be epigenetically regulated by DNA methylation and/or histone modifications. In addition, a subgroup of miRNAs, named epi-miRNAs, directly target enzymatic effectors involved in epigenetic modulation (11). Therefore, a regulatory loop between epigenetic modulation and miRNAs does exist, which could significantly affect transcription and enhance or slow-down aging and age-related diseases (11). The following miRNAs are either epigenetically regulated or represent epi-miRNAs involved in aging and in cardiovascular regeneration and are summarized in Fig. 1.

The miRNA-200 family

The miRNA-200 family consists of five members (i.e. miRNA-200a, miRNA-200b, miRNA-200c, miRNA-141,

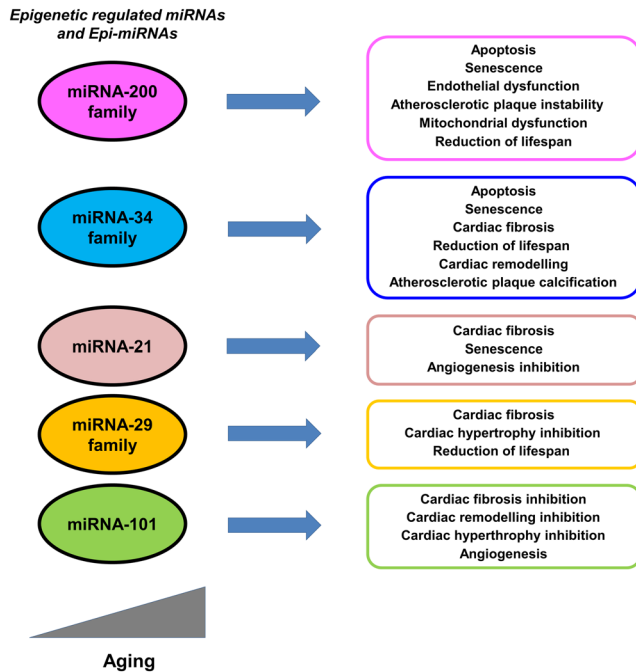


Figure 1
Biological effects of Epi-miRNAs.

and miRNA-429). MiRNA-200 plays a major role in the epigenetic control of tumorigenesis and Epithelial to Mesenchymal transition (EMT) (12). A negative feedback loop between miRNA-200c and DNA methyltransferase 3a (DNMT3a) was shown in gastric cancer cells (13). miRNA-200b has been shown to target *Suz12*, a subunit of polycomb repressive complex 2 (PCR2) decreasing H3 lysine 27 trimethylation at the promoters of target of E-cadherin, thereby causing transcriptional increase (14). The interaction between miRNA-200 and *Suz12* is highly conserved, and it seems to represent a regulatory mechanism to control the growth and function of stem cells.

miRNA-200 upregulation has been linked to aging. In particular, miRNA-200c and miR-141 expressions are increased in skeletal muscles and arteries of aging rhesus monkeys and mice (15, 16). miRNA-141 and miRNA-200c increase with aging in human liver (17). In addition, miRNA-200a was found upregulated in primary human keratinocytes from aging individuals (18). miRNA-200 upregulation with aging contributes to the impairment of mechanisms implicated in the repair of oxidative DNA damage in keratinocytes and endothelial cells (18, 19, 20). The most expressed and induced member, miRNA-200c, is responsible for apoptosis and senescence *via* downregulation of the transcription factor *ZEB1* and a *p53* and *pRb*-dependent mechanism (20).

Moreover, miRNA-200c upregulation induces endothelial dysfunction, since it disrupts the autoregulatory loop among three important proteins for endothelial function: Sirtuin 1 (*SIRT1*), forkhead box O1 (*FOXO1*), and the endothelial nitric oxide synthase (eNOS), directly targeting all of them (16). *SIRT1* is a major regulator of longevity and metabolic disorders (21). Its downregulation by miRNA-200c results in increased levels of acetylation of both *p53* and *FOXO1*. Acetylated *p53* is more active and further induces miRNA-200c. *FOXO1* acetylation inhibits its transcriptional activity, with the consequent decrease of catalase and Manganese Superoxide dismutase (MnSOD or SOD2), two ROS-scavengers, finally leading to oxidative stress upregulation (16). In addition, *SIRT1* deacetylates eNOS increasing NO bioavailability, while NO stabilizes the mRNA and protein of *SIRT1* (22). Since miRNA-200c targets both *eNOS* and *FOXO1*, their decrease further contributes to the disruption of the autoregulatory loop inducing endothelial dysfunction (16).

miRNA-200c also plays a major role in cardiovascular diseases (19). miRNA-200c is upregulated in plaques and plasma of atherosclerotic patients, being further enhanced in unstable vs stable carotid plaques (23) and in plasma of children with familiar hypercholesterolemia (24). miRNA-200c was found upregulated in cardiomyopathies induced by doxorubicin (DOX) in mice as well as in human cardiac mesenchymal progenitor cells exposed to DOX (25). Interestingly, stromal cell-derived factor-1 (SDF-1) administration was able to partially revert DOX-induced miRNA-200c and *p53* protein upregulation in mouse hearts, and to revert both the adverse remodelling and the impaired ventricular function (25). In addition, miRNA-200c is induced following acute hindlimb ischemia in mice (16, 20). Inhibition with anti-miRNA-200c increased post-ischemia reperfusion and reparative angiogenesis (16). Likewise, miRNA-200c inhibition restored endothelial function in diabetes (26) and attenuated cardiomyocyte hypertrophy in high glucose-treated cardiomyocytes (27).

miRNA-141 is involved in mitochondrial dysfunction and in cardiac abnormalities associated with type 1 diabetes (28). It targets the solute carrier family 25 member 3 (*SLC25A3*), an inner mitochondrial membrane phosphate transporter, which is essential for ATP production since it provides inorganic phosphate to the mitochondrial matrix. *SLC25A3* expression is decreased in type 1 diabetes, which has a deleterious effect on ATP production and cell viability (28). Interestingly, miRNA-141 also targets *ZMPSTE24*, which is involved in the post-translational maturation of lamin A (29).

ZMPSTE24 is responsible for the prelamin A accumulation in cellular senescence of human mesenchymal stem cells isolated from umbilical cord blood. Moreover, miRNA-141 is upregulated during senescence as a result of epigenetic regulation of its promoter region (29).

Altogether, these data indicate that miRNA-200 is deeply involved in the epigenetic control of aging and regeneration in different tissues and cells. Its manipulation can have beneficial effects on vascular and cardiac function.

The miRNA-34 family

The miRNA-34 family consists of three highly homologous miRNAs, miRNA-34a, b, and c. It is often epigenetically regulated in different diseases and tumors (30). A miRNA-34a activation has been associated with ethanol-linked hypomethylation of the miRNA-34a promoter (31). The miRNA-34a promoter is hypomethylated in preeclamptic placentas (32), and, in cholangiocarcinoma, miRNA-34a expression is silenced epigenetically by EZH2 and DNA methylation (33). In addition, miRNA-34a regulates epigenetic enzymes, such as deacetylases *SIRT1* and *HDAC1* (34, 35).

This miRNA family exhibits an age-dependent increase in different murine tissues, including liver, heart, and brain (36), in rat endothelial progenitor cells (37), and in human vasculature. miRNA-34a is thought to play an important pathogenic role in vascular smooth muscle cells senescence, calcification, and plaque formation through downregulation of *SIRT1* and AXL Receptor Tyrosine Kinase (38, 39). Accordingly, murine vascular smooth muscle cells lacking miRNA-34a are resistant to develop senescence and calcification (39). Experimental studies using anti-miRNA-34a in high fat diet (HFD)-induced ApoE^{-/-} mice showed reductions in atherosclerotic lesions and vascular cells apoptosis. Likewise, anti-miRNA-34a promoted cell viability and restrained apoptosis in ox-LDL-treated human aortic endothelial cells (40) and induced proliferation in human cardiac progenitor cells (41).

miRNA-34a has been associated to heart failure, where upregulation in cardiomyocytes targets pro-proliferative and pro-survival proteins CyclinD1 and Bcl2 (42). Several days post-MI, older mice exhibited a significant increase of miRNA-34a expression compared to young mice, resulting in a decline of cardiac performances and cardiac remodeling. Inhibition of either miRNA-34a or of the entire miRNA-34 family improves cardiac function in terms of fibrosis, cardiac remodeling, and

cardiomyocyte death in models of myocardial infarct (42). Higher miRNA-34a levels have been found in diabetes; in particular, miRNA-34a is elevated in plasma of diabetic patients and is upregulated in cardiac progenitor cells and cardiomyocytes exposed to high glucose, suggesting an inductive role of senescence in the diabetic heart (43).

miRNA-34 has been associated with reduction of lifespan. Interestingly, miRNA-34 is upregulated during *C. elegans* aging where it appears to antagonize longevity by repressing the autophagy gene *atg9* (44). In *Drosophila*, miRNA-34 has been shown to be essential for normal aging. Loss-of-function mutants of miRNA-34 display shorter lifespan through EIP74, an ecdysone-induced transcription factor (45).

In summary the miRNA-34 family plays a pivotal role in the epigenetic control of lifespan and is pathogenically involved in atherosclerosis, vascular calcification, cardiac fibrosis, and adverse remodeling in heart failure. Its inhibition can improve cardiovascular repair.

miRNA-21

miR-21 is epigenetically regulated in ovarian cancer and colorectal cancer (46, 47). miRNA-21 expression increases with aging in murine hearts and plays a major role in cardiac fibrosis in mice and in patients with aortic stenosis (48, 49). In a mouse model of acute cardiac allograft transplantation, it activates mitogen-activated protein kinase (MAPK) signaling in fibroblasts inducing cardiac fibrosis (50). Suppression of miRNA-21 prevents cardiomyocyte senescence induced by d-galactose and DOX (51) and promotes angiogenesis by restoring Ras homolog family member B (52).

The miRNA-29 family

The miRNA-29 family consists of three members, miRNA-29a, miRNA-29b, and miRNA-29c. It acts as a regulator of cell survival by inducing global DNA hypomethylation. It has been shown to directly target DNA methyltransferases *DNMT-3A* and *DNMT-3B* in lung cancer (53) and indirectly DNA methyl transferase *DNMT1* through the regulation of the trans-activator Sp1 in leukemia (54).

miRNA-29 is upregulated in aging hearts and its dysregulation after myocardial infarction induces the activation of TGF β causing cardiac fibrosis (55). MiRNA-29b administration inhibits TGF β /Smad3 signaling in hypertensive rats preventing cardiac fibrosis (56). An inverse correlation of circulating miRNA-29a with cardiac

fibrosis has been found in the plasma of patients with cardiac hypertrophy (57).

miRNA-29 modulates vascular function, as demonstrated by studies in diabetic models, in which intraluminal delivery of miRNA-29a or miRNA-29b restored normal endothelium-dependent vasodilation increasing NO availability. Thus, miRNA mimics may have therapeutic potential for cardiometabolic disorders (58). Interestingly, another miRNA-29 target is the prelamin A interacting protein (59). miRNA-29 is induced in a model of HGP syndrome, the *Zmpste24*-deficient mice, reflecting a p53-dependent compensatory response to DNA damage (59). miRNA-29 targets *Ppm1d* phosphatase, which in turn enhances p53 activity, inducing further miRNA-29 upregulation and eventually decreasing cell survival and proliferation (59).

miRNA-101

miRNA-101 is an epi-miRNA that targets both *EZH2* (60) and *EED* (61), two pivotal components of histone methyltransferase PCR2 complex, involved in cell proliferation and differentiation. Moreover, miRNA-101 increases with aging in brain (62). Following coronary artery ligation, miRNA-101 levels are downregulated in both rat cardiac fibroblasts and myocytes. miRNA-101 overexpression inhibits fibrosis and adverse cardiac remodelling through inhibition of c-FOS, a transcriptional modulator of TGF β (63). In a rat model of hypertrophy, miRNA-101 overexpression inhibited cardiomyocytes hypertrophy through the downregulation of its target small GTPase *Rab1a* (64). Finally, miRNA-101 is downregulated upon VEGF treatment, resulting in increased expression of the histone methyltransferase *EZH2* in endothelial cells and promotion of angiogenesis (65).

In summary, miRNA-101 modulates epigenetic and cardiovascular homeostasis, although its modulation with aging in cardiovascular tissues remains unexplored.

Noncoding RNAs that sponge miRNAs involved in aging and regeneration

In the last decades, other ncRNAs, such as long ncRNAs (lncRNAs) and circular RNAs (circRNAs), have been discovered and attracting attention in research. lncRNAs are ncRNAs that are 200 nt longer, usually polyadenylated, capped, and spliced (66). lncRNAs are transcribed from different genomic regions, such as intergenic regions, promoter upstream regions, enhancers, and the opposite

strand of protein-coding genes. Some lncRNAs are generated by unique biogenesis, such as RNase P cleavage and capping by snoRNA-protein (snoRNP) complexes at their ends (67).

CircRNAs are ncRNAs characterized by a covalently closed continuous loop without polarity or a polyadenylated tail (68). CircRNAs usually originate from protein-coding genes and complete exons (69) and are mainly produced during splicing. CircRNAs are resistant to exonucleases due to lack of free ends. They also have potential to roll circle amplification, constrain RNA folding, and rearrange genomic sequences (70). The most important function of circRNAs is miRNA sponge, where they regulate miRNAs. However, it is shown previously that some circRNAs are translated into polypeptides (69, 71).

Here we will discuss relevant lncRNAs and circRNAs known to sponge relevant miRNAs described previously that are known to have a role in cardiovascular aging and/or regeneration (Table 1).

Long noncoding RNAs (lncRNAs) and circular RNAs (circRNAs) that sponge miRNA-200 family

lncRNA MALAT1 (metastasis associated lung adenocarcinoma transcript 1) has been associated to miR-200 family in different studies related with cancers (72, 73, 74). Interestingly, its modulation has also been linked to CVD. Higher expression of MALAT1 was observed in both humans and rodents with MI, myocardial ischemia-reperfusion injury, and cardiomyocytes of arrhythmic rats (75, 76).

The role of MALAT1 in miR-200c/HMGB1 pathway was studied in arrhythmic rat model and shows that high-mobility group box 1 (HMGB1) acts as a downstream target of miR-200 family. miR-200 family inhibits *HMGB1* expression at both mRNA and protein levels. MALAT1 sponges miR-200c causing HMGB1 upregulation and thereby downregulating cardiac transient outward potassium current (Ito) associated arrhythmia (76). Further, in mice undergoing coronary artery ligation to produce acute MI, exogenous HMGB1 has been shown to promote cardiac repair, to improve cardiac function (77), and to inhibit adverse remodeling *via* an increase in miR-206 that directly targets Tissue Inhibitor of Metalloproteinase-3 (*TIMP3*) (78). Knockdown of MALAT1 or overexpression of miR-200c reduces HMGB1 expression level, indicating that MALAT1 sponges miR-200c to promote HMGB1 expression. Moreover, MALAT1 modulates outward potassium and repolarization currents

Table 1 Functions regulated by noncoding RNAs that sponge miRNAs.

Non-coding RNA	Associated miRNA	Disease	Modulation	Function	References
MALAT1	miR-200 family	MI, MI-reperfusion, heart arrhythmia	Upregulation	Cardiac transient outward potassium current	Zhu <i>et al.</i> (76)
circRNA_010567	miR-141	Myocardial fibrosis	Upregulation	Cardiomyocytes apoptosis	Sun <i>et al.</i> (79)
MEG3	miR-34a	MI, TAC and Cardiac Hypertrophy	Upregulation	Fibrosis induction	Zhou and Yu (83)
C2dat1	miR-34a	CAD	Upregulation	Endothelial dysfunction	Boon <i>et al.</i> (86)
HOTAIR	miR-34a	Diabetic cardiomyopathy	Downregulation	Inflammation and apoptosis	Tong <i>et al.</i> (88)
LincRNA 1700020114Rik	miR-34a	Diabetic Nephropathy	Upregulation	VSMC proliferation and migration	Wang <i>et al.</i> (91)
TALNEC2	miR-21	MI	Upregulation	Oxidative injury, inflammation, and apoptosis	Gao <i>et al.</i> (94)
TUG1	miR-21	Atherosclerosis and Hypoxia	Upregulation	Cell proliferation inhibition	Li <i>et al.</i> (95)
MEG3	miR-21	CAD	Downregulation	Hypoxic injury	Hao <i>et al.</i> (98)
TCONS_00024652	miR-21	Hypoxia-induced pulmonary hypertension	Downregulation	Endothelial cell apoptosis	Chen <i>et al.</i> (101)
H19	miR-29b	Atherosclerosis	Upregulation	Cell proliferation and migration	Wu <i>et al.</i> (102)
XIST	miR-101	Cardiac hypertrophy	Upregulation	Atherosclerotic plaque progression	Zhu <i>et al.</i> (97)
MI, myocardial ischaemia			Upregulation		Halimulati <i>et al.</i> (103)
			Upregulation	Tumorigenesis and metastasis	Lv MX <i>et al.</i> (105)
			Upregulation	EC and VSMC proliferation and apoptosis inhibition	Chen <i>et al.</i> (107)
			Upregulation	Hypertrophy	Xiao <i>et al.</i> (108)
			Upregulation	Tumorigenesis and metastasis	Chen <i>et al.</i> (109), Wu <i>et al.</i> (110)

that are involved in myocardial function. In hypoxia-induced cardiomyocytes, MALAT1 sponges miR-200a and upregulates programmed cell death 4 (PDCD4) expression, indicating that MALAT1-miR-200a-PDCD4 axis regulates cell cycle progression, proliferation, and apoptosis in myocardial cells (79).

LncRNAs and circRNAs that sponge miRNA-141

Several studies have shown the interaction of miR-141 with lncRNAs in different diseases including cancers (80, 81, 82). However, only few studies are available on lncRNAs and circRNAs that sponge miR-141 in CVD.

Among these circRNA_010567 is a fibrosis-promoting RNA upregulated in myocardium of diabetic mice and in cardiac fibroblasts (CFs) treated with Ang II (83). In db/db mice model, it was found that circRNA_010567 directly binds to miR-141. Indeed, silencing of circRNA_010567 results in upregulation of miR-141 and downregulation of its target TGF- β 1, thereby causing the suppression of fibrosis associated protein resection in cardiac fibroblasts. This indicates that circRNA_010567/miR-141/TGF- β 1 axis plays a major role in myocardial fibrosis (83).

LncRNAs and circRNAs that sponge miRNA-34 family

Maternally expressed gene 3 (MEG3) is a highly expressed lncRNA associated with myocardial infarction, transverse aortic constriction (TAC), and cardiac hypertrophy (84, 85). It contributes to endothelial dysfunction by mediating epigenetic regulations in aging (86). MEG3 inhibition in cardiac fibroblasts prevents cardiac fibrosis and improves diastolic performance (87). miR-34a is a direct target of MEG3 (88). *In vivo* studies on hepatocyte ischemia-reperfusion (HIR) injury revealed that MEG3 functions as a competing endogenous RNA for miR-34a. It downregulates miR-34a expression and protects hepatocytes from HIR injury mediated through 34a/Nrf2 signaling pathway (89). Further, in diabetic retinopathy, MEG3 targets miR-34a/SIRT1 axis and alleviates high glucose inducing apoptosis, as well as inflammation by inhibiting the NF- κ B signaling pathway (88).

Another lncRNA that shows a role in coronary artery disease (CAD) is C2dat1. LncRNA C2dat1 is a CaMK2D-associated transcript 1. LncRNA shows higher expression in CAD (90). C2dat1 binds to miR-34a and modulates its expression in CAD. Overexpression of C2dat1 suppresses miR-34a expression and upregulates its target protein SIRT1, increasing VSMC growth and migration in CAD (91).

LncRNA HOX transcript antisense RNA (HOTAIR) is a highly conserved and abundant lncRNA associated with various essential biological processes involved in different diseases. Increased expression of HOTAIR was observed in both plasma and cardiac tissues of patients with cardiac hypertrophy (92, 93). In contrast, diabetic mice showed a significant decrease in the expression of HOTAIR in heart tissues. HOTAIR knockdown in H9C2 rat cardiomyoblast cell line resulted in an elevated oxidative injury, inflammation, and apoptosis, whereas HOTAIR overexpression attenuated myocyte death and improved cardiac function. HOTAIR, in fact, functions as a competing endogenous RNA (ceRNA) of miR-34a. It has been demonstrated that HOTAIR sponges miR-34a, causing upregulation of SIRT1 expression and protecting mice from diabetic cardiomyopathy (94).

LincRNA 1700020I14Rik is another lncRNA that regulates miR-34a-5p/Sirt1/HIF-1 α signaling pathway by inhibiting cell proliferation, as well as fibrosis in diabetic nephropathy. Under elevated glucose condition, overexpression of lncRNA 1700020I14Rik prevents the expression of renal fibrosis markers and decreases cell proliferation, whereas its downregulation produces the opposite effect (95).

Although no studies have been published on lincRNA 1700020I14Rik and the heart and blood vessels, these observations raise the possibility of a link between lincRNA 1700020I14Rik and cardiovascular disease in diabetic patients with renal failure.

LncRNAs and circRNAs that sponge miRNA-21

miR-21 is a highly expressed microRNA in the cardiovascular system; it attenuates inflammation, maladaptive remodeling, and cardiac dysfunction in post myocardial infarction (96). Recent studies have reported that miR-21 expression can be modulated by other ncRNA associated with various molecular pathways (97).

TALNEC2 is a E2F1-regulated putative lncRNA, also known as Tumor Associated Long ncRNA Expressed on Chromosome 2. High expression of TALNEC2 was observed in myocardial ischemic patients. Studies on H9c2 cells showed that TALNEC2 modulates miR-21 expression under hypoxic condition. Overexpression of TALNEC2 aggravates hypoxic injury by downregulating miR-21 via the regulation of miR-21/PDCD4-mediated activation of the Wnt/ β -catenin signaling pathway in myocardial ischemic injury (98).

Long ncRNA taurine upregulated gene 1, also known as lncRNA TUG1, is known to promote VSMC proliferation

and to induce atherosclerosis progression. High expression of TUG1 was observed in atherosclerosis and hypoxia (99, 100). LncRNA TUG1 overexpression induces endothelial cell apoptosis, and its knockdown decreases apoptotic ratio and promotes cell survival (101). Further, lncRNA TUG1 competes with phosphatase and tensin homolog (PTEN) for miR-21 binding. Downregulation of TUG1 markedly inhibits the expression of miRNA-21, indicating that lncRNA TUG1 promotes VSMC proliferation and atherosclerosis by regulating miRNA-21/PTEN axis (99, 100).

LncRNA MEG3, as described previously, is a vastly expressed lncRNA associated with CVD (84, 85). In contrast, some studies showed the reduced expression of lncRNA MEG3 in CVD and Hypoxia-induced pulmonary hypertension (97, 102). It has been shown that miR-21 is a direct target of MEG3. In CAD, MEG3 is downregulated and negatively correlates with miR-21 levels. MEG3 overexpression suppresses EC proliferation and migration and reduces the expression of proteoglycan, type I, and V collagen through the decrease of miR-21 (102). Its inhibition promotes cell proliferation and migration in normal and hypoxic conditions through the miR-21/PTEN axis. Under hypoxic condition, MEG3 binds miR-21 and modulates PTEN expression resulting in cell proliferation and migration in pulmonary arterial smooth muscle cells (PASMCs) (97).

TCONS_00024652 is an endothelium-associated lncRNA involved in plaque angiogenesis and atherosclerosis progression. High expression of TCONS_00024652 was observed in TNF- α -stimulated HUVECs. The interaction of miR-21 with lncRNA TCONS_00024652 was determined using bioinformatics and molecular techniques. TNF- α treatment increases TCONS_00024652 expression in a dose dependent manner, and its knockdown inhibits proliferation and angiogenesis in endothelial cells. Binding of miR-21 to TCONS_00024652 reduces its expression and may be a potential method of improving endothelial dysfunction and plaque stabilization (103).

LncRNAs and circRNAs that sponge miRNA-29 family

Long ncRNA H19 is a paternally imprinted gene located on chromosome 11p15.5. Several studies reported the significant increase of lncRNA H19 in various human cancers associated with tumorigenesis and metastasis (104). The lncRNA H19 directly binds to miR-29b-3p and lowers the expression of DNA Methyltransferase 3 Beta (DNMT3B) epigenetic enzyme (105). Interestingly, lncRNA H19 has been reported to be upregulated in

atherosclerotic patients and to cause the increase of acid phosphatase 5 gene (106). The latter encodes a metalloenzyme named tartrate-resistant acid phosphatase (TRAP) that is significantly associated with cancer progression, and it is known to induce endothelial and VSMC proliferation and suppress apoptosis (107).

Although a direct link of RNA H19 and miR-29 family has not been described in CVD, it is possible that their association may modulate atherosclerosis progression.

LncRNAs and circRNAs that sponge miRNA-101

X-inactive specific transcript (XIST) is a lncRNA that regulates cardiac hypertrophy. Significantly upregulated expression of lncRNA XIST was observed in hypertrophic mouse heart, postmyocardial infarction cells, as well as in H9c2 cells treated with phenylephrine (PE) (108). Recently, it was shown that XIST competitively binds to miR-101 and promotes cardiac hypertrophy by enhancing the expression of Toll-like receptor 2 (TLR2) (108).

Further, in gastric and esophageal squamous cell carcinoma, lncRNA XIST modulates epigenetic mechanisms through the regulation of miR-101/EZH2 pathway causing cancer (109, 110).

Genetics of extreme longevity: can this inform new therapy sustaining health-span?

Although prolonged lifespan does not always correspond to health span, extreme longevity is often accompanied by reduced or delayed morbidity (111, 112). This led to the hypothesis that the aging-related decay is neither necessary nor irreversible (111). Genetic association investigation identified several candidate gene variants that segregate with exceptional longevity, which have been revised using a meta-analysis approach (113). Strength and limitations of the most powerful and used tools, such as genome-wide association study (GWAS) and whole-genome sequencing, have been recently reviewed by Giuliani *et al.* (114). The major results of this approach suggest that the genetics of longevity is highly population specific; small-effect alleles, pleiotropy, and the complex allele timing likely play a major role; and a close relationship between genetics of longevity and genetics of age-related diseases (including cardiovascular diseases) do exist. One important aspect in this as in other fields of research is reproducibility of the data. Some loci, such the one at chromosome 5q33.3, failed to show association in a replication analysis (115), while mutations located near *APOE/TOMM40* have been

replicated (116, 117, 118, 119) and confirmed in GWAS at genome-wide significance (115, 120, 121, 122). The e2e2 or e2e3 genotype is associated with significantly increased odds to reach extreme longevity, whereas the *APOEe4* allele is thought to contribute to morbidity and earlier and higher mortality (123).

Translation to therapy of promising genes emerging from GWAS

APOE4-targeted therapeutic approaches that have been developed in animal models of Alzheimer disease and myocardial ischemia and are close to be translated to humans (124). ApoE is a ligand for low-density lipoprotein LDL receptors, plays a role in lipid metabolism, and is pathogenically implicated in cardiovascular diseases. For instance, apoE4 can affect cerebrovascular integrity by several mechanisms, one of which consists of the accumulation of A β in the cerebral vasculature. Potential therapies targeting ApoE comprise of silencing the gene by CRISPR/Cas9 editing, anti-ApoE4 immunotherapy, structure correction or degradation, or use of molecules interacting with the protein or related signaling.

A GWAS performed on an Italian set of long living individuals (LLIs) and controls identified the BPI Fold-Containing Family B member 4 (*BPIFB4*) gene to be associated with lifespan, a result validated on two independent populations from Germany and USA (125). The minor allele rs2070325 is part of a SNP haplotype that codifies for a wild type variant (WT), a longevity-associated variant (LAV), and a rare variant (RV) of *BPIFB4*. The levels of immunoreactive BPIFB4 protein are reportedly higher in plasma and hematopoietic cells of healthy LLIs compared with diseased LLIs or young controls (126, 127). Overexpression of *LAV-BPIFB4* induced the activation of stress response-related heat-shock proteins, improved proteostasis, and increased nitric oxide availability in human cells (125). Studies in experimental models of cardiovascular disease showed that viral-vector mediated transfer of the human *LAV-BPIFB4* gene attenuates hypertension, atherosclerosis, and ischemic disease (125, 128, 129, 130, 131). Preclinical results on *BPIFB4* gene therapy provides compelling experimental evidence for the potential of transferring the healthy features of centenarians to individuals at risk. A major concept emerging from studies using *LAV-BPIFB4* is that gene transfer modified the molecular landscape without reversing it to that of normal controls. This is in keeping with the fact that centenarians experience age-related

diseases but cope better with them, possibly through amplification of unconventional molecular mechanisms able to override the pathological process. Clinical studies confirming safety and efficacy will determine if this novel modality may have a disruptive impact on current therapeutic modalities

Lessons from genetics of restricted lifespan

The Hutchinson–Gilford Progeria Syndrome (HGPS), a fast aging disorder, is characterized by an increased risk of atherosclerosis-induced complications, such as heart attack and stroke (132). The phenotypic effects are brought about by the mutation-induced expression of the progerin protein, a truncated splice variant of the nuclear architectural lamin A (132). This protein plays a role in nuclear structural stability and other nuclear functions such as DNA replication, DNA repair, and chromatin and nuclear pore complex organization. Maturation and stabilization of lamin A requires the cleavage of the farnesylated C-terminus by a zinc metalloprotease. Due to aberrant splicing, the lamin A variant progerin is truncated and remains stuck in its immature form. This leads to delocalization of nuclear envelope proteins, disorganized heterochromatin and nuclear pore complexes, disrupted nuclear morphology, and increased DNA damage and repair. Cells affected by progerin expression have increased nuclear rigidity and are more sensitive to mechanical strain. Phenotypic symptoms of HGPS are especially seen in tissues with high levels of mechanical stress such as bone, skeletal muscle, the heart, and blood vessels (132, 133).

Interestingly, progerin is not only present in the cases of HGPS patients, being also expressed in healthy aging individuals though to a lesser degree. This stands to reason that some of the modalities used to treat HGPS might be replicated or adapted to combat age-associated cardiovascular regenerative decline (134, 135). Progerin activates and dysregulates various downstream of Notch, including *HES1*, *HES5*, and *HEY* (135). It was therefore suggested that using inhibitors of the Notch signaling pathway could be a viable option to combat cardiovascular aging (135, 136). Recovery from an ischemic insult requires the activation of inflammatory cells and vascular cells and timely resolution of the initial sterile inflammation. In a murine model, we showed that targeting the Dll4/Notch signaling could have a relevant impact on coordinating reparative angiogenesis and inflammation under ischemic conditions (137).

More than 70% of UK adults aged 25 and over have low vitamin D levels. This can have an impact on precocious senescence. In fact, it was shown that HGPS cells have reduced expression of vitamin D receptor (VDR), and that reconstituting VDR signaling via $1\alpha,25$ -dihydroxyvitamin D3 treatment alleviates HGPS phenotypes, including nuclear morphological abnormalities, DNA repair defects, and premature senescence (138). Interestingly, vitamin D supplementation delayed epigenetic aging in overweight and obese non-HGPS individuals with suboptimal vitamin D status (139). Therefore, supplementation of this nutrient can be a viable method to improve health span in the general population.

Taking a closer look at the mechanisms by which progerin may be linked to physiological aging has revealed another mode of treatment (140). Progerin mRNA levels and levels of progerin do not increase with age; therefore, the hallmarks of aging are not related to an accumulation of progerin over time (140). Rather, the presence and expression of progerin mRNA in the nucleus leads to the delocalization and accumulation of wild type lamin A at the nuclear rim (140). *In vivo*, targeting the cryptic splice site in the gene with a morpholino oligonucleotide (Exo11) via electroporation of affected cells reversed the aberrant cellular phenotypes (140, 141).

Conclusions

Evidence from the literature highlights the complex molecular machinery involved in the dysregulation of cardiovascular function with aging. Investigation of mechanisms that allow the maintenance of health during aging or inhibit genetic programs of premature senescence could provide novel therapeutic modalities superior to current treatments. Much more research, refinement, and preclinical testing are, however, required before these treatments enter the clinical stage.

Declaration of interest

M C C and P M are Editors of *Vascular Biology*. M C C and P M were not involved in the review or editorial process of this paper, on which they are listed as authors. The other authors have nothing to disclose.

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