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Resveratrol Inhibits Aortic Root Dilatation in the Fbn1$^{C1039G/+}$ Marfan Mouse Model

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Objective—Marfan syndrome (MFS) is a connective tissue disorder caused by mutations in the fibrillin-1 gene. Patients with MFS are at risk of aortic aneurysm formation and dissection. Usually, blood pressure–lowering drugs are used to reduce aortic events; however, this is not sufficient for most patients. In the aorta of smooth muscle cell–specific sirtuin-1–deficient mice, spontaneous aneurysm formation and senescence are observed. Resveratrol is known to enhance sirtuin-1 activity and to reduce senescence, which prompted us to investigate the effectiveness of resveratrol in inhibition of aortic dilatation in the *Fbn1*$^{C1039G/+}$ MFS mouse model.

Approach and Results—Aortic senescence strongly correlates with aortic root dilatation rate in MFS mice. However, although resveratrol inhibits aortic dilatation, it only shows a trend toward reduced aortic senescence. Resveratrol enhances nuclear localization of sirtuin-1 in the vessel wall and, in contrast to losartan, does not affect leukocyte infiltration nor activation of SMAD2 and extracellular signal–regulated kinases 1/2 (ERK1/2). Interestingly, specific sirtuin-1 activation (SRT1720) or inhibition (sirtinol) in MFS mice does not affect aortic root dilatation rate, although senescence is changed. Resveratrol reduces aortic elastin breaks and decreases micro-RNA-29b expression coinciding with enhanced antiapoptotic Bcl-2 expression and decreased number of terminal apoptotic cells. In cultured smooth muscle cells, the resveratrol effect on micro-RNA-29b downregulation is endothelial cell and nuclear factor κB-dependent.

Conclusions—Resveratrol inhibits aortic root dilatation in MFS mice by promoting elastin integrity and smooth muscle cell survival, involving downregulation of the aneurysm-related micro-RNA-29b in the aorta. On the basis of these data, resveratrol holds promise as a novel intervention strategy for patients with MFS. **(*Arterioscler Thromb Vasc Biol*. 2016;36:1618-1626. DOI: 10.1161/ATVBAHA.116.307841.)**

Key Words: aortic aneurysm ■ extracellular matrix ■ Marfan syndrome ■ micro-RNAs ■ resveratrol ■ sirtuin-1

Marfan syndrome (MFS) is an autosomal connective tissue disorder caused by different mutations in the fibrillin-1 gene (*FBN1*) with an incidence of 1 of 5000 individuals.[1] Patients with MFS have extended bones, develop scoliosis and ectopia lentis. Another major clinical problem for patients with MFS is their increased risk to develop aortic aneurysms, and often, lethal dissections. Until now, the most effective treatment to prevent aortic dissections is prophylactic aortic surgery.[2] To evaluate the size and risk of aortic dissection, patients with MFS have their aorta monitored regularly by imaging. Current pharmacological treatment aims at blood pressure regulation using mostly β-blockers and losartan.[3]

An important role in the disease process has been attributed to angiotensin-II receptor type-1 (AT1R) signaling and subsequent overexpression of transforming growth factor-β (TGF-β), leading to canonical SMAD2 and noncanonical extracellular signal–regulated kinases 1/2 (ERK1/2) phosphorylation. Blockade of AT1R by losartan has a beneficial effect on aortic dilatation in the *Fbn1*$^{C1039G/+}$ and *Fbn1*$^{mgR/mgR}$ MFS mouse models.[4–6] However, in 3 out of 4 clinical trials in patients with MFS, losartan was not superior to β-blocker therapy.[7–10] Only in the COMPARE trial (Cozaar in Marfan Patients Reduces Aortic Enlargement), we observed a significant effect of losartan when used on top of standard medication,[7] which may be explained by the beneficial effect especially in MFS patients with a haploinsufficient *FBN1* mutation (one third of the patients with MFS).[11] This patient category was more sensitive to losartan treatment when compared with most patients with a

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Nonstandard Abbreviations and Acronyms

AT1R	angiotensin-II receptor type-1
ERK1/2	extracellular signal–regulated kinases 1/2
FBN1	fibrillin-1 gene
HUVECs	human umbilical cord endothelial cells
MFS	Marfan syndrome
miR	micro-RNA
NF-κB	nuclear factor κB
pERK1/2	phosphorylated ERK1/2
pSMAD2	phosphorylated SMAD family member-2
SIRT1	sirtuin-1
SMCs	smooth muscle cells
TGF	transforming growth factor
WT	wild-type

dominant-negative *FBN1* mutation (two third). These observations strongly support the hypothesis that besides AT1R signaling, additional pathways are responsible for pathological aortic changes in patients with MFS.

Spontaneous aneurysm development and senescence were observed in a proatherogenic mouse model with sirtuin-1 (SIRT1) deficiency specifically in smooth muscle cells (SMCs),[12] which may suggest that inhibiting senescence is a potential treatment approach to combat aneurysm development. Senescence is a cellular state of discontinued cell division, with a unique (inflammatory) cytokine profile. It can be induced by age and various stressors, such as DNA damage and oxidative stress.[13] Interestingly, cultured porcine abdominal aortic aneurysm SMCs show increased senescence compared with healthy SMCs.[14] In addition, enhanced oxidative stress is found in the aorta of MFS mice[15] as a potential inducer of vascular senescence. Together, these data suggest that senescence could play a role in aortic aneurysm formation. Resveratrol (a polyphenol in skin of red grapes) reduces vascular senescence by inhibiting nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity, thus decreasing oxidative stress, in a SIRT1-dependent fashion.[16] Therefore, we hypothesize that modulation of aneurysm progression in MFS mice may be possible with resveratrol, the SIRT1 agonist SRT1720, and the SIRT1 antagonist sirtinol. In this study, we show that resveratrol decreases aortic root dilatation in the *Fbn1*$^{C1039G/+}$ MFS mice, whereas specific SIRT modulation did not, which reveals a different mechanism of action than anticipated.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Senescence Is Correlated With Aortic Root Dilatation

To study aortic senescence, aortic arches of MFS mice were analyzed for senescence-associated (cytoplasmic) β-galactosidase activity, which is an accepted marker of senescence. Aortic senescence (blue) was mainly observed in the enlarged ascending aorta of MFS mice and seems to originate from the aortic root (Figure 1A). In cryosections of senescence-associated (cytoplasmic) β-galactosidase–stained aorta, the blue senescent cells were localized throughout the vessel wall in endothelial cells, medial SMCs, and adventitial fibroblasts (Figure 1A).

To assess if senescence correlates with aortic root dilatation rate, the aortic arches were incubated with fluorescent fluorescein di-β-D-galactopyranoside substrate to quantify senescence. We calculated the aortic root dilatation rate from the 2- and 4-month aortic root diameters as determined by quantitative morphometry. A strong positive correlation was found between the aortic root dilatation rate and aortic senescence (r=0.772; P<0.001; Figure 1B), suggesting that senescence may be involved in aneurysm development.

Resveratrol Reduces Aortic Root Dilatation

To counteract senescence, MFS mice were treated with resveratrol for 2 months starting at 2 months of age. Interestingly, 2-month-old MFS mice already had a larger aortic root diameter (tissue sections) than wild-type (WT) mice of the same age (0.62±0.031 versus 0.55±0.035 mm; P=0.003). Thus, drug treatment aims at controlling disease progression, not prevention. At 4 months of age, MFS mice showed the expected increase in aortic root dilatation rate when compared with WT mice (Figure 2A; P=0.004), which was significantly reduced by losartan (positive control; P=0.018). This is in line with previous findings in MFS mice, as measured by ultrasound.[4,17,18] Moreover, resveratrol treatment significantly reduced the aortic root dilatation rate (Figure 2A; P<0.001), even significantly better than losartan (P=0.007), suppressing it to WT level.

To relate our method of analysis of aortic root sections to in vivo diameters, we compared the histological diameters to the diameters obtained by ultrasound imaging in WT and MFS mice of different ages. Aortic root dimensions correlated well with ultrasound measurements in the same mice (Figure I in the online-only Data Supplement; r=0.709; P<0.001) although the diameter was 1.8-fold smaller in tissue sections, probably because of lack of arterial pressure and shrinkage on processing. Therefore, the absolute diameters obtained with ultrasound are considered as actual width. However, to monitor aortic dilatation and treatment efficacy, quantification via histology is reliable.

Resveratrol Has a Positive Effect on Aortic Wall Pathology

Given that resveratrol can activate SIRT1, we studied nuclear localization of SIRT1 as a measure of activation in the aortic root. Nuclear SIRT1 was similar in WT, MFS placebo, and MFS losartan-treated mice (Figure 2A); however, resveratrol treatment significantly increased the number of SIRT1-positive nuclei (Figure 2A and 2D; P<0.001). These data suggest that enhanced SIRT1 protects against aortic damage as resveratrol treatment diminished the aortic root dilatation rate.

Quantitative analysis of senescence in the aortic arch showed a significant increase in MFS placebo mice when compared with WT mice (Figure 2B; P=0.011). Interestingly, not only resveratrol- but also losartan-treated MFS mice showed a trend toward decreased senescence when compared with

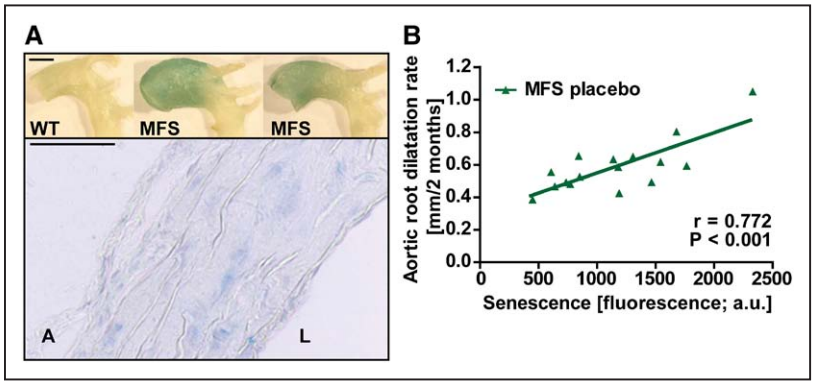

Figure 1. Aortic root dilatation correlates with senescence. **A**, Representative macroscopic photographs of senescence-associated (cytoplasmic) β-galactosidase–stained aortic arches from wild-type (WT) and Marfan syndrome (MFS) mice (**top**; ×6.5; scale bar, 1 mm) and a microscopic photograph of a cross section of an MFS mouse ascending aorta (**bottom**; ×200; scale bar, 50 μm). Blue cells are senescent cells. **B**, Correlation between aortic root dilatation rate (difference between 2- and 4-month diameters) and senescence as determined with a fluorescein di-β-D-galactopyranoside substrate (FDG) in arbitrary units (a.u.). A positive association between aortic dilatation rate and senescence is observed, with a correlation coefficient (*r*) of 0.772 (*P*<0.001). A indicates adventitia; and L, lumen.

the MFS placebo group (Figure 2B; *P*=0.087 and *P*=0.113, respectively).

Total medial area is known to increase in MFS mice (Figure 2B; *P*=0.002) because of excessive extracellular matrix production at sites of elastin loss, which is a sign of disease severity.[4] Compared with MFS placebo mice, the medial area was significantly smaller in resveratrol-treated mice (*P*=0.013), suggesting that disproportionate extracellular matrix production was reduced by resveratrol, whereas losartan did not improve medial thickness significantly.

A characteristic feature of MFS is elastic lamina fragmentation in the aorta because these lamellae consist of elastin and fibrillin-1[19,20]; of which, the latter protein is defective in MFS.[21] To determine if resveratrol has an effect

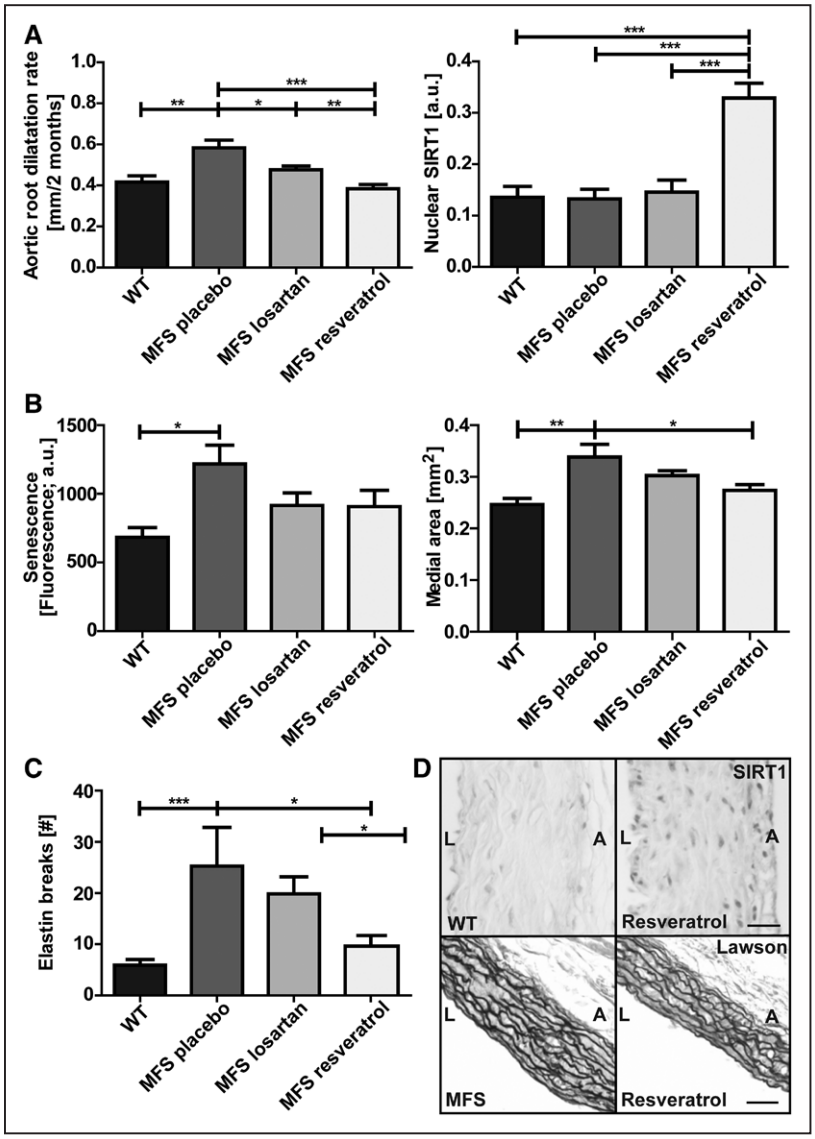

Figure 2. Resveratrol-mediated inhibition of aortic root dilatation and histological characteristics. **A**, **Left**, Aortic root dilatation rate is increased in Marfan syndrome (MFS) placebo mice compared with wild-type (WT) mice. Resveratrol and losartan effectively inhibit the aortic root dilatation rate. **Right**, Nuclear sirtuin-1 (SIRT1) staining is increased in resveratrol-treated mice, compared with other mouse groups. **B**, **Left**, Senescence is increased in MFS mice when compared with WT mice. Resveratrol and losartan-treated MFS mice do not show a significant increase in senescence compared with WT mice. **Right**, Medial area in aortic sections (area between internal and external elastic laminae in mm^2) is significantly larger in MFS mice when compared with WT mice. In resveratrol-treated mice, the medial area is normalized. **C**, MFS placebo mice show more elastin breaks when compared with WT mice. Resveratrol treatment results in a decrease of elastin breaks. **D**, Representative photographs of SIRT1 staining in aortic sections of WT and resveratrol-treated MFS mice (**top**; ×400; scale bar, 100 μm), indicating more nuclear SIRT1 staining in resveratrol-treated mice. Representative pictures of elastin fibers, visualized by Lawson staining from MFS placebo and resveratrol-treated mice (**bottom**; ×200; scale bar, 50 μm), showing decreased medial area and less elastin breaks. **P*≤0.05, ***P*≤0.01, and ****P*≤0.001. A indicates adventitia; a.u., arbitrary units; and L, lumen.

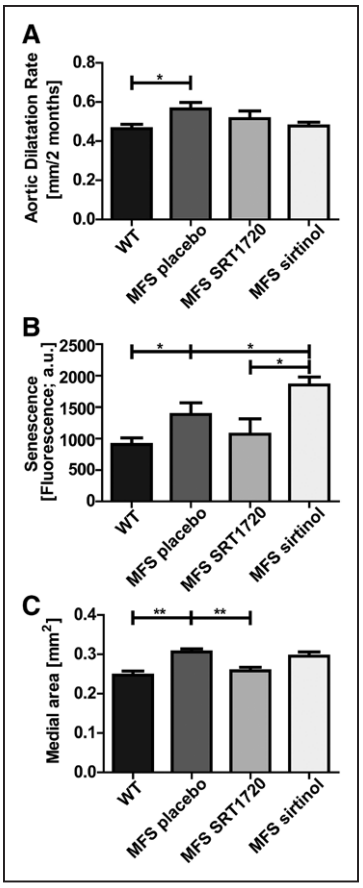

Figure 3. Sirtuin-1 (SIRT1) activation or inhibition does not affect aortic root dilatation, yet influences senescence and medial area in Marfan syndrome (MFS) mice. **A**, The aortic root dilatation rate is increased in MFS placebo mice when compared with wild-type (WT) mice. SRT1720 and sirtinol treatment both have no significant effect. **B**, Increased senescence is observed in MFS mice, whereas STR1720 treatment does not show a significant difference with WT mice. The SIRT1 inhibitor sirtinol increases senescence even more when compared with MFS placebo mice. **C**, In MFS placebo mice, the medial area is increased when compared with WT mice. Treatment with the SRT1720 decreases the medial area when compared with MFS placebo. a.u. indicates arbitrary units.

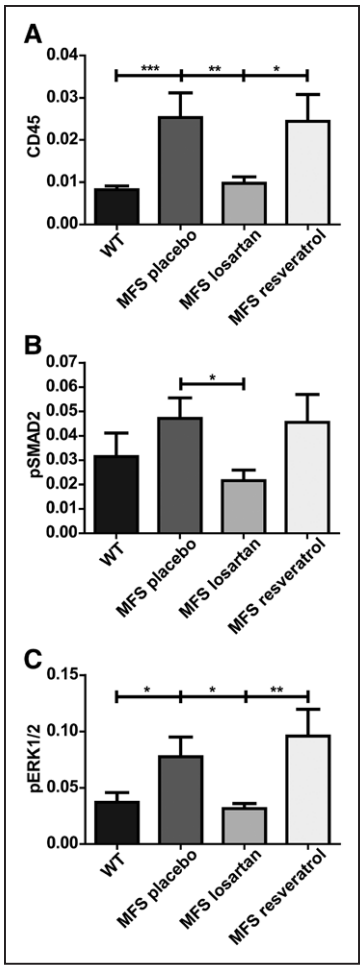

Figure 4. Resveratrol does not affect leukocyte infiltration or transforming growth factor-β (TGF-β)–mediated signaling. **A**, Morphometric analyses of CD45-stained aortic sections, to assess leukocyte accumulation in the aortic root, reveal increased inflammation in Marfan syndrome (MFS) when compared with wild-type (WT) mice. Losartan reduces the number of CD45-positive cells, whereas resveratrol does not. **B**, Nuclear phosphorylated SMAD family member-2 (pSMAD2) staining is increased in MFS and is reduced by losartan but not by resveratrol. **C**, Extracellular phosphorylated signal–regulated kinases 1/2 (pERK1/2) is increased when compared with WT mice, which is again only reduced by losartan.

on the integrity of these elastin fibers, the number of elastin breaks was quantified in aortic root sections. Although elastin breaks were high in MFS placebo (P=0.001), resveratrol treatment showed a decrease, even compared with losartan-treated MFS mice (Figure 2C and 2D; P=0.024 and P=0.021, respectively).

Resveratrol treatment showed the expected[16] decrease in weight gain (Figure II in the online-only Data Supplement; P=0.024), indicating that resveratrol was metabolized.

SIRT1 Modulation Does Not Alter Aortic Root Dilatation

To delineate the mechanism by which resveratrol inhibits aortic root dilatation in MFS mice, we performed an experiment with the SIRT1 activator SRT1720 and the SIRT1 inhibitor sirtinol,[22,23] as we observed increased nuclear SIRT1 staining in resveratrol-treated MFS mice. SRT1720 activates SIRT1 indirectly via intracellular increase of nicotinamide adenine dinucleotide (NAD).[22] This mechanism of SIRT1 induction is similar to that of resveratrol.[22] Sirtinol is known to enhance senescence through deactivation of SIRT1 and is used in cancer research to induce premature aging of cancer cells to limit tumor growth.[24] Unexpectedly, the aortic root dilatation rate was not changed significantly by SRT1720 or sirtinol (Figure 3A). Because the SIRT1 agonist and antagonist do not show opposing or significant effects on aortic root dilatation, we conclude that the positive resveratrol effect is not an SIRT1-dependent process.

To investigate treatment effectiveness, we measured senescence, medial area, and weight gain. On analysis of aortic senescence, we observed increased senescence by sirtinol (Figure 3B; P=0.050). Interestingly, the increase in senescence did not result in detrimental aortic growth. Significant reduction in medial area and weight gain was

measured upon SRT1720 treatment (Figure 3C; Figure III in the online-only Data Supplement; P=0.003 and P<0.001, respectively), which is similar to that shown for resveratrol. In conclusion, SRT1720 and sirtinol were provided in an effective dose; yet, they did not influence aortic root dilatation rate significantly.

Resveratrol Does Not Change Inflammation, TGF-β Signaling, and Cardiac Phenotype

Given that SIRT1 is not the mechanism whereby resveratrol inhibits aortic dilatation, we considered whether resveratrol reduces inflammation or TGF-β signaling. Because resveratrol is known to reduce inflammation[25] and inflammation is observed in MFS aortic tissues,[26,27] we therefore quantified aortic leukocytes (CD45). In addition, we quantified the amount of nuclear phosphorylated SMAD family member-2 (pSMAD2) and mitogen-activated protein kinase ERK1/2 (pERK1/2), representing the canonical and noncanonical TGF-β pathway[17,18] because TGF-β–mediated signaling is considered a typical feature of MFS. Leukocyte and pERK1/2 positive area were significantly increased in MFS placebo mice (Figure 4A and 4C; P<0.001 and P=0.015). Losartan effectively decreased the inflammatory state and nuclear pSMAD2 and pERK1/2 in the aortic root, as expected[4] (Figure 4A through 4C; P=0.005, P=0.039, and P=0.013), whereas the resveratrol-treated mice revealed a similar level of inflammation and pSMAD2 and ERK1/2 activation as the placebo MFS mice.

Because MFS may also affect the heart, the effect of resveratrol on cardiac stress markers atrial natriuretic peptide and brain natriuretic peptide was analyzed. No cardiac stress could be detected by in situ hybridization for atrial natriuretic peptide mRNA or serum brain natriuretic peptide in these relatively young MFS mice (Figure IV in the online-only Data Supplement).

These data thus demonstrate that resveratrol does not affect accumulation of inflammatory cells in the aortic root, leaves TGF-β signaling intact, and does not affect the cardiac phenotype, yet does reduce aortic root dilatation.

Resveratrol Affects Aneurysm-Related Micro-RNAs

Many relevant micro-RNAs (miRs) have been described for aortic aneurysm formation[28]; therefore, we investigated the effect of resveratrol on these miRs (Figure 5A). MiR-21a and miR-195 were upregulated after resveratrol treatment (P=0.014 and P=0.016), whereas miR-23b, miR-24, and miR-712 were unaffected. Interestingly, the miR-29 family members *a* to *c* were all downregulated by resveratrol (Figure 5A; P=0.030, P=0.038, and P=0.030, respectively). MiR-29b downregulation has actually been successful in preventing aneurysm formation in different murine abdominal and thoracic (MFS) aortic aneurysm models.[5,29–31] MiR-29b has been reported to increase SMC apoptosis; therefore, we measured expression of antiapoptotic factors Bcl-2 and Mcl-1.[5] Clearly, these prosurvival genes were more abundant after resveratrol treatment when compared with WT and MFS placebo mice (Figure 5C; P=0.010, P=0.041, P=0.002, and P=0.002, respectively), which may contribute to increased aortic integrity. Subsequently, apoptosis was investigated by performing a terminal deoxynucleotidyl transferase dUTP nick-end labeling staining. Resveratrol-treated MFS mice showed less apoptotic cells in the aortic root (Figure 5C; right; P=0.016), indicating reduced SMC loss.

Endothelial Cell–Dependent Effect of Resveratrol on SMC *miR-29b* Expression

To further delineate the mechanism whereby resveratrol modulates miR-29b expression, we cultured aortic mouse

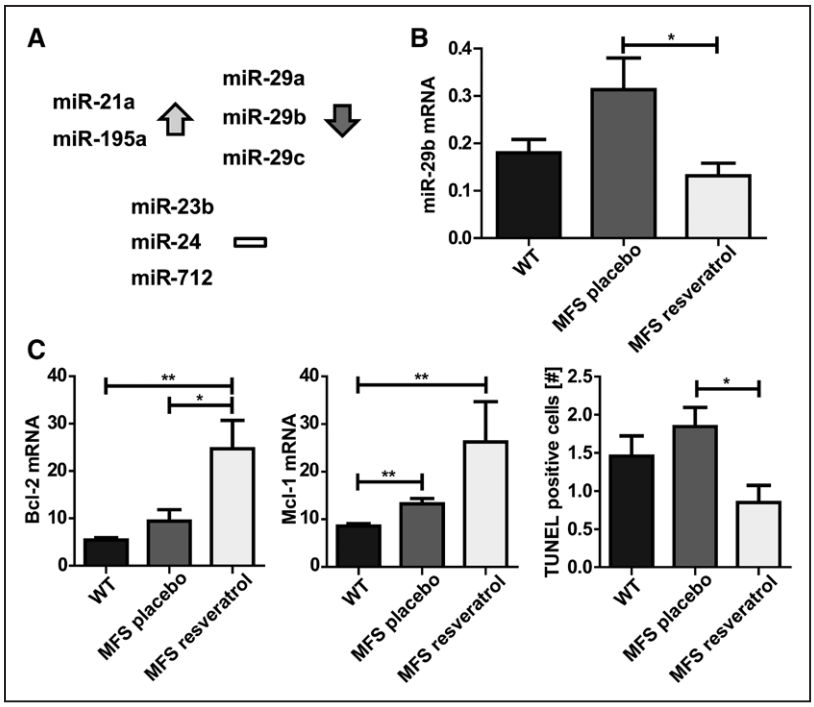

Figure 5. Resveratrol changes mRNA expression of micro-RNAs (miRs). **A**, Aneurysm-related miR expression profile indicated as upregulated (upward arrow), downregulated (downward arrow), or not changed (rectangle) by resveratrol treatment in ascending aorta samples. **B**, MiR-29b expression is decreased in the ascending aorta of resveratrol-treated mice when compared with Marfan syndrome (MFS) placebo mice. **C**, Resveratrol treatment upregulates Bcl-2 and Mcl-1 mRNA expression and reduces the number of terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL)–positive cells in the ascending aorta. WT indicates wild-type.

SMCs with resveratrol, as it is the major cell type in the vessel wall. However, no difference in miR-29b expression was observed (Figure VA in the online-only Data Supplement). Hereafter, human umbilical cord endothelial cells (HUVECs) were cultured with resveratrol as these cells communicate with SMCs and are dysfunctional in MFS.[32–35] Again, we did not observe a difference in expression of miR-29b upon direct resveratrol stimulation (Figure VB in the online-only Data Supplement). However, resveratrol-treated HUVECs expressed increased shear stress–responsive transcription factor KLF2, as also observed by others,[36,37] which represents an improved endothelial phenotype (Figure VC in the online-only Data Supplement; P=0.012). Subsequently, conditioned medium derived from resveratrol-treated HUVECs, given to SMCs (Figure 6A), did downregulate miR-29b and upregulate Bcl-2 expression in SMCs, similar to those in the resveratrol-treated mouse aortae (Figure 6A; P=0.011 and P=0.025, respectively). To delineate the role of KLF2 in SMC miR-29b expression, lentiviral overexpression of KLF2 in HUVECs was performed (Figure VIA in the online-only Data Supplement; P=0.044), and hereafter, SMCs were stimulated with the conditioned medium. No decrease of miR-29b or increase in Bcl-2 mRNA could be observed (Figure VIB and VIC), indicating no KLF2-dependent regulation of miR-29b.

To study apoptosis, SMCs were stimulated with HUVEC-conditioned medium +/− apoptosis-inducer staurosporin. SMCs showed a decrease in apoptotic cells when HUVECs were incubated with resveratrol (Figure 6A; ***P<0.001 and **P=0.004).

To investigate if nuclear factor κB (NF-κB) could be involved in the regulation of miR-29b expression, NF-κB activity was measured. NF-κB activity was downregulated in HUVECs and SMCs treated with resveratrol (Figure 6B; P<0.001 and P=0.05), as expected.[38] However, the effect of resveratrol-stimulated HUVEC-conditioned medium on SMCs was no longer significantly reduced (Figure 6B).

Pooled serum from resveratrol-treated mice also downregulated miR-29b expression in SMCs (Figure 6C; P=0.010), illustrating the indirect effect of resveratrol on SMCs via (among others) endothelial cells. NF-κB inhibitor Ikka is upregulated in SMCs stimulated with MFS placebo serum, whereas this effect was no longer seen in SMCs stimulated with resveratrol-treated mouse serum (Figure 6C; P=0.049). Typical downstream genes of NF-κB, cytokine Il6 and chemokine Il8, were downregulated in SMCs with MFS placebo mouse serum (Figure 6C, bottom; P=0.031, P=0.005, P<0.001, and P=0.005) and normalized with MFS resveratrol mouse serum, indicating reduced NF-κB activity in MFS placebo mice, which is rescued after resveratrol treatment. This suggests that enhanced NF-κB activity in SMCs may be responsible for the reduction in miR-29b, as shown before.[5]

Our data demonstrate that resveratrol has a protective effect on elastin integrity and SMC survival, presumably by increasing SMC NF-κB signaling and thereby reducing miR-29b expression, which protects against aortic dilatation (Figure 6D).

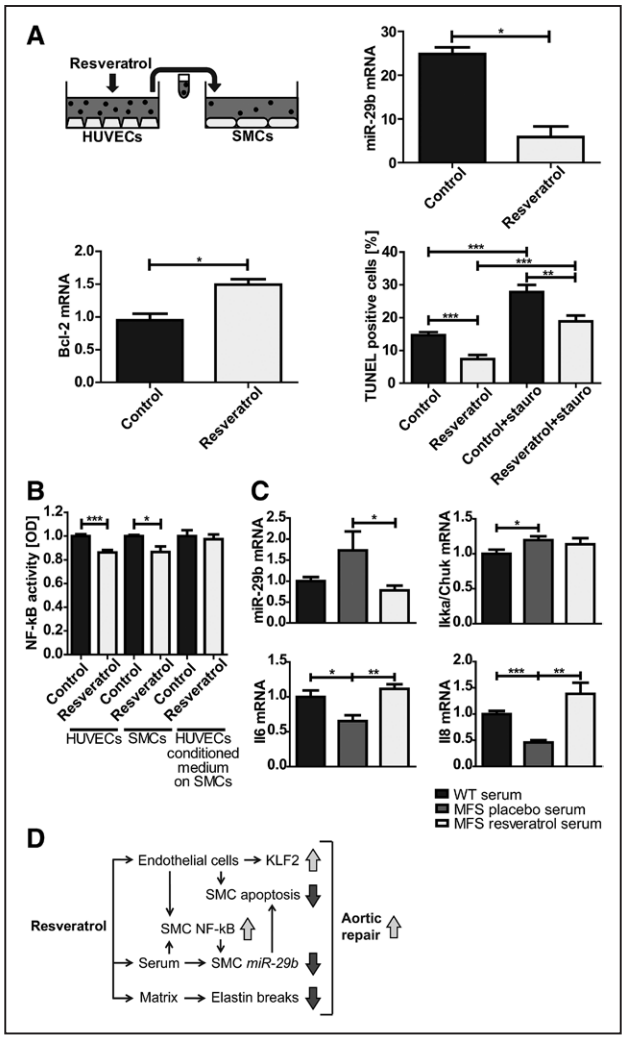

Figure 6. Resveratrol-mediated regulation of micro-RNA-29b (miR-29b) in the aorta. **A**, Scheme of experimental set-up; smooth muscle cells (SMCs) are stimulated with conditioned medium of human umbilical cord endothelial cells (HUVECs), which were treated with/without resveratrol. Medium of resveratrol-treated HUVECs inhibits miR-29b and upregulates Bcl-2 expression in cultured SMCs compared with medium of control HUVECs. The number of terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL)–positive cells is decreased by resveratrol-treated HUVEC medium, also after staurosporin (stauro) incubation. **B**, Nuclear factor κB (NF-κB) activity is decreased in HUVECs and SMCs, which are treated with resveratrol directly. In SMCs, treated with HUVEC-conditioned medium, no difference can be observed with/without resveratrol. **C**, SMCs stimulated with serum from wild-type (WT), Marfan syndrome (MFS) placebo, and resveratrol-treated MFS mice show an increase in miR-29b expression with MFS placebo serum, which is inhibited with MFS resveratrol serum. NF-κB inhibitor Ikka mRNA expression is increased with MFS serum, which is no longer significant with MFS resveratrol serum. Hallmark NF-κB downstream genes Il6 and Il8 are decreased in expression with MFS serum and normalized with MFS resveratrol serum. **D**, Resveratrol downregulates miR-29b in SMCs in an endothelial cell–dependent manner, probably via enhancing NF-κB signaling, resulting in decreased SMC apoptosis. This indirect effect could be mimicked when using serum from the different mouse groups. In addition, resveratrol improves the extracellular matrix integrity by decreasing the number of elastin breaks. Collectively, this leads to enhanced aortic repair. OD indicates optical density.

Discussion

In this study, we demonstrate a positive correlation between aortic senescence and aortic root dilatation. Treatment of *Fbn1*$^{C1039G/+}$ MFS mice with losartan and resveratrol inhibited aortic dilatation, with resveratrol having a more pronounced effect than losartan. Both compounds reduced aortic senescence such that it was not significantly different from the WT mice. Losartan treatment diminished vascular inflammation and pSMAD2 and pERK1/2 signaling, whereas resveratrol increased SIRT1 activation and reduced medial thickening, and elastin breaks. Yet, direct SIRT1 activation or inhibition did not affect aortic root dilatation, indicating that the beneficial effect of resveratrol is SIRT1 and senescence independent. Resveratrol did attenuate miR-29b expression in vivo and in vitro in SMCs in an indirect, endothelial cell–dependent manner. Interestingly, losartan has also been reported to reduce miR-29b, which was TGF-β dependent.[5] Given that TGF-β signaling (pSMAD2 and pERK1/2) remained unaltered in response to resveratrol, we conclude that resveratrol reduces miR-29b not via affecting TGF-β, yet via increasing NF-κB activity.[5]

In an abdominal aortic aneurysm model in rats, resveratrol effectively inhibited aortic dilatation by counteracting the inflammatory response.[25] We observed that losartan reduces vascular inflammation in MFS mice; however, resveratrol did not. This finding indicates that inflammation per se does not need to be reduced to inhibit aortic root dilatation. In line with these observations, we demonstrated that anti-inflammatory medication diminishes vascular inflammation, yet it did not reduce aortic root dilatation.[39] One may speculate that not the number of inflammatory cells is relevant but that the type of inflammatory cells in the vasculature is decisive on aortic growth outcome.

Interestingly, it has been described that resveratrol can suppress the expression of AT1R and thereby the detrimental pathways downstream of AT1R,[40] such as increased senescence.[16] However, if this would be the primary mechanism of action in our study, we would expect a similar outcome between the AT1R blocker losartan and resveratrol on vascular inflammation and phosphorylation of SMAD2 and ERK1/2, which we did not observe.

Excessive oxidative stress is detrimental for the vessel wall[41] and is observed in the *Fbn1*$^{C1039G/+}$ MFS mouse model.[15] Resveratrol was shown to inhibit aortic dilatation in the oxidative stress–induced $CaCl_2$ aneurysm mouse model by attenuation of inflammation, oxidative stress, and matrix degradation.[42] This is further illustrated in rat aortic tissue, where senescence is reduced by an SIRT1-dependent decrease in oxidative stress.[16] In the current study, we show a significant increase in nuclear SIRT1 and a modest decrease in aortic senescence after resveratrol. However, modulation of SIRT1 activity with SRT1720 or sirtinol did change senescence, yet did not change aortic root dilatation and thus seems insufficient as a drug target in MFS.

We demonstrated that resveratrol influenced aortic repair, indicated by decreased elastin degradation, increased cell survival (Bcl-2/Mcl-1/less apoptotic cells), and enhanced NF-κB signaling. These characteristics fit with the described features of inhibition of miR-29b in MFS mice[5] and thus may be considered as the working mechanism of resveratrol in MFS mice. Of interest, the increase in miR-21a and miR-195 after resveratrol may also contribute to the protective aortic phenotype,[29,43] but this requires further investigation in MFS.

In conclusion, resveratrol has a beneficial effect on the vasculature, resulting in improved elastin integrity and cell survival by downregulating miR-29b expression. With the knowledge that inhibition of miR-29b is effective in MFS mice,[5,29–31] it now becomes feasible to apply resveratrol as a novel treatment strategy.

In patients with MFS, blood pressure regulation is still the only pharmacological treatment available.[3] Here, we show that resveratrol is effective at inhibiting the aortic root dilatation rate in *Fbn1*$^{C1039G/+}$ MFS mice, affecting a mechanism different from AT1R or TGF-β signaling. Several resveratrol trials have been performed in humans, mostly in diabetic and obese men.[44,45] Positive effects of resveratrol were reported on reduced systolic blood pressure and body fat, affecting lipid profiles, inflammation markers, and glucose metabolism.[44] Taken together, no harmful effects were reported in these human studies, supporting the use of resveratrol as a potential drug candidate to treat patients with MFS.

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Disclosures

None.

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Highlights

- Resveratrol inhibits aortic root dilatation in the Marfan mouse (*Fbn1*$^{C1039G/+}$).
- The number of elastin breaks in the aortic wall is reduced by resveratrol.
- Micro-RNA-29b expression is downregulated by resveratrol.
- Resveratrol upregulates antiapoptotic micro-RNA-29b target Bcl-2 and decreases the number of apoptotic cells.
- Nuclear factor κB signaling is induced in smooth muscle cells by resveratrol-treated endothelial cell medium and mouse serum.