EndMT

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Endothelial mesenchymal transition (EndMT) is a complex biological process in which endothelial cells lose their specific markers, such as vascular endothelial cadherin (VE-cadherin), and acquire a mesenchymal or myofibroblastic phenotype, expressing specific products, such as α-smooth muscle actin (α-SMA) and type I collagen.

Keywords: endothelial mesenchymal transition ; cardiovascular diseases ; gasotransmitter ; fibroblast phenotype ; TGF-β

1. Introduction

Endothelial mesenchymal transition (EndMT) is a complex biological process in which endothelial cells lose their specific markers, such as vascular endothelial cadherin (VE-cadherin), and acquire a mesenchymal or myofibroblastic phenotype, expressing specific products, such as α-smooth muscle actin (α-SMA). From a histological point of view, during EndMT, the integrity of the endothelium is disrupted, manifesting a migratory, invasive and proliferative phenotype. Vascular endothelium can be viewed as a specialized form of epithelial tissue. Consistently, EndMT shares many molecular mechanisms with the best-known epithelial mesenchymal transition (EMT), a physiological reversible process, first described in the 1960s in chick embryos by Hay and today known to be required for normal embryonic patterning and organs' formation [1].

2. EndMT: Implications in Cardiovascular Fibrosis

Fibrosis is a complex process, in which tissue repair after damage becomes excessive and out of control, resulting in excessive formation of fibrous connective tissue. It can affect any tissue and cause organ dysfunction in different pathologies, such as heart disease, interstitial lung disease, liver cirrhosis, progressive systemic sclerosis and diabetic nephropathy [2].

Deposition of fibrotic scar tissue in the heart is typical after myocardial infarction in the post-ischemic stage, and in vessels of hypertensive subjects, it represents the way through which atherosclerosis develops. Other conditions can also induce and promote fibrotic processes in heart tissue, such as hypertensive cardiomyopathy, diabetic hypertrophic cardiomyopathy, idiopathic dilated cardiomyopathy and also physiological ageing [3][4].

Vascular fibrosis is associated with many diseases and their pathological progression, including atherosclerosis, which is one of the primary causes of the development of cardiovascular diseases. Indeed, atherosclerosis is characterized by accumulation of ECM proteins, primarily collagen and fibronectin, in the vascular media, contributing to structural vascular remodeling, proliferation of vascular smooth muscle cells and inhibition of matrix degradation, and is responsible for the thickness of the wall of arteries and formation of plaques [5]. Endothelial dysfunction is a critical event in the development of atherosclerosis; indeed, in physiological conditions, the endothelium is a monolayer squamous epithelium located in the luminal surface of the blood vessels and is a major regulator of vascular homeostasis. Proinflammatory cytokines contribute to increase the atherosclerotic region, through arterial plaque formation and additional cell apoptosis, leading to lipids's expulsion into adjacent plaque areas [6].

Interestingly, plaque formation during atherosclerosis has been associated with the accumulation of mesenchymal cells in the arterial intima, deriving from transition of endothelial cells. It is noteworthy that, in plaques of murine atherogenic models and in humans, endothelial and mesenchymal markers have been found, demonstrating the presence of a transitioning state of EndMT. Recently, it has been suggested that mesenchymal cells advance the progression of atherosclerosis, since they secrete proinflammatory molecules and synthetize ECM proteins and metalloproteases (MMPs), which facilitate plaque build-up and regulate plaque stability [7]. Interestingly, in endothelial cells, TGF-β predominantly binds ALK-1, promoting smad1 and smad5, and contributes to regulation of vascular homeostasis, cell proliferation and angiogenesis; in contrast, when TGF-β binds ALK5, it induces the activation of smad2 and smad3, which inhibit cell proliferation and facilitate EndMT [8][9].

Angiogenesis is a vascular remodeling process, promoted by VEGF and other factors that stimulate proliferation and migration of vascular endothelial cells to form new blood vessels. As previously described, TGF-β, beyond its role in physiological [10] and degenerative processes, has been recognized as one of the key factors inducing the expression of VEGF in endothelial cells via the ALK-5 pathway, thus creating a pro-angiogenic milieu for the tumor or for the EndMT [11]. However, FGF signaling is involved in angiogenesis modulation and in the maintenance of vascular integrity and endothelial function. In this regard, recent studies show that FGF inhibition at the endothelial level is associated with TGF-β activation [10].

Very interestingly, EndMT of the specialized brain endothelial cells, forming the blood–brain barrier, has been described in different conditions of neuroinflammation and neurodegeneration, typical of central nervous system pathologies, such as multiple sclerosis [12].

Cardiac fibrosis is a hallmark of the heart's pathological remodeling response to mechanical or biochemical stress. Indeed, cardiac fibrosis is characterized by increased stiffness of the heart valves, due to excessive proliferation of cardiac fibroblasts, accumulation of myofibroblasts and to deposition of ECM in the cardiac muscle. Nevertheless, unlike cardiomyocytes, cardiac fibroblasts are unable to generate an action potential and are not excitable (although they are able to electrically couple with each other and with neighboring cardiomyocytes), causing impaired mechanical–electrical coupling and arrhythmias [13]. On the other hand, impaired tissue function, myocardial dysfunction and ultimately heart failure are also observed [14].

Myocardial infarction is the most common cause of cardiac fibrosis. However, various other conditions, such as hypertension, diabetic hypertrophic cardiomyopathy, idiopathic dilated cardiomyopathy and ageing, may be responsible for reparative mechanisms, addressed to the replacement of dead cardiomyocytes with a collagen-based scar.

Another pathological condition promoting myocardial fibrosis is diabetic cardiomyopathy (DCM); interestingly, about 75% of patients with unexplained idiopathic dilated cardiomyopathy are found to be diabetic [15]. Endoplasmic reticulum stress, mitochondrial dysfunction, sympathetic nervous system activation, excessive oxidative stress, increased inflammation and abnormal coronary microcirculation are characteristic symptoms. These pathophysiological changes result in fibrosis, hypertrophy, diastolic/systolic dysfunction and ultimately systolic heart failure. In particular, cardiac interstitial fibrosis is a major feature of DCM [16], which includes the overproduction and deposition of myocardial interstitial collagen, resulting in myocardial stiffness and cardiac dysfunction.

Another aspect that can greatly affect the cardiovascular system is ageing. Ageing causes a decreased production of NO and a process of generalized endothelial dysfunction at the vascular level. At the cardiac level, consequently, with the progress of ageing, compensatory mechanisms addressed at hypertrophy [17][18][19][20][21] cause an increase in the left ventricular wall, which in the long term worsens the overall cardiac performance, and also triggers fibrotic processes as a final manifestation. Along with the mechanical consequences, cardiac fibrosis can also slow down the propagation of the electrical impulse and affect heart rate, suggesting that ageing can also promote cardiac arrhythmias [6].

hypothesized that EndMT is a process at the basis of cardiac fibrosis. Indeed, they observed that under stimulation, adult human coronary endothelial cells trans-differentiated into fibroblasts, and bone marrow cells could contribute to the cardiac fibroblast population. In fact, the treatment with rhBMP-7, a TGF-β antagonist, was able to preserve the endothelial phenotype and ameliorate the progression of fibrosis in cells as well as in a murine model of heart disease [22], demonstrating that it was responsible for the total pool of cardiac fibroblasts. According to this evidence, Goumans' group confirmed the key role of the growth factor in EndMT in different in vitro and in vivo models [23].

More recently, Kong et al. [24] reported that accumulation and production of collagen and cardiac fibroblasts in human pathological patients were related to the process of EndMT.

Kovacic and colleagues also demonstrated that EndMT plays a main role in cardiac fibrosis that progresses to heart failure under hypertrophic cardiomyopathy, diabetes-induced cardiac fibrosis and genetic deficiency of PAI-1 in aged mice [25].

This is a relevant aspect, since a significant number of non-myocytes are present in the heart. In particular, it has been reported that about 64% of the non-myocyte cell population in the mouse and 54% in the human heart are endothelial cells [26].

3. Contribution of Hydrogen Sulfide in Cardiovascular Fibrosis Associated with EndMT

Hydrogen sulfide (H2S) is an important endogenous mediator with a key role in the cardiovascular system. In this regard, it is noteworthy to underline that Mallat and co-workers have confirmed that inhibition of TGF-β signaling could promote the development of atherosclerosis with decreased collagen content in ApoE−/− mice [27]. Lu and colleagues have demonstrated in a recent study that the use of NaHS, a hydrophilic fast H2S release, could suppress the formation of atherosclerosis via the degradation of TGF-β, as well as the reduction of smad3 phosphorylation. Moreover, such effect is specific for H2S, since CSE inhibitors, such as propargylglycine, can conversely accelerate the atherosclerosis development [28].

Interestingly, CSE-knockout mice fed an atherogenic diet exhibited severe atherosclerosis, suggesting that the CSE/H2S pathway is somehow crucial to limit atherosclerosis development [29][30], which is also dependent on activation of inflammatory macrophages [31].

However, the mechanisms underlying the effects of H2S in the suppression of fibrosis are different and involve diverse pathways, and thus have to be found in its pleiotropic actions [32]. Although vasorelaxation can induce beneficial action over fibrosis per se, the inhibitory activity of H2S on ACE also abrogates vascular fibrosis associated with AngII proliferative pathways. Another alternative mechanism involving H2S signaling in the control of vascular fibrosis is associated with its anti-inflammatory properties, since H2S shows beneficial effects in cardiovascular remodeling through suppression of CD11b-positive leukocytes' migration [33]. Indeed, H2S shows protective effects in hypertensive cardiovascular disease and this effect is mediated by the eNOS/NO axis [34][35].

In addition, the endogenous CSE/H2S system has been shown to protect against the formation of UAAS via an alternative pathway involving the activation of protein kinase CbII (CPKCbII)/Akt signaling [36]. Thus, it should not be surprising that H2S biosynthesis as well as CSE expression drop down in conditions of vascular calcification [37] or following the development of neointimal hyperplasia associated with induction of balloon injury. Again, this effect is reversed by H2S treatment [38].

HHcy leads to vascular fibrosis, causing several disease conditions, including peripheral and cerebrovascular coronary occlusion as well as venous thromboembolism [39][40][41]. The interplay between Hcy and H2S has been shown by Sen and colleagues as they demonstrated that activation of both MMP-2 and MMP-9 by HHcy was controlled following H2S supplementation [42]. The accumulating evidence indicating H2S as a crucial controller of vascular function leads to the straightforward conclusion that a lack of it results in pathological conditions affecting vascular tissues, including hypertension and fibrosis. Thus, it is not surprising that SG1002, a donor of H2S, is able to normalize the histological and molecular scores for hypertrophy and fibrosis, leading to a decrease in collagen accumulation in HHcy mice and, consequently, preventing vascular fibrosis [43].

Indeed, Hcy induced the activation of MMP-9 together with the synthesis of collagen in endothelial cells, and this mechanism is driven by AT1-receptor activation by AngII [44]. Therefore, when an HHcy condition occurs, a reduction in H2S biosynthesis is observed, and this can undermine its ability to inhibit ACE, resulting in increased AngII-dependent profibrotic stimulation [45]. This aspect has also been investigated in pulmonary vasculature, where administration of NaHS is able to decrease the mean pulmonary arterial pressure and to inhibit smooth muscle cells' proliferation in the pulmonary artery wall [46]. In addition, administration of exogenous H2S also decreases the expression of collagen I and III in the pulmonary arteries, again suggesting that H2S plays an important role in the development of the fibrotic process and vascular structural remodeling, thus controlling homeostasis in pulmonary arterial pressure [46].

It is intuitive that the use of therapeutic tools aiming to rescue physiological H2S levels could represent a possible way to tackle the fibrotic process as well as other vascular-based impairments such as hypertension at different levels (Figure 3). Indeed, H2S could modulate activation of the EndMT process as well as interfere with intermediate steps towards fibrosis.

Necrosis of cardiomyocytes in ischemic hearts triggers a strong inflammatory response and promotes interstitial and perivascular fibrosis due to biochemical, geometric and biomechanical changes of the ventricular wall not affected by the lesion [47]. The high production of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) accompanies myocardial damage and hypertrophic tissue remodeling, contributing to the deposition of fibrotic tissue.

Interestingly, a reduction of H2S levels and of CSE expression has been found in post-ischemic hearts [48]. Furthermore, H2S levels have been found to be inversely correlated with the severity of coronary heart disease [49].

Indeed, H2S shows cardioprotective activity against I/R injury and it is considered an important modulator of the ischemic preconditioning process. The mechanisms of action responsible for the effects of H2S are heterogeneous, for instance, protein sulfhydration of mitoKATP and mitochondrial voltage-gated potassium channels (mitoKv7). It is noteworthy that Kv7.4 channels have been recently described in cardiac mitochondria, where they seem to play a cardioprotective role, in inhibition of mast cell degranulation and inflammatory processes, as well as in antioxidant and pro-angiogenic action [50] [51]. H2S seems to also be involved in suppression of pro-fibrotic mechanisms associated with myocardial ischemia through the involvement of different signaling pathways, such as Nrf2 [52][53][54], signaling pathways involving miRNAs [48] [55][56] and mitochondrial protection mechanisms [57][58][59][60][61].

Experimental evidence shows that NaHS and slow-release H2S donors such as AP39, diallyl disulfide (DADS) and GYY4137 are able to reduce oxidative stress and apoptotic mechanisms [62][63][64], mitochondrial permeability transition pore (mPTP) opening [58][59][60], inflammatory responses and cardiomyocyte death [65] and iNOS expression in experimental models of myocardial infarction [66]. NaHS is also able to increase heme oxygenase-1 (HO-1) expression [66], to promote pathways of GSK-3β/β-catenin [67], cGMP-dependent PKG/phospholamban [68] and to promote angiogenesis [69] and autophagy in elderly hearts [70][71].

GYY4137 preserves cardiac function, attenuates adverse remodeling and can exert post-ischemic cardioprotective effects. Indeed, hearts treated with GYY4137 had left ventricular fibrosis significantly lower than untreated hearts after myocardial infarction. Greater blood vessel density was found in the left ventricular scar area of the GYY4137-treated animals compared to all other infarcted groups. Despite preserved left ventricular structure and function, treatment with GYY4137 increased the levels of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) in association with increased cGMP levels, parallel to higher cGMP-type

Daily intraperitoneal administration of GYY4137 for 4 weeks to spontaneously hypertensive rats (SHR) decreased systolic blood pressure and inhibited myocardial fibrosis. This kind of treatment reduced the collagen deposition in the left ventricle, the ratio of perivascular collagen area vs. lumen area in perivascular regions and the concentration of hydroxyproline, collagen I and III mRNA expression and cross-linked collagen. GYY4137 also inhibited AngII-induced neonatal rat cardiac fibroblast proliferation, reduced the number of S-phase fibroblasts, decreased the expression and protein synthesis of collagen I and III mRNA, attenuated oxidative stress and suppressed α-SMA, by modulating the expression of TGF-β1 and the phosphorylation of smad2. These results showed that GYY4137 improved myocardial fibrosis, possibly through a mechanism involving inhibition of oxidative stress, blockade of the TGF-β1/smad2 signaling pathway and decreased expression of α-SMA in cardiac fibroblasts [64].

Even more innovative H2S donors, such as ADT-OH (H2S-aspirin hybrid molecule) or ZYZ-802 (a cysteine derivative), were able to positively intervene in the ischemic lesion by activating the AMPK signaling pathway [60] and reducing the miRNA-30 family [48].

Polhemus and co-workers demonstrated that administration of diallyl trisulfide (DATS), a long-acting H2S-donor organic polysulfide compound present in garlic, could attenuate left ventricular dilatation and dysfunction in a model of pressure-overload heart failure, attenuating the development of perivascular and intermuscular fibrosis and then cardiac hypertrophy [72].

Finally, it is interesting that SG-1002, an orally active H2S donor, is able to positively act in the fibrotic damage following a heart failure condition and in general of myocardial dysfunction, favoring the adiponectin-AMPK signaling pathway [73] and increasing the bioavailability of NO [74].

The metabolic dysregulation characteristic of diabetes, including hyperglycemia, hyperlipidemia and oxidative stress, causes the death of cardiomyocytic cells. The early stages of cardiac remodeling following diabetes are generally asymptomatic, with myocardial changes and damage almost exclusively at the molecular level. In the middle phase of remodeling, progressive hypertrophy of cardiomyocytes and myocardial fibrosis result in a reduced ejection fraction [75].

In patients with diabetes, as well as in rats treated with STZ, downregulation of the gene expression of the enzymes involved in the endogenous biosynthesis of H2S, with consequent reduction of circulating levels of H2S, was frequently found [76][77][78]. Growing evidence suggests that H2S exogenous administration could exert cardioprotective effects in these pathological conditions. There is evidence that the JAK/STAT signaling pathway participates in the protective effects of exogenous H2S against myocardial fibrosis in diabetes mellitus, though other studies point to an involvement of the H2S-forkhead box protein O1 (FoxO1) pathway in the pathogenesis of diabetic cardiomyopathy. This evidence suggests that H2S could attenuate diabetes-induced cardiac fibrosis through the modulation of MMP/TIMP expression, the regulation of TGF-β1 and other possible intracellular pathways, including the regulation of the PKC-ERK1/2/MAPK signaling pathway and the inhibition of inflammatory reactions [79][80][81].

Subsequent studies confirmed the protective effect of H2S against diabetes-induced myocardial fibrosis and demonstrated that it was also associated with the attenuation of autophagy through upregulation of the PI3K/Akt1 signaling pathway [82].

References

1. Trelstad, R.L.; Hay, E.D.; Revel, J.-P. Cell contact during early morphogenesis in the chick embryo. Dev. Biol. 1967, 16, 78–106.
2. Wynn, T.A. Cellular and molecular mechanisms of fibrosis. J. Pathol. A J. Pathol. Soc. Great Br. Irel. 2008, 214, 199–210.
3. Jellis, C.; Martin, J.; Narula, J.; Marwick, T.H. Assessment of nonischemic myocardial fibrosis. J. Am. Coll. Cardiol. 2010, 56, 89–97.
4. Disertori, M.; Masè, M.; Ravelli, F. Myocardial fibrosis predicts ventricular tachyarrhythmias. Trends Cardiovasc. Med. 2017, 27, 363–372.
5. Lan, T.-H.; Huang, X.-Q.; Tan, H.-M. Vascular fibrosis in atherosclerosis. Cardiovasc. Pathol. 2013, 22, 401–407.
6. Testai, L.; Citi, V.; Martelli, A.; Brogi, S.; Calderone, V. Role of hydrogen sulfide in cardiovascular ageing. Pharmacol. Res. 2020, 160, 105125.
7. Souilhol, C.; Harmsen, M.C.; Evans, P.C.; Krenning, G. Endothelial–mesenchymal transition in atherosclerosis. Cardiovasc. Res. 2018, 114, 565–577.
8. Krenning, G.; Barauna, V.G.; Krieger, J.E.; Harmsen, M.C.; Moonen, J.-R.A. Endothelial plasticity: Shifting phenotypes through force feedback. Stem Cells Int. 2016, 2016, 9762959.
9. Goumans, M.-J.; Valdimarsdottir, G.; Itoh, S.; Lebrin, F.; Larsson, J.; Mummery, C.; Karlsson, S.; Ten Dijke, P. Activin receptor-like kinase (ALK) 1 is an antagonistic mediator of lateral TGFβ/ALK5 signaling. Mol. Cell 2003, 12, 817–828.
10. Chen, P.-Y.; Simons, M. Fibroblast growth factor–transforming growth factor beta dialogues, endothelial cell to mesenchymal transition, and atherosclerosis. Curr. Opin. Lipidol. 2018, 29, 397–403.
11. Hao, Y.-M.; Yuan, H.-Q.; Ren, Z.; Qu, S.-L.; Liu, L.-S.; Yin, K.; Fu, M.; Jiang, Z.-S. Endothelial to mesenchymal transition in atherosclerotic vascular remodeling. Clin. Chim. Acta 2019, 490, 34–38.
12. Troletti, C.D.; de Goede, P.; Kamermans, A.; de Vries, H.E. Molecular alterations of the blood–brain barrier under inflammatory conditions: The role of endothelial to mesenchymal transition. Biochim. Biophys. Acta Mol. Basis Dis. 2016, 1862, 452–460.
13. Sacks, D.; Baxter, B.; Campbell, B.C.; Carpenter, J.S.; Cognard, C.; Dippel, D.; Eesa, M.; Fischer, U.; Hausegger, K. Multisociety consensus quality improvement revised consensus statement for endovascular therapy of acute ischemic stroke. Int. J. Stroke 2018, 13, 612–632.
14. Ma, J.; Sanchez-Duffhues, G.; Goumans, M.-J.; Ten Dijke, P. Tgf-β-induced endothelial to mesenchymal transition in disease and tissue engineering. Front. Cell Dev. Biol. 2020, 8, 260.
15. Tarquini, R.; Lazzeri, C.; Pala, L.; Rotella, C.M.; Gensini, G.F. The diabetic cardiomyopathy. Acta Diabetol. 2011, 48, 173–181.
16. Asbun, J.; Villarreal, F.J. The pathogenesis of myocardial fibrosis in the setting of diabetic cardiomyopathy. J. Am. Coll. Cardiol. 2006, 47, 693–700.
17. Stern, S.; Behar, S.; Gottlieb, S. Aging and diseases of the heart. Circulation 2003, 108, e99–e101.
18. Dai, D.-F.; Chen, T.; Johnson, S.C.; Szeto, H.; Rabinovitch, P.S. Cardiac aging: From molecular mechanisms to significance in human health and disease. Antioxid. Redox Signal. 2012, 16, 1492–1526.
19. Antelmi, I.; De Paula, R.S.; Shinzato, A.R.; Peres, C.A.; Mansur, A.J.; Grupi, C.J. Influence of age, gender, body mass index, and functional capacity on heart rate variability in a cohort of subjects without heart disease. Am. J. Cardiol. 2004, 93, 381–385.
20. Lakatta, E.G.; Levy, D. Arterial and cardiac aging: Major shareholders in cardiovascular disease enterprises: Part II: The aging heart in health: Links to heart disease. Circulation 2003, 107, 346–354.
21. Cheitlin, M.D. Cardiovascular physiology—changes with aging. Am. J. Geriatr. Cardiol. 2003, 12, 9–13.
22. Zeisberg, E.M.; Tarnavski, O.; Zeisberg, M.; Dorfman, A.L.; McMullen, J.R.; Gustafsson, E.; Chandraker, A.; Yuan, X.; Pu, W.T.; Roberts, A.B. Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. Nat. Med. 2007, 13, 952–961.

23. Goumans, M.-J.; van Zonneveld, A.J.; ten Dijke, P. Transforming growth factor β–induced endothelial-to-mesenchymal transition: A switch to cardiac fibrosis? Trends Cardiovasc. Med. 2008, 18, 293–298.

24. Kong, P.; Christia, P.; Frangogiannis, N.G. The pathogenesis of cardiac fibrosis. Cell. Mol. Life Sci. 2014, 71, 549–574.

25. Kovacic, J.C.; Mercader, N.; Torres, M.; Boehm, M.; Fuster, V. Epithelial-to-mesenchymal and endothelial-to-mesenchymal transition: From cardiovascular development to disease. Circulation 2012, 125, 1795–1808.

26. Pinto, A.R.; Ilinykh, A.; Ivey, M.J.; Kuwabara, J.T.; D'antoni, M.L.; Debuque, R.; Chandran, A.; Wang, L.; Arora, K.; Rosenthal, N.A. Revisiting cardiac cellular composition. Circ. Res. 2016, 118, 400–409.

27. Mallat, Z.; Gojova, A.; Marchiol-Fournigault, C.; Esposito, B.; Kamaté, C.; Merval, R.; Fradelizi, D.; Tedgui, A. Inhibition of transforming growth factor-β signaling accelerates atherosclerosis and induces an unstable plaque phenotype in mice. Circ. Res. 2001, 89, 930–934.

28. Lu, X.; Wang, S.; Feng, S.; Li, H. CSE/H 2 S system alleviates uremic accelerated atherosclerosis by regulating TGF-β/Smad3 pathway in 5/6 nephrectomy ApoE−/−mice. BMC Nephrol. 2020, 21, 1–9.

29. Mani, S.; Untereiner, A.; Wu, L.; Wang, R. Hydrogen sulfide and the pathogenesis of atherosclerosis. Antioxid. Redox Signal. 2014, 20, 805–817.

30. Mani, S.; Li, H.; Untereiner, A.; Wu, L.; Yang, G.; Austin, R.C.; Dickhout, J.G.; Lhoták, Š.; Meng, Q.H.; Wang, R. Decreased endogenous production of hydrogen sulfide accelerates atherosclerosis. Circulation 2013, 127, 2523–2534.

31. Kavurma, M.M.; Rayner, K.J.; Karunakaran, D. The walking dead: Macrophage inflammation and death in atherosclerosis. Curr. Opin. Lipidol. 2017, 28, 91.

32. Sun, H.-J.; Wu, Z.-Y.; Nie, X.-W.; Bian, J.-S. Role of endothelial dysfunction in cardiovascular diseases: The link between inflammation and hydrogen sulfide. Front. Pharmacol. 2020, 10, 1568.

33. Wu, T.; Li, H.; Wu, B.; Zhang, L.; Wu, S.-w.; Wang, J.-n.; Zhang, Y.-e. Hydrogen sulfide reduces recruitment of CD11b + DG Gr-1 + DG cells in mice with myocardial infarction. Cell Transplant. 2017, 26, 753–764.

34. Testai, L.; D'Antongiovanni, V.; Piano, I.; Martelli, A.; Citi, V.; Duranti, E.; Virdis, A.; Blandizzi, C.; Gargini, C.; Breschi, M.C. Different patterns of H2S/NO activity and cross-talk in the control of the coronary vascular bed under normotensive or hypertensive conditions. Nitric Oxide 2015, 47, 25–33.

35. Jin, S.; Teng, X.; Xiao, L.; Xue, H.; Guo, Q.; Duan, X.; Chen, Y.; Wu, Y. Hydrogen sulfide ameliorated L-NAME-induced hypertensive heart disease by the Akt/eNOS/NO pathway. Exp. Biol. Med. 2017, 242, 1831–1841.

36. Zhang, H.; Bai, Z.; Zhu, L.; Liang, Y.; Fan, X.; Li, J.; Wen, H.; Shi, T.; Zhao, Q.; Wang, Z. Hydrogen sulfide donors: Therapeutic potential in anti-atherosclerosis. Eur. J. Med. Chem. 2020, 205, 112665.

37. WU, S.y.; PAN, C.s.; Geng, B.; Zhao, J.; Yu, F.; PANG, Y.z.; TANG, C.s.; QI, Y.f. Hydrogen sulfide ameliorates vascular calcification induced by vitamin D3 plus nicotine in rats 1. Acta Pharmacol. Sin. 2006, 27, 299–306.

38. Meng, Q.H.; Yang, G.; Yang, W.; Jiang, B.; Wu, L.; Wang, R. Protective effect of hydrogen sulfide on balloon injury-induced neointima hyperplasia in rat carotid arteries. Am. J. Pathol. 2007, 170, 1406–1414.

39. McCully, K.S. Vascular pathology of homocysteinemia: Implications for the pathogenesis of arteriosclerosis. Am. J. Pathol. 1969, 56, 111.

40. Tyagi, S.C. Homocyst (e) ine and heart disease: Pathophysiology of extracellular matrix. Clin. Exp. Hypertens. 1999, 21, 181–198.

41. Boers, G.H.; Smals, A.G.; Trijbels, F.J.; Fowler, B.; Bakkeren, J.A.; Schoonderwaldt, H.C.; Kleijer, W.J.; Kloppenborg, P.W. Heterozygosity for homocystinuria in premature peripheral and cerebral occlusive arterial disease. New Engl. J. Med. 1985, 313, 709–715.

42. Sen, U.; Basu, P.; Abe, O.A.; Givvimani, S.; Tyagi, N.; Metreveli, N.; Shah, K.S.; Passmore, J.C.; Tyagi, S.C. Hydrogen sulfide ameliorates hyperhomocysteinemia-associated chronic renal failure. Am. J. Physiol. Ren. Physiol. 2009, 297, F410–F419.

43. Citi, V.; Martelli, A.; Gorica, E.; Brogi, S.; Testai, L.; Calderone, V. Role of hydrogen sulfide in endothelial dysfunction: Pathophysiology and therapeutic approaches. J. Adv. Res. 2020, 27, 99–113.

44. Sen, U.; Herrmann, M.; Herrmann, W.; Tyagi, S.C. Synergism between AT1 receptor and hyperhomocysteinemia during vascular remodeling. Clin. Chem. Lab. Med. 2007, 45, 1771–1776.

45. Sen, U.; Mishra, P.K.; Tyagi, N.; Tyagi, S.C. Homocysteine to hydrogen sulfide or hypertension. Cell Biochem. Biophys. 2010, 57, 49–58.

46. Hongfang, J.; Bailin, C.; Bin, Z.; Chunyu, Z.; Xinmin, L.; Weijin, Z.; Ying, S.; Chaoshu, T.; Junbao, D. Effects of hydrogen sulfide on hypoxic pulmonary vascular structural remodeling. Life Sci. 2006, 78, 1299–1309.

47. Talman, V.; Ruskoaho, H. Cardiac fibrosis in myocardial infarction—From repair and remodeling to regeneration. Cell Tissue Res. 2016, 365, 563–581.

48. Shen, Y.; Shen, Z.; Miao, L.; Xin, X.; Lin, S.; Zhu, Y.; Guo, W.; Zhu, Y.Z. miRNA-30 family inhibition protects against cardiac ischemic injury by regulating cystathionine-γ-lyase expression. Antioxid. Redox Signal. 2015, 22, 224–240.

49. Jiang, H.; Wu, H.; Li, Z.; Geng, B.; Tang, C. Changes of the new gaseous transmitter H2S in patients with coronary heart disease. Di 1 Jun Yi Da Xue Xue Bao Acad. J. First Med Coll. PLA 2005, 25, 951–954.

50. Testai, L.; Marino, A.; Piano, I.; Brancaleone, V.; Tomita, K.; Mannelli, L.D.C.; Martelli, A.; Citi, V.; Breschi, M.C.; Levi, R. The novel H2S-donor 4-carboxyphenyl isothiocyanate promotes cardioprotective effects against ischemia/reperfusion injury through activation of mitoKATP channels and reduction of oxidative stress. Pharmacol. Res. 2016, 113, 290–299.

51. Testai, L.; Barrese, V.; Soldovieri, M.V.; Ambrosino, P.; Martelli, A.; Vinciguerra, I.; Miceli, F.; Greenwood, I.A.; Curtis, M.J.; Breschi, M.C. Expression and function of Kv7. 4 channels in rat cardiac mitochondria: Possible targets for cardioprotection. Cardiovasc. Res. 2016, 110, 40–50.

52. Shimizu, Y.; Nicholson, C.K.; Lambert, J.P.; Barr, L.A.; Kuek, N.; Herszenhaut, D.; Tan, L.; Murohara, T.; Hansen, J.M.; Husain, A. Sodium sulfide attenuates ischemic-induced heart failure by enhancing proteasomal function in an Nrf2-dependent manner. Circ. Heart Fail. 2016, 9, e002368.

53. Xie, L.; Gu, Y.; Wen, M.; Zhao, S.; Wang, W.; Ma, Y.; Meng, G.; Han, Y.; Wang, Y.; Liu, G. Hydrogen sulfide induces Keap1 S-sulfhydration and suppresses diabetes-accelerated atherosclerosis via Nrf2 activation. Diabetes 2016, 65, 3171–3184.

54. Tsai, C.-Y.; Wang, C.-C.; Lai, T.-Y.; Tsu, H.-N.; Wang, C.-H.; Liang, H.-Y.; Kuo, W.-W. Antioxidant effects of diallyl trisulfide on high glucose-induced apoptosis are mediated by the PI3K/Akt-dependent activation of Nrf2 in cardiomyocytes. Int. J. Cardiol. 2013, 168, 1286–1297.

55. Rodriguez, A.M.; Yin, V.P. Emerging roles for immune cells and microRNAs in modulating the response to cardiac injury. J. Cardiovasc. Dev. Dis. 2019, 6, 5.

56. Toldo, S.; Das, A.; Mezzaroma, E.; Chau, V.Q.; Marchetti, C.; Durrant, D.; Samidurai, A.; Van Tassell, B.W.; Yin, C.; Ockaili, R.A. Induction of microRNA-21 with exogenous hydrogen sulfide attenuates myocardial ischemic and inflammatory injury in mice. Circ. Cardiovasc. Genet. 2014, 7, 311–320.

57. Zhou, X.; An, G.; Lu, X. Hydrogen sulfide attenuates the development of diabetic cardiomyopathy. Clin. Sci. 2015, 128, 325–335.

58. Li, H.; Zhang, C.; Sun, W.; Li, L.; Wu, B.; Bai, S.; Li, H.; Zhong, X.; Wang, R.; Wu, L. Exogenous hydrogen sulfide restores cardioprotection of ischemic post-conditioning via inhibition of mPTP opening in the aging cardiomyocytes. Cell Biosci. 2015, 5, 1–17.

59. Chatzianastasiou, A.; Bibli, S.-I.; Andreadou, I.; Efentakis, P.; Kaludercic, N.; Wood, M.E.; Whiteman, M.; Di Lisa, F.; Daiber, A.; Manolopoulos, V.G. Cardioprotection by H2S donors: Nitric oxide-dependent and-independent mechanisms. J. Pharmacol. Exp. Ther. 2016, 358, 431–440.

60. Karwi, Q.G.; Bornbaum, J.; Boengler, K.; Torregrossa, R.; Whiteman, M.; Wood, M.E.; Schulz, R.; Baxter, G.F. AP39, a mitochondria-targeting hydrogen sulfide (H2S) donor, protects against myocardial reperfusion injury independently of salvage kinase signalling. Br. J. Pharmacol. 2017, 174, 287–301.

61. Elrod, J.W.; Calvert, J.W.; Morrison, J.; Doeller, J.E.; Kraus, D.W.; Tao, L.; Jiao, X.; Scalia, R.; Kiss, L.; Szabo, C. Hydrogen sulfide attenuates myocardial ischemia-reperfusion injury by preservation of mitochondrial function. Proc. Natl. Acad. Sci. USA 2007, 104, 15560–15565.

62. Geng, B.; Chang, L.; Pan, C.; Qi, Y.; Zhao, J.; Pang, Y.; Du, J.; Tang, C. Endogenous hydrogen sulfide regulation of myocardial injury induced by isoproterenol. Biochem. Biophys. Res. Commun. 2004, 318, 756–763.

63. Meng, G.; Wang, J.; Xiao, Y.; Bai, W.; Xie, L.; Shan, L.; Moore, P.K.; Ji, Y. GYY4137 protects against myocardial ischemia and reperfusion injury by attenuating oxidative stress and apoptosis in rats. J. Biomed. Res. 2015, 29, 203.

64. Meng, G.; Zhu, J.; Xiao, Y.; Huang, Z.; Zhang, Y.; Tang, X.; Xie, L.; Chen, Y.; Shao, Y.; Ferro, A. Hydrogen sulfide donor GYY4137 protects against myocardial fibrosis. Oxidative Med. Cell. Longev. 2015, 2015.

65. Zhu, C.; Su, Y.; Juriasingani, S.; Zheng, H.; Veramkovich, V.; Jiang, J.; Sener, A.; Whiteman, M.; Lacefield, J.; Nagpal, D. Supplementing preservation solution with mitochondria-targeted H2S donor AP 39 protects cardiac grafts from prolonged cold ischemia–reperfusion injury in heart transplantation. Am. J. Transplant. 2019, 19, 3139–3148.

66. Hua, W.; Chen, Q.; Gong, F.; Xie, C.; Zhou, S.; Gao, L. Cardioprotection of H2S by downregulating iNOS and upregulating HO-1 expression in mice with CVB3-induced myocarditis. Life Sci. 2013, 93, 949–954.

67. Ge, N.; Liu, C.; Li, G.; Xie, L.; Zhang, Q.; Li, L.; Hao, N.; Zhang, J. Hydrosulfide attenuates acute myocardial ischemic injury through the glycogen synthase kinase-3β/β-catenin signaling pathway. Int. J. Mol. Med. 2016, 37, 1281–1289.
68. Bibli, S.-I.; Andreadou, I.; Chatzianastasiou, A.; Tzimas, C.; Sanoudou, D.; Kranias, E.; Brouckaert, P.; Coletta, C.; Szabo, C.; Kremastinos, D.T. Cardioprotection by H2S engages a cGMP-dependent protein kinase G/phospholamban pathway. Cardiovasc. Res. 2015, 106, 432–442.
69. Qipshidze, N.; Metreveli, N.; Mishra, P.K.; Lominadze, D.; Tyagi, S.C. Hydrogen sulfide mitigates cardiac remodeling during myocardial infarction via improvement of angiogenesis. Int. J. Biol. Sci. 2012, 8, 430.
70. Chen, J.; Gao, J.; Sun, W.; Li, L.; Wang, Y.; Bai, S.; Li, X.; Wang, R.; Wu, L.; Li, H. Involvement of exogenous H2S in recovery of cardioprotection from ischemic post-conditioning via increase of autophagy in the aged hearts. Int. J. Cardiol. 2016, 220, 681–692.
71. Kang, S.C.; Sohn, E.-H.; Lee, S.R. Hydrogen sulfide as a potential alternative for the treatment of myocardial fibrosis. Oxidative Med. Cell. Longev. 2020, 2020, 4105382.
72. Polhemus, D.J.; Kondo, K.; Bhushan, S.; Bir, S.C.; Kevil, C.G.; Murohara, T.; Lefer, D.J.; Calvert, J.W. Hydrogen sulfide attenuates cardiac dysfunction after heart failure via induction of angiogenesis. Circ. Heart Fail. 2013, 6, 1077–1086.
73. Barr, L.A.; Shimizu, Y.; Lambert, J.P.; Nicholson, C.K.; Calvert, J.W. Hydrogen sulfide attenuates high fat diet-induced cardiac dysfunction via the suppression of endoplasmic reticulum stress. Nitric Oxide 2015, 46, 145–156.
74. Polhemus, D.J.; Li, Z.; Pattillo, C.B.; Gojon Sr, G.; Gojon, G., Jr.; Giordano, T.; Krum, H. A novel hydrogen sulfide prodrug, SG 1002, promotes hydrogen sulfide and nitric oxide bioavailability in heart failure patients. Cardiovasc. Ther. 2015, 33, 216–226.
75. Pappachan, J.M.; Varughese, G.I.; Sriraman, R.; Arunagirinathan, G. Diabetic cardiomyopathy: Pathophysiology, diagnostic evaluation and management. World J. Diabetes 2013, 4, 177.
76. Jain, S.K.; Bull, R.; Rains, J.L.; Bass, P.F.; Levine, S.N.; Reddy, S.; McVie, R.; Bocchini, J.A., Jr. Low Levels of Hydrogen Sulfide in the Blood of Diabetes Patients and Streptozotocin-Treated rats Causes Vascular Inflammation? Mary Ann Liebert Inc.: New Rochelle, NY, USA, 2010.
77. Liu, M.; Li, Y.; Liang, B.; Li, Z.; Jiang, Z.; Chu, C.; Yang, J. Hydrogen sulfide attenuates myocardial fibrosis in diabetic rats through the JAK/STAT signaling pathway. Int. J. Mol. Med. 2018, 41, 1867–1876.
78. Jin, S.; Pu, S.-X.; Hou, C.-L.; Ma, F.-F.; Li, N.; Li, X.-H.; Tan, B.; Tao, B.-B.; Wang, M.-J.; Zhu, Y.-C. Cardiac H2S generation is reduced in ageing diabetic mice. Oxidative Med. Cell. Longev. 2015, 2015, 758358.
79. Long, J.; Liu, M.; Liu, S.; Tang, F.; Tan, W.; Xiao, T.; Chu, C.; Yang, J. H2S attenuates the myocardial fibrosis in diabetic rats through modulating PKC-ERK1/2MAPK signaling pathway. Technol. Health Care 2019, 27, 307–316.
80. Xiao, T.; Zeng, O.; Luo, J.; Wu, Z.; Li, F.; Yang, J. Effects of hydrogen sulfide on myocardial fibrosis in diabetic rats: Changes in matrix metalloproteinases parameters. Bio-med. Mater. Eng. 2015, 26, S2033–S2039.
81. Yang, R.; Jia, Q.; Ma, S.F.; Wang, Y.; Mehmood, S.; Chen, Y. Exogenous H2S mitigates myocardial fibrosis in diabetic rats through suppression of the canonical Wnt pathway. Int. J. Mol. Med. 2019, 44, 549–558.
82. Xiao, T.; Luo, J.; Wu, Z.; Li, F.; Zeng, O.; Yang, J. Effects of hydrogen sulfide on myocardial fibrosis and PI3K/AKT1-regulated autophagy in diabetic rats. Mol. Med. Rep. 2016, 13, 1765–1773.