Plants and Their Bioactive Compounds with the Potential to Enhance Mechanisms of Inherited Cardiac Regeneration

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Abstract
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This article reviews the current progress and research indications in the application of natural plant compounds with the potential for the treatment of cardiovascular diseases. Our understanding of how to apply natural plant compounds to enhance mechanisms of inherited cardiac regeneration, which is physiologically pertinent to myocyte turnover or minor cardiac repair, for substantial cardiac regeneration to repair pathological heart injuries is discussed. Although significant progress has been made in the application of natural plant compounds for therapy of heart diseases, the understanding or the application of these compounds specifically for enhancing mechanisms of inherited cardiac regeneration for the treatment of cardiovascular diseases is little. Recent recognition of some natural plant compounds that can repair damaged myocardial tissues through enhancing mechanisms of inherited cardiac regeneration has offered an alternative for clinical translation. Application of natural plant compounds, which show the activity of manipulating gene expressions in such a way to enhance mechanisms of inherited cardiac regeneration for cardiac repair, may provide a promising strategy for the reconstruction of damaged cardiac tissues due to cardiovascular diseases.

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Introduction
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Cardiovascular disease (CVD) is a class of diseases caused mainly by disorders of the heart and blood vessels, including coronary heart disease (CHD), congenital heart disease, arrythmias, heart failure, cardiomyopathy, aorta disease, and peripheral artery disease [1,2]. CVD is the leading cause of death around the world. Due to worldwide population growth and an increasing elderly population, the death toll of CVD increases each year and is estimated to reach 23 million by 2030 according to the World Health Organization [3].

Among all types of CVD, CHD is the most common one that accounts for one-third of the total CVD-related deaths [4]. CHD results from partial or complete occlusion of the coronary artery and subsequent cardiac ischemia in the affected territory. Complete occlusion of the coronary artery leads to a massive loss of cardiac tissues, including cardiac myocytes and coronary vasculature in the distribution territory of the affected artery [5,6]. Conventional treatments for CHD, including medication (e.g., beta-blockers, diuretics, etc.) and surgical intervention (e.g., angioplasty, coronary artery bypass grafting), can only alleviate symptoms and slow down the deterioration of the disease without pathological modifying effects [7]. Although heart transplantation may serve as a final resort for end-stage heart failure patients, it is not only costly, but also limited by donor availability and host immune rejection. Recent striking advances in stem cell research and regeneration medicine have provided the hope for therapeutic cardiac repair through cell transplantation-based strategies. Stem cells of particular interest include embryonic stem cells (ESCs) [8,9], induced pluripotent stem cells (iPS) [10,11], bone marrow-derived mesenchymal stem cells (MSCs) [12,13], and umbilical cord blood cells [14,15]. However, there is still a long way to go before the successful clinical translation of stem cell transplantation-based therapy due to the yet to be solved drawbacks and complications, including host immune rejection, risk of tumorigenesis, and low cardiogenic efficiency.

Thus, searching for alternative strategies for an effective treatment of CVD has attracted increasing research interest, especially following the recent discovery of the inherited ability of heart regener-

ation in the adult mammalian heart [16–19]. With the growth of our understanding, it was soon realized that mechanisms of inherited cardiac regeneration pertinent to heart homeostasis and possible minor cardiac injury repair are far from effective for the repair of pathological damages of the heart [14, 15]. Nonetheless, the realization of this limitation hints for an alternative strategy for substantial treatment of CVD through enhancing the inherited cardiac regeneration ability. This review is dedicated to the emerging research topic on how natural plant compounds can be used to enhance mechanisms of inherited cardiac regeneration for effective treatment of injured hearts due to CVD.

Mechanisms of Inherited Cardiac Regeneration
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Cardiac regeneration capacity is largely retained throughout the lifespan in lower vertebrates, such as amphibians and fish [20–22]. By contrast, mammals still retain some cardiac regeneration capacity at their neonatal stage, which is then lost shortly after birth [23]. The classic dogma that the adult mammalian heart is a post-mitotic organ without renewal ability has preset the field for decades [24, 25]. However, this traditional view has been challenged by increasing new research findings that the adult mammalian heart still contains limited renewal ability. BrdU incorporation experiment in a rat model demonstrated a descending trend of BrdU positive cells in the heart throughout the whole life span from ~ 37% on day 3 to ~ 11% on day 5, ~ 5% on day 13 after birth, and 0.2 ~ 2% in an adult [26].

Growing data supporting this new concept that the adult mammalian heart contains limited renewal ability have been documented for human hearts [27, 28]. Using incorporated ^{14}C in DNA obtained from human adult hearts, Bergmann et al. provided direct evidence that human adult myocytes continue to regenerate during the whole lifetime, though the capacity for myocyte regeneration decreases with age [29]. It was soon found that a subpopulation of replicating myocytes was preserved in the postnatal, adult, or senescent heart with the ability of repopulating parenchymal myocytes [27, 30–33]. The existence of cardiac resident stem cells (CSCs), in line with stem cell criteria of self-renewing, clonogenic, multipotent, giving rise to myocytes, smooth muscle, and endothelial cells, was demonstrated [34–39]. These identified CSCs were further grouped according to their surface markers, such as c-kit, Sca-1, Isl1, and MDR1 positive cells. Of them, the c-kit and Sca-1 positive CSCs were identified as the predominant CSC subpopulations in the heart stem cell pool [35, 36, 40]. These CSCs are likely involved in the cell turnover-mediated cardiac cell (e.g., myocytes, smooth muscle cells, and vessel endothelial cells) replacement and possible cardiac minor repair during myocardial homeostasis [31, 33, 35, 36, 40, 41]. Consistent with this notion, the number of CSCs increased significantly in response to myocardial infarction (MI) [42] and the application of c-kit-positive CSCs produced a substantial engraftment and regenerated cardiac tissues in an MI animal model [31, 43]. In the physiological condition of human adult life, the death rate of myocytes during cardiac homeostasis accounts for the loss of ~ 3 × 10^6 myocytes per day, which is putatively a close match to that of regenerated myocytes through mechanisms of inherited cardiac regeneration [15]. Unfortunately, this ability to repopulate the lost myocytes during cardiac homeostasis is severely limited in pathological cardiac repair [15]. Therefore, maneuvers to enhance mechanisms of inherited cardiac regeneration would accomplish the aim for substantial treatment of CVD.

Plant Compounds Used to Enhance Cardiac Angiogenesis and Myocardial Regeneration
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As aforementioned, current available strategies for the treatment of CVD, including drugs, surgical interventions, and cell transplantation-based therapies, have their innate limitations, some of which even cause severe or life-threatening complications. Therefore, the development of more effective and safer strategies for substantial treatment of CVD is highly desirable. In light of the newly discovered mechanisms of inherited cardiac regeneration and the realization of its low efficiency in pathological cardiac repair, it is reasonable to postulate that maneuvers that can enhance mechanisms of inherited cardiac regeneration could potentially achieve pathology-modifying and functional repair of diseased heart.

To potentiate such repair of a diseased heart, the creation of an inductive microenvironment for cardiogenesis in the territory is a corequisite [15]. The requirement of an inductive microenvironment for cardiogenesis, such as timely delivery of oxygen, necessary nutrients, variants of growth factors, and circulating stem cells to the territory, must be satisfied in order for cell trafficking, survival, growth, and differentiation in the previously deprived region. Therefore, rapid reconstitution of the damaged vasculature in the territory is primarily vital for the creation of such an inductive microenvironment for any myocardial regeneration. For substantial repair of acute cardiac injury, such as MI, replacement of the dead cardiac tissues with newly regenerated myocardium is a therapeutic ideal. Only under such an aforementioned cardiogenesis-inductive microenvironment may myocardial regeneration be fulfilled through enhanced (i) proliferation and differentiation of resident CSCs; (ii) or/and cardiogenic differentiation of bone marrow-derived circulating stem cells that migrate to the site of damage; (iii) transient dedifferentiation and proliferation of terminally differentiated myocytes under defined conditions.

One such maneuver that can help create such a cardiogenesis-inductive microenvironment and promote cardiogenesis is natural plant compounds with known functions, such as traditional Chinese medicinal herbs that are related to cardiac regeneration. Although this is an emerging research field, several research teams, including our laboratory, have already made significant progress with promising findings. Listed below are several such examples of these natural plant compounds with potential for substantial cardiac regeneration.

The Beneficial Effects of *Rehmannia glutinosa* on Cardiac Angiogenesis and Cardiogenesis
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Rehmannia glutinosa (*R. glutinosa*, also known as Dihuang), a member of the Scrophulariaceae family, is a well-known traditional Chinese medicine widely used in Asian societies [44]. The safety of *R. glutinosa* administration has been proven by its practice in traditional medicine over thousands of years. Due to its beneficial effects on Yin and the kidney, *R. glutinosa* has been used commonly for the treatment of diseases associated with hypodynamia. In the last several decades, studies have shown that *R. glutinosa* has multiple functions in the cardiovascular system [45, 46], though the underlying molecular mechanisms are yet to be revealed.

Some research results demonstrated that *R. glutinosa* extract (RGE) may exert its specific effect on hematogenesis in mice by

enhancing proliferation and differentiation of bone marrow-derived hematopoietic stem cells [47]. Other recent studies reported that the oral administration of RGE (1–1.5 g/kg/day) to MI model rats not only increased the quantities of endothelial progenitor cells (EPCs), potential targets for cardiac repair, both in blood and in bone marrow, but also promoted their mobilization to peripheral blood, migration to injured heart tissues, and activation of their function for angiogenesis [48]. It was further found that RGE (25–50 µg/ml culture medium) could also stimulate EPC proliferation, migration, and capillary-like tube formation, probably through the activation of the stromal-derived factor-1α/receptor (SDF-1α/CXCR4) cascade [48].

Other angiogenesis-associated factors, such as vascular endothelial growth factor receptor 2 (VEGFR2) and CD133 (a hematopoietic stem and progenitor cell marker), were also upregulated by RGE. These properties of RGE may help create a cardiogenesis inductive microenvironment in the territory of cardiac damage that favors the ensuing myocardial regeneration.

In addition to the potential angiogenic effect of RGE, the activity of RGE in the prevention of caspase-3 activation-induced cardiac myocyte death *in vitro*, probably by increasing Bcl-2 expression and inhibiting Bax expression, was also suggested [49]. More importantly, RGE treatment could lead to significant functional improvement in the chronic stage after acute MI including an increased left ventricle eject fraction, a reduced ischemic area, and a decreased apoptotic index in the infarct myocardium. Despite the fact that the chemical compositions of RGE await further clarification, these findings render RGE a promising candidate for further drug development towards the treatment of CVDs.

The Multi-Beneficial Effects of Ginseng on Cardiovascular Disease

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Ginseng, a member of the Araliaceae family, is one of the most well-known and wildly used herbal medicines around the world. It is routinely used for the general human well-being and is considered to have multi-beneficial effects on pathological conditions and diseases related to the immune system, endocrine system, central nervous system, and cardiovascular system [50–52]. During the past few decades, an increasing application of ginseng has been observed not only in oriental society, but also in Western countries. There are various species of ginseng, among which Asian ginseng (*Panax ginseng*) and North American ginseng (*Panax quinquefolius*) are the two major ones [53, 54]. Ginseng reportedly contains multiple active constituents, including ginsenosides, quinquefolans, polysaccharides, pyridoxine, and fatty acids [55]. Among them, ginsenosides are considered to be the main active compounds that are attributed to the multi-beneficial effects of ginseng [54, 56]. Ginsenosides are a special group of triterpenoid saponins that are found almost exclusively in *Panax* species (ginseng), and up to now, more than 150 naturally occurring ginsenosides have been isolated from roots, leaves/stems, fruits, and/or flower heads of ginseng. The protopanaxadiol (e.g., Ra1 and Rb1) and protopanaxatriol groups (e.g., Rg1 and Re) are the two main groups of ginsenosides (◉ **Fig. 1 a, b**). The considerable variety of ginsenosides and their multiple functions make it hard to dissect out their individual functions. Nevertheless, extensive investigations of several major ginsenosides were performed, owing to a renewed interest in exploring the mechanistic nature underlying ginseng's beneficial effects for possible novel drug development.

Rg1, one of the most active compounds isolated from ginseng, has attracted many research interests. *In vitro* studies on human umbilical vein endothelial cells (HUVECs) demonstrated that Rg1 treatment significantly promoted the migration, proliferation, and capillary-like tube formation of the cultured HUVECs [57–59]. Downregulation of microRNA-214 and microRNA-15b, which in turn leads to increased VEGFR-2 expression, was suggested to underlie Rg1-induced angiogenesis [60, 61]. Consistent with these *in vitro* results, some *in vivo* studies demonstrated that Rg1 could improve cardiac function, reduce infarct size, and increase capillary density in the infarct area of a rat MI model and in a transverse aortic constriction-induced left ventricular hypertrophy model, probably through upregulating expressions of vascular endothelial growth factor (VEGF), CD31, and hypoxia inducible factor-1α (HIF-1α) [62, 63]. Moreover, further *in vitro* studies found that Rg1 exerted a significant effect on preventing apoptosis [62, 63]. Consistent with these findings, it was reported that Rg1 could reduce apoptosis of cultured H9c2 cardiomyocytes [64] and dose-dependently increase cell viability in a cardiomyocyte hypoxia/reoxygenation model [65].

Rb1, another major ginsenoside isolated from ginseng, also showed a protective effect of cardiomyocytes from apoptosis [66–68]. Some studies indicated that the PKA signaling pathway and caspase-9 pathway might be involved in an Rb1-mediated antiapoptotic effect [69]. Many other investigations with myocardial remodeling, cardiac ischemia and reperfusion, angiogenesis, and cardiomyopathy animal models also consistently demonstrated the cardiovascular beneficial effects of Rg1 and Rb1 [70–73]. Therefore, it would be highly rewarding to investigate whether ginsenosides can enhance cardiogenesis in the adult mammalian heart under both physiological and pathological conditions.

The Application of *Salvia miltiorrhiza* Bunge and its Isolated Compounds for the Treatment of Cardiovascular Disease

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Salvia miltiorrhiza Bunge (*S. miltiorrhiza* Bunge, Danshen in Chinese), a member of the Labiatae family, is extracted from the dried root of a perennial plant, *S. miltiorrhiza* Bunge, and is widely used for promoting blood circulation and removing blood stasis in Chinese herbal medicine practice. Cardiovascular beneficial effects of *S. miltiorrhiza* Bunge whole extract have been studied extensively. These studies showed that *S. miltiorrhiza* Bunge could promote angiogenesis, protect cardiomyocytes against ischemia-induced apoptosis, and enhance the transdifferentiation of MSCs into cardiomyocytes [74–78]. Due to its various cardiovascular beneficial effects, *S. miltiorrhiza* Bunge has been commonly used for the clinical treatment of CVD in China.

To date, more than 80 chemical components, including water-soluble phenolic acids and lipophilic tanshinones, have been identified and isolated from *S. miltiorrhiza* Bunge [78, 79]. The main lipophilic compounds from *S. miltiorrhiza* Bunge include tanshinone I (tan I), tanshinone IIA (tan IIA), tanshinone IIB (tan IIB), and cryptotanshinone, while the major hydrophilic compounds include salvianolic acid A, salvianolic acid B, danshensu, and protocatechuic aldehyde.

Tan IIA (◉ **Fig. 1 c**) is one of the most abundant and active lipophilic diterpenes found in *S. miltiorrhiza* Bunge. Multiple beneficial effects of tan IIA, including antioxidant, anti-inflammatory, antiapoptosis, and proangiogenic effects, have been demonstrat-
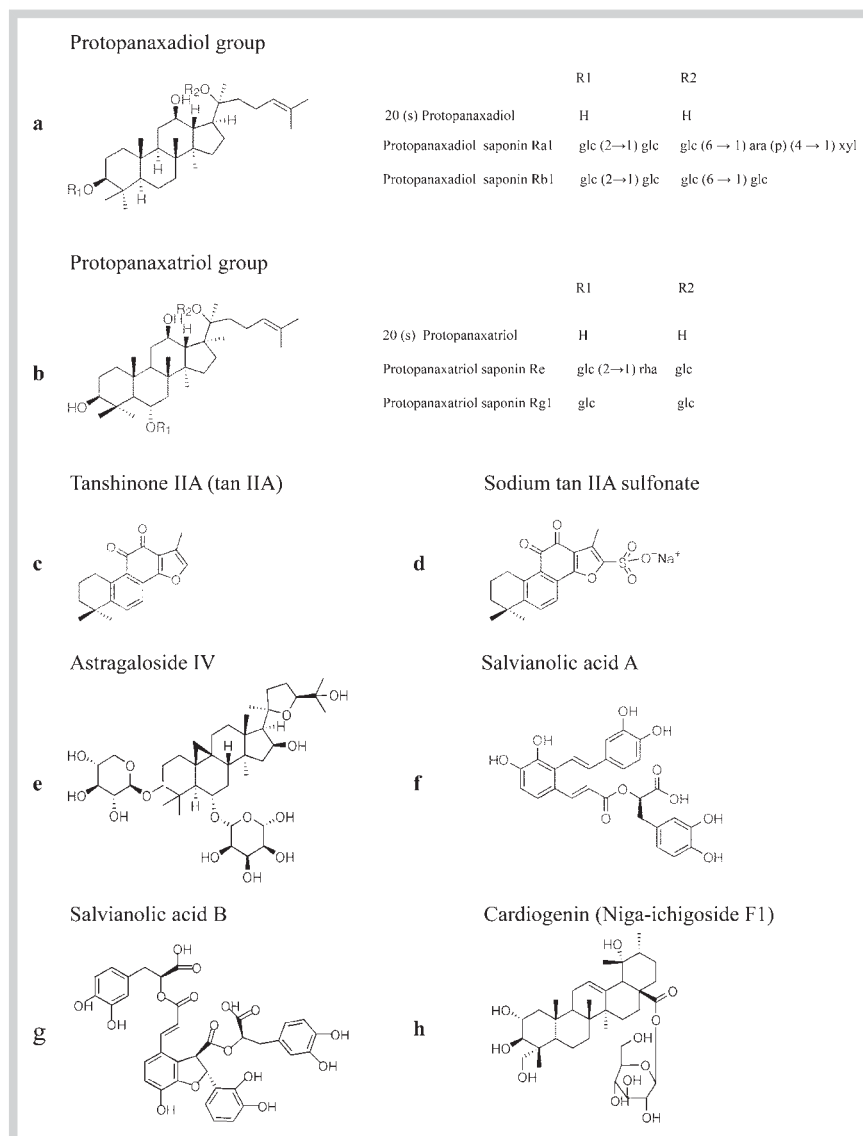
Fig. 1 Chemical structures of the cited compounds isolated, respectively, from ginsenosides, *S. miltiorrhiza* Bunge and *G. japonicum* Thunb. vax. chinense F. Bolle.

ed [80,81]. As a result, tan IIA has long been used effectively to prevent and treat various CVD clinically in China. Although the precise mechanisms underlying the effects of tan IIA await more detailed and systematic experimental and clinical studies, recent studies have started to uncover some interesting beneficial effects of tan IIA on the cardiovascular system.

It has been found that tan IIA can improve the local microenvironment of a damaged heart area through promoting angiogenesis. Studies using rat MI models showed that administration of tan IIA resulted in improved cardiac function, reduced infarct size presumably through upregulating VEGF expression, and consequent angiogenesis [82]. Further studies also consistently demonstrated that treatment of acute MI animals with a water-soluble derivative of tan IIA, sodium tan IIA sulfonate (○ **Fig. 1 d**), significantly reduced the infarct size and decreased the number of apoptotic cardiomyocytes in the infarct zone [83].

In a heart failure rat model induced by thoracic aorta constriction, tan IIA injection for 12 weeks decreased myocardial apoptosis, assessed by the TUENL method, likely through upregulating the mRNA and protein levels of Bcl-2 and miR-133 [84]. Regulation of miR-133 levels by tan IIA was further confirmed in cultured neonatal cardiomyocytes under hypoxic condition showing

that tan IIA treatment increased the miR-133 level by activating the MAPK ERK1/2 pathway [85]. Using both *in vitro* and *in vivo* studies, Fu et al. showed that tan IIA could protect myocytes against apoptosis triggered by oxidative stress involving Bcl-2 regulation [80]. The mechanism underlying the action of tan IIA in preventing cardiac apoptosis is presumably via regulation of the ratio of Bcl-2/Bax [86] and downregulating of the miR-1 level [87,88].

Interestingly, recent evidences indicated that tan IIA could also enhance the migration of bone marrow MSCs to the infarct region following MI [89,90]. As mentioned above, both endogenous and transplanted MSCs can improve cardiac functions after MI, though with limited efficacy, by transdifferentiation into cardiomyocytes directly and/or through the paracrine mechanism indirectly. Tong et al. showed that combination treatment of tan IIA and MSCs in a rat MI model exhibited better effects than that with MSCs alone in terms of infarct size reduction and cardiac function improvement [89]. Tan IIA treatment significantly increased the number and the survival rate of MSCs in the infarct region, likely through the upregulation of the SDF-1/CXCR4 axis, which is essential for tan IIA to enhance the recruitment of MSCs to the infarct zone [89]. Consistent with this finding, using both

in vitro experiments and a rat acute MI model, Xie et al. [90] reported that treatment of tan IIA and astragaloside IV (○ **Fig. 1 e**) could promote the migration and homing of exogenous MSCs to the ischemic region, at least partially, through the upregulation of CXCR4 expression.

Besides tan IIA, some other compounds isolated from *S. miltiorrhiza* Bunge also showed cardioprotective activities. For example, salvianolic acid A and salvianolic acid B (○ **Fig. 1 f, g**) could enhance angiogenesis both *in vitro* and in rat MI models [91–93]. Mechanistic studies in a rat MI model showed that salvianolic acid A enhanced ischemia-induced angiogenesis, likely by upregulating VEGF and VEGFR-2 levels, and promoting the migration and vasculargenesis of EPCs [91]. Recent studies further indicated that the extract of *S. miltiorrhiza* Bunge not only promoted angiogenesis and protected cardiomyocytes against ischemia-induced apoptosis, but also enhanced the transdifferentiation of MSCs into cardiomyocytes [74–78] and myocardial regeneration in the infarct zone in MI animal models (authors' unpublished data). Although *S. miltiorrhiza* Bunge has been commonly used for the treatment of CVD based on its various cardiovascular beneficial effects, the dissection of the effect of individual compounds of *S. miltiorrhiza* Bunge remains to be elucidated due to the complexity of its extract.

Potentiating Mechanisms of Inherited Cardiac Repair for the Treatment of Cardiovascular Disease by *Geum japonicum*
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Several recent studies have demonstrated that an organic extract of *Geum japonicum* Thunb. vax. chinense F. Bolle (EGJ) exerted dual actions on angiogenesis and myogenesis leading to the substantial repair of infarct hearts and damaged muscles in animal models [13, 94, 95]. Two active fractions (Angio-T & AFGJ), isolated from EGJ, showed remarkable activities in promoting the growth of coronary collateral vessels in CHD rat models [96, 97]. The most significant merits of these studies are the clear demonstrations of the types, densities, and distribution of the newly grown coronary collaterals in the ischemic heart [97]. The results showed that AFGJ, which is mainly comprised of polyphenols, could significantly induce the growth of small coronary arteries, including arterioles (21–63 µm) and microarteries (63–210 µm), in ischemic hearts. The densities of the arterioles and the microvesseles in the hearts of AFGJ-treated animals were significantly higher than those in the hearts of the vehicle-treated group (* $p < 0.05$; ○ **Fig. 2 A, B**) [97]. However, the densities of vessels (with diameters less than 21 µm or greater than 210 µm) showed no significant difference between the two groups. The 2D evaluation of the unit area of vessels in ROI showed that the average density of vessels of AFGJ-treated hearts was significantly higher than that of the vehicle-treated hearts (*** $p < 0.001$). More interestingly, quantitative volumetric measurements of heart vascular angiogenesis demonstrated that both the values of vascular volume (VV) and total volume (TV) in AFGJ-treated ischemic hearts
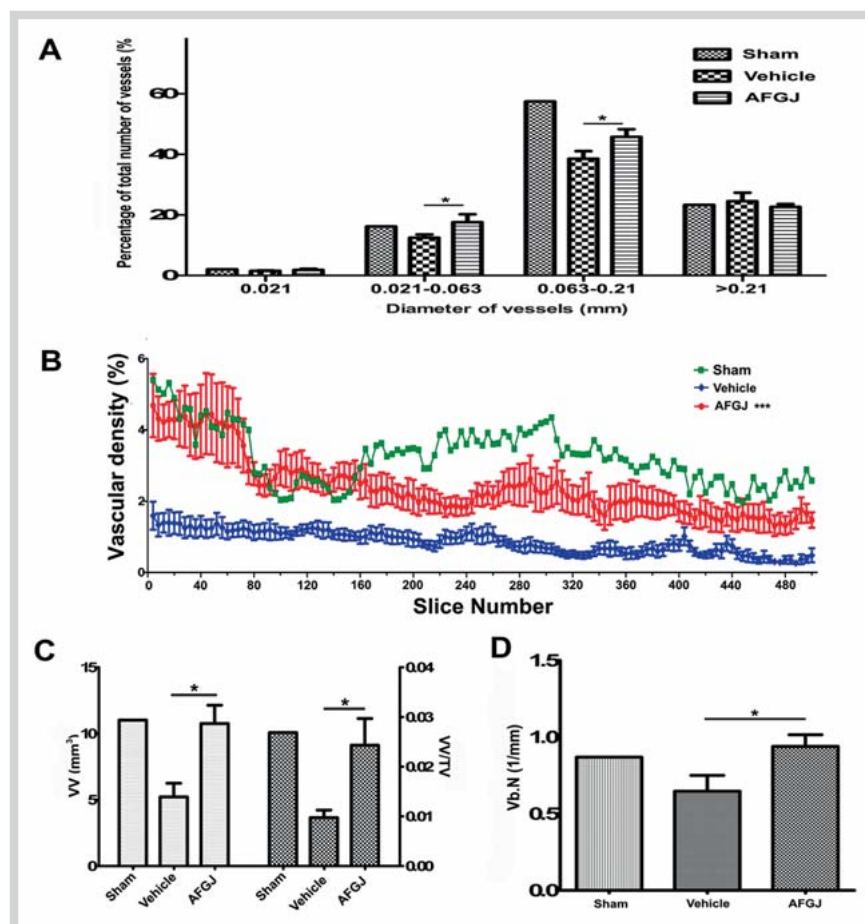

Fig. 2 MicroCT-based quantitative analysis (n = 6). **A** The distribution of different diameters of vessels. **B** Representative 2D evaluation of the unit area of vessels in ROI that was derived from 125 data sets of 500 slices. **C** Quantitative volumetric measurements of heart vascular angiogenesis. The left y-axis represents the vascular volume (VV) of the ischemic area. **D** Quantitative measurement of AFGJ-induced therapeutic angiogenesis represented as vessel branching points ([97], with the permission of the authors). (Color figure available online only.)

were significantly increased compared with those in the vehicle-treated hearts (* p < 0.05), and were virtually close to those of the nonischemic hearts in the sham operated group (mean: 10.754 vs. 11.020 and 0.024 vs. 0.027), indicating that AFGJ treatment effectively reconstituted the lost coronary vessels due to infarction, especially the microvessels in the ischemic region of the hearts (○ **Fig. 2C**). More importantly, an analysis of a number of intersections between vessel and non-vessel components per total length (vb. N) of vessels in volume of interest (VOI), which can provide information on vessel branching points, demonstrated that substantially more branching points in the AFGJ-treated heart compared with those in the vehicle-treated heart (mean ± SEM: 0.9392 ± 0.07 615 vs. 0.6462 ± 0.1036; * p < 0.05), implying the formation of new substantial collateral vessels in AFGJ-treated ischemic hearts (○ **Fig. 2D**). Both the quantitative MicroCT and quantitative histological analysis results consistently demonstrated the significantly increased vascular density and collateral branching points in the ischemic region of AFGJ-treated hearts. MicroCT analysis also provided visible evidence of the vascular network of the experimental hearts.

Taken together, AFGJ appears to promote therapeutic angiogenesis through the induction of growth of new coronary collaterals (with diameter 0.021–0.21 mm) in adult ischemic hearts. AFGJ-induced growth of new collaterals in ischemic hearts is of therapeutic significance evidenced by the improved functional performance of the CHD hearts. More importantly, AFGJ-induced growth of coronary collaterals into the ischemic region of CHD hearts should address the root pathology of the disease and provide a novel therapeutic method for effective/curative treatment of chronic CHD.

In addition to the effect of EGJ or AFGJ on the stimulation of substantial growth of coronary collateral vessels in ischemic hearts, several other studies also demonstrated that another active fraction identified/isolated from EGJ showed the property in stimulating myocardial regeneration in an acute MI animal model through topical injection (0.3 mg) or intragastric administration (300–500 mg/kg body weight) [13,94,98,99]. These findings prompted further investigations for the identification and isolation of the active component from the fraction and demonstration of the activity of the active component. Consequently, a cardiogenic compound (i.e., cardiogenin; ○ **Fig. 1h**) was isolated from the active fraction with activities in enhancing cardiogenic differentiation efficiency of MSCs and regenerating myocardial tissues in MI animal models [13,98,99]. These remarkable results showed that both the active fraction and cardiogenin can promote cardiogenic differentiation of MSCs in culture with approximately 40–50% of the cultured MSCs elongated, forming rod-like phenotypes with MEF2 (3-day treatment) or MHC (7-day treatment) positively stained. More importantly, the indication of the effect of the active fraction or cardiogenin in promoting cardiogenic differentiation of MSCs *in vitro* can be translated into regeneration of a substantial amount of myocyte-like cells with colocalized ki67 positive nuclei and MHC positive cytoplasm observed throughout the whole infarct region *in vivo*, which should contribute to the significantly improved cardiac function [13,99]. These newly regenerated myocytes replaced the dead cardiac tissues. Computated planimetric analysis demonstrated that the scar area of the hearts in the active fraction or cardiogenin-treated MI hearts were approximately ¼–½ smaller than that in the vehicle-treated rats (p < 0.01).

The identification of male cardiac cells in female donor hearts, which were transplanted to male recipients, raised the thought that these male cardiac cells might be the progeny of circulating stem cells of the recipient's bone marrow origin [100,101]. Some recent studies have also shown agreeable evidences for the active participation of bone marrow-derived MSCs in myocardial regeneration [13,96,99]. In these studies, it was found that although bone marrow-derived MSCs can migrate to the infarct zone after acute MI, their cardiogenic differentiation efficiency is too low to produce meaningful repair to the damaged heart. The experiment using cardiogenin to treat MI rats that were subjected to bone marrow transplantation of labeled MSCs prior to MI production provided evidence that considerable bone marrow-derived MSCs participated in myocardial regeneration [13,96,99]. Many vessels (yellow-circled) and DiI- (orange-labeled cytoplasm) MEF2 (red-stained nuclei) colocalized cells (green-circled) were observed throughout the infarct zone (○ **Fig. 3**). Some of the myocyte-like cells in the central infarct zone (blue arrows), which were positively stained by both Ki67 (nuclei) and MHC (cytoplasm), were found with some of Ki67 positive nuclei of the vessel endothelial cells observed (green arrows). The normal myocytes along the infarct rim were MHC positive, but Ki67 negative (red circles). Some of the regenerating myocytes joined together in tandem forming myocardium-like tissue (blue arrows). The importance of these studies is the demonstration that endogenous MSCs could be an important progenitor cell pool for regeneration of new myocardium on the condition that their cardiogenic differentiation potential is triggered. The supreme weight of using natural plant compounds to potentiate mechanisms of inherited cardiac repair for treatment of cardiac damage in these studies is that activation of endogenous stem cells by natural plant compounds for repair of impaired hearts would eliminate the possible teratoma formation, immunorejection reaction, and many other possible complications that are associated with the use of ESCs or other exogenous progenitor cells [102–105]. The mechanistic study indicated that cardiogenin or the active fraction derived from EGJ may interact with G proteins initially, which subsequently trigger the sequential cascade of cardiogenic differentiation-associated intracellular events, such as suppression of miRNA-9 expression, which consequently upregulates its target genes, including the G protein family, zinc finger proteins, E-cadherin, and other growth factors such as BMPs, TGFβ, FGF, and EGF to a level sufficient for an enhanced cardiogenic differentiation efficiency of MSCs and myocardial regeneration [13,99].

Cardiac Repair by the Active Fraction Isolated from *Rosa laevigata* Michx
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The total flavonoids (TFs) and some other compounds isolated from *Rosa laevigata* Michx fruit have been reported to have a potent antioxidant activity in both *in vitro* and *in vivo* experiments [106,107]. These studies showed that TFs not only exhibited a high scavenging effect, but also significantly decreased the levels of total blood cholesterol (45.02%), triglycerides (33.86%), and low-density lipoprotein cholesterol (73.68%) in a hyperlipemia mouse model [107]. The authors therefore inferred that the properties of TFs in antioxidant and hypolipidemic activities might render TFs a potential medicine for CVD.

A recent study from our laboratory has again demonstrated the ability of an active fraction isolated from the Chinese herb *R. laevigata* Michx (i.e., aFRLM) in promoting myocardial regeneration in an acute MI rat model. It was found that daily oral administration of aFRLM (300 mg/kg body weight) to an acute MI ani-
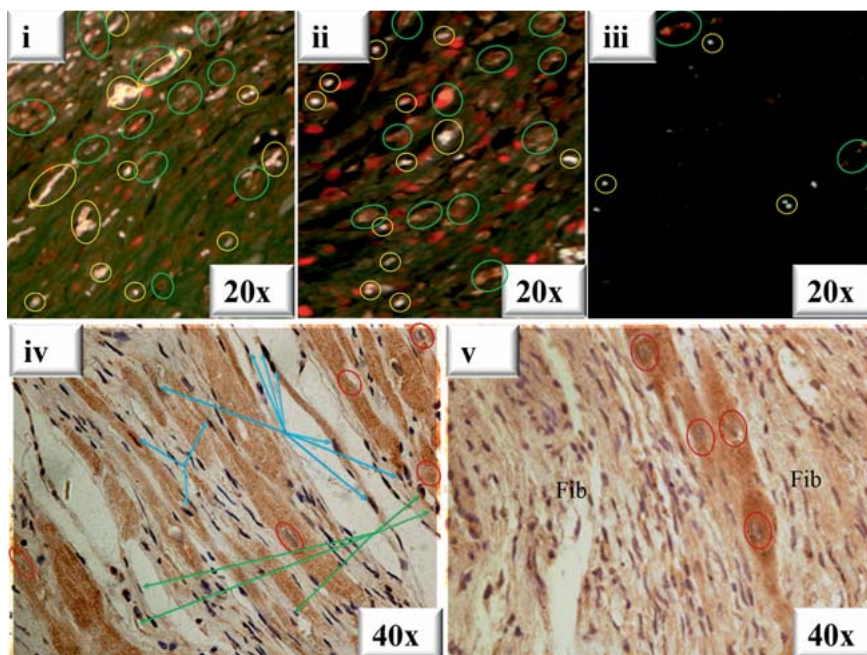
Fig. 3 The possible cellular origin of the regenerating myocytes. Representative counter-staining (DiI and MEF2) micrographs of the MI heart, which was subject to BMT with DiI-labeled MSCs 1 week prior to LAD ligation. **i** One week post-cardiogenin treatment. **ii** Two weeks post-treatment, significantly more MEF2 positive cells colocalized with the DiI labeling (green circles) were found. **iii** There were less vessels (yellow circles) and DiI-MEF2 colocalized cells (green circles) observed in the vehicle-treated heart. **iv** Ki67 and MHC double immunostained LV section of the cardiogenin-treated heart. Blue arrows, newly formed myocyte-like cells. Green arrows, Ki67 positively stained vessel endothelial cells. Red circles, the normal myocytes with MHC positively, but Ki67 negatively stained. **v** Ki67 and MHC double immunostained LV section of the vehicle-treated control heart. Fibrous scar replacement (Fib) ([99], with the permission of the authors). (Color figure available online only.)

mal for a month progressively improved the cardiac function of the experimental animals. Morphological analysis of the therapeutic effect of aFRLM on MI demonstrated that the infarcted cardiac tissues were replaced by many well-arranged, red-stained myocyte-like cell clusters in the central infarct zone. By comparison, a large area of fibrous scar was found throughout the whole infarct region in the vehicle-treated MI heart with few myocyte-like cell clusters observed. The result of Masson's trichrome staining further demonstrated that although the infarcted cardiac tissues were replaced by blue-stained fibrous scar tissues, many red-stained myocyte-like cell clusters were found in the central infarct region. By contrast, in the vehicle-treated MI heart, the blue-stained fibrous scar replacement of the infarcted cardiac tissues was found throughout the whole infarcted region with few red-stained myocyte-like cells clusters observed. Some of the myocyte-like cells were positively stained by both Ki-67 (brown nuclei) and MHC (yellow cytoplasm) specific antibodies in aFRLM-treated MI hearts. Some of the regenerating myocytes joined together in tandem forming myocardium-like tissue. By contrast, fibrous scar replacement was found throughout the infarct region with few Ki-67 and MHC positively stained myocyte-like cells found (◉ **Fig. 4**) (author's unpublished data). Although the cellular origin of the regenerating myocytes remains to be verified, based on the ability of aFRLM (40 µg/ml culture medium) to induce cardiogenic differentiation of MSCs (20–30%) *in vitro*, and the one-third smaller sizes of the newly regenerated cardiac myocytes in aFRLM-treated MI hearts, the regenerating myocytes are likely derived from circulating MSCs or CSCs [108–110].

Occlusion of a major coronary artery would cause a significant loss of functional cardiac myocytes in its distribution territory through necrosis, intrinsic and extrinsic apoptosis pathways, and autophagy. Repair of the dead cardiac tissues with newly regenerated cardiac myocytes remains a mission impossible. Therefore, this report may provide an alternative method for the repair of infarct hearts through enhancing mechanisms of inherited cardiac repair.

Conversion of Fibroblasts in Cardiac Scar into Myocytes by Natural Plant Compounds
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Although in fetal life the ability of regeneration of tissues to repair wounds is retained with no scar tissue formed, in postnatal life, wound healing occurs at the expense of function with a fibrous scar formed to repair the wound [111]. MI is naturally repaired by the formation of a fibrous scar that patches up the cardiac wound, sustaining the integrity of the heart. However, the scars are mainly composed of disordered fibrous tissues having no resemblance to the original cardiac tissues being replaced. Therefore, although the integrity and about 70% of the strength of the affected myocardium is attained, the scar tissues are not functional, which may eventually cause heart failure [112]. Therefore, conversion of the cardiac fibroblasts in the scar tissues into myocytes may be an ideal alternative for the treatment of chronic MI.

Although conversion of fibroblasts in heart scar tissues into myocytes would be a very intriguing therapy for the disease, solid supporting evidences are few. A recent study reported that the introduction of a combination of Gata4, Mef2c, and Tbx5 genes into cardiac resident fibroblasts (CRFs) could convert them into cardiac myocyte-like cells both *in vitro* and *in vivo* [113, 114]. A subsequent study from another laboratory soon reported that the conversion of CRFs by this combination of transcription factors was not only inefficient, but also resulted in decreased cell survival *in vivo* [115]. Some other studies indicated that delivery of TGFβ or MyoD into CRFs may convert them into myocyte phenotypes [116, 117]. Compared with the method associated with gene combination delivery, treatments with natural compounds, such as a drug-like small molecule or compound combination, that can manipulate expressions of cardiac differentiation-associated genes or a gene combination, such as MEF2, GATA4, and TBX5 in treated CRFs, would be more convenient and practical alternatives, so that when the drug-like molecule or compound combination is administered, the drug-like molecule-mediated actions may override the post-mitotic phenotype of the fibroblasts in the scar tissues and covert them into cardiac myocytes.
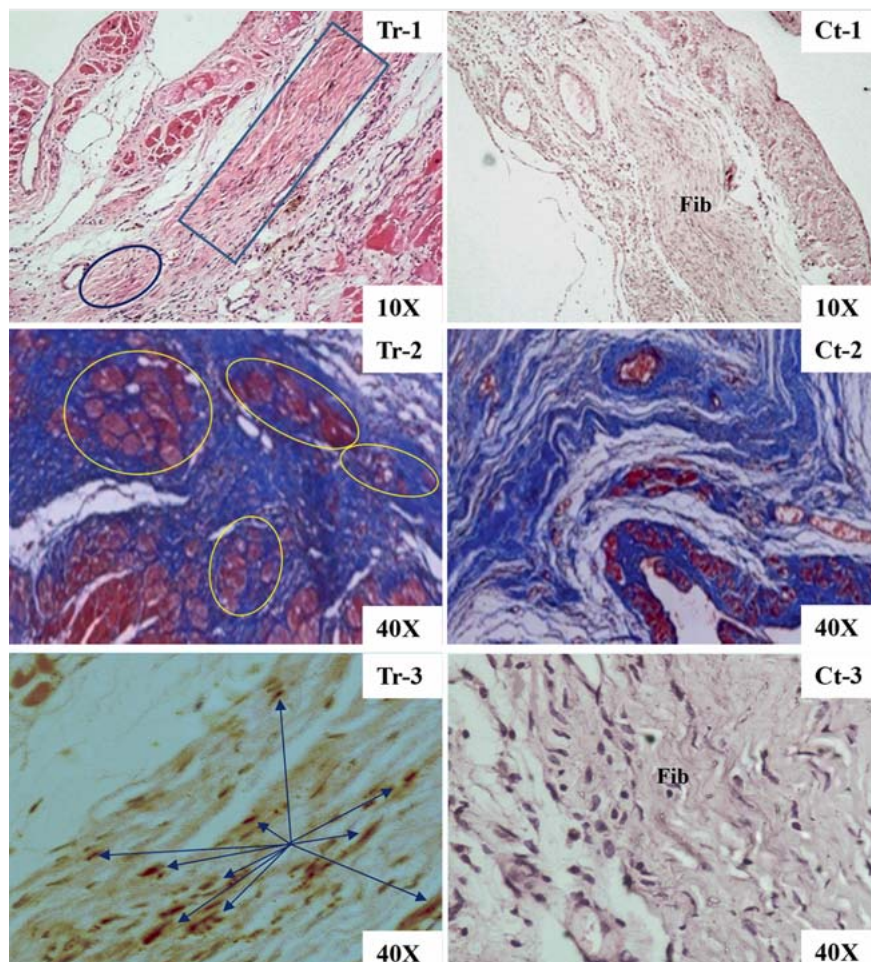
Fig. 4 Morphological assessment of the therapeutic effect of aFRLM on MI. Tr-1, representative micrographs of the aFRLM-treated (4 weeks) MI heart showing many red-stained myocyte-like cell clusters (blue rectangular surrounded) formed in the central area of the infarct. Ct-1, a fibrous scar was found throughout the whole infarct region (Fib) in the vehicle-treated MI heart. Tr-2, Masson's trichrome staining demonstrated the formation of many red-stained myocyte-like cell clusters in the aFRLM-treated heart (yellow-circled). Ct-2, the blue-stained fibrous scar was formed in the vehicle-treated MI heart. Tr-3, some of the myocyte-like cells (blue arrow heads) were positively stained by both Ki-67 (brown nuclei) and MHC (yellow cytoplasm) specific antibodies in the aFRLM-treated MI hearts. Ct-3, fibrous scar replacement was found throughout the infarct region (Fib). (Color figure available online only.)

This assumption has been recently tested in our laboratory by treating dermal fibroblasts or CRFs *in vitro* with a combination of compounds. These treatments could transiently upregulate the expressions of certain cell dedifferentiation-related genes, such as Oct4, c-Myc, Sox2, or Klf4, as well as promote the expression of cardiogenic conversion-associated transcription factors, such as MEF2, GATA4, and TBX5 genes, in treated dermal fibroblasts or CRFs with some beating myocytes of rat-tail-derived fibroblast origin observed [15]. More importantly and consistently, when rats with chronic MI were treated with the combination of compounds, myocardial conversion of the fibroblast in the scar region was observed with the global cardiac function significantly improved [15].

Taken together, we have provided examples of natural plant extracts or compounds, such as *R. glutinosa* (RGE), ginseng (e.g., Rg1 and Rb1), *S. miltiorrhiza* Bunge (e.g., tan IIA, salvianolic acid A and salvianolic acid B), *G. japonicum* Thunb. vax. chinense F. Bolle (e.g., EGJ, AFGJ, and cardiogenin), *R. laevigata* Michx fruit (e.g., aFRLM), and compound combinations for conversion of CRFs. These treatments can promote angiogenesis, prevent myocyte apoptosis, enhance the proliferation, migration, cardiogenic differentiation, and cardiogenic conversion of EPCs, MSCs, and CRFs for the treatment of CVD. Many other natural products are found to possess similar properties as well. For instance, Xiongshao Capsule, extracted from *Rhizoma Ligusticum Wallichii* and *Radix Paeonia Rubra*, can promote angiogenesis via upregulating VEGF and basic fibroblast growth factor (bFGF) expressions [118]. YiQiFuMai injection, a Chinese medicine with ginsenosides as its major constituents, was reported to exert cardioprotective effects to treat chronic heart failure [119]. Shuanglong formula (SLF), a Chinese medicine composed of *P. ginseng* and *S. miltiorrhiza*, was reported to have a therapeutic effect on MI in clinical practice. SLF was further simplified through a bioactivity-guided screening to finally attain a minimized composition (new formula NSLF6) while maintaining its therapeutic effect for MI. It was found that the administration of NSLF6 for the treatment of MI resulted in synergistic therapeutic efficacies between total ginsenosides and total salvianolic acids, probably due to its actions in promoting cardiac cell regeneration, therapeutic angiogenesis, and antagonizing myocyte oxidative damage [120]. It should be noted that a single compound that targets a single gene or gene product may not be sufficient to elicit a curative treatment for most CVDs since the pathology-modifying treatment of a disease, MI for example, involves especially complicated multifactor coordinated processes. However, a rational combination of compounds with their respective activities may achieve a curative treatment effect through a well-coordinated synergistic mechanism.

Conclusions
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1. Under physiological conditions of an adult human life, the regeneration rate of myocytes through mechanisms of inherited cardiac regeneration during cardiac homeostasis accounts for

the loss of ~ 3 × 10^6 myocytes per day, which is, however, severely limited in pathological cardiac repair [15].

2. Cardiovascular beneficial effects of the application of some natural plant compounds, such as *R. glutinosa*-derived RGE, ginseng-derived ginsenosides (Rg1, Rb1), *S. miltiorrhiza* Bunge-derived tan IIA, salvianolic acid A and salvianolic acid B, *G. japonicum* Thunb. vax. chinense F. Bolle-derived EGJ, AFGJ, and cardiogenin, *R. laevigata* Michx fruit-derived aFRLM, and active compound combinations, to enhance mechanisms of inherited cardiac regeneration include promoting angiogenesis, preventing myocyte apoptosis, enhancing the proliferation, migration, and cardiogenic differentiation of the progenitor cells and cardiogenic conversion of EPCs and CRFs.

3. Although previous studies have provided promising evidence of natural plant compounds to enhance mechanisms of inherited cardiac regeneration for the treatment of CVD, future studies in this field should focus more efforts on the isolation of the single compound possessing a particular activity contained in the active fraction or extract of the plant and on the elucidation of the underlying mechanism of a particular action of such a compound, so that a single compound with a specific activity or compound combination with desired multiple activities that are required for curative treatment of a particular disease can be developed.

Acknowledgements
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Conflict of Interest
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The authors claim no conflicts of interest.

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