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Research article

Transplantation of induced pluripotent stem cells-derived cardiomyocytes combined with modified Taohong Siwu decoction improved heart repair after myocardial infarction

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ABSTRACT

Objective: This study aimed to study whether modified Taohong Siwu decoction (MTHSWD) combined with human induced pluripotent stem cells-derived cardiomyocytes (iPS-CMs) transplantation can promote cardiac function in myocardial infarction (MI) nude mouse model and explore its possible mechanism.

Methods: The MI mouse model was established by the ligation of left anterior descending coronary artery. After 4 weeks of gavage of MTHSWD combined with iPS-CMs transplantation, the changes in heart function of mice were examined by echocardiography. The histological changes were observed by Masson's trichrome staining. The survival and differentiation of transplanted cells were detected by double immunofluorescence staining of human nuclear antigen (HNA) and cardiac troponin T (cTnT). The number of c-kit-positive cells in the infarct area were evaluated by immunofluorescent staining. The levels of stromal cell-derived factor 1 (SDF-1), stem cell factor (SCF), vascular endothelial growth factor (VEGF) and basic fibroblast growth factor in infarcted myocardium tissues were detected by ELISA.

Results: MTHSWD combined with iPS-CMs transplantation can improve the heart function of MI mice, reduce the infarct size and collagen deposition in infarct area. By immunofluorescence double-label detection of HNA and cTnT, it was found that MTHSWD combined with iPS-CMs transplantation can improve the survival and maturation of iPS-CMs. In addition, MTHSWD combined with iPS-CMs transplantation can activate more endogenous c-kit positive cardiac mesenchymal cells, and significantly increase the content of SDF-1, SCF and VEGF in myocardial tissues.

Conclusions: The combination of MTHSWD with iPS-CMs transplantation promoted cardiac function of nude mice with MI by improving the survival and maturation of iPS-CMs in the infarct area, activating the endogenous c-kit positive cardiac mesenchymal cells, and increasing paracrine.

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1. Introduction

Cardiovascular disease is a major occasion of age-related morbidity and mortality in the world, among which myocardial infarction (MI) is the most frequent cardiovascular disease [1]. The blockage of coronary artery is a major contributor to myocardial infarction, which induced local myocardial hypoxia and ischaemia, apoptosis and necrosis and collagen deposition in the infarct area, and finally lead to the decrease of myocardial contractility and even heart failure [2,3]. The reversibility of cardiac ischemic injury caused by coronary artery blockage mainly depends on the ability and time of reperfusion [4]. The survival of ischemic tissue can be reversed and the prognosis of patients can be significantly improved by medical treatment or percutaneous coronary intervention. However, due to the weak regeneration ability of cardiomyocytes, there is no treatment to regenerate new cardiomyocytes [5]. The only treatment for heart failure upon MI is heart transplantation, nevertheless, the immune rejection and lack of donors limited its clinical application [6]. Therefore, it is urgent to find novel treatment strategy for MI.

Traditional Chinese medicine (TCM) has a multi-target, multi-channel and multi-level overall regulatory effect to prevent and treat cardiovascular diseases, with mild efficacy and little side effects. Mounting studies have been administered in recent decades to estimate the effectiveness and safety of TCM in MI treatment. Studies have shown that TCM can inhibit cardiomyocyte apoptosis and inflammation, while enhance myocardial angiogenesis, reduce cardiac remodeling and improve heart function [7,8]. Taohong Siwu Decoction (THSWD) play an important role in prevention and treatment of myocardial injury [9]. Our previous studies also showed that THSWD can improve the local ischemic microenvironment and reduce mitochondrial fission, so as to enhance heart function upon MI [10]. However, the effects of THSWD on improvement of heart function after MI was moderate. Based on the understanding of etiology and pathogenesis of MI in TCM, four additional TCM herbs were added into THSWD to constitute modified THSWD (MTHSWD). Recently, we found that MTHSWD improved heart function after myocardial ischaemia and reperfusion through enhancing the mobilization of endogenous stem cell and regulating metabolites [11].

In recent years, instead of traditional treatment, the stem cell therapy has been paid more and more attention in MI therapy. Stem cells are a variation of primitive cells with a variety of differentiation potential that can differentiate into a variety of cells in specific environment. Plenty of researches have shown that stem cells can repair necrotic myocardium and improve heart function to a certain extent upon MI [12,13]. Induced pluripotent stem cell (iPS) is a stem cell type similar to embryonic stem cell (ESC), which is generated by the transfection of the four transcription factors (Oct4, Sox2, KLF4 and c-myc) into differentiated somatic cells. In similar with ESC, iPS has the potential of self-renewal and multi-directional differentiation. Besides, iPS cells can avoid ethical problems and immune rejection as homologous transplantation, which have gradually become a propitious source of cells for transplantation. Current research suggested that transplantation of iPS-derived cardiomyocytes (iPS-CMs) improved heart function and reduce infarct area upon MI [14,15]. The therapeutic efficacy of mesenchymal stem cells (MSCs) could be enhanced by Danhong Injection after MI [16]. In addition, we have found that Guanxin Danshen Formulation improved the effect of MSCs transplantation for the treatment of MI probably via enhancing the engraftment [17].

Therefore, based on previous research, this study aimed to investigate whether the MTHSWD combined with iPS-CMs transplantation could further improve the heart function after MI and explore the potential mechanisms.

2. Materials and methods

2.1. LC-MS analysis of MTHSWD extracts

Ultimate 3000 UHPLC system (Thermo Fisher, Orbitrap Elite, United States) was used to analyze the component of MTHSWD extracts. All chromatographic separations were performed on ACQUITY UPLC HSS T3 (100 × 2.1 mm 1.7 μm; Thermo Fisher Scientific). The mobile phase consisted of acetonitrile (A) and 0.1% formic acid aqueous solution at a flow rate of 0.3 ml/min, with gradient as 2–5% Mobile phase B over 10 min, 5–50% Mobile phase B over 10–20 min, 50–95% Mobile phase B over 20–25 min, 95% Mobile phase B over 25–30 min, 2% Mobile phase B over 30.5–35 min. The column temperature was set to 40 °C. A positive ion spray voltage of 3.8 k eV and a negative ion spray voltage of 3.2 k eV was used for electrospray ionization source (ESI). The capillary temperature was 350 °C; the sheath gas flow rate was 35 arb, the aux gas flow rate was 15 arb, the sweep gas flow rate was 1 arb; The mass scanning range was m/z 100~1000.

2.2. The induction of hiPS cell differentiation

hiPS (line number: DYR0100, kindly provided by Stem Cell Bank, Chinese Academy of Sciences) were subcultured in 12 well plates at the ratio of 1:6 and cultured in a 37 °C with 5% CO_2 incubator. When the cell density achieved 90%, iPS cells were induced with induction differentiation reagent I (CA2004500-1, Cellapy Biological Technology, Beijing, China) for 48 h and induction differentiation reagent II (CA2004500-2, Cellapy Biological Technology, Beijing, China) for additional 48 h. Then the iPS cells were incubated with induction differentiation reagent III (CA2004500-3, Cellapy Biological Technology, Beijing, China) until them grow into beating cells, and the cell medium was then changed to cardiomyocyte culture medium.

2.3. Animal grouping and treatment

The SPF level adult male nude mice (weighing 20–25 g), provided by the animal ethics committee of Shanghai University of TCM

(approval number: [PZSHUTCM190628003]), were randomly divided into 5 groups (12 mice in each group): 1) Sham group: thoracotomy was performed under anesthesia with isoflurane but the left anterior descending artery (LAD) was not ligated, and the mice were fed routinely every day. 2) MI group: after being anesthetized with isoflurane, the LAD was ligated to establish MI model and the mice were fed routinely every day. 3) MTHSWD group: after the successful development of MI model, nude mice were treated with MTHSWD by gavage for 4 weeks. The extraction of MTHSWD were the same as that in our published article [11]. The dosage of MTHSWD was. 4) iPS-CMs transplantation group: after the successful development of MI model, 10^5 iPS-CMs in 9 μL of DMEM were injected into the border of infarct area of nude mice at three separated points averagely and fed routinely every day. 5) MTHSWD + iPS-CMs transplantation group: after the successful establishment of MI model, 10^5 iPS-CMs in 9 μL of DMEM were injected into the border of infarct area of nude mice at three separated points averagely, and the mice were fed with MTHSWD by gastric infusion every day for 4 weeks.

2.4. Echocardiography

After 4 weeks of MTHSWD administration combined with iPS-CMs transplantation, the changes in cardiac function were detected by echocardiography. The nude mice were anesthetized with isoflurane and examined with a commercial echocardiography system (Vevo Visualsonics 2100, VisualSonics, Toronto, ON, Canada). Each measurement was obtained from three continuous measurements in M mode. The ejection fraction (EF, %) and fractional shortening (FS, %) of left ventricle (LV), as well as the left-ventricular end-systolic (LVESV) and end-diastolic volume (LVEDV) were measured by the echocardiography software. The operator who performed echocardiography was blinded to the animal treatments.

2.5. Masson's trichrome staining

After the echocardiography measurements, the mice were sacrificed and the hearts were cut into two parts transversally along the center of the infarct zone. The part close to cardiac base was fixed with 4% paraformaldehyde, embedded with optimal cutting temperature (OCT) compound (Sakura, USA), and then sliced into 10 μm sections on a cryomicrotome (HM525, Thermofisher, USA) at −20 °C. After being washed with the phosphate buffer solution (PBS, 0.01 M, pH 7.2~7.4), the sections were stained with Masson's trichrome according to the manufacturer's recommendations (BP-DL022, Senbeijia Biotechnology, China) and the images were evaluated with Image-Pro Plus software (Bethesda, USA). The infarct size and collagen content in the infarct area was calculated as below: infarct size (%) = (epicardial + endocardial infarct circumference)/(total LV epicardial + endocardial circumferences) × 100%. Collagen content (%) = blue area/total area in a filed × 100%.

2.6. Immunofluorescence staining

The sections were incubated with 0.5% Triton X-100 at room temperature for 15 min to promote the permeabilization of cell membrane. After being washed with 0.01 M PBS for three times, the nonspecific binding sites were blocked with normal goat serum at 37 °C for 30 min. Next, the sections were incubated with the primary antibody HNA (ab191181, Abcam, 1:100), c-kit (ab273119, Abcam, 1:200) and cTnT (ab209813, Abcam, 1:200) overnight at 4 °C and followed by reaction with corresponding secondary antibody (1:200; Invitrogen, USA) at 37 °C for 1 h. After being washed with 0.01 M PBS for three times, the sections were photographed under an IX53 fluorescence microscope (Olympus, Japan). For the verification of iPS and iPS-CM, immunofluorescence staining for OCT4, Nanog and cTnT were carried out.

2.7. ELISA detection

The samples close to cardiac apex were washed with 0.01 M PBS, weighed and added to the corresponding volume of PBS (weight/volume ratio: 1:9). After that, the tissues were homogenized and the supernatant was obtained by centrifuging (5000×*g*, 10 min) at 4 °C. For the detection with the ELISA kits (ml037712, ml063287, ml037273, ml037702; Enzyme-linked Biotechnology, China), each standard well was added with 50 μL standard sample, and the sample well was added with 50 μL tissue sample. Apart from the blank wells, 100 μL horseradish peroxidase labeled antibody was added into per well and incubated in an oven at 37 °C for 60 min. Then the liquid was poured out, and each well was washed with 300 μL washing solution for 5 times. Subsequently, each well was supplemented with 50 μL of substrate A and 50 μL of substrate B, and incubated at 37 °C in dark for 15 min. Eventually, the recreation was terminated by adding 50 μL termination solution, and the optical density (OD) value at 450 nm was determined.

2.8. Statistical analysis

Data were presented as means ± standard deviation. The statistical differences between groups were analyzed via One-way analysis of variance (ANOVA) with Scheffe's post hoc multiple-comparison analysis through IBM SPSS Statistics V23.0. $P < 0.05$ was deemed as statistically significant.

3. Results

3.1. HPLC analysis identified constituents of MTHSWD extracts

The mass scanning range was *m/z* 100~1000. All of the data were processed using Compound Discoverer (version 2.1). The retention time (RT) and mass spectrometric data of each chemical component in MTHSWD were obtained after UPLC-QTOF-MS detection, and 165 components were resolved from the collected data, as shown in Fig. 1A (positive ion modes) and Fig. 1B (negative ion modes) and Supplementary Table 1.

3.2. Characteristic and cardiomyocyte differentiation of iPS cells

hiPS cells grew clonally *in vitro*, with high nucleocytoplasmic ratio and obvious nucleolus. Through immunocytofluorescense staining, we found that iPS cells were positive for stem cell markers Oct4 and Nanog (Fig. 2A). After the induction towards cardiomyocytes, most of iPS-CMs positively expression the cardiomyocyte markers cTnT (Fig. 2B). The purity of hiPSC-CMs was 80%–90%, which proved that the cells can be used in subsequent *in vivo* transplantation experiments.

3.3. Effect of MTHSWD combined with iPS-CMs transplantation on cardiac function in MI nude mice

According to the data of the echocardiography, MTHSWD or iPS-CMs transplantation alone, and MTHSWD combined with iPS-CMs

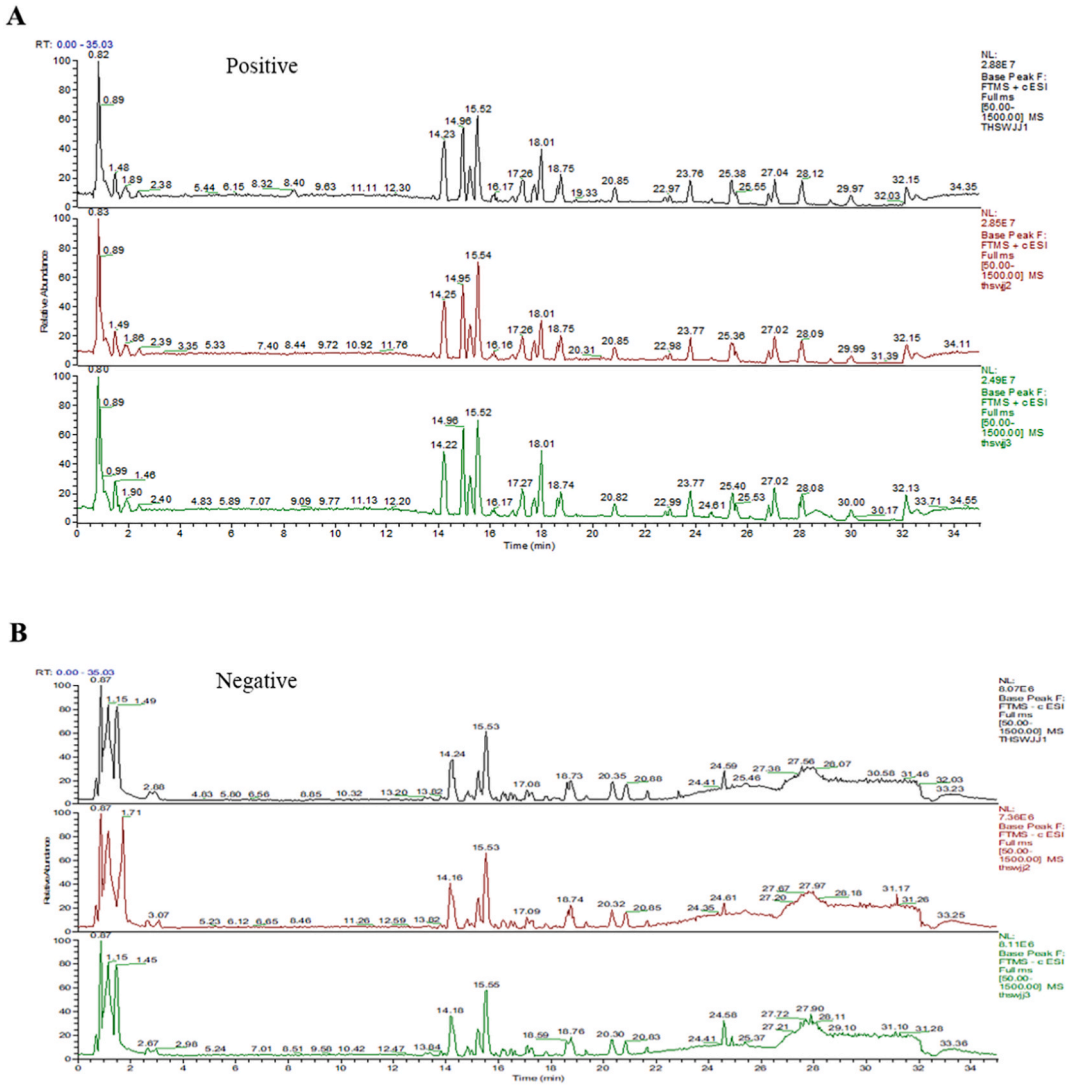

Fig. 1. Chemical composition analysis of MTHSWD. (A) The positive ion modes. (B) The negative ion modes.

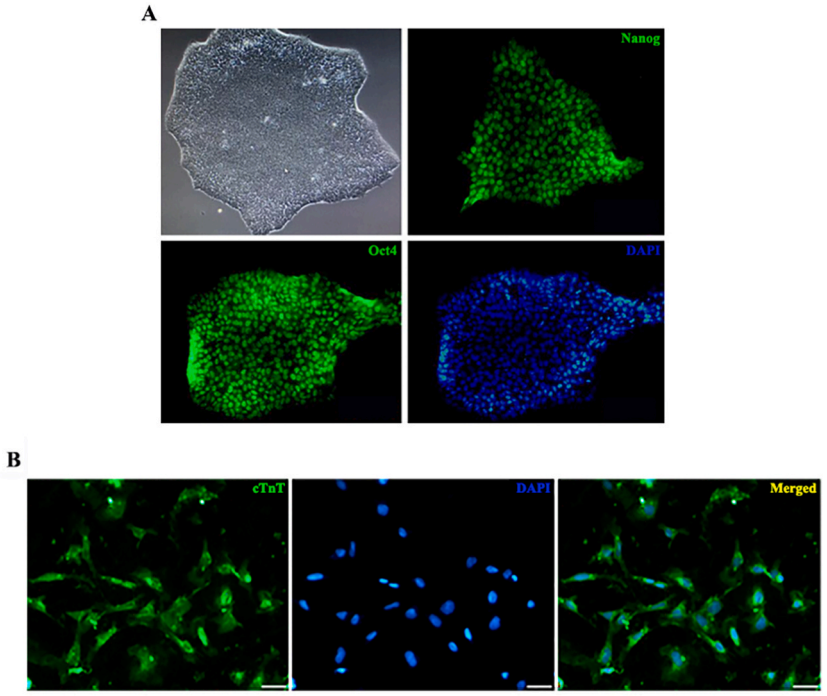

Fig. 2. Identification of iPS and the differentiation of iPS towards cardiomyocytes. (A) The expression of stem cell markers Oct4 and Nanog were detected by immunocytofluorescense staining. (B) Immunofluorescence staining for cTnT in iPS-CMs. Scale bar = 25 μm (B) or 50 μm (C).

transplantation (hereinafter referred to as the combined group) could increase the values of EF and FS in the hearts of nude mice (Fig. 3A–C). The values of EF and FS in the combined group were significantly higher than those in MTHSWD and iPS-CMs transplantation group (Fig. 3B and C). The comparison of LVIDs and LVESV values among groups were similar to the results of EF and FS (Fig. 3E–G). Compared with the model group, the MTHSWD group, iPS-CMs transplantation group and combined group could reduce the LVIDd and LVEDV values, but there was no significant difference among the MTHSWD group, iPS-CMs transplantation group and combined group (Fig. 3D–F).

3.4. MTHSWD combined with iPS-CMs transplantation reduced infarct area and collagen deposition in infarct area

According to Masson's trichrome staining, MTHSWD, iPS-CMs transplantation or combined treatment could reduce the infarct area and collagen deposition in infarct area. The infarct area and collagen deposition in the combined group were the smallest, which was statistically different from the MTHSWD group and iPS-CMs transplantation group (Fig. 4A–D). Compared with the MI group, the MTHSWD group, iPS-CMs transplantation group and combined group could reduce the ratio of heart weight to body weight, whereas no significant difference was observed in the three groups (Fig. 4E). The ventricular wall thickness of infarct area was measured and it was found that both MTHSWD and iPS-CMs transplantation treatment could increase the ventricular wall thickness of infarct area. In addition, no significant difference was obtained between the MTHSWD group and the iPS-CMs transplantation group. Moreover, the ventricular wall thickness of infarct area was the highest in the combined group, which was significantly higher than that in MTHSWD group and iPS-CMs transplantation group (Fig. 4F).

3.5. MTHSWD enhanced the survival of iPS-CMs after transplantation

As the iPS cells were generated from human, the survival of transplanted iPS-CMs was detected by immunofluorescence staining of human nuclear antigen (HNA). The number of transplanted cells in the combined group was markedly elevated in comparison to iPS-CMs group. By double immunofluorescence detection of HNA and cTnT, it was found that most transplanted cells were positive for cTnT. Interestingly, compared with iPS-CMs transplantation group, there were more transplanted cells expressed cardiomyocyte marker cTnT in the combined group (Fig. 5A–C). These data suggested that intragastric administration of MTHSWD not only promoted the survival of iPS-CMs in the hostile microenvironment, but also was beneficial to cardiomyocyte differentiation of iPS after transplantation.

3.6. MTHSWD combined with iPS-CMs transplantation increased endogenous c-kit cells in the infarct area

Previous research has proposed that c-kit positive cells are involved in tissue repair and regeneration after MI even though they

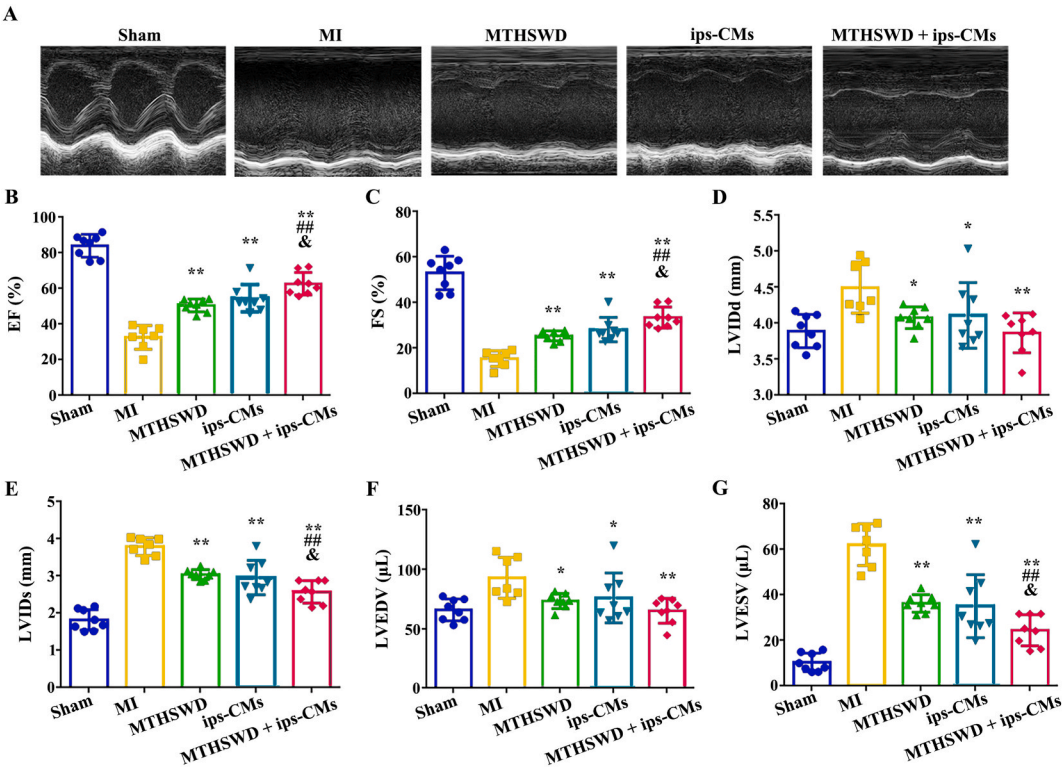

Fig. 3. Comparison of cardiac function of nude mice in each group after 4 weeks of treatment. (A) The changes in cardiac function were detected by echocardiography. (B–G) The values of EF, FS, LVIDs, LVIDd, LVEDV and LVESV were determined in each group. $^{*}P < 0.05$ and $^{**}P < 0.01$, vs the MI group; $\#\#P < 0.01$, vs the MTHSWD group; $\&P < 0.05$, vs the iPS-CMs group; MI indicates myocardial infarction; MTHSWD indicates modified Taohong Siwu Decoction. n = 7–8.

can't differentiate into cardiomyocytes. It was reported that although c-kit + cardiac mesenchymal cells cannot differentiate into cardiomyocytes, they obviously enhanced cardiac function by regulating the immune response after MI [18]. After MI, c-kit positive cells could be seen in the infarct area, but the number was not large. In comparison with the MI group, the number of c-kit positive cells in the infarct area was significantly increased in the MTHSWD group, iPS-CMs transplantation group and combined group (Fig. 6A). In addition, the amount of c-kit positive cells in the infarct area in the combined group was the largest, which was remarkably higher than that in the MTHSWD group and iPS-CMs transplantation group, whereas it was similar between the MTHSWD group and the iPS-CMs transplantation group (Fig. 6B).

3.7. Regulation of MTHSWD combined with iPS-CMs transplantation on paracrine

To investigate the effects of MTHSWD combined with iPS-CMs transplantation on paracrine after MI, the contents of SDF-1, SCF, VEGF and bFGF in the infarcted tissues were detected by ELISA. In comparison to the MI group, the contents of SDF-1, VEGF and bFGF in the MTHSWD group or iPS-CMs transplantation group was significantly elevated, as well as in the combined group (Fig. 7A–C, D). Compared with the MTHSWD group, the contents of SDF-1, SCF and VEGF, but not bFGF, in the combined group were significantly increased (Fig. 7A–D). Compared with iPS-CMs transplantation group, the levels of SDF-1 and VEGF in the combined group upregulated significantly, whereas no significant difference was found in the contents of SCF and bFGF (Fig. 7A–D).

4. Discussion

After MI, myocardial cells are ischemic and hypoxic in the infarct area, and the ischemic and hypoxic microenvironment is not conducive to the survival of transplanted cells. Hence, optimizing the local microenvironment might be an effective means to strengthen the effectivity of transplanted stem cells [19,20]. The microenvironment contains a large number of cytokines and extracellular matrix. These bioactive components can interact with stem cells and affect their survival, proliferation and differentiation. It was reported that cyclin D2 overexpression enhanced the efficacy of hips-CMs for myocardial repair after MI [21]. In addition, the survival of iPS or ES derived CMs post transplantation could be enhanced through many other strategies such as injection with single cells [22], transplantation with patch with large cell dose [23]. Recently, we found that MTHSWD could improve heart function and decrease infarct size after myocardial ischaemia and reperfusion through enhancing endogenous stem cell mobilization and

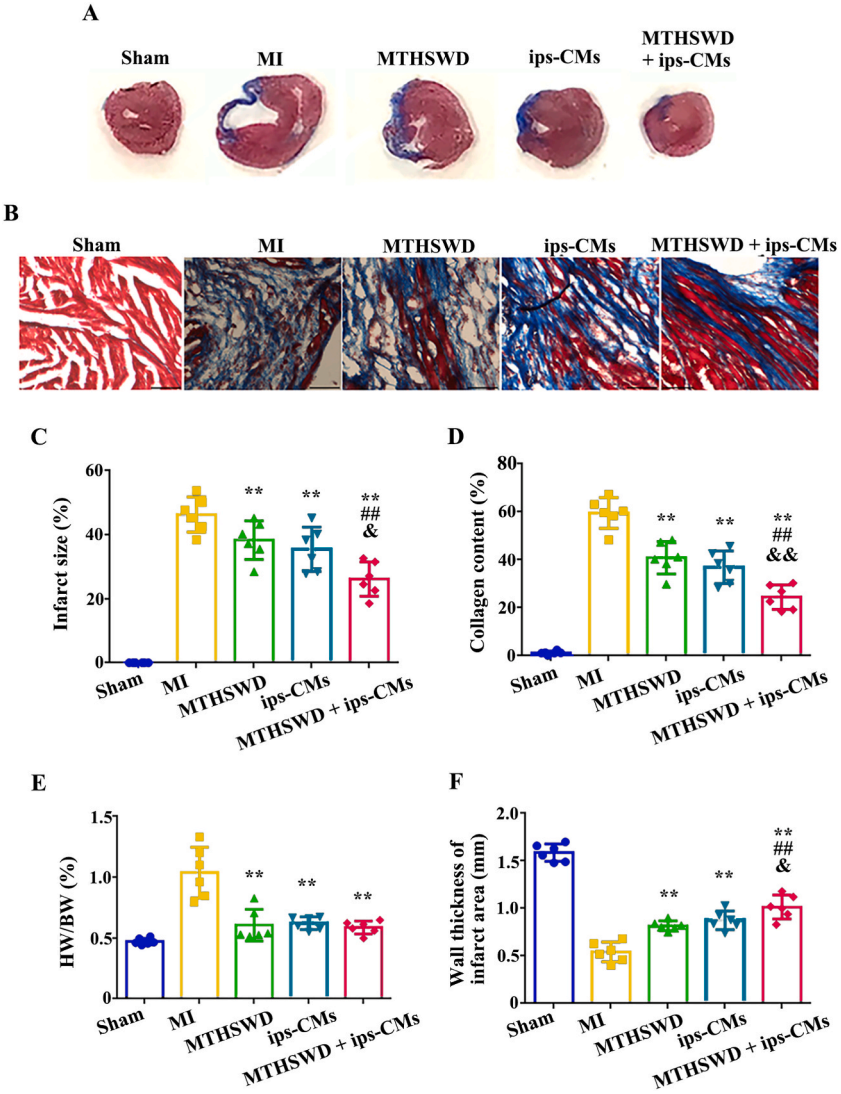

Fig. 4. Masson's trichrome staining and statistical analysis after 4 weeks of treatment. (A–D) The infarct area and collagen deposition in infarct area of each group was examined by Masson's trichrome staining. (E) The ratio of heart weight to body weight for each group. (F) The ventricular wall thickness of infarct area for each group. $^{**}P < 0.01$, vs the MI group; $\#\#P < 0.01$, vs the MTHSWD group; $\&P < 0.05$ and $\&\&P < 0.01$, vs the iPS-CMs group; Scale bar = 50 μm. n = 6.

regulating metabolites [11]. This study found that MTHSWD can improve the microenvironment after MI by regulating inflammatory factors and chemokines as well as enhancing angiogenesis, which was not only conducive to the protection of host cardiomyocytes, but also conducive to the survival of transplanted iPS-CMs. In fact, natural compounds play an effective role in stimulating and activating stem cells for myocardial regeneration [24]. Although the traditional Chinese medicine has been proved to be effective in the clinical practice, however the specific content that take effect are largely unclear. Recently, we found that some ingredients of MTHSWD including ginsenoside Rb3, tanshinone I, danshensu, dihydrotanshinone I, and tanshinone IIA could reduce the apoptosis of H9c2 in H_2O_2 injury model [25]. In this study, which possible content of MTHSWD extracts may play a role in enhancing cell survival or differentiation after transplantation is a key point to study in future.

The mechanism of MTHSWD combined with iPS-CMs transplantation to improve cardiac function may be related to promoting the survival of iPS-CMs, activating the endogenous progenitor cells, and enhancing paracrine. iPS-CMs maturation is very important to the success of stem cell transplantation, as the matured iPS-CMs may have the advantage of more normal physiological functions. It was found that cardiomyocytes produced by hiPS can express proteins such as Cx43 and myosin chain complex, which are necessary for phenotypic development of adult ventricular myocytes [26], and cardiomyocytes derived from iPS can beat spontaneously and were sensitive to adrenergic stimulation [27]. We found that hiPS-CMs that induced *in vitro* can express myocardial specific markers cTnT, which provided the basis for cell replacement after transplantation. Several studies have found that iPS-CMs can effectively enhance the activity of H9C2 cardiomyocytes and inhibit their apoptosis [28]. iPS-CMs can reduce the apoptosis of cardiomyocytes in diabetic

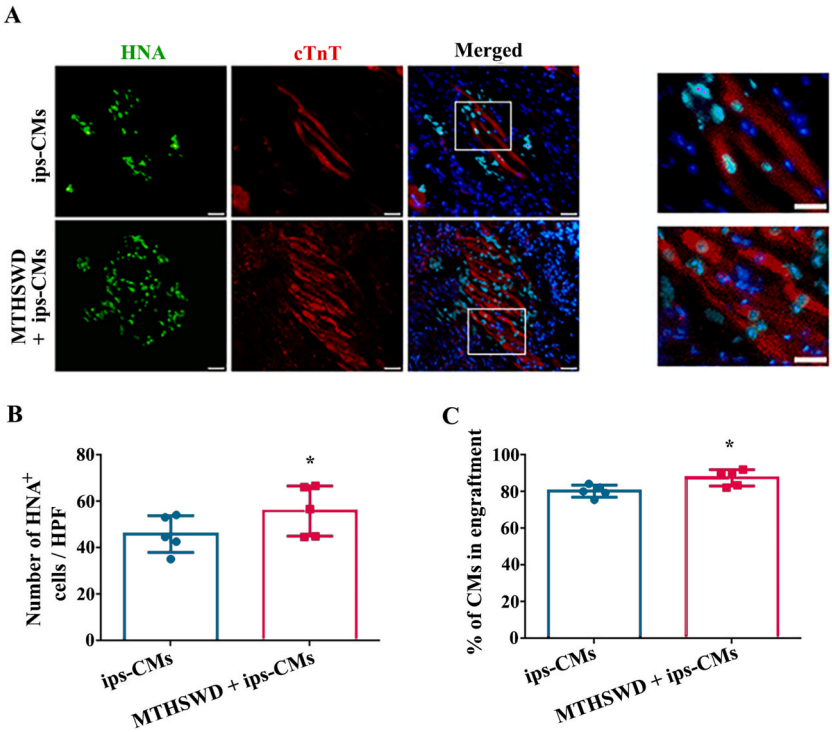

Fig. 5. Effect of MTHSWD on the survival and differentiation of transplanted cells. (A) Double immunofluorescence detection of HNA and cTnT. Scale bar = 25 μm. (B, C) The number of HNA positive cells and percentage of cTnT positive injected cells were analyzed. *$P < 0.05$, vs the iPS-CMs group. n = 5.

cardiomyopathy rats [29]. In this study, an obvious myocardial tissue and less collagen deposition were observed at the infarct site after the treatment of MTHSWD + ips-CMs. Most of these cardiomyocytes were compensated by iPS-CMs transplantation, and some survived cardiomyocytes may be related to the protection of host cells by MTHSWD combined with iPS-CMs transplantation.

Current studies believe that the paracrine pathway is a main mechanism of cell transplantation to improve cardiac function, which is related to inhibiting cardiomyocyte apoptosis [30], reducing inflammation [31] and promoting angiogenesis [32]. Studies have found that the supernatant of cultured stem cells secreted cytokines such as bFGF, NGF, VEGF and IGF-1, which can improve cell activity [33–35]. Paracrine factors of iPSCs were capable of inhibiting stress-derived apoptosis of H9C2 cells by impeding p53-p21 and p16-pRb signaling pathways [36]. Gene expression and immunohistochemical analysis showed that iPS-CMs transplantation could significantly upregulate TNF-α and VEGF expression [37]. Zhao et al. [38] suggested that hypoxia treated cells could express more VEGF and IGF-1, suggesting that paracrine pathway is involved in the mechanism of iPS-CMs transplantation in the treatment of MI. In present research, we found that MTHSWD combined with iPS-CMs transplantation significantly increased the level of SDF-1 in myocardial tissue. SDF-1 belongs to chemokine CXC and exerts crucial regulatory role in stem cell mobilization and recruitment and myocardial protection. After SDF-1 binds to its receptor CXCR4, it can activate a variety of signal transduction pathways to regulate cell chemotaxis, movement, adhesion and angiogenesis. SDF-1 combined with CXCR4 receptor can elevate the level of CD9, thereby affecting the mobilization of stem cells to damaged muscles, so as to improve skeletal muscle regeneration [39]. The therapeutic efficacy of the SDF-1/CXCR4 interaction for MSC transplantation could be improved through some strategies, such as the combination of chemokines and cytokines, hormones and drugs, hypoxia, gene engineering, and biomaterials [40]. Transplantation of iPS-derived neural progenitor cells with SDF-1 overexpression can promote the repair of endogenous nerves and vessels after ischemic stroke in mice [41]. Studies have shown that overexpression of SDF-1 can protect cardiac function upon acute MI in diabetic mice [42]. Herein, the contents of SCF, VEGF and bFGF were significantly increased in the combined group. As an acidic glycoprotein produced by stromal cells, SCF mainly functions in early hematopoietic stem cells and primitive hematopoietic progenitor cells, and can affect the survival, proliferation and differentiation of stem cells. PP2A regulates SCF-induced the migration of stem cells by interacting with p38/MAPK pathway [43]. VEGF and bFGF can promote angiogenesis. Therefore, MTHSWD combined with iPS transplantation can promote the release of cytokines such as SDF-1, SCF, VEGF and bFGF through paracrine pathway, so as to improve the microenvironment after MI and enhance the survival of transplanted cells in the infarct area.

Previous research reported that although c-kit + cardiac mesenchymal cells cannot differentiate into cardiomyocytes, they can enhance cardiac function by regulating the immune response after MI [18]. After MI, c-kit positive cells can be seen in the infarct area although the number is small. It has been found that IGF-1 could interact with miR-193a to enhance the proliferation and migration of c-kit-positive cells by triggering PI3K/Akt/DNMT signaling pathway [44]. Transplantation of c-kit positive cardiac progenitor cell (CPC) sheet promoted angiogenesis and improved myocardial remodeling in rats with MI [45]. We found that compared with the MI

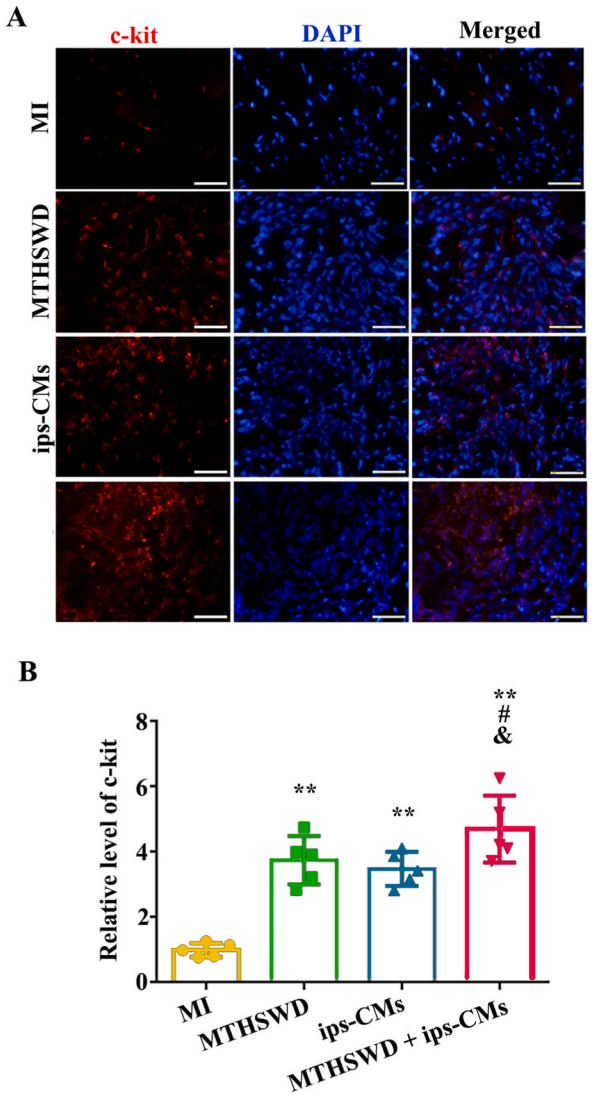

Fig. 6. Comparison of endogenous c-kit positive cells in infarct area after 4 weeks of treatment. (A) The expression of c-kit in each group were detected by immunofluorescence staining. (B) The expression of c-kit was analyzed in each group. $^{**}P < 0.01$, vs the MI group; $\#P < 0.05$, vs the MTHSWD group; $\&P < 0.05$, vs the iPS-CMs group; Scale bar = 25 μm. n = 5.

group, the number of c-kit positive cells was significantly increased in the infarct area of the MTHSWD group, iPS-CMs transplantation group and combined group, especially in the combined group. According to the significant paracrine, we speculated that the elevated number of c-kit positive cells was related to the high level of SDF-1 and SCF in the combined group. Co-injection of CPC and bone marrow MSC can improve the recruitment of donor cells and promote the secretion of a variety of angiogenic factors, and the effect of co-transplantation of CPC and bone marrow MSC on improving cardiac function is significantly better than that in single CPC or MSC transplantation group [46]. Therefore, activating endogenous stem cell migration may also be another possible mechanism of MTHSWD combined with iPS-CMs transplantation to improve cardiac function.

5. Conclusion

In summary, current study showed that MTHSWD combined with iPS-CMs transplantation improved the survival and maturation of iPS-CMs in the infarct area, activated endogenous c-kit positive cells, enhanced the paracrine pathway, thereby reducing the infarct area and improving cardiac function after MI. This study provided a novel strategy for the treatment of cardiovascular diseases such as MI.

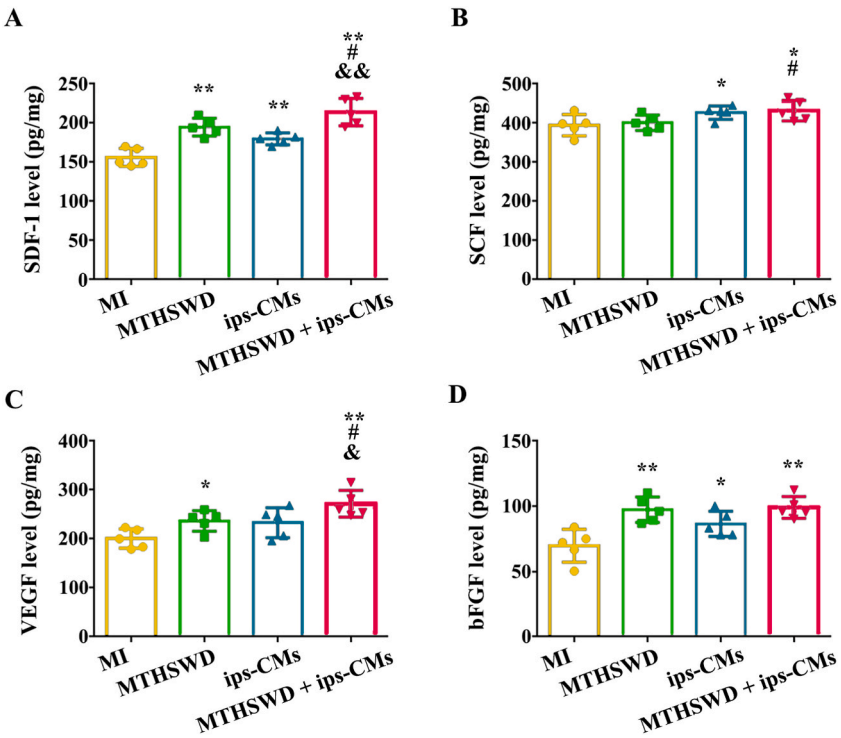

Fig. 7. The expression of cytokines in each group was detected by ELISA. The contents of SDF-1 (A), SCF (B), VEGF (C) and bFGF (D) were detected respectively. $^{*}P < 0.05$ and $^{**}P < 0.01$, vs the MI group; $\#P < 0.05$, vs the MTHSWD group; $\&P < 0.05$ and $\&\&P < 0.01$, vs the iPS-CMs group. n = 5.

Ethics declarations

This study was reviewed and approved by the animal ethics committee of Shanghai University of TCM, with the approval number: [PZSHUTCM190628003].

Data availability

Data are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Zhi-rong Luo: Writing – original draft, Methodology, Investigation. **Wan-ting Meng:** Validation, Methodology, Investigation. **Han Li:** Validation, Resources, Methodology. **Yu Wang:** Software, Investigation. **Ya-chao Wang:** Validation, Methodology. **Yue Zhao:** Investigation. **Ping-ping Lu:** Resources, Methodology. **Yuan Yuan:** Writing – review & editing, Supervision, Project administration. **Wei Huang:** Writing – review & editing, Validation, Supervision, Investigation. **Hai-dong Guo:** Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26700.

References

[1] J.A. Finegold, P. Asaria, D.P. Francis, Mortality from ischaemic heart disease by country, region, and age: statistics from World Health Organisation and United Nations, Int. J. Cardiol. 168 (2013) 934–945.
[2] L.H. Opie, P.J. Commerford, B.J. Gersh, M.A. Pfeffer, Controversies in ventricular remodelling, Lancet 367 (2006) 356–367.
[3] J.L. Anderson, D.A. Morrow, Acute myocardial infarction, N. Engl. J. Med. 376 (2017) 2053–2064.
[4] I.M. Seropian, S. Toldo, B.W. Van Tassell, A. Abbate, Anti-inflammatory strategies for ventricular remodeling following ST-segment elevation acute myocardial infarction, J. Am. Coll. Cardiol. 63 (2014) 1593–1603.
[5] S.T. Ji, H. Kim, J. Yun, J.S. Chung, S.M. Kwon, Promising therapeutic strategies for mesenchymal stem cell-based cardiovascular regeneration: from cell priming to tissue engineering, Stem Cell. Int. 2017 (2017) 3945403.
[6] T. Ma, J. Sun, Z. Zhao, W. Lei, Y. Chen, X. Wang, J. Yang, Z. Shen, A brief review: adipose-derived stem cells and their therapeutic potential in cardiovascular diseases, Stem Cell Res. Ther. 8 (2017) 124.
[7] X.H. Wang, G.P. Li, W.S. Yang, Z.Q. Jiao, H.M. Liu, Y.P. Ni, Cardioprotective effects of traditional Chinese medicine Guanmaitong on acute myocardial infarction, Exp. Ther. Med. 12 (2016) 3927–3933.
[8] D.P. Xu, D.Z. Zou, H.L. Qiu, H.L. Wu, Traditional Chinese medicine ShenZhuGuanXin Granules Mitigate cardiac Dysfunction and promote myocardium angiogenesis in myocardial infarction rats by upregulating PECAM-1/CD31 and VEGF expression, Evid. Based Complement Alternat. Med. 2017 (2017) 5261729.
[9] C.L. Shao, G.H. Cui, H.D. Guo, Effects and mechanisms of Taohong Siwu decoction on the prevention and treatment of myocardial injury, Front. Pharmacol. 13 (2022) 816347.
[10] Z.R. Luo, H. Li, Z.X. Xiao, S.J. Shao, T.T. Zhao, Y. Zhao, F.F. Mou, B. Yu, H.D. Guo, Taohong Siwu decoction exerts a beneficial effect on cardiac function by possibly improving the microenvironment and decreasing mitochondrial fission after myocardial infarction, Cardiol. Res. Pract. 2019 (2019) 5198278.
[11] W.T. Meng, Z.X. Xiao, H. Li, Y.C. Wang, Y. Zhao, Y. Zhu, H.D. Guo, Modified Taohong Siwu decoction improves cardiac function after myocardial ischaemia and reperfusion in rats by promoting endogenous stem cell mobilization and regulating metabolites, Pharm. Biol. 60 (2022) 1721–1731.
[12] F. Huang, M.L. Li, Z.F. Fang, X.Q. Hu, Q.M. Liu, Z.J. Liu, L. Tang, Y.S. Zhao, S.H. Zhou, Overexpression of MicroRNA-1 improves the efficacy of mesenchymal stem cell transplantation after myocardial infarction, Cardiology 125 (2013) 18–30.
[13] X. Li, Y.D. Hu, Y. Guo, Y. Chen, D.X. Guo, H.L. Zhou, F.L. Zhang, Q.N. Zhao, Safety and efficacy of intracoronary human umbilical cord-derived mesenchymal stem cell treatment for very old patients with coronary chronic total occlusion, Curr. Pharmaceut. Des. 21 (2015) 1426–1432.
[14] H. Masumoto, T. Ikuno, M. Takeda, H. Fukushima, A. Marui, S. Katayama, T. Shimizu, T. Ikeda, T. Okano, R. Sakata, J.K. Yamashita, Human iPS cell-engineered cardiac tissue sheets with cardiomyocytes and vascular cells for cardiac regeneration, Sci. Rep. 4 (2014) 6716.
[15] O. Iglesias-García, S. Baumgartner, L. Macrí-Pellizzeri, J.R. Rodriguez-Madoz, G. Abizanda, E. Guruceaga, E. Albiasu, D. Corbacho, C. Benavides-Vallve, M. Soriano-Navarro, S. González-Granero, J.J. Gavira, B. Krausgrill, M. Rodriguez-Mañero, J.M. García-Verdugo, C. Ortiz-De-Solorzano, M. Halbach, J. Hescheler, B. Pelacho, F. Prósper, Neuregulin-1β induces mature ventricular cardiac differentiation from induced pluripotent stem cells contributing to cardiac tissue repair, Stem Cell. Dev. 24 (2015) 484–496.
[16] J. Chen, J. Wei, Y. Huang, Y. Ma, J. Ni, M. Li, Y. Zhu, X. Gao, G. Fan, Danhong Injection enhances the therapeutic efficacy of mesenchymal stem cells in myocardial infarction by promoting angiogenesis, Front. Physiol. 9 (2018) 991.
[17] X.J. Han, H. Li, C.B. Liu, Z.R. Luo, Q.L. Wang, F.F. Mou, H.D. Guo, Guanxin Danshen Formulation improved the effect of mesenchymal stem cells transplantation for the treatment of myocardial infarction probably via enhancing the engraftment, Life Sci. 233 (2019) 116740.
[18] R.J. Vagnozzi, M. Maillet, M.A. Sargent, H. Khalil, A. Johansen, J.A. Schwanekamp, A.J. York, V. Huang, M. Nahrendorf, S. Sadayappan, J.D. Molkentin, An acute immune response underlies the benefit of cardiac stem cell therapy, Nature 577 (2020) 405–409.
[19] M. Hu, G. Guo, Q. Huang, C. Cheng, R. Xu, A. Li, N. Liu, S. Liu, The harsh microenvironment in infarcted heart accelerates transplanted bone marrow mesenchymal stem cells injury: the role of injured cardiomyocytes-derived exosomes, Cell Death Dis. 9 (2018) 357.
[20] J.E. Oh, C. Jung, Y.S. Yoon, Human induced pluripotent stem cell-derived vascular cells: recent progress and future directions, J. Cardiovasc. Dev. Dis. 8 (2021) 148.
[21] M. Zhao, Y. Nakada, Y. Wei, W. Bian, Y. Chu, A.V. Borovjagin, M. Xie, W. Zhu, T. Nguyen, Y. Zhou, V. Serpooshan, G.P. Walcott, J. Zhang, Cyclin D2 overexpression enhances the efficacy of human induced pluripotent stem cell-derived cardiomyocytes for myocardial repair in a swine model of myocardial infarction, Circulation 144 (2021) 210–228.
[22] Y. Shiba, S. Fernandes, W.Z. Zhu, D. Filice, V. Muskheli, J. Kim, N.J. Palpant, J. Gantz, K.W. Moyes, H. Reinecke, B. Van Biber, T. Dardas, J.L. Mignone, A. Izawa, R. Hanna, M. Viswanathan, J.D. Gold, M.I. Kotlikoff, N. Sarvazyan, M.W. Kay, C.E. Murry, M.A. Laflamme, Human ES-cell-derived cardiomyocytes electrically couple and suppress arrhythmias in injured hearts, Nature 489 (2012) 322–325.
[23] E. Querdel, M. Reinsch, L. Castro, D. Köse, A. Bähr, S. Reich, B. Geertz, B. Ulmer, M. Schulze, M.D. Lemoine, T. Krause, M. Lemme, J. Sani, A. Shibamiya, T. Stüdemann, M. Köhne, C.V. Bibra, N. Hornaschewitz, S. Pecha, Y. Nejahsie, F. Weinberger, Human engineered heart tissue patches remuscularize the injured heart in a dose-dependent manner, Circulation 143 (2021) 1991–2006.
[24] C. Carresi, M. Scicchitano, F. Scarano, R. Macrì, F. Bosco, S. Nucera, S. Ruga, M.C. Zito, R. Mollace, L. Guarnieri, A.R. Coppoletta, M. Gliozzi, V. Musolino, J. Maiuolo, E. Palma, V. Mollace, The potential properties of natural compounds in cardiac stem cell activation: their role in myocardial regeneration, Nutrients 13 (2021) 275.
[25] Y.C. Wang, H. Wang, C.L. Shao, X.Y. Li, J. Cui, H.D. Guo, Screening and identification of effective components from modified Taohong Siwu decoction for protecting H9c2 cells from damage, In Vitro Cell. Dev. Biol. Anim. 59 (2023) 346–355.
[26] C. Gardiner, D. Di Vizio, S. Sahoo, C. Théry, K.W. Witwer, M. Wauben, A.F. Hill, Techniques used for the isolation and characterization of extracellular vesicles: results of a worldwide survey, J. Extracell. Vesicles 5 (2016) 32945.
[27] D.K. Jeppesen, M.L. Hvam, B. Primdahl-Bengtson, A.T. Boysen, B. Whitehead, L. Dyrskjøt, T.F. Orntoft, K.A. Howard, M.S. Ostenfeld, Comparative analysis of discrete exosome fractions obtained by differential centrifugation, J. Extracell. Vesicles 3 (2014) 25011.
[28] X. Guo, X. Gu, S. Hareshwaree, X. Rong, L. Li, M. Chu, Induced pluripotent stem cell-conditional medium inhibits H9C2 cardiomyocytes apoptosis via autophagy flux and Wnt/β-catenin pathway, J. Cell Mol. Med. 23 (2019) 4358–4374.
[29] S. Neel, D.K. Singla, Induced pluripotent stem (iPS) cells inhibit apoptosis and fibrosis in streptozotocin-induced diabetic rats, Mol. Pharm. 8 (2011) 2350–2357.
[30] L. Ye, Y.H. Chang, Q. Xiong, P. Zhang, L. Zhang, P. Somasundaram, M. Lepley, C. Swingen, L. Su, J.S. Wendel, J. Guo, A. Jang, D. Rosenbush, L. Greder, J. R. Dutton, J. Zhang, T.J. Kamp, D.S. Kaufman, Y. Ge, J. Zhang, Cardiac repair in a porcine model of acute myocardial infarction with human induced pluripotent stem cell-derived cardiovascular cells, Cell Stem Cell 15 (2014) 750–761.
[31] S.W. Kim, M. Houge, M. Brown, M.E. Davis, Y.S. Yoon, Cultured human bone marrow-derived CD31(+) cells are effective for cardiac and vascular repair through enhanced angiogenic, adhesion, and anti-inflammatory effects, J. Am. Coll. Cardiol. 64 (2014) 1681–1694.
[32] T. Kinnaird, E. Stabile, M.S. Burnett, C.W. Lee, S. Barr, S. Fuchs, S.E. Epstein, Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms, Circ. Res. 94 (2004) 678–685.
[33] S.H. Ranganath, O. Levy, M.S. Inamdar, J.M. Karp, Harnessing the mesenchymal stem cell secretome for the treatment of cardiovascular disease, Cell Stem Cell 10 (2012) 244–258.
[34] C.A. Ribeiro, J.S. Fraga, M. Grãos, N.M. Neves, R.L. Reis, J.M. Gimble, N. Sousa, A.J. Salgado, The secretome of stem cells isolated from the adipose tissue and Wharton jelly acts differently on central nervous system derived cell populations, Stem Cell Res. Ther. 3 (2012) 18.
[35] S. Pluchino, C. Cossetti, How stem cells speak with host immune cells in inflammatory brain diseases, Glia 61 (2013) 1379–1401.
[36] Y. Zhang, D. Wang, K. Cao, M. Chen, X. Yang, Y. Tao, Rat induced pluripotent stem cells protect H9C2 cells from cellular senescence via a paracrine mechanism, Cardiology 128 (2014) 43–50.

[37] A. Tachibana, M.R. Santoso, M. Mahmoudi, P. Shukla, L. Wang, M. Bennett, A.B. Goldstone, M. Wang, M. Fukushi, A.D. Ebert, Y.J. Woo, E. Rulifson, P.C. Yang, Paracrine effects of the pluripotent stem cell-derived cardiac myocytes salvage the injured myocardium, Circ. Res. 121 (2017) e22–e36.
[38] X. Zhao, H. Chen, D. Xiao, H. Yang, I. Itzhaki, X. Qin, T. Chour, A. Aguirre, K. Lehmann, Y. Kim, P. Shukla, A. Holmström, J.Z. Zhang, Y. Zhuge, B.C. Ndoye, M. Zhao, E. Neofytou, W.H. Zimmermann, M. Jain, J.C. Wu, Comparison of non-human primate versus human induced pluripotent stem cell-derived cardiomyocytes for treatment of myocardial infarction, Stem Cell Rep. 10 (2018) 422–435.
[39] E. Brzoska, K. Kowalski, A. Markowska-Zagrajek, M. Kowalewska, R. Archacki, I. Plaskota, W. Stremińska, K. Jańczyk-Ilach, M.A. Ciemerych, Sdf-1 (CXCL12) induces CD9 expression in stem cells engaged in muscle regeneration, Stem Cell Res. Ther. 6 (2015) 46.
[40] Q. Jiang, K. Huang, F. Lu, S. Deng, Z. Yang, S. Hu, Modifying strategies for SDF-1/CXCR4 interaction during mesenchymal stem cell transplantation, Gen. Thorac. Cardiovasc. Surg. 70 (2022) 1–10.
[41] M. Chau, T.C. Deveau, M. Song, Z.Z. Wei, X. Gu, S.P. Yu, L. Wei, Transplantation of iPS cell-derived neural progenitors overexpressing SDF-1α increases regeneration and functional recovery after ischemic stroke, Oncotarget 8 (2017) 97537–97553.
[42] M.E. Mayorga, M. Kiedrowski, P. Mccallinhart, F. Forudi, J. Ockunzzi, K. Weber, W. Chilian, M.S. Penn, F. Dong, Role of SDF-1:CXCR4 in impaired post-myocardial infarction cardiac repair in diabetes, Stem Cells Transl. Med. 7 (2018) 115–124.
[43] Y. Wang, Y. Xia, D. Kuang, Y. Duan, G. Wang, PP2A regulates SCF-induced cardiac stem cell migration through interaction with p38 MAPK, Life Sci. 191 (2017) 59–67.
[44] Y. Sun, R. Xu, J. Huang, Y. Yao, X. Pan, Z. Chen, G. Ma, Insulin-like growth factor-1-mediated regulation of miR-193a expression promotes the migration and proliferation of c-kit-positive mouse cardiac stem cells, Stem Cell Res. Ther. 9 (2018) 41.
[45] K. Dergilev, Z. Tsokolaeva, P. Makarevich, I. Beloglazova, E. Zubkova, M. Boldyreva, E. Ratner, D. Dyikanov, M. Menshikov, A. Ovchinnikov, F. Ageev, Y. Parfyonova, C-kit cardiac progenitor cell based cell sheet improves vascularization and attenuates cardiac remodeling following myocardial infarction in rats, BioMed Res. Int. 2018 (2018) 3536854.
[46] L. Bao, Q. Meng, Y. Li, S. Deng, Z. Yu, Z. Liu, L. Zhang, H. Fan, C-kit positive cardiac stem cells and bone marrow-derived mesenchymal stem cells synergistically enhance angiogenesis and improve cardiac function after myocardial infarction in a paracrine manner, J. Card. Fail. 23 (2017) 403–415.