Stem/Progenitor Cells and Their Therapeutic Application in Cardiovascular Disease

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Cardiovascular disease is the leading cause of death in the world. The stem/progenitor cell-based therapy has emerged as a promising approach for the treatment of a variety of cardiovascular diseases including myocardial infarction, stroke, peripheral arterial disease, and diabetes. An increasing number of evidence has shown that stem/progenitor cell transplantation could replenish damaged cells, improve cardiac and vascular functions, and repair injured tissues in many pre-clinical studies and clinical trials. In this review, we have outlined the major types of stem/progenitor cells, and summarized the studies in applying these cells, especially endothelial stem/progenitor cells and their derivatives, in the treatment of cardiovascular disease. Here the strategies used to improve the stem/progenitor cell-based therapies in cardiovascular disease and the challenges with these therapies in clinical applications are also reviewed.

Keywords: stem cells, progenitor cells, endothelial progenitor cells, cardiovascular disease, cell therapy

INTRODUCTION

According to the recent study, cardiovascular diseases (CVDs) are highly prevalent globally and produce immense health and economic burdens in the United States and the world (Writing Group Members et al., 2016). The pathophysiological and physiological changes accompanied with vascular aging lead to compromised cardiovascular functions and elevated risks of CVDs including atherosclerosis, hypertension, and diabetes in elder population (El Assar et al., 2012). Peripheral arterial disease (PAD) and coronary heart disease including myocardial infarction (MI) account for most of all CVDs (Writing Group Members et al., 2016). Except for genetic defects, most CVDs can be attributed to unhealthy lifestyle factors such as high fat diet, high salt diet, and smoking (Writing Group Members et al., 2016).

With the advances in our understanding of the underlying mechanisms of CVDs, breakthrough has been achieved in diagnosis and intervention, such as percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG), and heart transplantation. However, to some degree, these approaches can only delay the heart failure (Trevelyan et al., 2005). This burden of disease has driven the investigation of stem/progenitor cell-based therapy for the CVDs. Experimental studies suggested that administration of endogenous stem/progenitor cells may contribute to functional regeneration of infarcted myocardium and repair damaged/injured endothelial cells (Xu, 2006).

Over the past decades, stem or progenitor cell-based therapy has emerged as a promising approach for the treatment of various CVDs, such as MI, heart failure, and PAD (Wollert and Drexler, 2010). The efficacy of various stem/progenitor cells including endothelial progenitor cells (EPCs) (Leistner et al., 2011), hematopoietic stem cells (HSCs) (Perin et al., 2012), cardiac stem cells...
(CSCs) (Makkar et al., 2012), and bone-marrow derived mononuclear cells (MNCs) (Wollert et al., 2017) in treating CVDs has already been evaluated in clinical trials. The potential therapeutic applications of stem/progenitor cells, such as embryonic stem cells (ESCs) (Shiba et al., 2012) and mesenchymal stem cells (MSCs) (Min et al., 2002), have been investigated in experimental and preclinical studies. As endothelial dysfunction is one of the major problems for CVDs, there is an increasing interest and ongoing efforts to study EPCs and other stem/progenitor cell-derived endothelial cells as potential sources for cell therapy (Reed et al., 2013). Here we summarize the studies using endothelial stem/progenitor cells and their derivatives, or sometimes oversimplified as “EPCs,” in the treatment of CVDs.

**CELL SPECTRUM OF STEM/PROGENITOR CELL DERIVED ENDOTHELIAL CELLS**

**MSCs**

Mesenchymal stem cells were originally identified and characterized by Friedenstein et al. (1976) in the 1970s. MSCs have been found in multiple organs throughout the body including bone marrow (BM), umbilical cord, placenta, dental pulp, and adipose tissue and their characteristics have been reviewed recently (Karantalis and Hare, 2015). MSCs derived from BM, adipose tissue, and umbilical cord have been widely used in preclinical and clinical trials. It has been reported that adipose-derived stromal/stem cells (ASCs) possess strong angiogenic potential and paracrine activities (Bura et al., 2014).

Early phase clinical trials have shown that ASC transplantation has improved rest pain, ulcer surface, walking distance, pain-free walking time, and transcutaneous oxygen pressure in PAD patients (Lee et al., 2012). MSCs possess several advantages as one of the promising candidates for stem/progenitor cell-based therapy: First, MSCs are easy to isolate and expand; Second, MSCs can secrete growth factors or directly differentiate into vascular cells or myocytes to contribute to arteriogenesis and angiogenesis (Wingate et al., 2014); Third, MSCs hold an immunoregulatory capacity and immunosuppressive effect indicating their potential of autotransplantation (De Miguel et al., 2012). These advantages enable MSCs to improve the neovascularization and blood flow in PAD and MI related ischemic tissues (Iwase et al., 2005; Gnecchi et al., 2006). Though with limitations such as the low retention and survival of transplanted MSCs (Mueller-Ehmsen et al., 2006), the cell pretreatment and genetic engineering approaches will provide a promising future for MSC based therapy (Li et al., 2007).

**iPSCs**

Induced pluripotent stem cells (iPSCs), which exhibit pluripotent differentiation and self-renewal potential that are similar to that of ESCs, was originally reported by Takahashi et al. (2007). By introducing four essential transcription factors (Oct3/4, Sox2, c-Myc, and Klf4) into fibroblasts, Yamanaka and colleagues have reprogrammed the cells into pluripotent stem cells. Using this technique, they and others have shown that iPSCs can be differentiated into endothelial cells (Sivarapatna et al., 2015). Studies have shown that iPSC derived endothelial cells are capable of angiogenesis and reendothelialization to form vascular networks in vitro (Suzuki et al., 2012). Preclinical studies also showed the promising therapeutic potential of iPSCs (Gu et al., 2012). Although teratoma formation (Seminatore et al., 2010) and the potential of tumorigenicity of transplanted cells (Yamanaka, 2012) are challenges in the clinical applications of iPSCs, iPSCs generated via non-genetic based techniques (Rhee et al., 2011) will improve the safety to overcome those disadvantage. Because iPSCs can be derived from mature somatic cells, the cell source is easy to obtain. Furthermore, the source of iPSCs can be autologous, so there is no need for immunosuppression when delivery. These features make iPSCs an attractive cell source for regenerative medicine.

**AFSCs**

Amniotic fluid derived stem cells (AFSCs) have been documented to be a special type of stem cells that possess a comprehensive multi-differentiation potential (Romani et al., 2015). Preclinical studies have shown that AFSCs can differentiate into vascular cell lineages to improve blood supply (Maraldi et al., 2013) or promote the regeneration of myocytes through their paracrine effects (Bollini et al., 2011). Besides, AFSCs also possess several advantages which make them a potential therapeutic approach. First, AFSCs are easy to be obtained from amniocentesis specimens which are used for prenatal genetic diagnosis. Second, the obtained ASFCs, which are c-Kit positive, can be readily expanded ex vivo with a doubling time of 36 h. Third, ASFCs can be differentiated into cell types including adipogenic, osteogenic, myogenic, endothelial, neuronal, and hepatic lineages (Romani et al., 2015). More importantly, it has been recently reported that AFSCs can induce immunosuppressive activities of regulatory T cells (Tregs) to promote allograft survival in animal models of allogeneic transplantation (Romani et al., 2015). With more extensive studies being conducted, detailed molecular mechanisms have been proposed. A most recent study has demonstrated that several properties of AFSCs including immunoregulatory functions, cell differentiation toward multiple lineages, and migratory potency are regulated by sphingosine-1-phosphate (S1P) (Romani et al., 2018).

**MNCs**

Mononuclear cells, which can be isolated from BM and peripheral blood, are extensively studied in tissue engineering and regenerative medicine. They can be harvested from BM and peripheral blood by density gradient centrifugation with no need for ex vivo expansion. Moreover, MNCs are heterogeneous which contain several types of stem/progenitor cells such as MSCs and EPCs. These cells are capable of differentiating into vascular and/or myocytes, or secrete growth factors improving the regeneration of injured tissues (Karantalis et al., 2012). These features allow quick autologous application after harvest, so MNCs are widely used as therapeutic cells in CVDs (Goumans et al., 2014). However, recent systemic review and meta-analysis...
of the clinical efficacy of MNC transplantation only reveal modest clinical benefit. For PAD, improvements could be achieved in wound healing, amputation-free survival, pain-free walking, resting pain, and ulcer healing, but administration of MNCs could not improve the primary end-point of limb amputation compared with placebo (Rigato et al., 2017; Qadura et al., 2018). Another recent meta-analysis consisting of 2037 patients with acute MI has shown that MNC therapy only modestly improved left ventricular ejection fraction (LVEF) and infarct size (de Jong et al., 2014). Despite the publication bias and possible lack of statistical power, several aspects during MNC administration could be improved to achieve better clinical results, for instance, refinement of cell delivery strategy to enhance cell survival and function. Recent progress made in the decellularized scaffolds, which create the scaffolds enriched in structural extracellular matrix components that support cell attachment and infiltration in vitro and in vivo (Crapo et al., 2011), stimulates great interest. Moreover, current genomic sequencing and proteomic techniques could also be utilized to identify essential pathways to improve the survival and function of transplanted cells.

CPCs

After the introduction of cardiac progenitor cells (CPCs), researchers began to determine the possibility of the experimental and clinical usage of CPCs as a potential therapeutic agent. CPCs are a group of heterogeneous cells residing in the cardiac tissue (Senyo et al., 2013). After the identification of CPCs, researchers have discovered different cardiac resident cellular pools in human or murine heart, showing a variety of stem cell markers, including c-Kit$, stem cell antigen-1$ (Sca-1$)$, Islet 1$ (Isl-1$)$, stage-specific embryonic antigen-1$ (SSEA-1$)$, cardiospheres (CS), cardiospheres-derived (CD), and side population (SP), which has recently been reviewed extensively by Bianconi et al. (2017, 2018). CPCs can self-renew, and they can also differentiate into three different cardiac cell types including cardiomyocytes, smooth muscle cells and endothelial cells (Sturzu and Wu, 2011; Bianconi et al., 2018). It has been reported that embryonic heart tubes derived CPCs can differentiate into pacemaker-like cells through endothelin-1 factor involved signaling (Zhang et al., 2012). Recently, engineered cardiac pacemakers containing both CPC-derived pacemaker-like cells and EPCs have demonstrated the promising potential to ameliorate sinus node malfunction (Zhang et al., 2017). Meanwhile, accumulating studies have shown that CPCs promote cardiac tissue restoration after CVD by releasing anti-apoptotic and angiogenic signals in a paracrine manner (Ibrahim et al., 2014). It has been shown that CPC-derived exosomes promoted angiogenesis, cardiomyocyte survival and proliferation, and reduced cell apoptosis (Marban, 2014). Analysis of CPC-based clinical trials has revealed that patients suffering from heart-related diseases benefit from CPC-based therapy (Bianconi et al., 2018).

EPCs

Asahara et al. (1997) initially isolated angioblasts with endothelial lineage potential from human peripheral blood and named them “EPCs.” They also found that these EPCs can differentiate into endothelial-like cells in vitro and participate in neovascularization in animal models of ischemia. Later, EPCs have been shown to migrate to peripheral blood from BM to participate in repairing dysfunctional endothelia and decreasing cardiovascular risk factor related endothelial injury by directly infusing into and forming new vessels or secreting pro-angiogenic growth factors or cytokines (Asahara et al., 2011). Although an increasing number of reports have been documented to identify EPCs, there is still a lack of unambiguous and consistent definition of EPCs. Generally, EPCs are a group of cells which are characterized by positively expressing VEGFR2 Flk1, CD133/AC133, and CD34 at early stages; while at late stages when they gradually differentiate into endothelial cells, EPCs start to express endothelial markers including VE-cadherin, VWF, and endothelial nitric oxide synthase (eNOS) (Ambasta et al., 2017). Accumulating studies indicates that early EPCs promotes angiogenesis in a paracrine manner, and the late stage EPCs directly participate in endothelial neovascularization (Ambasta et al., 2017).

EPC BASED CELL THERAPY IN CVDs

It is well-known that the integrity and functional activity of the endothelial monolayer are maintained by replication and migration of neighboring mature endothelial cells under physiological conditions. However, a series of clinical and preclinical studies have provided the evidence that in conditions of endothelial injury, regeneration of endothelial monolayer is assisted by EPCs homing to the artery wall. A critical early event in CVDs is endothelial dysfunction, which is perpetuated during the exposure of cardiovascular risk factors including hypercholesterolemia, metabolic syndrome, diabetes, hypertension, dyslipidemia, aging, and smoking. It has been reported that the number of circulating EPCs is inversely correlated with the presence of cardiovascular risk factors (Mannarino and Pirro, 2008; Pirro et al., 2015). Over the last two decades, extensive investigations in clinical and preclinical studies indicate that EPCs are a promising option to treat CVDs such as MI. The findings of EPC cell therapy for MI in animal studies have been summarized in Table 1. Accumulating clinical trials have evaluated the safety and efficacy of EPCs for CVDs treatment, as summarized in Table 2. As revealed in Table 2, the clinical outcomes of the stem/progenitor cell-based therapy only achieved modest benefits, so more strategies should be employed to improve the stem/progenitor cell-based therapy.

EPC BASED THERAPY FOR ISCHEMIC VASCULAR DISEASES

The therapeutic efficacy of EPCs was not only documented in the studies of CVDs but also in the peripheral artery diseases (PAD). PAD is commonly referred to as the ischemia of limbs associated with atherosclerotic occlusion (Ouriel, 2001). Peripheral arteries supply oxygenated blood and nutrients to the legs and feet and narrowing of these arteries results
TABLE 1 | Stem cell/EPC therapy in animal models of MI.

| Animal model | Transplanted cell type | Delivery strategy | Outcomes | Reference |
|--------------|------------------------|-------------------|----------|-----------|
| Mouse MI     | Mouse BM-EPCs          | Intravenous injection | EPC incorporated into neovascularization foci at infarct border | Asahara et al., 1999 |
| Mouse MI     | Bone marrow derived mouse Lin− c-kit+ | Intramyocardial injection | Newly formed myocardium occupied 68% of the infarcted portion of the ventricle were observed | Orlic et al., 2003 |
| Rat MI       | Human peripheral blood EPCs | Intravenous injection | EPCs incorporated into foci of neovascularization, smaller ventricular dimensions and ventricular scarring; increased fractional shortening, capillary density | Kawamoto et al., 2001 |
| Rat MI       | Human peripheral blood CD34+ cells | Tail vein injection | Decreased apoptosis of hypertrophied myocytes in the peri-infarct region, reduced collagen deposition, increased myocardium survival and cardiac function | Kocher et al., 2001 |
| Pig MI       | Pig MNCs               | Trans-endocardial injection | Increased systolic function, regional blood flow, collateral vessel formation, and decreased ischemic area | Kamihata et al., 2002 |
| Pig MI       | Pig MSCs               | Intramyocardial injection | Decreased degree of contractile dysfunction and wall thinning | Shake et al., 2002 |
| Rat MI       | Rat MSCs transduced Akt1 | Intramyocardial injection | Inhibited the process of cardiac remodeling, restored myocardial volume | Mangi et al., 2003 |
| Rat MI       | Human peripheral blood CD34+ angioblasts (EPCs) | Tail vein injection | Dose-dependent neovascularization with development of larger-sized capillaries; improve cardiac function through inhibiting apoptosis and promoting proliferation of cardiomyocytes | Schuster et al., 2004 |
| Rat MI       | Rat ASCs               | Sheet technology (monolayered cell graft placed on the surface of the anterior scar) | ASCs reversed wall thinning in scar area and improve cardiac function. ASCs triggers angiogenesis and differentiate into vessels and cardiomyocytes | Miyahara et al., 2006 |
| Rat MI       | Rat umbilical cord blood CD133+ cells | Intravenous infusion | Scar thinning and LV systolic dilatation were prevented | Leor et al., 2006 |
| Pig MI       | Pig CD34+              | Intracoronary injection | Improved cardiac repair and collateral vessel formation | Zhang et al., 2007 |
| Rat MI       | Human EPCs accompanied with SDF-1 | Intramyocardial injection | Improved fractional shorting, left ventricular developing pressure, coronary flow rates, and neovascularization. Reduced the number of inflammatory cells and the rate of apoptotic cells | Schuh et al., 2008 |
| Mouse MI     | Human myoendothelial cells | Intramyocardial injection | Improved left ventricular function. Increased angiogenesis. Stimulated proliferation and survival cardiomyocytes. Reduced scar tissue | Okada et al., 2008 |
| Rat MI       | ECM scaffold supplemented with EPCs primed with SDF-1 | Sutured to the anterolateral left ventricular wall | Increased VEGF level, vessel density, microvascular perfusion, vasculogenic response, and decreased scar formation | Frederick et al., 2010 |
| Rat MI       | Rat peripheral blood EPCs transduced with IGF-1 | Intramyocardial injection | Increased cardiac function, cardiomyocyte proliferation, and capillary density, decreased cardiac apoptosis | Sen et al., 2010 |
| Pig MI       | Human embryonic stem cells | Fibrin-cell path applied to the LV anterior wall of the MI area | Improved left ventricular function and neovascularization | Xiong et al., 2011 |

in PAD. The most common symptom of PAD is the pain with walking which is also known as intermittent claudication (Ouriel, 2001). Critical limb ischemia (CLI) is the most severe clinical manifestation of PAD affecting a limb, if not interrupted, CLI could lead to ischemic ulcerations or even gangrene (Ouriel, 2001). In preclinical studies, the most adopted animal model is the hindlimb ischemia model (HLI) (Niiyama et al., 2009). In the HLI model, the femoral artery is ligated to reduce the blood supply to the lower leg which induces the angiogenesis to compensate for the reduced blood flow (Limbourg et al., 2009). The therapeutic efficacy of EPCs have been evaluated by this model by many groups, and Table 3 summarizes the preclinical animal studies of EPC cell therapy for PAD.
| Trial design | Disease | Cell type | Delivery strategy | Outcomes | Reference |
|--------------|---------|-----------|-------------------|----------|-----------|
| 22 Bilateral ischemia patients, 25 unilateral ischemia patients, within-patient controls | CLI | MNCs derived from BM or peripheral blood (PB) | Intramuscular injection | Improved transcutaneous oxygen pressure (TcPO2), rest pain, pain-free walking time, and ankle-brachial index (ABI) | Tateishi-Yuyama et al., 2002 |
| 7 Patients, no controls | CLI | BM derived MNCs | Intramuscular injection | Improved ABI, TcPO2, pain-free walking time, and leg blood flow | Higashi et al., 2004 |
| 6 Patients, no controls | Acute myocardial infarction (AMI) | PB CD34+ cells | Intracoronary injection | Improved wall motion score index | Blocklet et al., 2006 |
| 44 Cell-injected patients, 22 control | AMI | BM-MNCs | Intracoronary injection | Increased LVEF and peak systolic velocities the infarcted wall longitudinal contraction | Meluzin et al., 2006 |
| 41 Cell-injected patients, 45 control | ST-segment elevation MI | BM-MNCs | Intracoronary injection | Increased LVEF, no improvement of myocardial viability of infarcted area | Cao et al., 2009 |
| 7 Patients, non-randomized control | Anterior MI | PB CD34+ cells | Transcoronary, intracoronary infusion | Decreased end-systolic volume | Dedobbeleer et al., 2009 |
| 7 Patients, no controls | AMI | PB CD34+ cells | Intracardiac infusion | Increased LVEF, vascularization, and the regeneration of myocardial structure | Pasquet et al., 2009 |
| 28 Patients, no controls | CLI | CD34+ CD133+ EPCs | Intramuscular injection | Improved limb salvage rate and attenuated pain scale | Lara-Hernandez et al., 2010 |
| 25 Cell-injected patients, 25 placebo-injected patients; Randomized double-blinded trial | Chronic myocardial ischemia | BM-MNCs | Intramyocardial infusion | Improved E/e' and E/A ratios, increased LVEF | van Ramshorst et al., 2011 |
| 112 Cell-injected patients, 56 placebo-injected patients; Phase II, prospective, double-blinded, randomized trial | Refractory angina | CD34+ cells | Intramyocardial infusion | Improved exercise tolerance | Losordo et al., 2011 |
| 71 Cell-injected patients, 71 placebo-injected patients; Phase III, randomized, double-blinded trial | MI | CD133+ cells | Intramyocardial infusion | Patients received CD133+ cell injection had higher LVEF | Donndorf et al., 2012 |
| 17 Patients, no control, Phase I/II clinical trial | CLI | Granulocyte-colony stimulating factor (GCSF) mobilized CD34+ cells | Intramuscular injection | Improved toe brachial pressure index and TcPO2, pain scale, ulcer size, and exercise tolerance | Kinoshita et al., 2012 |
| 25 Patients, no control | CLI | GCSF mobilized PB CD34+ cells | Intramuscular injection | Improved pain-free walking time, ABI, TcPO2, and decreased pain score | Dong et al., 2013 |
| 11 Patients, no control; Phase II clinical trial | CLI | GCSF mobilized PB CD34+ cells | Intramuscular injection | Increased pain scale, skin perfusion pressure, TcPO2, total walking distance, toe brachial pressure index, and CLI-free ratio | Fujita et al., 2014 |
| 49 Patients, no control | CLI | BM-MNCs | Intramuscular and intraarterial injection | Limb amputations were delayed; Improved ABI, rest pain, and ulcer healing | Franz et al., 2015 |
**TABLE 3 | Stem cell/EPC therapy in animal studies of PAD.**

| Animal model | Transplanted cell type | Delivery strategy | Outcomes | Reference |
|--------------|------------------------|-------------------|----------|-----------|
| Mouse and rabbit HLI | Human CD34⁺; mouse Flk-1⁺ | Tail vein injection | EPC incorporated into sites of active angiogenesis | Asahara et al., 1997 |
| Mouse HLI | Human EPC | Intracardiac injection | Ischemic hindlimb blood flow increased, capillary density increased, limb loss rate decreased | Kalka et al., 2000 |
| Rat HLI | Human CD34⁺ NMC (EPCs) | Intramuscular injection | Neovascularization and blood flow increased in ischemic hindlimb | Murohara et al., 2000 |
| Mouse HLI | Human CD34⁺ cells | Intramuscular injection | Blood flow restored in diabetic mice but not in non-diabetic mice | Schattenman et al., 2000 |
| Rabbit HLI | Rabbit BM-MNCs | Intramuscular injection | More angiographically detectable collateral vessel, improved blood perfusion | Shintani et al., 2001 |
| Mouse HLI | VEGF gene transduced Human EPCs | Tail vein injection | Neovascularization and blood flow recovery improved, and limb necrosis was reduced | Iwaguro et al., 2002 |
| Mouse HLI | Human EPCs accompanied with SDF-1 | Intramuscular SDF-1 and intravenous EPC injection | Improved local accumulation of EPCs in ischemic muscle, ischemic tissue perfusion, and capillary density | Yamaguchi et al., 2003 |
| Mouse HLI | Human cord blood CD34⁺ KDR⁺ or CD34⁺ KDR-cells | Intramuscular injection | CD34⁺ KDR⁺ cells significantly improved limb salvage and neovascularization, reduced endothelial cell apoptosis and interstitial fibrosis compared with CD34⁺ KDR-cells | Madeddu et al., 2004 |
| Mouse HLI | Human umbilical cord blood CD133⁺ EPCs | Tail vein injection | Increased neovascularization and improved ischemic limb salvage | Yang et al., 2004 |
| Rat HLI | Human peripheral blood CD133⁺ progenitor cells | Intramuscular injection | Increased arteriole and capillary density | Suuronen et al., 2006 |
| Mouse HLI | Human EPCs and smooth muscle progenitor cells | Intravenous injection | Vessel density and foot perfusion increased | Foubert et al., 2008 |
| Mouse HLI | Mouse MNCs | Intramuscular injection | Increased blood flow ratio and capillary density; improved ankle-brachial index value, walking distance, pain scale, and TcPO2 | Zhang et al., 2008 |
| Mouse HLI | Human iPSC-ECS | Intramuscular injection | Increased capillary density and blood perfusion ratio | Rufaihah et al., 2011 |
| Mouse HLI | Human HUVECs and umbilical cord MSCs | Intramuscular injection | Blood perfusion recovered, increased vessel formation | Chen et al., 2013 |
| Mouse HLI | Human MNCs, ESC, and iPSC | Intramuscular injection | Increased neovascularization and decreased hindlimb ischemia | Lai et al., 2013 |
| Mouse HLI | Human AFSCs | Intramuscular injection | Increased limb salvage, limb blood perfusion, and capillary and arteriole density | Liu et al., 2013 |

**APPROACHES FOR ENHANCING EPC THERAPY IN DISEASES**

Although EPCs possess exciting therapeutic potency, their limited plasticity and amount in patients with ischemic cardiac or ischemic vascular disease have become the obstacle to the success in EPC therapy. It has been reported that compromised EPC availability and repair potential to regenerate the injured endothelial monolayer mainly resulted from the influence of cardiovascular risk factors such as aging, smoking, diabetes, hypertension, and hypercholesterolemia (Pirro et al., 2008, 2012). As summarized, the clinical outcome of EPC based therapy was modest, and large-scale clinical trials have not been conducted. One of the reasons is that there is a lack of suitable transplantation models. Studies in animal models suggested that BM-MNCs or EPCs could home to ischemic tissues and restore the blood supply, however, during atherosclerosis acute surgical resection has little resemblance to chronic occlusion (Qadura et al., 2018). Moreover, because of the heterogeneity between patients, in clinical trials, the selection of patient population for stem/progenitor cell-based therapy may not be optimized. These problems should be addressed before the clinical transfer of EPC based cell therapy. Therefore, an increasing number of studies have been focusing on the strategies to enhance the therapeutic efficacy of EPCs (Penn and Mangi, 2008). Various modifiers including chemokine receptors, growth factors, signaling molecules or factors, medicines, and physical exercise have been demonstrated to enhance the therapeutic effects of EPCs.

The key factors shown to enhance the cell-based therapeutics in CVDs include but not limited to: chemokine receptors such as CXCR2 (Hou et al., 2015), CXCR4 (Jujo et al., 2013), CX3CR1 (Herlea-Pana et al., 2015), CXCR7 (Zhang et al., 2014), and CCR5 (Zhang et al., 2015); growth factors and their receptors...
such as VEGF1/2/3 (Shintani et al., 2006; Smadja et al., 2007), PDGF (Rosell et al., 2013), FGF-1/2 (Rosell et al., 2013; Chien et al., 2016), and so on. Signaling molecules and factors such as eNOS/nitric oxide (Kaur et al., 2009; Cui et al., 2011), AMP-activated protein kinase (AMPK) (Wang X.R. et al., 2011), heme-oxygenase-1 (HO-1) (Sambuceti et al., 2009), and manganese superoxide dismutase (MnSOD) (Marrotte et al., 2010), have also been shown to play important roles in EPC biology. Additionally, several pathways have been found to be important in angiogenesis. Studies have shown that exercise training, as well as the cell survival after delivery are also needed to be investigated to address the limitations of EPC transplantation. Moreover, the findings of the positive effects of medications and physical exercise provide additional options to enhance the efficacy of EPC therapy in cost-efficient manner.

CONCLUSION

Although clinical trials and preclinical studies have shown that EPCs and other stem cell and progenitor cells based therapy possess great therapeutic potential to improve cardiac function and blood perfusion in MI and PAD, obstacles still exist to be overcome before widespread application of EPCs in the treatment of CVD (Roediger, 1980). Cell isolation, characterization, modification, and processing strategies must be further studied and refined to achieve enhanced therapeutic efficacy. For instance, there is still lack of consistent definition of EPCs, so further study is needed to standardize methods to define EPCs, through both lineage tracing and functional analysis (Masuda et al., 2011). Meanwhile, upregulation of certain circulating progenitor cells such as circulating osteoprogenitor cells may result in vascular calcification which is a cardiovascular risk factor (Pirro et al., 2013). Moreover, the cell infusion approach, dosing regimens, as well as the cell survival after delivery are also needed to be improved to achieve optimal outcomes (Freyman et al., 2006). Due to the possibility of occurrence of teratoma formation and tumorigenesis, especially during the transplantation of iPSCs, the safety of the stem/progenitor cell-based therapies should also be monitored (Yamanaka, 2012). In summary, previous clinical trials and preclinical studies have shed light on the EPC based therapy for treating CVDs. With more efforts to understand the biology of stem/progenitor cells and continued commitment to preclinical and clinical studies, stem/progenitor cell-based therapy may present an integral part of routine regenerative therapy for CVDs in the future.

AUTHOR CONTRIBUTIONS

YH wrote the manuscript and participated in edits. CL revised and edited the entire manuscript.

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REFERENCES

Ambasta, R. K., Kohli, H., and Kumar, P. (2017). Multiple therapeutic effect of endothelial progenitor cell regulated by drugs in diabetes and diabetes related disorder. J. Transl. Med. 15:185. doi: 10.1186/s12967-017-1280-y

Ashara, T., Kamimoto, A., and Masuda, H. (2011). Concise review: circulating endothelial progenitor cells for vascular medicine. Stem Cells 29, 1650–1655. doi: 10.1002/stem.745

Ashara, T., Masuda, H., Takahashi, T., Kalka, C., Pastore, C., Silver, M., et al. (1999). Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ. Res. 85, 221–228. doi: 10.1161/01.RES.85.3.221

Asahara, T., Murohara, T., Silver, M., Van Der Zee, R., Li, T., et al. (1997). Isolation of putative progenitor endothelial cells for angiogenesis. Science 275, 964–967. doi: 10.1126/science.275.5302.964

Bianconi, V., Fallarino, F., Mannarino, M. R., Bagaglia, F., Kararoudi, M. N., Aragona, C. O., et al. (2017). Autologous cell therapy for vascular regeneration: the role of proangiogenic cells. Curr. Med. Chem. [Epub ahead of print]. doi: 10.2174/092986732466617011211603

Bianconi, V., Sahbekar, A., Kovanen, P., Bagaglia, F., Ricciuti, B., Calabro, P., et al. (2018). Endothelial and cardiac progenitor cells for cardiovascular repair: a controversial paradigm in cell therapy. Pharmacol. Ther. 181, 156–168. doi: 10.1016/j.pharmthera.2017.08.004

Blocket, D., Tourgouz, M., Berkenboom, G., Lambermont, M., Unger, P., Preumont, N., et al. (2006). Myocardial homing of non-mobilized peripheral blood CD34 + cells after intracoronary injection. Stem Cells 24, 333–336. doi: 10.1634/stemcells.2005-2021

Bollini, S., Cheung, K. K., Riegler, J., Dong, X., Smart, N., Ghionzoli, M., et al. (2011). Amniotic fluid stem cells are cardioprotective following acute myocardial infarction. Stem Cells Dev. 20, 1985–1994. doi: 10.1080/10641261003704042

Brunt, K. R., Wu, J., Chen, Z., Poeckel, D., Dercho, R. A., Melo, L. G., et al. (2012). Ex vivo Akt/HO-1 gene therapy to human endothelial progenitor cells enhances myocardial infarction recovery. Cell Transplant. 21, 1443–1461. doi: 10.3727/096368912X653002

Bura, A., Planat-Benard, V., Bourin, P., Silvestre, J. S., Gross, F., Grolleau, J. L., et al. (2014). Phase I trial: the use of autologous cultured adipose-derived stroma/stem cells to treat patients with non-revascularizable critical limb ischemia. Cytototherapy 16, 245–257. doi: 10.1016/j.jcyt.2013.11.011

Cao, F., Sun, D., Li, C., Narsinh, K., Zhao, L., Li, X., et al. (2009). Long-term myocardial functional improvement after autologous bone marrow mononuclear cells transplantation in patients with ST-segment elevation myocardial infarction: 4 years follow-up. Eur. Heart J. 30, 1986–1994. doi: 10.1093/eurheartj/ehp220

Chen, D. Y., Wei, H. J., Lin, K. J., Huang, C. C., Wang, C. C., Wu, C. T., et al. (2012). An amniotic fluid-derived mesenchymal stem cell line for myocardial repair. J. Vasc. Surg. 58, e403. doi: 10.1016/j.jvs.2013.01.037

Donnadou, P., Kaminski, A., Tiedemann, G., Kuntz, B., and Steinhoff, G. (2012). Validating intramyocardial bone marrow stem cell therapy in combination with coronary artery bypass grafting, the PERFECT Phase III randomized multicenter trial: study protocol for a randomized controlled trial. Trials 13:99. doi: 10.1186/1477-7220-13-99

Dedobbeleer, C., Blocklet, D., Tourgouz, M., Lambermont, M., Unger, P., Degauve, J. P., et al. (2009). Myocardial homing and coronary endothelial function after autologous blood CD34 + progenitor cells intracoronary injection in the chronic phase of myocardial infarction. J. Cardiovasc. Pharmacol. 53, 480–485. doi: 10.1097/FJC.0b013e318147b572

Dong, Z., Chen, B., Fu, W., Wang, X., Guo, D., Wei, Z., et al. (2013). Transplantation of purified CD34 + cells in the treatment of critical limb ischemia. J. Vasc. Surg. 58:e403. doi: 10.1016/j.jvs.2013.01.037

Fernandes, T., Nakamura, J. S., Magalhaes, F. C., Roque, F. R., Lavini-Ramos, C., Schettert, I. T., et al. (2012). Exercise training restores the endothelial progenitor cells number and function in hypertension: implications for angiogenesis. J. Hypertens. 30, 2133–2143. doi: 10.1097/1HJH.0b013e32838588d6

Foubert, P., Matrone, G., Soutouh, B., Lere-Dean, C., Barateau, V., Plouet, J., et al. (2008). Coadministration of endothelial and smooth muscle progenitor cells enhances the efficiency of proangiogenic cell-based therapy. Circ. Res. 103, 751–760. doi: 10.1161/CIRCRESAHA.108.175083

Franz, R. W., Shah, K. J., Pin, R. D., Hanksins, T., Hartman, J., and Wright, M. L. (2015). Autologous bone marrow mononuclear cell implantation therapy is an effective limb salvage strategy for patients with severe peripheral arterial disease. J. Vasc. Surg. 62, 673–680. doi: 10.1016/j.jvs.2015.02.059

Friederick, J. R., Fitzpatrick, J. R. III, McMormick, R. C., Harris, D. A., Kim, A. Y., Muenzer, J. R., et al. (2010). Stromal cell-derived factor-1alpha activation of tissue-engineered endothelial progenitor cell matrix enhances ventricular function after myocardial infarction by inducing neovascularization. Circulation 122, S107–S117. doi: 10.1161/CIRCULATIONAHA.109.930404

Gnecchi, M., He, H., Noiseux, N., Liang, O. D., Zhang, L., Morello, F., et al. (2006). Intravenous delivery of adipose-derived stromal/stem cells to treat patients with non-revascularizable critical limb ischemia. Circulation 114, 2137–2146. doi: 10.1161/CIRCULATIONAHA.105.560375

Herlea-Pana, O., Yao, L., Heuser-Baker, J., Wang, Q., Wang, Q., Georgescu, C., et al. (2009). Myocardial progenitor cells for cardiovascular repair: a basic science behind cardiovascular cell-based therapies. Circulation 110, 882–893. doi: 10.1161/CIRCULATIONAHA.109.626901

Hou and Li
Roediger, W. E. (1980). The colonic epithelium in ulcerative colitis: an energy-deficiency disease? Lancet 2, 712–715. doi: 10.1016/S0140-6736(80)91934-0

Romani, R., Manni, G., Donati, C., Pirisini, L., Bernacchioni, C., Gargaro, M., et al. (2018). SIP promotes migration, differentiation and immune regulatory activity in amniotic-fluid-derived stem cells. Eur J Pharmacol 833, 173–182. doi: 10.1016/j.ejphar.2018.06.005

Romani, R., Pirisini, I., Calvitti, M., Pallotta, M. T., Gargaro, M., Bistoni, G., et al. (2015). Stem cells from human amniotic fluid exert immunoregulatory function via secreted indoleamine 2,3-dioxygenase1. J Cell Mol Med 19, 1593–1605. doi: 10.1111/jcmm.12534

Rosell, A., Morancio, A., Navarro-Sobrino, M., Martinez-Saez, E., Hernandez-Guillamon, M., Lopez-Piedraflata, S., et al. (2013). Factors secreted by endothelial progenitor cells enhance neurorepair responses after cerebral ischemia in mice. PLoS One 8:e73244. doi: 10.1371/journal.pone.0073244

Rufaihal, A. J., Huang, N. F., Jame, S., Lee, J. C., Nguyen, H. N., Byers, B., et al. (2011). Endothelial cells derived from human iPSCs increase capillary density and improve perfusion in a mouse model of peripheral arterial disease. Arterioscler. Thromb. Vasc. Biol. 31, e72–e79. doi: 10.1161/ATVBAHA.111.230938

Sambuceti, G., Morbelli, S., Vanella, L., Kusmic, C., Marinii, C., Massolli, M., et al. (2009). Diabetes impairs the vascular recruitment of normal stem cells by oxidant damage, reversed by increases in pAMPK, heme oxygenase-1, and adiponectin. Stem Cells 27, 399–407. doi: 10.1634/stemcells.2008-0800

Schatteman, G. C., Hanlon, H. D., Jiao, C., Dodds, S. G., and Christy, B. A. (2000). Blood-derived angioblasts accelerate blood-flow restoration in diabetic mice. J Clin Invest. 106, 571–578. doi: 10.1172/JCI08907

Schulz, A., Liehn, E. A., Sasse, A., Hristov, M., Sobota, R., Kelm, M., et al. (2008). Transplantation of endothelial progenitor cells improves neovascularization and left ventricular function after myocardial infarction in a rat model. Basic Res Cardiol. 103, 69–77. doi: 10.1007/s00395-007-0685-9

Schuster, M. D., Kocher, A. A., Seki, T., Martens, T. P., Xiang, G., Homma, S., et al. (2004). Myocardial neovascularization by bone marrow angioblasts results in cardiomyocyte regeneration. Am J Physiol Circ Physiol. 287, H525–H532. doi: 10.1152/ajpheart.00058.2004

Seminatore, C., Polentes, J., Eliby, D., Kozubenko, N., Itier, V., Tine, S., et al. (2010). The postischemic environment differentially impacts teratoma or tumor formation after transplantation of human embryonic stem cell-derived neural progenitors. Stroke 41, 153–159. doi: 10.1161/STROKEAHA.109.563015

Sen, S., Merchon, J., Dean, J., Mi, G., Gavin, M., Silver, M., et al. (2010). Autologous transplantation of endothelial progenitor cells genetically modified by adenovirus-associated viral vector delivering insulin-like growth factor-1 gene after myocardial infarction. Hum Gene Ther 21, 1327–1334. doi: 10.1089/hum.2010.0006

Senyo, S. E., Steinhauser, M. L., Pizzimboni, C. L., Yang, V. K., Cai, L., Wang, M., et al. (2013). Mammalian heart renewal by pre-existing cardiomyocytes. Nature 493, 433–436. doi: 10.1038/nature11682

Shiba, J. G., Gruber, P. J., Bauernfeind, W. A., Senechal, G., Meyers, J., Redmond, J. M., et al. (2002). Mesenchymal stem cell implantation in a swine myocardial infarct model: engraftment and functional effects. Am J Physiol Cell Physiol. 287, C1925–C1926. doi: 10.1152/ajpcell.00060.2001

Shiba, Y., Fernandes, S., Zhu, W. Z., Filice, D., Muskheli, V., Kim, J., et al. (2012). Human ES-cell-derived cardiomyocytes electrically couple and suppress arrhythmias in injured hearts. Nature 489, 322–325. doi: 10.1038/nature11317

Shintani, K., Kusano, K., Mi, I., Iwakura, A., Heyd, L., Curry, C., et al. (2006). Synergistic effect of combined intramyocardial CD34 + cells and VEGF2 gene therapy in MI. Nat Clin Pract Cardiovasc Med. 3(Suppl. 1), S123–S128. doi: 10.1038/npcardio0430

Shintani, S., Murohara, T., Ikeda, H., Ueno, T., Sasaki, K., Duan, J., et al. (2001). Augmentation of postnatal neovascularization with autologous bone marrow transplantation. Circulation 103, 897–903. doi: 10.1161/01.CIR.103.6.897

Sivaparapatna, A., Ghaedi, M., Le, A. V., Mendez, J. J., Qyang, Y., and Niklason, L. E. (2015). Arterial specification of endothelial cells derived from human induced pluripotent stem cells in a biomimetic flow bioreactor. Biomaterials 53, 621–633. doi: 10.1016/j.biomaterials.2015.02.121

Smadja, D. M., Bieche, I., Helley, D., Laurendeau, I., Simonin, G., Muller, L., et al. (2004). Increased VEGFR2 expression during human late endothelial progenitor cells expansion enhances in vitro angiogenesis with up-regulation of integrin alpha(6). J Cell Mol Med 11, 1149–1161. doi: 10.1111/j.1582-4934.2007.00090.x
Sturzu, A. C., and Wu, S. M. (2011). Developmental and regenerative biology of multipotent cardiovascular progenitor cells. *Circ. Res.* 108, 353–364. doi: 10.1161/CIRCRESAHA.110.227066

Suuronen, E. J., Veinot, J. P., Wong, S., Kapila, V., Price, J., Griffith, M., et al. (2006). Tissue-engineered injectable collagen-based matrices for improved cell delivery and vascularization of ischemic tissue using CD133 + progenitors expanded from the peripheral blood. *Circulation* 114, I138–I144. doi: 10.1161/CIRCULATIONAHA.105.01081

Suzuki, H., Shibata, R., Kito, T., Yamamoto, T., Iishi, M., Nishio, N., et al. (2012). Comparative angiogenic activities of induced pluripotent stem cells derived from young and old mice. *PLoS One* 7:e39562. doi: 10.1371/journal.pone.0039562

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., et al. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131, 861–872. doi: 10.1016/j.cell.2007.11.019

Tateishi-Yuyama, E., Matusbara, H., Murohara, T., Ikeda, U., Shintani, S., Masaki, H., et al. (2002). Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. *Lancet* 360, 427–435. doi: 10.1016/S0140-6736(02)09670-8

Trevelyan, J., Needham, E. W., Morris, A., and Mattu, R. K. (2005). Comparison of the effect of enalapril and losartan in conjunction with surgical coronary revascularisation versus revascularisation alone on systemic endothelial function. *Heart* 91, 1053–1057. doi: 10.1136/hrt.2004.036897

van Ramsorst, J., Antoni, M. L., Beers, S. L., Roos, S. D., Delgado, V., Rodrigo, S. F., et al. (2011). Intramyocardial bone marrow-derived mononuclear cell injection for chronic myocardial ischemia: the effect on diastolic function. *Circ. Cardiovasc. Imag.* 4, 122–129. doi: 10.1161/CIRCIMAGING.110.957548

Wang, W., Lang, J. K., Suzuki, G., Canty, J. M. Jr., and Cimato, T. (2011). Statins enhance clonal growth of late outgrowth endothelial progenitors and increase myocardial capillary density in the chronically ischemic heart. *PLoS One* 6:e24868. doi: 10.1371/journal.pone.0024868

Wang, X. R., Zhang, M. W., Chen, D. D., Zhang, Y., and Chen, A. F. (2011). WIFI-activated protein kinase rescues the angiogenic functions of endothelial progenitor cells via manganese superoxide dismutase induction in type 1 diabetes. *Am. J. Physiol. Endocrinol. Metab.* 300, E1135–E1145. doi: 10.1152/ajpendo.00001.2011

Wingate, K., Floren, M., Yan, T., Tseng, P. O., and Tan, W. (2014). Synergism of matrix stiffness and vascular endothelial growth factor on mesenchymal stem cells for vascular endothelial regeneration. *Tissue Eng. Part A* 20, 2503–2512. doi: 10.1089/ten.tea.2013.0249

Wolffert, K. C., and Drexler, H. (2010). Cell therapy for the treatment of coronary heart disease: a critical appraisal. *Nat. Rev. Cardiol.* 7, 204–215. doi: 10.1038/nrcardio.2010.1

Wolffert, K. C., Meyer, G. P., Muller-Ehmsen, J., Tischop, C., Bonarjee, V., Larsen, A. L., et al. (2017). Intracoronary autologous bone marrow cell transfer after myocardial infarction: the BOOST-2 randomised placebo-controlled clinical trial. *Eur. Heart J.* 38, 2936–2943. doi: 10.1093/eurheartj/ehx188

Writing Group Members, Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., et al. (2016). Heart disease and stroke statistics-2016 update: a report from the American heart association. *Circulation* 133, e38-360.

Wu, G., Rana, J. S., Wykrzykowska, J., Du, Z., Ke, Q., Kang, P., et al. (2009). Exercise-induced expression of VEGF and salvage of myocardium in the early stage of myocardial infarction. *Am. J. Physiol. Heart Circ. Physiol.* 296, H389–H395. doi: 10.1152/ajpheart.01393.2007

Xia, W. H., Li, J., Su, C., Yang, Z., Chen, L., Wu, F., et al. (2012). Physical exercise attenuates age-associated reduction in endothelium-reparative capacity of endothelial progenitor cells by increasing CXCR4/JAK-2 signaling in healthy men. *Aging Cell* 11, 111–119. doi: 10.1111/j.1474-9726.2011.00758.x

Xiong, Q., Hill, K. L., Li, Q., Suntharalingam, P., Mansoor, A., Wang, X., et al. (2011). A fibrin patch-based enhanced delivery of human embryonic stem cell-derived vascular cell transplantation in a porcine model of postinfarction left ventricular remodeling. *Stem Cells* 29, 367–375. doi: 10.1002/stem.580

Xu, Q. (2006). The impact of progenitor cells in atherosclerosis. *Nat. Clin. Pract. Cardiovasc. Med.* 3, 94–101. doi: 10.1038/nccardiio0396

Yamaguchi, J., Kusano, K. F., Masuo, O., Kawamoto, A., Silver, M., Murasawa, S., et al. (2003). Stromal cell-derived factor-1 effects on ex vivo expanded endothelial progenitor cell recruitment for ischemic neovascularization. *Circulation* 107, 1322–1328. doi: 10.1161/01.CIR.0000055313.77510.22

Yamazaki, S. (2012). Induced pluripotent stem cells: past, present, and future. *Cell Stem Cell* 10, 678–684. doi: 10.1016/j.stem.2012.05.005

Yang, C., Zhang, Z. H., Li, Z. J., Yang, R. C., Qian, G. Q., and Han, Z. C. (2004). Enhancement of neovascularization with cord blood CD133 + cell-derived endothelial progenitor cell transplantation. *Thromb. Haemost.* 91, 1202–1212. doi: 10.1160/TH03-06-0378

Zhang, H., Zhang, N., Li, M., Feng, H., Jin, W., Zhao, H., et al. (2008). Therapeutic angiogenesis of bone marrow mononuclear cells (MNCs) and peripheral blood MNCs: transplantation for ischemic hindlimb. *Ann. Vasc. Surg.* 22, 238–247. doi: 10.1016/j.avsg.2007.07.037

Zhang, L., Li, X., Yu, X., Li, Y., Sun, A., Huang, C., et al. (2017). Construction of vascularized pacemaker tissues by seeding cardiac progenitor cells and endothelial progenitor cells into Matrigel. *Life Sci.* 179, 139–146. doi: 10.1016/j.lfs.2017.05.007

Zhang, S., Ge, J., Zhao, L., Qian, J., Huang, Z., Shen, L., et al. (2007). Host vascular niche contributes to myocardial repair induced by intracoronary transplantation of bone marrow CD34 + progenitor cells in infarcted swine heart. *Stem Cells* 25, 1195–1203. doi: 10.1634/stemcells.2006-06065

Zhang, X., Guo, J. P., Chi, Y. L., Liu, Y. C., Zhang, C. S., Yang, X. Q., et al. (2012). Endothelin-induced differentiation of Nkx2.5(+) cardiac progenitor cells into pacemaking cells. *Mol. Cell. Biochem.* 366, 309–318. doi: 10.1007/s11010-012-1309-8

Zhang, X. Y., Su, C., Cao, Z., Xu, S. Y., Xia, W. H., Xie, W. L., et al. (2014). CXCR7 upregulation is required for early endothelial progenitor cell-mediated endothelial repair in patients with hypertension. *Hypertension* 63, 383–389. doi: 10.1161/HYPERTENSIONAHA.113.02273

Zhang, Z., Dong, J., Lobe, C. G., Gong, P., Liu, J., and Liao, L. (2015). CCR5 facilitates endothelial progenitor cell recruitment and promotes the stabilization of atherosclerotic plaques in ApoE-/- mice. *Stem Cell Res. Ther.* 6:36. doi: 10.1186/s13287-015-0026-0

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