



Improving stem cell engraftment to enhance functional efficacy in cardiovascular disease: where are we now?

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Abstract

Stem cell therapy is a promising therapy for repairing damaged tissue. A growing body of research shows that stem cells work effectively in several diseases such as cardiovascular disease, hepatic disease, and diabetes. It has been shown that stem cells not only differentiate into functional cells and replace dead cells, but also release growth factors and cytokines which can recruit autologous cells. The most significant barrier to achieve clinical relevance of this treatment mode is the poor survival rate of injected cells. To improve transplantation and enhance functional outcome, investigations of gene transfection (overexpression of anti-apoptotic and antioxidant proteins), growth factor supplementation, and scaffolding matrices are being conducted. In this review, we will focus on methods to increase cell survival in stem cell transplantation as a novel treatment for cardiovascular disease.

Keywords

Cardiovascular disease treatment; Cell transplantation improvement; Stem cell therapy

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Introduction

Cardiovascular disease is the leading cause of death globally. The World Health Organization (WHO) reported that about 17.5 million people died from this disease in 2012. Among many therapies and interventions that have been developed to treat the disease, stem cell therapy is one of the greatest potential candidates for treatment (Fuster, 2014). For instance, unlike heart replacement, various sources of progenitor cells/stem cells are available - umbilical cord derived stem cells, bone marrow derived mesenchymal stem cells, adipose derived stem cells, embryonic stem cells, cardiac stem cells, and induced pluripotent stem cells. Therefore, it is possible to have a suitable cell type for a specific patient. Moreover, the therapy does not require a surgery, so it is safer, more affordable and requires less recovery time and resources. Cells can be injected into the heart by catheter or needle and syringe. Patients do not even need to undergo anesthesia. This can reduce morbidity and mortality in an already high-risk population. As a consequence, there have been several clinical trials using cell therapy to treat both acute myocardial infarction and chronic ischemia in human (Fisher et al., 2014; Fisher et al., 2015).

The heart lacks self-regeneration ability. After infarction, hypoxia-sensitive cardiomyocytes die and an inflammatory response ensues, removing the debris. Fibroblasts then proliferate and occupy the space, creating a collagenous, non-contractile scar which continues to remodel, causing negative effects on cardiac function. The hope in using stem cells resides in the idea that they not only become heart cells, but also recruit autologous stem/progenitor cells, mobilized to the infarcted area, to recapitulate the highly-vascularized environment to support the metabolic demands. Additionally, many cytokines and chemokines are secreted to induce paracrine effects.

The goals are that cell therapy can repair the damaged heart by mediating some combination of the following mechanisms:

Differentiate to cardiomyocytes

Many stem cell types have been showed that they can express markers of cardiomyocytes and cardio-progenitor cells (Boheler et al., 2002; Quevedo et al., 2009; Toma et al., 2002). For example, cardiomyocytes could be derived from embryonic stem cells when scientists cultivated embryoid body to day 7 and/or under the induction of several factors. When beating area appeared, they collected the cells from the population by enzyme (like collagenase) (Laflamme et al., 2007; Maltsev et al., 1993; Maltsev et al., 1994). Then, they adjusted the expression of cardio-specific proteins like myosin heavy chain (MHC), α -actin, desmin, and cardiac troponin T (Boheler et al., 2002). In addition to embryonic stem cells, mesenchymal stem cells has also been studied the differentiation

ability into cardiomyocytes both *in vitro* and *in vivo* (Toma et al., 2002; Xu et al., 2004). At the present, adipose tissue-derived stem cells (ADSC) and induced-pluripotent stem cells (iPSC) are the main focus. 5-azacytidine is used as an effective induced factor to start the differentiation process by activating transcription factor genes like GATA4, Nkx2.5 which then stimulate expression of cardiac-specific genes (BurrIDGE and Zambidis, 2013; Carvalho et al., 2013; Gherghiceanu et al., 2011; Li et al., 2015). As a result of this potential, stem cell therapy has become a promising therapy to treat cardiovascular disease, especially diseases relevant to myocardial injury.

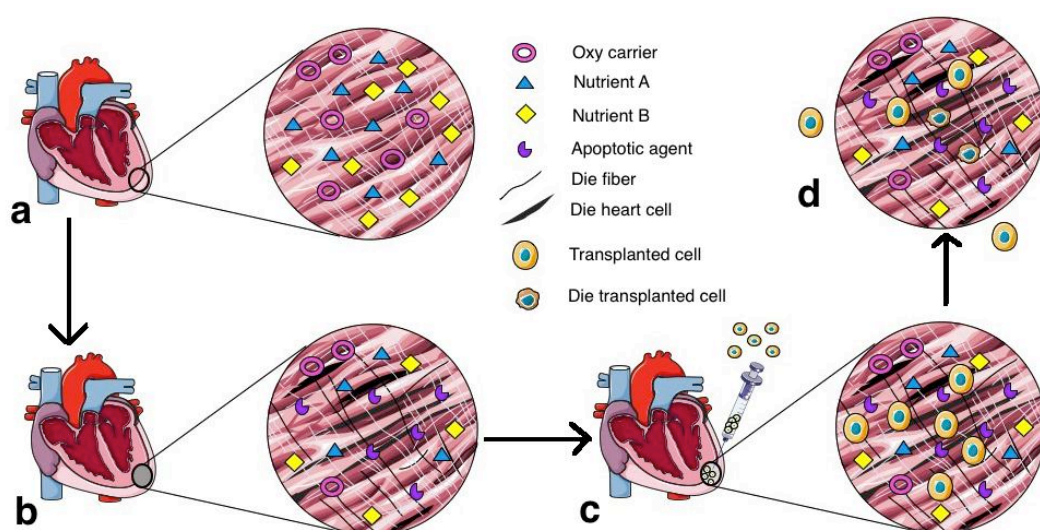


Figure 1. Reasons for cell loss after transplantation. (a) Normally, heart is supplied enough oxygen, nutrients as well as a good matrix for its life; (b) In ischemic condition, the infarcted area lacks of blood leading to the decreasing of these essential factors and the increasing of apoptotic factors; (c & d) When delivered to the damaged heart area, the number of transplanted cells/stem cells could be lost by two ways: the cells die causing by harsh environment in the infarcted zone and/or go out of the injured tissue causing by weak adhesion to a poor extra cellular matrix.

Release of paracrine factors

In addition to differential ability, transplanted stem cells also release paracrine signaling and recruit endogenous cardiac stem cells (Gnecchi et al., 2008). A growing body of research has shown that the mechanism which stem cells can regenerate the damaged heart was the cells release soluble factors that stimulated the cardiac regeneration, neovascularization, and cardiac modeling after myocardial infarction (Gnecchi et al., 2008; Kinnaird et al., 2004; Zhang et

al., 2007). These factors could activate pro-survival signaling pathway like PI3K/Akt or STAT3 which protect cardiomyocytes and enhance cardiac regeneration (Gnecchi et al., 2006). They contain factors such as bFGF, IGF-2, VEGF, IL-11. Additionally, these factors also recruit endogenous cardiac stem cells which, although the existence is controversial and if so, not present in significant numbers, have strong abilities to become heart cells, vascular smooth muscle cells, and endothelial cells *in vivo* (Leri et al., 2005). These factors like IGF-1 and HGF released by transplanted Mesenchymal stem cells-MSCs were shown that they could activate endogenous cardiac stem cells (Gnecchi et al., 2008). The broad spectrum of abilities of stem cells has been a significantly important issue that could explain the cardio-protection and remodeling of stem cell transplantation.

Cell fusion

Moreover, cell fusion between transplanted cells and domestic cells also has been used to explain the mechanism of damaged heart renewal by stem cell transplantation (Doppler et al., 2013). Injected cells were demonstrated to fuse with host cardiomyocytes and stimulate the regeneration of injured hearts. These stem cell types were cardiac progenitor cells (Oh et al., 2003), bone marrow stem cells (Nygren et al., 2004), and adipose tissue-derived stem cells (Metzele et al., 2011). This potential of stem cells has contributed to benefits of stem cell therapy for cardiovascular disease.

Although stem cell transplantation is a promising therapy and has been under development for a long time since the first study was published (Marelli et al., 1992), it also contains many challenges when cells are injected to hearts (Fig. 1). Firstly, poor cell survival caused by harsh environment in the infarcted area (Zhang et al., 2001). When cells are transplanted to injured heart, they are immediately embedded in the ischemic condition which lacks oxygen, nutrients, and contain many apoptotic signals, causing an abundance of cell death as soon as the first hour after transplantation (Haider and Ashraf, 2008; Muller-Ehmsen et al., 2002; Zhang et al., 2001). Secondly, cell engraftment has also been a major concern of stem cell therapy for heart disease. As a result of tenuous cell retention and cell survival, injected cells have difficulty integrating with the host tissue. The saline solution which is regularly used as a vehicle for delivery cannot keep the cells in the heart tissue because it does not contain a matrix for cell adherence of survival factors (Li et al., 2016). More studies are needed to optimize the transplant conditions.

To solve these two main problems in stem cell therapy for heart disease, scientists have suggested many solutions focusing on three approaches: gene transfection, growth factor introduction, and creation of hydrogel scaffolding (Fig. 2).

Gene transfection

Akt

Overexpressing Akt helps stem cells (like MSCs) exhibit a significantly enhanced intramyocardial retention and engraftment. It is believed that Akt is necessary and sufficient for cell survival because it not only targets apoptotic family members, but also increases glucose metabolism and releases energy during hypoxia (Datta et al., 1999). In light of these advantages of Akt protein, Akt-modified MSCs genetically restore significantly greater myocardial volume than equal numbers of control cells (Mangi et al., 2003). In addition to Akt signal transduction, many investigators work with hypoxia inducible factor 1 alpha (HIF-1 α) and Pim-1 kinase.

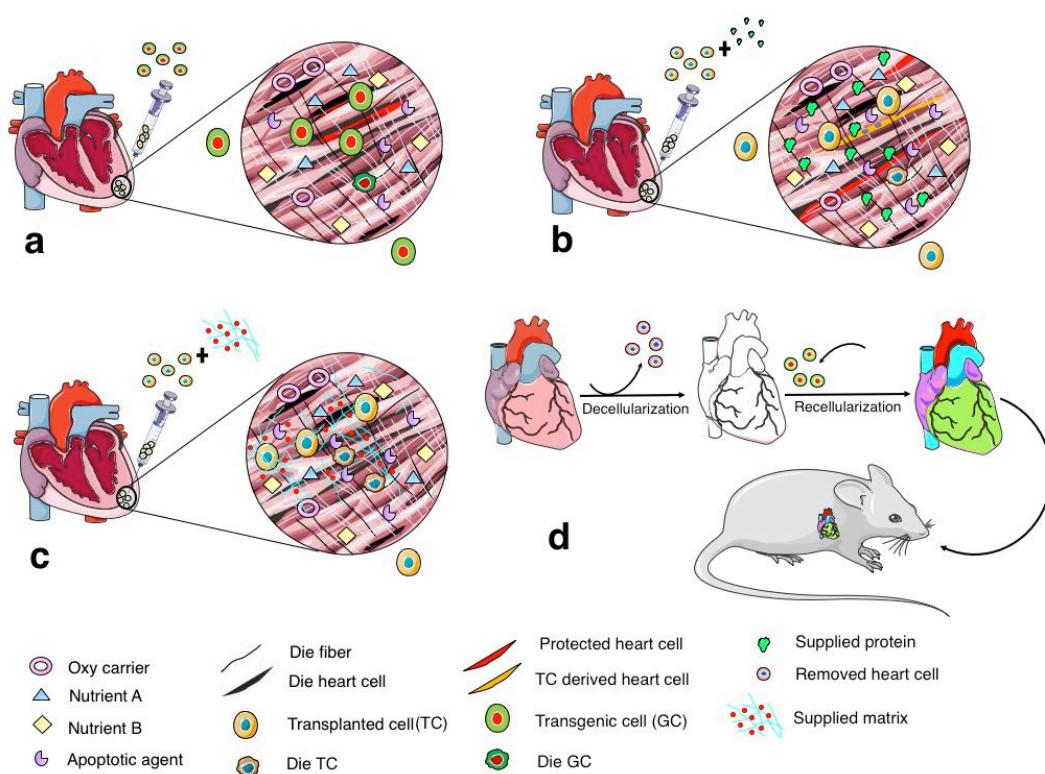


Figure 2. Stem cell engraftment improving methods. (a) The transgenic stem cells released helpful factors to protect recipient cardiomyocytes and themselves from a harsh environment that contributed to increase the number of survival cells after injection; (b) Together with transplanted cells, supplied proteins promoted the differentiation of cells into myocardial cells and protected cells from ensuing lesions; (c) In order to enhance the adhesion of transplanted cells, the matrix was also added to the cell solution for implantation (c) Heart was removed all cells to form a natural heart scaffold which was then seeded with suitable cells to form a perfect heart for transplantation in vivo.

HIF-1 alpha

HIF-1 alpha is a pro-angiogenic transcription factor which regulates the expression of many genes such as vascular endothelial growth factor (VEGF), angiopoietin 1 (ANGPT1) and ANGPT2, placental growth factor (PGF), and platelet-derived growth factor B (PDGFB) (Dai et al., 2007; Manalo et al., 2005). Under hypoxic conditions, hydroxyl reaction does not occur, causing HIF-1 protein to escape from inhibition and be activated, resulting in transcription of many genes and stimulates angiogenesis (Pugh and Ratcliffe, 2003). Basing on the important function of HIF 1 in hypoxic cells, it's expression was introduced in MSCs, which was expected that these MSCs could protect co-culture cardiomyocytes through HIF pathway (Dai et al., 2007).

Pim-1

Pim-1 is a proto-oncogenic serine-threonine kinase which was first described in hematopoietic cells. The kinase is responsible for cell survival, proliferation, and differentiation in the cell type (Wang et al., 2001). According to Aho et al, Pim-1 kinase inhibits pro-apoptotic Bad protein and increases Bcl-2 activity, promoting cell survival (Aho et al., 2004). Recently, it is also demonstrated that Pim-1 plays an important role in cardioprotection downstream of Akt signaling (Muraski et al., 2007). Fischer et al proves that Pim-1 overexpress – cardiac progenitor cells (Pim-1 – CPCs) survive and proliferate better than control CPCs (Fischer et al., 2009). In this study, Pim-1-CPCs improve cardiac repair and regeneration through increasing in vascular density, cell proliferation, and persistence (Fischer et al., 2009).

Protein

While genes must be introduced to cells before injection, growth factors can be supplied to the injected cells before or at the same time with transplantation. The following proteins have demonstrated promise as adjuncts to cellular therapy:

Ephrin

The Ephrin/Eph receptor tyrosine kinase (RTK) family is the largest of the RTK families. Unlike the other members of RTK family, Ephs specially can activated intracellular signals in both receptor exposing cells and ligand exposing cells that allows them to participate in many important biological processes such as development, proliferation, differentiation, and migration. There are two subclasses in Ephs family, including EphAs and EphBs that are segregated by their abilities to bind with the glycosylphosphatidylinositol-linked Ephrin-A ligands or the transmembrane-bound ephrin-B ligands. Typically, the receptors of specific groups respond to ligands of their corresponding groups, ie receptor

EphA binds to ligand ephrinA. They are less or not bind to ligands of opposing groups, except ephrin B3 can attach and activate EphA4 and ephrin A5 can bind and activate EphB2 (O'Neal et al., 2013). Ephrin as a target for cardiovascular treatment was first described by Mansson-Broberg et al. in 2008 (Mansson-Broberg et al., 2008). Since then, a few publications about the EphrinA/EphA-R signaling system in the heart have been produced (Frieden et al., 2010; Goichberg et al., 2011; O'Neal et al., 2013). Goichberg et al showed that motility and migration of human cardiac stem cells (hCSCs) were enhanced by the increasing of interactions between EphA2 on hCSCs and ephrinA1 which presented on cardiomyocytes (Goichberg et al., 2011). The Virag laboratory performed many studies to clarify the effects of this protein in cardio-protection after myocardial infarction (Dries et al., 2011; DuSablón et al., 2014; O'Neal et al., 2014). They demonstrated that mice lacking EphA2 receptor expression caused negative effects on survival after myocardial infarction and made the remodeling process stagnant (DuSablón et al., 2014). Furthermore, they demonstrated that EphrinA1 signaling could modulate inflammatory response and limit the infarct size (O'Neal et al., 2014). Unlike cancer treatment (Boyd et al., 2014), the approach using Ephrin signaling in heart disease treatment is still novel and more research is warranted to be assess clinical relevance.

Bone morphogenetic protein - BMP

BMP belongs to the transforming growth factor-beta (TGF- β) super family (Chen et al., 2004). It is a key protein in mesoderm development and cardiac differentiation (Chen et al., 2008). A growing body of research has shown that BMP is needed for pluripotent stem cells differentiating into cardiac cells. When supplied to the growth medium, BMP expands embryonic stem cells and has been demonstrated to promote cardiomyocyte differentiation (Pal and Khanna, 2007). Using BMP4 and Activin A to induce ESCs to differentiate into cardiomyocytes, Laflamme et al presented a cocktail of pro-survival factors to enhance post-transplantation cell survival. This approach significantly improved ESC engraftment in myocardial infarction treatment. For example, it prevented death pathways to protect the cells on the transplanted grafts and helped the grafts survive 1 to 4 weeks after implantation. Therefore, the grafts could cause positive effects on the cardiac function like remuscularization, wall thickening, ventricular dilation decreasing, global function improving, and wall motion enhancement (Behfar et al., 2002; Laflamme et al., 2007). With the advantages, BMP should be researched to apply in cell therapy for heart disease treatment.

Fibroblast growth factor – FGF

More than 20 years ago, FGF was shown to be cardioprotective, especially after ischemia-reperfusion injury by Kardami et al. (Kardami et al., 1993). FGF is a member of the family of heparin-binding growth factors and is involved in several important developmental processes (Thisse and Thisse, 2005). In the heart, FGF works as cardioprotective and angiogenic agent (Detillieux et al., 2003). It not only maintains cardiomyocyte survival, but also stimulates functional

recovery after injury (Rosenblatt-Velin et al., 2005), leading to the notion that using FGFs is a feasible approach to improve stem cell transplantation effectiveness. Recently, to pre-treat stem cells with FGF before transplantation, scientists combined it in a cocktail of growth factors such as TGF β , BMP-2, Activin A, etc. (Behfar et al., 2010; Hahn et al., 2008). In the future, FGFs might be applied in both stem cell culture and global tissue restoration after heart damaged.

Insulin-like growth factor 1– IGF-1

IGF-1 has been shown to reduce the doubling time of mesenchymal stem cells (MSC), improve MSC proliferation, and enhance CXCR4 expression (Huang et al., 2012). IGF-1 also a demonstrated role in modulating cardiovascular function. It facilitates cardiac development, enhances cardiac contractility, and protects cardiac tissue after myocardial infarction (Ren et al., 1999). Therefore, using IGF-1 accompany with transplanted cells has become a promising strategy. Davis et al. has reported that, when the combination of cardiomyocytes and IGF-1 in a “biotin sandwich” transplanted into injured hearts resulted in recovery of heart function (Davis et al., 2006). This result suggested that IGF-1 could cause positive effects on both cardiac and injected cells and using IGF-1 in cell therapy needs further exploration.

Scaffold

To improve cell survival and cell engraftment in stem cell therapy for heart disease treatment, scaffolding to support cells is a valuable tool. Scaffolding helps us not only control the cell distribution, but also provide growth factors and structural support for transplanted cells.

Hydrogel

Although gene and growth factor strategies can improve cell survival, they do not contribute substantially to cell distribution and engraftment. To improve the stem cell transplantation effectiveness, a new approach called Matrix-Assisted Cell Transplantation (MACT) which creates a materials-based environment that enhances pro-survival paracrine signaling and then increase cell engraftment with the host tissues was born (Parisi-Amon et al., 2013; Prestwich, 2008). There are several materials that can be used in the strategy including natural and synthetic types like hydrogel, alginate, chitosan, collagen, etc. (Benoit et al., 2008; Kang et al., 2014). Subsequently, some scientists suggested that they will use hydrogel as a MACT to secrete growth factors and improve cell survival after transplantation (Jha et al., 2015). Hyaluronic acid –based hydrogel is a promising candidate which contains key advantages such as biocompatibility, tunable properties, and native biofunctionality (Highley et al., 2016; Travan et al., 2016). It is wide used for biomedical applications, especially in heart disease treatment

therapy (Abdalla et al., 2013; Bonafe et al., 2014; Jha et al., 2015). More research is needed to validate its clinical potential as an adjunct to cellular engraftment therapies.

Natural heart scaffold

Decellularization of the heart is also an attractive and challenging approach. Scientists collect heart from deceased patients and used detergents like Triton-X-100 or SDS to decellularize the hearts. They pushed detergents through a tube to the heart's aorta to remove DNA, soluble protein, lipid, and other cellular material to perform a natural matrix. This matrix was then used to seed cells to create an engineered heart (Ott et al., 2008). Finally, they transplant the heart to animal model (Maher, 2013). The time and energy required to decellularize the tissue, seed the cells, grow the tissue to achieve functionality, and transplant it into an animal is an extremely labor- and time-intensive endeavor. However, "this train has left the station" and so there are several investigators that continue to focus on refining and expediting this strategy and the results are heartening (Lu et al., 2013; Tapias and Ott, 2014). Although it has tremendous challenges, it still holds remarkable advantages such as providing an excellent model to research cell development and differentiation in 3D, and improving our understanding of the complex, unique, and dynamic nature of the myocardial scaffolding. This strategy needs to be explored to optimize the decellularization process, which cell type(s) can be used, and the functional range of blood-pumping capacity.

Cell-derived matrices

Scaffold created from cell-derived matrices is also an attractive approach. Instead of using directly natural tissue (such as heart from the newly dead) to create scaffold, people used cells/stem cells to form matrices which mimicked natural extracellular matrices. Then, these cells would be removed. According to Fitzpatrick and McDevitt review, several approaches in using these scaffold for cardiovascular and other organs (like skeletal tissue or skin) regeneration have been conducted (Fitzpatrick and McDevitt, 2015). For example, to perform heart valve or blood vessel, scientists cultured fibroblast or smooth muscle cells in sheets, then these sheets were wrapped around a mandrel to have a tubular structure (Quint et al., 2012). Although there were several clinical trials using this strategy (McAllister et al., 2009; Quint et al., 2012; Wystrychowski et al., 2011), it needs to be explored more about engineered cells and decellularization techniques (Fitzpatrick and McDevitt, 2015) before applying on patients.

Conclusions and future directions

As stem cells provide a hopeful treatment for cardiovascular disease patients, despite controversial opinions about the potential of this therapy (Abbott, 2014),

there are numerous pre-clinical trials and clinical trials using them (Kastrup et al., 2016; Krishna et al., 2011; Westerdahl et al., 2016). Our group has also reported that umbilical cord blood derived stem cells can differentiate into cardiac progenitor cells and that injection of induced cells into damaged heart reduced scar size, stabilized blood pressure, and maintained heart function (Pham et al., 2015). Van der Spoel et al. has a systematic review and meta-analysis about stem cell therapy for ischemic heart disease in large animals (van der Spoel et al., 2011). According to this review, stem cell therapy is safe and improves left ventricular ejection fraction (LVEF) after injury. Currently, Poulin and colleagues also have a similar review on 29 studies which showed that stem cell therapy could enhance LVEF, reduce infarcted size, and increase myocardial viability (Poulin et al., 2016). In the future, stem cell therapy with its safety, effective, and affordable price will be a promising therapy to save heart disease patients and improve the quality of life. Improving the outcome of the therapy by enhancing transplanted cell survival, distribution, and engraftment need further modifications and refinements to ensure optimal conditions before they can be routinely applied.

Abbreviations

ADSC: Adipose tissue-derived stem cell; Akt: Serine/threonine-specific protein kinase; ANGPT1, 2: Angiopoietin 1, 2; Bcl-2: B-cell lymphoma 2; bFGF: Basic fibroblast growth factor; BMP: Bone morphogenetic protein; CXCR4: Chemokine receptor type 4; Ephs: Ephrins protein; FGF: Fibroblast growth factor; HGF: Hepatocyte growth factor; HIF: Hypoxia inducible factor; IGF-2: Insulin-like growth factor 2; IL-11: Interleukin 11; iPSC: induced-Pluripotent stem cell; LVEF: left ventricular ejection fraction; MACT: Matrix-Assisted Cell Transplantation; MHC: Myosin heavy chain; MSC: Mesenchymal stem cell; PDGFB: Platelet-derived growth factor B; PGF: Placental growth factor; PI3K: Phosphoinositide 3-kinase; RTK: The Ephrin/Eph receptor tyrosine kinase; STAT3: Signal transducer and activator of transcription 3; TGF-beta: Transforming growth factor beta; VEGF: vascular endothelial growth factor.

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Author contribution

Anh Thi Van Bui created the outline and wrote the manuscript, Dr. Truc Le Buu Pham draw 2 figures and edited the manuscript, Assistant Professor Jitka Virag organized and edited the manuscript.

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