

## REVIEW

# The pi3k/Akt Pathway Is Associated With Angiogenesis, Oxidative Stress and Survival of Mesenchymal Stem Cells in Pathophysiologic Condition in Ischemia

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## Summary

Ischemic diseases are characterized by reduced blood supply to a tissue or an organ due to obstruction of blood vessels. The most serious and most common ischemic diseases include ischemic heart disease, ischemic stroke, and critical limb ischemia. Revascularization is the first choice of therapy, but the cell therapy is being introduced as a possible way of treatment for no-option patients. One of the possibilities of cell therapy is the use of mesenchymal stem cells (MSCs). MSCs are easily isolated from bone marrow and can be defined as non-hematopoietic multipotent adult stem cells population with a defined capacity for self-renewal and differentiation into cell types of all three germ layers depending on their origin. Since 1974, when Friedenstein and coworkers (Friedenstein *et al.* 1974) first time isolated and characterized MSCs, MSC-based therapy has been shown to be safe and effective. Nevertheless, many scientists and clinical researchers want to improve the success of MSCs in regenerative therapy. The secret of successful cell therapy may lie, along with the homing, in secretion of biologically active molecules including cytokines, growth factors, and chemokines known as MSCs secretome. One of the intracellular signalling mechanism includes the activity of phosphatidylinositol-3-kinase (phosphoinositide 3-kinase) (PI3K) - protein kinase B (serine-threonine protein kinase Akt) (Akt) pathway. This PI3K/Akt pathway plays key roles in many cell types in regulating cell proliferation, differentiation, apoptosis, and migration. Pre-conditioning of MSCs could improve efficacy of signalling mechanism.

## Key words

Ischemic diseases • Critical limb ischemia • Mesenchymal stem cells • Regenerative therapy • Moming • MSCs secretome • PI3K/Akt pathway

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## Introduction

Ischemic diseases are characterized by reduced blood supply to a tissue or an organ due to obstruction of blood vessels. The most serious and most common ischemic diseases include ischemic heart disease, ischemic stroke, and critical limb ischemia. They seriously impair quality of patient's life and present high risk of cardiovascular events and death (Uccioli *et al.* 2018). Reperfusion therapy (pharmaceutical therapy, surgery, or endovascular intervention) is usually used to counteract these diseases. Care management of patients is also related to their increased risk of any cardiovascular event.

Therefore, best medical approach includes cardiovascular risk factors management comprising pharmacological therapy as well as non-pharmacological

approach, such as smoking cessation, healthy diet, and regular physical exercise. The pharmacological component includes antihypertensive, lipid-lowering, and antithrombotic drugs (Aboyans *et al.* 2017). Although both the pharmacological and surgical treatment may restore the functions of arteries, they are not able to promote regeneration and functional recovery of the surrounding tissues affected by ischemia (Kastrup *et al.* 2016). The use of unconventional techniques, such as gene or cell therapy, may represent a possible way to treat no-option patients. However, there are major questions to explore the mechanisms governing MSCs therapies and mode of action. Another question is the possibility to improve the regenerative/repair effect of MSCs therapy by pre-conditioning of MSCs with pharmacological factors.

Phosphatidylinositol-3-kinase is a lipid kinase and produces phosphatidylinositol-3,4,5-trisphosphate. This trisphosphate is a second messenger essential for the translocation of Akt to the plasma membrane, where Akt is phosphorylated and activated by phosphoinositide-dependent kinase (PDK 1) and phosphoinositide-dependent kinase 2 (PDK 2). Activation of Akt plays a crucial role in basic cellular functions such as cell proliferation and survival (Osaki *et al.* 2004, Wang *et al.* 2019, Zálešák *et al.* 2015, Zálešák *et al.* 2016).

## Mesenchymal stem cells

MSCs can be defined as multipotent adult stem cells population with a defined capacity for self-renewal and differentiation into cell types of all three germ layers depending on their origin (Youssef *et al.* 2017). They can be isolated from various locations of the human body, e.g. bone marrow, adipose tissue, umbilical cord, and others (Kariminekoo *et al.* 2016). The International Society for Cellular Therapy (ISCT) proposed minimal criteria to define multipotent mesenchymal stromal cells. They should be adherent to plastic, positive for CD73, CD90, and CD105, negative for CD11b or CD14, CD19 or CD79 $\alpha$ , CD34, CD45, and HLA-DR and differentiate into osteoblasts, adipocytes, and chondroblasts *in vitro* (Meirelles *et al.* 2009, Mahla 2016).

In bone, MSCs are located around sinusoids and along perivascular network in the stroma, where they are involved in the heterogenous system of bone marrow microenvironment. MSCs together with pericytes, adventitial cells, fibroblasts, marrow adipocytes, endothelial cells, hematopoietic, and immune cells create a dynamic compartment by establishing cell-to-cell

interactions and producing soluble factors, which possess autocrine and paracrine functions (Sobacchi *et al.* 2017). In physiopathological conditions, such as ischemia, MSCs secrete a variety of biologically active molecules, including cytokines, growth factors and chemokines, known as MSCs secretome. At least 54 different factors and molecules were identified to be present in the secretome. Recently, the concept of MSCs secretome is believed to play an essential role in the reparative processes, because therapeutic effects of MSCs persist even if they do not engraft or differentiate into tissue-specific cells (Ferreira *et al.* 2018). Table 1 shows MSCs secretome and its reparative and regenerative functions, which may be used in reparative mechanisms in ischemic disease.

**Table 1.** MSC secretome with reparative and regenerative effects

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### Immunomodulatory

(Meirelles *et al.* 2009, Di Nicola 2002, Martinet *et al.* 2009)

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prostaglandin E2 (PGE2)  
transforming growth factor beta (TGF- $\beta$ )  
hepatocyte growth factor (HGF)  
indoleamine 2,3 dioxygenase (IDO)  
inducible nitric oxide synthase (iNOS)  
leukemia inhibitory factor (LIF)  
interleukin 6 (IL-6)  
interleukin 10 (IL-10)

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### Angiogenic

(Hung *et al.* 2007, Kinnaird *et al.* 2004)

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fibroblast growth factors (FGF)  
vascular endothelial growth factor (VEGF)  
placental growth factor (PIGF)  
monocyte chemoattractant protein 1 (MCP-1)  
IL-6

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### Anti-apoptotic

(Rehman *et al.* 2004, Tögel *et al.* 2007)

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VEGF  
HGF  
insulin-like growth factor 1 (IGF1)  
stanniocalcin 1 (STC1)  
TGF  
FGF  
granulocyte-macrophage colony-stimulating factor (GM-CSF)

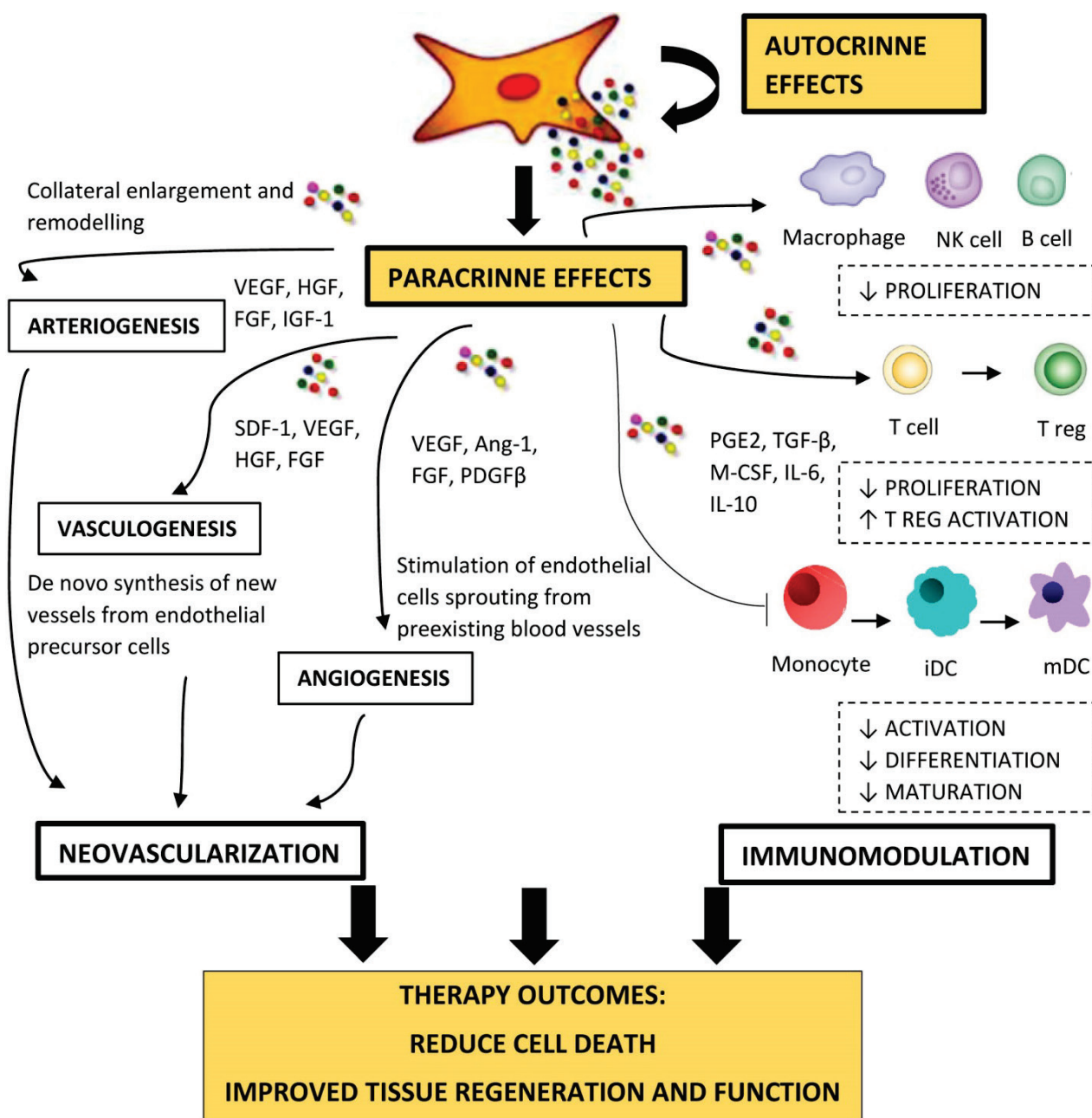
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### MSCs and ischemic diseases

The characteristics of MSCs are that what make them interesting for cell therapy and tissue engineering. They can be isolated and expanded *ex vivo* and used for an autologous transplantation, avoiding the problem of finding a compatible donor. MSCs are hypoimmunogenic, since they lack the expression of HLA class II and co-stimulatory molecules.

It has been shown that MSCs prevent T cell response indirectly through modulation of dendritic cells and directly by suppressing natural killer cell function of

CD8+ and CD4+ T cells. In addition, MSCs induce a suppressive local microenvironment by producing prostaglandins and interleukins. Other advantageous characteristic of MSCs is that they are easy to modify *in vitro* using viral vectors (Gnecchi *et al.* 2008). Besides this, it has been shown that MSCs are able to suppress activation and function of leukocytes, which are actively involved in a process of atherosclerosis. This reflects their great potential in repairing injured blood vessel, preventing the tissue ischemia. The main mechanism of paracrine and autocrine effects of human MSCs is shown in Fig. 1.



**Fig. 1.** Main mechanism of human MSCs in the treatment of ischemic tissue

MSCs can secrete angiogenic molecules, especially vascular endothelial growth factor (VEGF) and differentiate into endothelial cells to induce angiogenesis in ischemic regions and promote regeneration of injured tissue. Although positive outcomes have been demonstrated in preclinical trials using animal models of ischemic diseases, there remains a lack of published clinical trials verifying the effectiveness of MSCs application in ischemic diseases, such as myocardial infarction, ischemic stroke, and critical limb ischemia. They can be potentially used in clinical applications in vessel repair and ischemic disease, thanks to unique properties they possess.

There exist several questions related to the safety, effectiveness, and main mechanism of stem cell therapy due to different aspects of cell dosage, cell source or cell administration methods, and timing prior to clinical trials (Kode *et al.* 2009, Singh *et al.* 2016, Yong *et al.* 2018). However, it is true that the number of registered clinical trials of MSC-based therapy increases. The benefit and regenerative potential can be greatly influenced by diverse extrinsic factors which include pharmacological pre-conditioning, for example with statin - atorvastatin. Statins are lipid lowering drugs, inhibitors of 3-hydroxy-3-methyl-glutaryl-CoA reductase, the rate-limiting enzyme in the synthesis of cholesterol in the liver. They belong to a class of medications used in the treatment of hyperlipidemia and are currently widely used in the primary and secondary prevention of cardiovascular diseases (Carson *et al.* 2018). Nevertheless, statins have additional cholesterol-independent effects which include endothelium protection, immunomodulation, anti-inflammation, increasing bioavailability of nitric oxide, anti-oxidant activity, anti-cancer and stem cell modulating capacities (Xu *et al.* 2013).

These non-cholesterol effects of statin could be mediated by inhibiting mevalonate synthesis, where statins block the synthesis of isoprenoid intermediates, such as farnesylpyrophosphate and geranylgeranylpyrophosphate. These isoprenoid intermediates serve as important lipid attachments for the posttranslational modification of proteins, including small GTP-binding proteins. This modification is crucial for intracellular trafficking and function of small GTP-binding proteins belonging to the family of Ras, Rho, Rap and Rab GTPases (Rikitake and Liao 2005). Besides mentioned Rho-GTPase pathway, there are several others proposed intracellular signaling mechanisms accounting for statin

non-cholesterol effects, including PI3K/Akt pathway or nitric oxide pathway.

### **The PI3K/Akt pathway, angiogenesis and nitric oxide pathway**

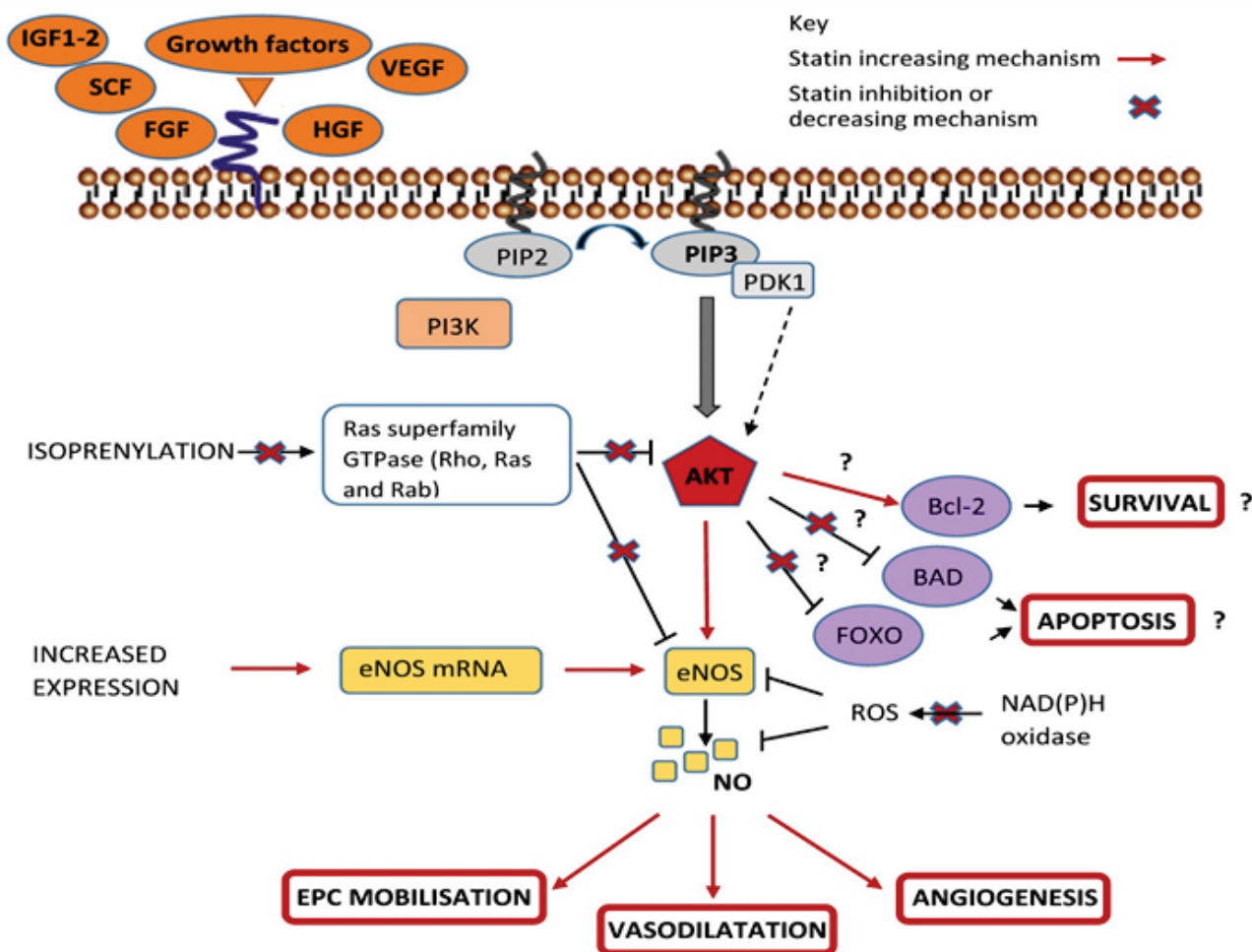
The PI3K/Akt pathway is a survival pathway regulating cell proliferation, differentiation, apoptosis, and migration. This pathway plays key roles in the physiology and pathophysiology of many cell types. Once activated, signalling through Akt can be propagated to a diverse array of substrates with different effects. Abnormal increase or decrease in the pathway activity leads to many diseases, such as diabetes, stroke, cancer, or neurodegenerative diseases. Activation of PI3K can occur through tyrosine kinase growth factor receptors such as insulin-like growth factor-1 receptor, c-kit receptor, receptor for vascular endothelial growth factor, and others.

The key enzyme of the pathway, PI3K, converts phosphatidylinositol 4,5-bisphosphate into phosphatidylinositol 3,4,5-trisphosphate, which binds Akt and PDK 1. This action allows PDK 1 to phosphorylate Akt, which is the primary direct downstream target protein of PI3K. The activation of Akt causes a cascade of responses of downstream targets that regulate cellular functions (Chen *et al.* 2013, Wang *et al.* 2019).

### **The PI3K/Akt pathway, angiogenesis and nitric oxide pathway**

The angiogenic process in ischemic tissue is a complex issue and involves a regional increase on growth factors able to promote the new blood vessel growth and immunomodulation of tissue repair, and regeneration of cell activity (Ko and Bandyk. 2014). MSCs can help vessels to grow and invade from neighbouring tissues by promoting the formation of tubes by endothelial cells. MSCs can recruit pericytes and smooth muscle cells to promote maturation of newly formed blood vessels. In addition, MSCs themselves can also differentiate into endothelial cells to increase vascularization (Chen *et al.* 2013, Wang *et al.* 2019).

The PI3K/Akt pathway is associated with angiogenesis through the regulation of the NO signalling pathway. The PI3K pathway releases a group of angiogenic factors including VEGF. Receptor 2 for VEGF has a central role in VEGF-induced angiogenesis. VEGF is required for the migration of endothelial cells and allows



**Fig. 2.** The impact of statins in cells.

formation of capillary like structures *via* PI3K/Akt dependent manner. Nitric oxide (NO) is released from the endothelium and represents the primary mediator of smooth muscle tone that causes vasodilatation through the activity of endothelial-type nitric oxide synthase (eNOS). NO may function as a cellular signalling molecule, an angiogenic factor involved in stimulation, promotion, and stabilization of new blood vessels together with VEGFs, fibroblast growth factors (FGFs), angiopoetin, various integrins, and others.

Reduced bioavailability of NO is characterized for endothelial dysfunction, which could be caused by reduced expression of endothelial NO synthase, impairment in its activation, or rapid inactivation of NO by oxidative stress. Statins are able to increase eNOS expression by inhibiting isoprenylation of Rho GTPase. Statins can also rapidly induce the phosphorylation and activation of eNOS *via* PI3K/Akt pathway (Gazzerro *et al.* 2012, Sandhu *et al.* 2017).

### The PI3K/Akt pathway, ROCKs and oxidative stress

The PI3K/Akt intracellular pathway is associated with inhibition of the Rho kinases (ROCKs), which are serine/threonine kinases and a downstream effector of the small GTPase Rho. ROCKs were initially characterized by their ability to mediate the formation of RhoA induced stress fibres and focal adhesions through the increased phosphorylation of myosin light chain. The effect of statin is the blockage of isoprenoids synthesis with subsequent geranylgeranylation of the Rho GTPase. Isoprenylation, as one of the post-translational modifications, is crucial for intracellular trafficking and functioning of small GTP-binding proteins. Another member of small GTPase subfamily is Rac, monomeric G-protein. The Rac signalling pathway affects actin cytoskeletal remodelling and generation of reactive oxygen species (ROS). Rac1 influences multiple actin cytoskeletal remodelling proteins and leads to the

activation of the NADPH oxidase system and subsequent generation of ROS (Zhou and Liao 2009).

Oxidative stress has a central role in cardiovascular diseases and a process of atherosclerosis too. LDL oxidation is a result of a chain reaction of free radicals converting polyunsaturated fatty acids into lipid peroxides and formation of active aldehydes. Oxidation of LDL causes senescence of endothelial progenitor cells (EPCs), whereas high density lipoprotein is regarded as atheroprotective, due to its antioxidant properties. In addition, ROS interact and decrease NO formation. Statins have antioxidant pleiotropic effect, which can include indirect mechanism increasing NO bioavailability accounting for antioxidant properties. Secondly, statin therapy has also been shown to inhibit activation of NAD(P)H oxidase and ROS release and activation of catalase and thioredoxin ROS scavenging mechanisms (Sandhu *et al.* 2017).

### The PI3K/Akt pathway and survival of MSCs

The PI3K/Akt pathway has a role in the survival of MSCs, evidenced by the effects of overexpression of key components of this pathway. Overexpression of Akt1 in MSCs causes improving of MSCs survival after transplantation into the hearts in rats. In MSC overexpressing Akt, the level of target proteins downstream of Akt, such as anti-apoptotic protein Bcl-2, was increased, whereas the level of the pro-apoptotic protein Bax was decreased. The overexpression of Bcl-2 has been shown to increase survival of MSCs and their function in the treatment of myocardial infarction (Mangi *et al.* 2003).

The impact of statins on cell apoptosis is doubtful (Fig. 2). Some literature reports statins can induce apoptosis through decreasing levels of anti-apoptotic proteins Bcl-2 and Bcl-xL and upregulating the

activation of pro-apoptotic molecules Bax, Bad and Caspases 3, 8, and 9 and these effects are used in the treatment of certain types of cancers (Beckwitt *et al.* 2018, Goc *et al.* 2012). However, other studies demonstrate no or minimal influence of statins on inducing cancer cell apoptosis. A different effect was observed in normal cell lines where statins reduced apoptosis and increased Bcl-2 expression. Thus, exact mechanism of statin-induced or reduced apoptosis on different cell types remains unclear (Wood *et al.* 2013).

### Conclusions

Ischemic disease accounts for the highest mortality globally. The pharmacological and surgical treatment is not successful in no-option patients. The only possibility is the use of the unconventional techniques – cell or gene therapy.

Although MSCs therapy reached notable admiration, some problems need to be overcome in order to establish it as a successful technique. MSCs are dynamic compartment, which engages in autocrine and paracrine functions, secrete biologically active molecules – secretome. The secretome may be influenced by pre-conditioning of MSCs by different factors and conditions.

In general, the PI3K/Akt pathway plays key roles in the physiology and pathophysiology of many cell types and represents a survival point regulating cell proliferation, differentiation, apoptosis, and migration. The PI3K/Akt pathway has a role in the survival of MSCs, evidenced by the effects of overexpression of key components of this pathway. Pre-conditioning of MSCs by pharmacological factors, such as statins could be a critical gap for quality and quantity of MSCs secretome, paracrine and autocrine signals.

### Conflict of Interest

There is no conflict of interest.

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