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Review Article

Pleiotropic effects of Erythropoietin. Influence of Erythropoietin on processes of mesenchymal stem cells differentiation

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Abstract

Structure and synthesis of Erythropoietin: Erythropoietin (EPO) is a glycoprotein hormone.

Recombinant Erythropoietin (Epoetin): Human recombinant erythropoietin is characterised as a factor which stimulates differentiation and proliferation of erythroid precursor cells, and as a tissue protective factor.

Anti-ischemic effects of recombinant Erythropoietin: Erythropoietin is one of the most perspective humoral agents which are involved in the preconditioning phenomenon.

Erythropoietin receptors and signal transduction pathways: Erythropoietin effects on cells through their interconnection with erythropoietin receptors, which triggers complex intracellular signal cascades, such as JAK2/STAT signaling pathway, phosphatidylinositol 3-kinase (PI3K), protein kinase C, mitogen-activated protein kinase (MAPK), and nuclear factor (NF)-κB signaling pathways.

Mechanisms of the effect of Erythropoietin on hematopoietic and non-hematopoietic cells and tissues: In addition to regulation of haemopoiesis, erythropoietin mediates bone formation as it has an effect on hematopoietic stem cells and osteoblastic niche, and this illustrates connection between the processes of haematopoiesis and osteopoiesis which take place in the red bone marrow.

The effect of Erythropoietin on mesenchymal stem cells and process of bone tissue formation: Erythropoietin promotes mesenchymal stem cells proliferation, migration and differentiation in osteogenic direction. The evidence of which is expression of bone phenotype by cells under the influence of EPO, including activation of bone specific transcription factors Runx2, osteocalcin and bone sialoprotein.

Conclusion: Erythropoietin has a pleiotropic effect on various types of cells and tissues. But the mechanisms which are involved in the process of bone tissue restoration via erythropoietin are still poorly understood.

Keywords

angiogenesis, erythropoietin, mesenchymal stem cells, osteogenesis.

Structure and synthesis of Erythropoietin

Erythropoietin (EPO) is a glycoprotein hormone which belongs to type I Cytokine Superfamily (Coleman and Brines 2004). EPO is most similar to cytokines, which modulate growth and inflammation (Erbayraktar et al. 2003). EPO is made up of about 60% protein and 40% carbohydrate (Rölfing 2014). Molecular weight of the EPO is 30.4 kDa (Jelkmann 2013).

The feature of erythropoietin is its multifunctionality because, depending on conditions, this protein can act as cytokine, hormone and growth factor. Erythropoietin realizes its cytokine effect through its interaction with specific receptors located on the surface of the target cells, which leads to the signal transduction from external to internal medium of the target cells. Erythropoietin acts as a classic hormone because it is synthesized in one organ (interstitial cells of the peritubular capillary bed of the renal cortex in adults or perisinusoidal cells of the liver during the embryonic and perinatal period), then it is released into the blood flow and effects on remote structures, including the hematopoietic system (red blood cells progenitors and precursors found in human bone marrow). And, finally, as a growth factor, erythropoietin is essential for mature red blood cells formation from multipotent progenitor cells (Uversky and Redwan 2017).

The gene of human EPO codes the precursor protein which includes 193 aminoacidic residues (Lappin et al. 2002, Sasaki et al. 2000, Vazquez-Mellado et al. 2017). Removal of 27-aminoacid sequence results in formation of mature protein, which is subjected to the N-glycosylation at three aspartic residues, O-glycosylation at serine residue; C-terminal arginine is removed to create final circulating form which includes 165 aminoacidic residues. Thus, mature EPO is heavily glycosylated (Jelkmann 2007, Ridley et al. 1994, Vazquez-Mellado et al. 2017), has three N-linked and one O-linked acidic oligosaccharide side chains (Jelkmann 2013, Ridley et al. 1994, Vazquez-Mellado et al. 2017). Four anti-parallel α-helixes (αA, αB, αC and αD in order from N-terminal to C-terminal) with adjacent cycles determine spatial tertiary structure of EPO (Desai 2012, Lappin et al. 2002). Glycosylated chains maintain the biological activity of EPO and ensure stability of the structure (Maiese et al. 2008, Toyoda et al. 2000, Vazquez-Mellado et al. 2017). The EPO molecule is hydrophobic and requires presence of the disulphide bridges for its activity (Ridley et al. 1994).

EPO production depends on the transcription rate of the EPO gene (located in the 7th chromosome). The process of EPO gene transcription involves few transcription factors. The EPO gene promoter is inhibited by GATA-2 and nucleus factor κB (NF-κB), which probably are responsible for a decrease in EPO expression during inflammatory diseases (Bunn et al. 1998, La Ferla et al. 2002, Jelkmann 2013).

Kidneys are the primary site of EPO production. During hypoxia, the EPO gene expression increases in the kidney peritubular fibroblasts via hypoxia-induced transcription factor, and EPO is subsequently released into the circulation (Rölfing 2014). Before the birth, the liver is a primary site of EPO production, in adults approximately 10% of EPO is synthesized by the liver (Dame et al. 1998, Jelkmann 2007, Rankin et al. 2007, Rölfing 2014, Zanjani et al. 1977). Predominant expression of EPO occurs in kidneys and liver, though transcripts can be also found in other tissues, such as brain, heart, and lungs (Elliott and Sinclair 2012).

Recombinant Erythropoietin (Epoetin)

Biotechnological achievements have made it possible to clone and produce a great amount of human recombinant erythropoietin (rhEPO) (Flaharty et al. 1990) and its analogues (erythropoiesis-stimulating agents (ESAs)) (Elliot and Sinclair 2012, Jacobs et al. 1985, Rölfing 2014). Endogenous erythropoietin and recombinant erythropoietin are identical, except for some minor differences in glycosylation (Koury and Bondurant 1992, Rölfing 2014). Human recombinant erythropoietin was first characterised as a hematopoietic factor (Ye et al. 2010). It was shown that this substance stimulated differentiation and proliferation of erythroid precursor cells (Broudy et al. 1991, Ye et al. 2010). Recombinant forms of erythropoietin are epoetin-alfa (human erythropoietin which is produced in cell culture using recombinant DNA technology), epoetin-beta (which is a synthetic recombinant form of EPO) and long-term acting analogue darbepoetin-alfa (which is also a synthetic form of erythropoietin) (Arcasoy 2008, Uversky and Redwan 2017). Use of recombinant forms of erythropoietin was characterised to treat anaemia during pregnancy (Sanchez-Gonzalez et al. 2016), chronic kidney disease (Tögel et al. 2016) or chemotherapy-induced anaemia during oncological diseases (Arcasoy 2008, Elliot and Sinclair 2012, Suk et al. 2008).

Ubiquitous expression of erythropoietin receptors on non-erythroid cells is the evidence that the erythropoietin plays a lot of biologically important roles in non-hematopoietic tissues (Arcasoy 2008, Arcasoy 2010).

Recombinant erythropoietin was characterized as a tissue protective factor (Elliot and Sinclair 2012, Rocha et al. 2015, Vinberg et al. 2015). Protective effect of erythropoietin is dose-dependent and receptor-dependent. This circumstance has resulted in pharmacological synthesis of various types of erythropoietin: Asialoerythropoietin has half-life under 2 minutes (whereas for erythropoietin this index is approximately 5–10 hours); Carbamyl-erythropoietin (CEPO) is characterised by presence of a few NH2 groups. It was shown that CEPO has bigger protective effect in such injuries, as spinal cord compression, diabetic neuropathy or experimental autoimmune encephalitis without the erythropoiesis stimulation effect (Buemi et al. 2009).

There were identified some properties of recombinant erythropoietin in regulation of genes expression and their function similar to vascular endothelial growth factor (VEGF), main angiogenic factor (Suk et al. 2008). It is known that erythropoietin is able to stimulate proliferation of endothelial cells *in vitro* and mobilize endothelial progenitor cells from bone marrow (Bahlmann et al. 2003, Bahlmann et al. 2004, Heeschen et al. 2003, Klopsch et al. 2009), increase adhesive and proliferative characteristics of circulating endothelial progenitor cells (George et al. 2005, Klopsch et al. 2009) and induce neovascularization of ischemic tissue *in vivo* (Ye et al. 2010). Under the influence of erythropoietin, embryonic stem cells can differentiate into cardiomyocytes; erythropoietin also facilitates proliferation of cardiomyocytes (Broudy et al. 1991, Sachinidis et al. 2002, Ye et al. 2010). Erythropoietin is able to influence differentiation of neuronal stem cells into neuronal cells (Chen et al. 2009, Kretz et al. 2005, Ye et al. 2010).

Anti-ischemic effects of recombinant Erythropoietin

In a pharmacological aspect, erythropoietin is one of the most perspective humoral agents which are involved in the preconditioning phenomenon. A number of studies showed that erythropoietin has a protective effect on ischemia-reperfusion in various organs and tissues (Danilenko 2013). The opportunity of using recombinant erythropoietin in suberythropoietic doses is justified for pharmacological preconditioning; influence of recombinant erythropoietin on ischemic tissues survival was revealed and it was concluded that this effect was similar to that from remote preconditioning (Kolesnik et al. 2010). Recombinant human erythropoietin is of increased interest in regard to pharmaceutical simulation of ischemic preconditioning because it proved its efficacy in protection of heart, brain and liver tissues from pathological influence of ischemia-reperfusion (Alehin et al. 2015).

Erythropoietin is highly effective in prevention and treatment of ischemic-reperfusion-induced acute kidney injury. Derivatives of erythropoietin (asialated erythropoietin, carbamylated darbepoetin) have nephroprotective effects (Elagin et al. 2018).

Recombinant erythropoietin was found to stimulate neovasculogenesis in ischemic muscle (Kolesnik et al. 2011, Pokrovskiy et al. 2012).

A possibility of pharmacological correction of ischemia and reperfusion injuries of small intestine and liver tissues with recombinant erythropoietin in a suberythrostimulating dose in rats was investigated. It was shown that injection of recombinant erythropoietin in a suberythrostimulating dose in rats had a significant effect on microcirculation recovery both in liver and small intestine tissues. Erythropoietin is considered as a preconditioning agent which realizes its effect via the early preconditioning mechanisms (Alekhin et al. 2011).

Using of recombinant erythropoietin Darbepoetin alpha is characterized by development of endothelium- and cardioprotective effect (Denisyuk et al. 2015, Denisyuk and Pokrovsky 2016, Korokina et al. 2009). In the experiments on animals' (rats') hearts, it was shown that application of erythropoietin led to improved coronary perfusion. This effect is caused by activation of endothelial NO synthase and Akt (Protein kinase B) activating phosphorylation, which leads to long NO-dependent vasodilatation. Some evidence was obtained that recombinant erythropoietin is characterized by a protective effect in L-NAME-induced endothelial disfunction and ischemia of limbs, abdominal organs and retina (Denisyuk et al. 2015).

An independent cardioprotective effect of erythropoietin was proven, as well as a decrease in the myocardial infarction zone and improvement of heart function in ischemia-reperfusion, irrespective of changes in haemoglobin, red blood cell count and oxygen tension in blood. Erythropoietin is a trigger of ischemic preconditioning and may cause pharmacological preconditioning; realisation of natural mechanisms of protection from ischemia is carried out through the activation of ATP-dependent potassium channels and biosynthesis of nitric oxide, pharmacological mimetics of preconditioning which induce a reproducible and stable cardioprotective reply and which lack any serious side effects (Danilenko 2013). Some studies show that decreased apoptosis is one of the possible most important mechanism of erythropoietin's protective effect (Danilenko et al. 2012, Danilenko 2013, Denisyuk and Pokrovsky 2016, Denisyuk 2016).

Using recombinant erythropoietin leads to a significant increase in anti-inflammatory effect (Danilenko et al. 2011).

Erythropoietin receptors and signal transduction pathways

Erythropoietin is known to bind to membrane receptors (EPOR) localized on the surface of the target cells (Jelkmann 2007). EPOR belongs to Class I of Cytokine superfamily receptors, which are characterized by the presence of extracellular N-terminal sequence. Mature human EPOR is a glycoprotein which includes 484 aminoacidic residues and 1 N-glycan. Molecular weight of human EPOR is 52.6 kDa, and because of glycosylation and phosphorylation it increases to 60 kDa (Jelkmann 2007).

In hematopoietic cells, two molecules of erythropoietin receptor (EPOR) form a homodimer (EPOR/EPOR) with high affinity to its ligand erythropoietin (Rölfing et al. 2014). Binding with erythropoietin induces conformational changes and leads to tight conjunction of two monomer EPOR molecules. As a result, Janus kinases 2 (JAK2) molecules (Witthuhn et al. 1994), which contact with EPOR in the juxtamembrane domain, converge and subsequently transphosphorylate (Elliott and Sinclair 2012). Activation of JAK2 leads to phosphorylation of a few EPOR tyrosine residues, which serve as sites for

signal molecules binding, such as Src homology-2 (SH2) domain-containing proteins and signal transducer, and activator of transcription (STAT) 1, 3 and 5 (Carter-Su et al. 2016, Kirito et al. 2002, Klingmuller et al. 1996, Kwon et al. 2014, Li et al. 2015, Ma et al. 2016, Rafiee et al. 2005, Vazquez-Mellado et al. 2017, van der Kooij et al. 2008, Ye et al. 2010). Activated JAK2 also induces signaling pathways via nuclear factor kappa B (NF-κB) (Ma et al. 2016). Erythropoietin is known to be the main activating factor of JAK2/STAT3 signaling pathway, which is confirmed to be launched in blood diseases and cardiac cerebral vascular diseases (Chen et al. 2009, Piuhola et al. 2008, Ye et al. 2010). When STAT3 signaling transduction pathway is activated, the activated STAT3 transfers the signal directly into the nucleus and regulates multiple genes (Ye et al. 2010). STAT5 and NF-κB translocate into the nucleus and act as transcription factors for Bcl-2 (Correia et al. 2015, Ma et al. 2016) and Bcl-xL (Ma et al. 2016, Schwartz et al. 2015), which are antiapoptotic genes (Ma et al. 2016). In addition to activation of JAK/STAT pathway, erythropoietin also stimulates other signaling pathways, including phosphatidylinositol-3 kinase/Akt (PI3K/Akt) pathway, mitogen-activated protein kinase/extracellular signal-related kinase (MAPK/ ERK1/2) pathway and protein kinase C, ensuring the survival of the cell and its proliferation (Bao et al. 1999, Damen et al. 1995, Hiram-Bab et al. 2015, Hiram-Bab et al. 2017, Kwon et al. 2014, Vazquez-Mellado et al. 2017). The effect of erythropoietin is terminated by the influence of haematopoietic cell phosphatase (HCP) which catalyzes JAK2 dephosphorylation (Jelkmann 2007).

Unlike hematopoietic cells, in non-hematopoietic cells erythropoietin carries out its functions through the interaction with a heterodimeric receptor (EPOR/CD131) (Rölfing et al. 2014). In comparison with homodimeric EPOR, which is present in the hematopoietic system, the heterodimeric receptor is characterised by lower affinity to its ligand, erythropoietin. Due to this, for erythropoietin to realize its protective effect towards tissues and other pleiotropic effects, a bigger concentration of the hormone is required than during erythropoiesis stimulation (Rölfing 2014).

It is known that on the surfaces of the cells which are sensitive to erythropoietin, EPOR and a common receptor subunit β (βcR), also known as CD131, are co-expressed. The common receptor subunit β provides for an increase in the binding affinity of the ligand to the receptor complex and is also a signal-transducing component common to the granulocyte-macrophage colony stimulating factor (GM-CSF), IL-3 and IL-5 receptors. Furthermore, tissue protection is known to be realized through this heteroreceptor complex containing EPOR and βcR (Brines et al. 2004).

Non-hematopoietic intracellular signaling pathways include production of nitrogen oxide and signaling through JAK2, STAT3/5, PI3K/Akt, as well as MAPK pathways (Rölfing et al. 2014). Some functions are activated when specific pathways are activated, whereas for erythropoietin to exercise its full pleiotropic effect, all signalling intracellular pathways need to be activated (Rölfing et al. 2014).

Mechanisms of effect of Erythropoietin on hematopoietic and non-hematopoietic cells and tissues

The effect of Erythropoietin on hematopoietic tissues

Erythropoietin was first identified as a hormone whose main function was to stimulate differentiation of erythroid cell precursors in bone marrow into functional erythroblasts (Coleman and Brines 2004, Debeljak et al. 2014, Flaharty et al. 1990, Ganz 2018, Perreault and Venters 2018, Ridley et al. 1994, Tojo et al. 2015), which makes it possible to regulate delivery of oxygen to the tissues (Semenza and Wang 1992). Erythropoietin increases the number of developing erythroid precursors and accelerates release of reticulocytes from bone marrow, but does not influence on the duration of the cell cycle or the number of meioses which take place during the cell differentiation (Ridley et al. 1994). Erythropoietin increases the pool of cells capable of differentiating into erythroid cells. It is an important hormone which determines differentiation of cells in the erythroid direction, interacts with early-stage (burst forming unit-erythroid, BFU-E) and late-stage (colony forming erythroid unit, CFU-E) erythroid precursors, cells committed to erythroid differentiation. Erythropoietin apparently stimulates proliferation of proerythroblasts and basophilic erythroblasts, which are the first cells to be morphologically recognized as erythroid elements; however, erythropoietin is not an obligatory differentiation growth factor in the erythroid direction at this stage (Ridley et al. 1994). Regulation of red blood cells production is mediated via presence of a specific cellular receptor for erythropoietin on the surface of cells (Arcasoy 2008).

The effect of Erythropoietin on non-hematopoietic tissues

For a long period of time, there was an opinion that erythropoietin influenced only on erythroid precursor cells (Lappin et al. 2002). At present, it is known that erythropoietin is a pleiotropic growth factor and, besides its classic role in facilitating erythropoiesis, this hormone carries out a number of biological functions (Wang et al. 2018), including activity towards multiple cells and tissues (Coleman and Brines 2004, Debeljak et al. 2014, Holstein et al. 2011). This hormone is able to stimulate bone tissue recovery after damage (Holstein et al. 2007, Holstein et al. 2011, Garcia et al. 2011, Klontzas et al. 2016), wound healing due to erythropoietin-mediated stimulation of cell proliferation and angiogenesis (Gupta and Wish 2017) which is related to increased expression of vascular endothelial growth factor (VEGF), endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) (Holstein et al. 2011). Erythropoietin is known to have a neuroprotective effect (Bramlett et al. 2016, Juul

and Pet 2015, Liu et al. 2017, Marelli et al. 2016, Modrak et al. 2017, Ott et al. 2015) and to provide liver regeneration (Kedarisetty et al. 2015).

Expression of erythropoietin was found in multiple tissues and types of cells outside the kidney tissue, including astrocytes, neurones, female genital tracts, male genitals, mammary glands, placenta trophoblasts, bone marrow macrophages and erythroid precursors (Arcasoy 2010, Bernaudin et al. 1999, Conrad et al. 1996, Juul et al. 2000, Kobayashi et al. 2002, Magnanti et al. 2001, Marti et al. 1996, Masuda et al. 2000, Sato et al. 2000, Stopka et al. 1998, Vogt et al. 1989). mRNA of erythropoietin was also revealed in liver, spleen, lungs; besides, erythropoietin could be produced in small amounts in these organs (Jelkmann 2013). The research results showed that the receptors for erythropoietin are expressed on various cells of the fetus and the adult organism, including cells of central nervous system, digestive canal, kidney, muscle tissue (smooth, skeletal and cardiac), uterus, retina, pancreas, gonads and lungs (Ammarguellat et al. 1996, Anagnostou et al. 1994, Arcasoy 2010, Erbayraktar et al. 2003, Grimm et al. 2002, Liu et al. 1997, Ogilvie et al. 2000, Shiozawa et al. 2010, Westenfelder et al. 1999, Wu et al. 1999). Due to this, erythropoietin can influence non-hematopoietic cells and tissues (Debeljak et al. 2014).

Thus, it was revealed that erythropoietin, in addition to its basic role in the regulation of erythropoiesis in mammals, influences proliferation and apoptosis of various non-hematopoietic cells through the erythropoietin receptors (Arcasoy 2008). The characteristic of non-erythropoietic biological effects of erythropoietin and understanding the mechanisms of signal activation of the erythropoietin/erythropoietin receptor systems in non-hematopoietic organs and types of cells are important for future development of new methods of applying erythropoietin and its derivatives (Arcasoy 2008).

The effect of Erythropoietin on mesenchymal stem cells and process of bone tissue formation

Mesenchymal stem cells (MSCs) are primary multipotent stem cells with an ability of self-renewal and differentiation into multiple cell lines. MSCs have fibroblast-like morphology and express stromal markers, including CD73, CD105, CD29, CD44 and CD90, but not hematopoietic cell markers (such as CD34, CD44 and CD45) (Chen et al. 2013, Ye et al. 2010).

Bone-marrow-derived mesenchymal stem cells are suitable for transplantation and are widely used in tissue engineering and cellular therapy because they are characterised by simple selection and obtaining, multipotent potential, immunosuppression and stability during autologous transplantation (Li et al. 2017, Rennert et al. 2012, Ye et al. 2010).

However, the population of MSCs in bone marrow is not large (about 0.001–0.01% of all nuclear cells), thus it is necessary to increase the number of MSCs in culture for subsequent use during scientific experiments or clinical application (Ye et al. 2010). Whereas some studies showed that MSCs tended to lose pluripotency and proliferative capacity when cultivated *in vitro* (Banfi et al. 2000, Digirolamo et al. 1999, Wagner et al. 2008, Ye et al. 2010), MSCs are also known to be capable of spontaneous differentiation during *in vitro* passaging in the absence of special inducing factors (Deng et al. 2006, Sachinidis et al. 2002, Ye et al. 2010). In this way, instability of cultured MSCs and their decreased stemness limit their wide application in clinic (Ye et al. 2010).

The indicator of undifferentiated state and multiple differentiation potential of MSCs is the mRNA expression level of embryonic transcription factors OCT4, Nanog, Sox2 and TERT (Boyer et al. 2005, Greco et al. 2007, Loh et al. 2006, Wang et al. 2006, Ye et al. 2010). It was shown that erythropoietin regulates the mRNA expression of TERT, OCT4, Nanog, Sox2 genes (Ye et al. 2010, Saei Arezoumand et al. 2017), which are linked with a cell differentiation delay. In this case, the effect of erythropoietin depends on duration of its action and concentration. It is proved that erythropoietin could maintain MSCs in undifferentiated state for 12 hours at a concentration of 5 U/ml (Ye et al. 2010).

Erythropoietin is also known to promote MSCs proliferation (Minguell et al. 2001, Ye et al. 2010, Zheng et al. 2009). Thus, influencing proliferation, erythropoietin is able to increase the number of MSCs while preserving their multiple differentiation potential, to improve MSCs expansion in cell culture and to maintain culture *in vitro* (Ye et al. 2010).

Despite the achievements in transplantation of bone-marrow-derived mesenchymal stem cells, only a tiny fraction of transplanted cells migrates to the target sites. This is why it is vital to develop a strategy of more effective delivery of MSCs to appropriate structures (Ye et al. 2010). It is known that erythropoietin activates a directed migration of bone-marrow-derived mesenchymal stem cells into the organs which were damaged, for example, kidneys and heart (Lin et al. 2008, Liu et al. 2013, Vazquez-Mellado et al. 2017). Erythropoietin promotes mobilization of bone-marrow-derived mesenchymal stem cells by increasing the stromal cell-derived factor-1 (SDF-1) chemokine levels (Satoh et al. 2006, Vazquez-Mellado et al. 2017).

In addition, the important problem of applying MSCs in tissue engineering is their low viability and functionality, as well as their potential capacity to differentiate after transplantation. The majority of MSCs undergo apoptosis after transplantation. This is why improving MSCs viability and functionality is necessary to enhance their potential application efficiency. A few strategies aiming at improving the ability of MSCs to form tissues have been studied, including those with applying growth factors, overexpression of regulatory genes by stem cells and with improving scaffolds biomaterials. All the approaches used were connected with activation of PI3K/Akt signaling pathway. This pathway plays key regulatory roles in MSCs survival, proliferation, migration, angiogenesis, cytokine production and differentiation processes (Chen et al. 2013).

The effect of Erythropoietin on osteogenic differentiation of MSCs

MSCs are able to differentiate into the osseous, cartilaginous and adipose tissue lineages after stimulation in certain conditions. When being cultivated in the presence of glucocorticoid dexamethasone, ascorbic acid and beta-glycerophosphate (factors of osteogenic differentiation), MSCs differentiate in osteogenic direction and form bone-like nodules with mineralized extracellular matrix containing hydroxyapatite. One of the potential pathways of signaling transduction which can participate in regulation of proliferation and differentiation of MSCs in osteogenic direction is MAP kinase pathway. Members of MAP kinase family are extracellular signal-regulated kinases (ERK1 and ERK2), c-Jun N-terminal kinase (JNK), which is also known as stress-activated protein kinase, and p38-reactivating kinase (p38 RK or simply p38). Transforming growth factor-beta stimulates ERK and p38 and acts as a physiological regulator of osteoblasts differentiation and bone tissue remodelling by regulation and coordination of osteoblasts and osteoclasts activity. It was found out that differentiation of cells in osteoinductive culture medium activates ERK2, JNK2 and p38 in time-dependent manner. ERK2 is activated via phosphorylation during differentiation of cells (Jaiswal et al. 2000). Elevated activity of NF-κB (which belongs to the Rel transcription factor family) in human mesenchymal stem cells was found to stimulate differentiation of cells in osteogenic direction, whereas decreased NF-κB signaling does not prevent the osteogenic differentiation (Hess et al. 2009).

It was shown that microRNA (miRNA) promotes osteogenic differentiation via gene regulation, for example miR-2861 maximizes the Runx2 activity (Meng et al. 2015). It is known that PI3K/Akt and β-catenin pathways are able to regulate bone development. In this case, increased levels of β-catenin lead to enhancement of Runx-2 expression and finally facilitated bone formation. There is evidence that miR-21 can regulate PI3K/Akt pathway, and also regulates β-catenin pathway (Meng et al. 2015).

BMP-2 and BMP-4 factors are known to stimulate MSCs differentiation in osteogenic direction. Moreover, MSCs, which produce BMP-2, improve bone tissue repair in the sites of defects (Levy et al. 2010).

Activation of STAT proteins, which are inactive cytoplasmic transcription factors, occurs as a result of stimulation of a variety of receptor types, and is usually accompanied by STAT tyrosine and serine phosphorylation. Members of the JAK tyrosine kinase family usually mediate STAT tyrosine phosphorylation, which leads to activation of STAT, its dimerization and translocation into nucleus. Transcriptional activity of STAT is significantly regulated by serine phosphorylation, which usually leads to transcription of proliferation-related genes (Levy et al. 2010).

The role of JAK-STAT signaling in MSCs is not properly studied yet; perhaps JAK-STAT signaling turns on in response to the influence of BMP-2 and BMP-4 (Levy et al. 2010).

Based on pleiotropic effects of erythropoietin on bone tissue, this hormone is attractive for increasing efficiency of bone tissue repair (Omlor et al. 2016). However, there have been a few studies to investigate the influence of erythropoietin on bone tissue repair *in vivo* (Bozlar et al. 2006, Garcia et al. 2011, Holstein et al. 2007, Holstein et al. 2011, Omlor et al. 2016, Rölfing et al. 2012, Shiozawa et al. 2010, Sun et al. 2012). At present, the effect of erythropoietin on bone tissue is not fully understood (Balaian et al. 2018).

A positive effect of erythropoietin on differentiation of mesenchymal stem cells in osteogenic direction attracts a significant interest (Wang et al. 2018). It is known that mesenchymal stem cells immediately after fracture migrate to site of injury and differentiate into osteoblasts preceding the bone regeneration (Nair et al. 2013).

Most scientific papers note that erythropoietin triggers differentiation of mesenchymal stem cells into osteoblasts *in vitro* (Balaian et al. 2018, Kim et al. 2011, Li et al. 2015, Nair et al. 2013, Shiozawa et al. 2010).

On the surface of human MSCs, there are EPOR and CD131 receptors, which form EPO heterodimer receptor (EPOR/CD131) (Brines et al. 2004, Rölfing et al. 2014, Ye et al. 2010, Zwezdaryk et al. 2007). According to data obtained by Rölfing et al. (2014), there is no exclusive intracellular pathway responsible for the osteogenic differentiation of erythropoietin-stimulated MSCs (Rölfing et al. 2014). It is known that osteogenic effect of erythropoietin on the mesenchymal stem cells is realized through the mammalian target of rapamycin (mTOR), JAK2 and PI3K signaling pathways (Rölfing et al. 2014).

It was shown that PI3K/Akt pathway is important in the process of MSCs differentiation into different cell types. However, the role of the pathway is complicated because of the opposite effects. PI3K/Akt signaling pathway regulates proliferation of cells, apoptosis, differentiation and migration. The factors which can stimulate the pathway activation include various growth hormones, cytokines and other molecules (Chen et al. 2013). The key enzyme of the pathway is PI3K, which converts phosphatidylinositol 4,5-biphosphate into phosphatidylinositol 3,4,5-triphosphate, which binds Akt and 3-phosphoinositide-dependent protein kinase 1 (PDK1), and allows PDK1 to phosphorylate Akt. Class 1A PI3K consists of two subunits: p110 catalytic subunit and p85 regulatory subunit. There are four isoforms of p110 subunit: p110-alpha, -beta, -gamma and –sigma and three isoforms of p85 subunit: p85-alpha, -beta and -gamma. The main target protein of the PI3K pathway is Akt. There are three isoforms of Akt: Akt1, Akt2, Akt3. Activation of Akt triggers a cascade of responses which regulate the cell functions. For instance, Akt regulates migration of cells by means of Rac1 and rhoA, increases viability through bcl-2, elevates angiogenesis via VEGF, and enhances proliferation by activation of mammalian target of rapamycin (Chen et al. 2013).

Activation of PI3K/Akt signaling pathway in MSCs is applied both in cellular therapy and in tissue engineering (Chen et al. 2013).

It is proved that the ability of MSCs to secrete cytokines is regulated by means of activity of PI3K/Akt signaling pathway. In this case, MSCs are able to secrete a number of cytokines which can regulate other cells and contribute to tissue recovery. These cytokines include vascular endothelial growth factor, fibroblast growth factor (FGF), monocyte chemoattractant protein-1, hepatocyte growth factor (HGF), insulin-like growth factor 1 (IGF-1), stromal cell-derived factor 1 (SDF-1), and thrombopoietin. Overexpression of Akt in MSCs increases expression of genes VEGF, FGF-2, HGF and IGF-1 and thus enhances secretion of these cytokines. These cytokines influence MSCs, increasing their functionality. For example, MSCs are able to express receptor to HGF, c-Met (tyrosine-protein kinase Met or hepatocyte growth factor receptor (HGFR)). HGF at a concentration of 20 ng/ml promotes osteogenic differentiation by activating c-Met, Akt pathways and enhanced expression of the cell-cycle inhibitor p27 and osteogenic transcription factors Runx2 and Osterix, whereas at high concentrations (100 ng/ml), HGF promotes proliferation and suppresses osteogenic differentiation by activating the ERK1/2 pathway and inhibiting the Akt pathway (Chen et al. 2013).

IGF-1 is found to be expressed in MSCs to increase osteogenesis for further bone repair. The mechanism includes activation of insulin receptor substrate-1 and phosphatidylinositol-3-kinase signaling pathway (IRS1-PI3K). Also, IGF-1 can activate the MAPK pathway. Some studies show that inhibition of MAPK increases rather than decreases the rate of osteogenesis (Higuchi et al. 2002, Osyczka and Leboy 2005, Payne et al. 2010, Wang et al. 2012), proving that the PI3K/Akt pathway plays a key role in IGF-induced osteogenesis. It was shown that mechanically induced activation of Akt also increases the rate of osteogenesis. There are also opposite data about the role of the PI3K/Akt pathway in the process of osteogenesis, according to which, imatinib stimulates osteogenesis by inhibiting platelet-derived growth factor receptors/PI3K/Akt (PDGFR/PI3K/Akt) signaling in MSCs. However, the reason of this phenomenon is not completely understood yet. It is known that BMP-2 enhances osteogenesis by activating the PI3K/Akt pathway. At present, a number of signaling pathways are known to be involved in the process of cell differentiation in certain direction (Chen et al. 2013, Higuchi et al. 2002, Osyczka and Leboy 2005, Payne et al. 2010, Wang et al. 2012).

Erythropoietin is known to stimulate bone tissue formation and to increase the number of osteoblasts *in vivo* (Balaian et al. 2018, Mihmanli et al. 2009, Rolfing et al. 2012, Sun et al. 2012), especially if the ephrinB2/EphB4 signaling pathway is activated (Balaian et al. 2018, Li et al. 2015,). Erythropoietin regulates expression of EphB4 receptor of bone marrow mesenchymal stem cells (Balaian et al. 2018). Interaction of ephrinB2 with EphB4 receptor influencing erythropoietin-activated MSCs, triggers differentiation of cells (Balaian et al. 2018). Erythropoietin enhances osteoblast activity through the EphB4 signaling pathway, and also increases the number of ephrinB2-expressed osteoclasts but decreases their resorption activity. The signals activated by erythropoietin through ephrinB2/EphB4 result in bone formation (Balaian et al. 2018).

Erythropoietin was found to act in collaboration with ephrinB2 and to induce osteoblast differentiation, but it can also influence this process through other potential mechanisms (Balaian et al. 2018). In the mouse cell line RAW264.7, erythropoietin significantly increases expression of Nfatc1, which is an obligatory transcription factor for osteoclastogenesis and regulates differentiation and fusion of osteoclasts. Moreover, erythropoietin significantly decreases expression of Mmp9 and Ctsk, which are involved in osteoclast-mediated resorption of organic components of the bone (Balaian et al. 2018). Furthermore, erythropoietin enhances expression of ephrinB2 on the cell line RAW264.7, which can indicate that erythropoietin increases the number of osteoclasts which are less capable of bone tissue resorption, but express a higher level of ephrinB2 (Balaian et al. 2018).

Erythropoietin can stimulate bone tissue formation due to increased expression of vascular endothelial growth factor (Holstein et al. 2011).

It was revealed that erythropoietin activates hematopoietic stem cells to produce BMP factors (BMP2, BMP6). In addition, it was discovered that erythropoietin influences directly on bone marrow mesenchymal stem cells and induces their differentiation in osteogenic direction (Kim et al. 2011, Shiozawa et al. 2010). It is important that in *in vivo* conditions erythropoietin mediates bone formation by influencing simultaneously on both hematopoietic stem cells and the osteoblast niche. Thus, erythropoietin regulates bone formation directly and indirectly, which illustrates a link between the hematopoiesis and osteopoiesis processes in bone marrow (McGee et al. 2012, Shiozawa et al. 2010).

The evidence of the fact that erythropoietin causes differentiation of bone marrow mesenchymal stem cells in osteogenic direction is bone phenotype expression by cells, including activation of the bone-specific transcription factor Runx2, osteocalcin (OCN) and bone sialoprotein (BSP) under the influence of erythropoietin. Moreover, erythropoietin mediates an increase in mineral accumulation by the cell cultures and enhanced expression of alkaline phosphatase (ALP) (Shiozawa et al. 2010).

For erythropoietin-mediated osteoblast differentiation of human mesenchymal stem cells, the mTOR signaling pathway needs to be activated (Kim et al. 2011). It was also found out that erythropoietin in a combination with receptor activator of nuclear factor kappa-B ligand (RANKL) increased the number of osteoclasts formed from mouse marrow mononuclear cells (mMMCs) and mouse macrophage cell line RAW264.7. However, erythropoietin inhibits osteoclasts activity by decreasing cathepsin K regulation in mTOR-independent manner (Kim et al. 2011). During osteoblastogenesis, mTOR signaling may influence proliferation and differentiation of osteoblasts (Kim et al. 2011).

The effect of Erythropoietin on angiogenesis

It is well known that osteogenesis and angiogenesis are interconnected processes which are both required for bone formation (Wu et al. 2014). Angiogenesis is a complex process of new blood vessels formation from pre-existing ones. The process of angiogenesis includes several various stages: production and release of angiogenic factors, binding with endothelial cell receptors and subsequent activation of proliferation, directed migration, extracellular matrix remodelling, preparation and stabilization of the vessels (Buemi et al. 2009).

Erythropoietin takes part in regulation of angiogenesis processes including new blood vessels formation from pre-existing ones. Physiological angiogenesis occurs actively in developing embryo, in female reproductive organs to maintain cyclic tissue renewal, and in wound healing (Arcasoy 2008). The role of erythropoietin is described as that of a pro-angiogenic factor comparable with classical pro-angiogenic factors, such as vascular endothelial growth factor and basic fibroblast growth factor (bFGF) (Zwezdaryk et al. 2007).

Expression of erythropoietin receptors on the various types of vascular endothelial cells is connected with the ability of erythropoietin to stimulate migration and proliferation of endothelial cells (Arcasoy 2008). One of the supposed mechanisms of erythropoietin-mediated stimulation of tissue neo-vascularisation is mobilization of bone marrow endothelial precursor cells into the circulatory bed (Arcasoy 2008, Bahlmann et al. 2004, George et al. 2005, Heeschen et al. 2003, Satoh et al. 2006, Vazquez-Mellado et al. 2017).

Human mesenchymal stem cells derived from bone marrow are known to express erythropoietin receptors and can promote angiogenesis following an erythropoietin treatment. Thus, human MSCs promote angiogenesis not only by direct participating in the process like endothelial cells which form new vessels, but also by secretion and recruiting of the components which are necessary for this process (Liu et al. 2011, Liu et al. 2013, Zwezdaryk et al. 2007).

Conclusions

Erythropoietin has the pleiotropic effect on multiple number of cells and tissues; it also influences the processes of remodelling and recovery of bone tissue. It is known that the effects of erythropoietin on bone tissue are linked with increased osteogenesis, osteoclastogenesis and angiogenesis. At the same moment, the mechanisms involved in the process of bone tissue build-up via erythropoietin are still not fully understood.

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