Review

Erythropoietin, a Novel Versatile Player Regulating Energy Metabolism beyond the Erythroid System

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Abstract

Erythropoietin (EPO), the required cytokine for promoting the proliferation and differentiation of erythroid cells to stimulate erythropoiesis, has been reported to act as a pleiotropic cytokine beyond hematopoietic system. The various activities of EPO are determined by the widespread distribution of its cell surface EPO receptor (EpoR) in multiple tissues including endothelial, neural, myoblasts, adipocytes and other cell types. EPO activity has been linked to angiogenesis, neuroprotection, cardioprotection, stress protection, anti-inflammation and especially the energy metabolism regulation that is recently revealed. The investigations of EPO activity in animals and the expression analysis of EpoR provide more insights on the potential of EPO in regulating energy metabolism and homeostasis. The findings of crosstalk between EPO and some important energy sensors and the regulation of EPO in the cellular respiration and mitochondrial function further provide molecular mechanisms for EPO activity in metabolic activity regulation. In this review, we will summarize the roles of EPO in energy metabolism regulation and the activity of EPO in tissues that are tightly associated with energy metabolism. We will also discuss the effects of EPO in regulating oxidative metabolism and mitochondrial function, the interactions between EPO and important energy regulation factors, and the protective role of EPO from stresses that are related to metabolism, providing a brief overview of previously less appreciated EPO biological function in energy metabolism and homeostasis.

Key words: Erythropoietin, pleiotropic cytokine, erythroid system

Introduction

EPO, the important cytokine, is required and necessary for the growth, survival and differentiation of red blood progenitor cells. EPO exerts its physiological role by binding to its specific cell surface receptor (EpoR). The mice lacking EPO or EpoR are embryonic lethal in utero due to severe anemia, suggesting its primary role of EPO in the regulation of red cell production [1]. Human EPO was first purified in 1977 and cloned in 1985 [2-4], which lead to the production of recombinant EPO. For the past two decades, human recombinant EPO has been widely used clinically in the treatment of anemia and associ-ated conditions including chronic kidney disease and chemotherapeutic cancer patients. However, the reason for EPO attracts more researchers' attention is not only its primary role in erythropoiesis but also a range of actions of EPO in nonhematopoietic system. The discovery of expression of EPO and EpoR in a number of nonhematopoietic tissues provides the strong support for the various biological function of EPO. EPO production has been detected in the brain, retina, reproductive tract, and skeletal muscle myoblasts. EpoR even can be detected in more widespread tissues including brain, endothelial cells, retina, muscle

progenitor cells, lung, heart, liver, myocardium, adipocytes, macrophages and pancreas [5-7]. Importantly, EPO and EpoR are both hypoxia inducible, suggesting the potential protective role of EPO in dealing with stress. Accumulating evidences have demonstrated the neuroprotective activity during hypoxic conditions, antiapoptotic effect and increased angiogenesis properties of EPO. Consistently, EPO and EpoR knockout mice also show impaired angiogenesis and defective brain and heart development [8-10]. More recently, some new findings promote people to realize that EPO may act as a novel regulator of energy homeostasis. For example, the mice with EpoR restricted in erythroid system exhibited significant increase in body weight gain due to increased fat mass and a decrease in total activity and energy expenditure compared with Wild type (WT) mice [6]. These mice were also glucose intolerant and became insulin resistant with the development of obesity [6]. EPO treatment can protect against diabetes development in db/db mouse model (a model of obesity, diabetes, and dyslipidemia wherein leptin receptor activity is deficient) and streptozotocin-induced type 1 and type 2 diabetes mouse models via the direct antiapoptotic, anti-inflammatory, and angiogenic effects within the islets [7]. The loss of EpoR in adipocytes negatively regulates mitochondrial function of adipocytes and leads to obese mice with decreased energy expenditure [11]. In contrast, EPO administration in wild type mice protects the mice from high fat induced obesity and improves glucose intolerance and insulin resistance [6]. Moreover, the crosstalk between EPO/EpoR and the important energy sensors such as PGC-1α, Sirt1 and AMPK also provides evidence for EPO acting as an energy metabolism regulator [11-14]. In this review, we will discuss the important activity of EPO in nonhematopoietic system especially to highlight the tissue protective role and metabolic activity regulation function of EPO. We will also describe the novel activity of EPO in the metabolism related diseases including obesity, metabolism disorders and diabetes and the involvement of EPO in the energy sensing network.

The production of EPO and the expression of EpoR in different tissues

EPO protein and tissue specific production

EPO is a glycoprotein hormone and a single polypeptide of 166 amino acids folded into four α-helix with two dissulphide brides between cysteines 6 and 161 and between cysteines 29 and 33 [3, 4, 15]. EPO shares structural homology with growth hormone and other members of the hematopoietic cytokine superfamily. In erythroid progenitors, EPO

binds to its cell surface receptor (EpoR) to activate the JAK2 and downstream signal transduction pathways including STAT5, PI3K and MAPK [16, 17]. EPO/EpoR is required for erythroid progenitor survival, proliferation and differentiation. Definitive erythropoiesis takes place in the fetal liver during development. Therefore, the major place of EPO production is fetal liver. In humans, EPO is also expressed in kidney begins from about 18 weeks before birth and then increases dramatically after 30 weeks of gestation. The EPO producing cells change from central veins surrounding hepatocytes to peritublar interstitial cells with neural characteristics [18-20]. After birth, the kidney becomes the principal EPO producing organ with the site of hematopoiesis switches to the bone marrow. However, EPO expression in liver is inducible and can be induced to 20%-50% of total body EPO mRNA by hypoxia in mice [21, 22]. In addition, in adult kidney, the EPO is also inducible. Hypoxia inducible factor (HIF) plays a primary regulatory role in hypoxia induced EPO expression in both liver and kidney although the regulation of EPO expression in the liver and kidney requires different flanking regulatory elements, which are located 3' for liver expression and 5' for kidney expression, respectively [23-25].

Besides the kidney and fetal liver, EPO production is also detected in the brain, female organs and skeletal muscle myoblasts. In the brain, EPO is produced by astrocytes and neurons [26-28]. EPO production in the brain is also hypoxia-inducible [27, 29]. In the human brain, EPO is observed in the fetal CNS and in the spinal fluid of normal preterm and term infants [30, 31]. EPO production in female reproductive organs can be stimulated by estrogen and contributes to the regulation of the cyclic changes in female reproductive organs [32-34]. Also, hypoxia can induce EPO production in female organs [35]. Reduced oxygen tension induced EPO is also detected in skeletal muscle myoblasts, which contributes to muscle maintenance and repair [36-38]. The expression of EPO in non-hematopoietic cells provides evidence for its pleiotropic biological function.

EpoR expression and regulation

The EPO response in hematopoietic cells relies not only on the EPO concentration but also on the extent of EpoR expression. EpoR is expressed in a cell-restricted fashion and expression depends on the stage of differentiation. EpoR expresses at a relative low level on early erythroid progenitor cells or BFU-E (erythroid burst-forming unit) stage [39]. The expression is then increased during erythroid differentiation by more than tenfold on the erythroid progenitor cells by the CFU-E stage, which requires EPO signaling for

protection against apoptosis. During late erythropoiesis, EpoR expression is downregulated and EPO/EpoR signaling is no longer required for the erythroid cell survival [39]. EpoR expression is also regulated by erythroid specific transcription factors including GATA1, SCL/TAL1 and EKLF (KLF1) [40-42].

The expression of EpoR now has been demonstrated beyond hematopoietic cells, which provides the possibility for EPO function generally in proliferation, protective/survival activity in multiple tissue development, maintenance and or repair. The two well-documented roles of EPO beyond hematopoietic system are angiogenesis and neuroprotection that are related to the findings of EpoR expression in endothelial cells and brains (Figure 1). Expression of EpoR in nonhematopoietic tissues was first detected in endothelial cells [43, 44] and the expression can be induced by hypoxia when combined with EPO treatment [45, 46]. The expression of EpoR in endothelial cells mediates increased angiognesis in vascular system (Figure 1), promotes survival of primary human endothelial cells and also importantly, contributes to cardioprotective effects of EPO in animal model [47-49]. In the developing mouse brain during mid gestation, EpoR was found to localize to the neural tube in the neuroepithelium that contains proliferating neuroprecursors [50, 51]. EpoR is expressed at a high level in the neural tube at E10.5, which is comparable to adult hematopoietic tissue in mice [51].With the neural progenitor cell differentiation to mature neurons, EpoR expression was down-regulated [51]. EpoR level persists at low levels through adulthood [51]. In adult brain from humans, nonhuman primates and rodents, EPO binding experiments provided the evidence of EpoR localization in the hippocampus, cortex and midbrain [52, 53]. In addition to neurons, EpoR is also expressed in astrocytes and microglia [54]. Harmful stress, such as inflammation and ischemia can up-regulate neuronal EpoR, suggesting that the EPO/EpoR signaling can provide preconditional protection against severe ischemia. Hypoxia also induced EpoR expression in neuronal cells and increases sensitivity to EPO (2007 review 7, 28). However, no evidence shows EpoR can be directly regulated by hypoxia inducible factor (HIF). Similar to the situation in erythroid cells, EpoR expression in neural NT2 cells can be transactivated by GATA-3, a transcription factor required for brain development and inducible by EPO (2007 review 7,32).
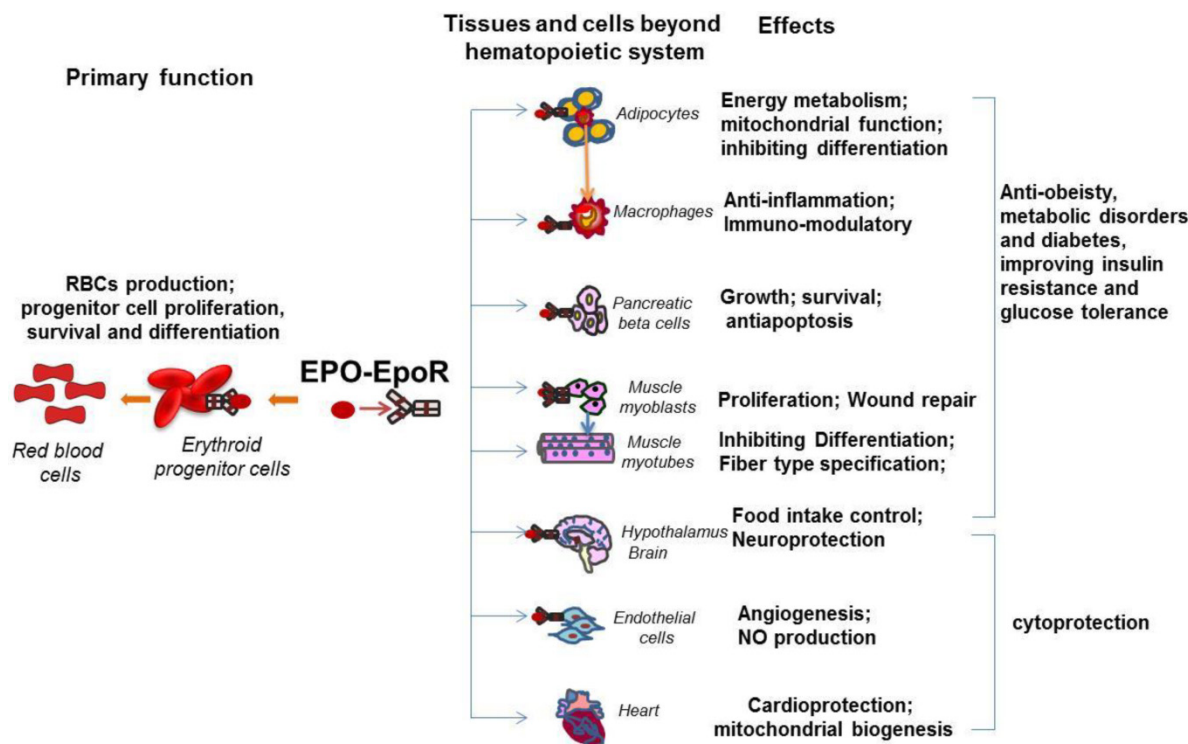

Figure 1. The pleiotropic activity of EPO in multiple tissues beyond hematopoietic tissues. EpoR expression was detected on erythroid cells, adipocytes, immune system cells such as macrophages, pancreatic beta cells, skeletal muscle myoblasts, neural cells, hypothalamus neurons and endothelial cells. The primary function of the EPO/EpoR system is to stimulate erythroid progenitor cell proliferation, survival and differentiation to provide adequate red blood cells. The well documented non hematopoietic effect of EPO is cytoprotection including cardioprotection and neuroprotection, which are also contributed by the EPO activity in endothelial cells such as angiogenesis. The newly revealed biological activity of EPO includes prevention from obesity and metabolic disorders and improvement of insulin resistance and glucose intolerance. These effects are contributed by EPO promoted energy metabolism in adipocytes, anti-inflammation in macrophages, antiapoptosis in pancreatic beta cells, and the central control of energy intake in hypothalamus neurons.

In addition to the important role of EPO in angiogenesis and neuroprotection, recently, the novel and important function of EPO has drawn researchers' attention, the effect of EPO in metabolism and energy homeostasis. This function is tightly related to the expression of EpoR in metabolism related tissues such as skeletal muscle, adipocytes, adipose tissue macrophage, POMC neurons, and pancreas (Figure 1). The widespread distribution of EPO and EpoR among a variety of organs beyond erythroid system suggests EPO is not only a required cytokine for erythropoiesis but also an important factor that is involved in many physiological activities and possibly, potential diseases therapeutic applications.

EPO/EpoR system activity in tissues with high energy demands

EPO/EpoR in adipose tissue

EpoR expression in adipocytes

Compared with other non-hematopoietic tissue, we found that EpoR is expressed at high level in white adipocyte tissue (WAT) (around 60% of hematopoietic tissue) (Figure 1). Also, the expression of EpoR in adipocytes is comparable to the expression in the stromal vascular cells in white adipose tissue [6]. In vitro, EpoR protein is also detected in cultured 3T3-L1 adipocytes and EpoR expression is increased during preadipocyte differentiation and significantly decreased in dexamethasone-induced insulin resistant 3T3-L1 adipocytes [55, 56]. Upregulated EpoR expression and activated PI3K/AKT and JAK/STAT5 pathways under EPO treatment were also observed in adipocytes [55]. And EpoR siRNA blocked EPO-induced activation of AKT and STAT5 [55]. In addition, the loss of EpoR in adipose tissue leads to the obese phenotype of mice with increased body weight and white fat accumulation [6, 11]. In contrast, EPO treatment in wild type mice, EPO over expression in skeletal muscle and mice expressing high transgenic levels of EPO demonstrated reduced body weight and adipose tissue mass [6, 57, 58]. These observations raise the possibility that endogenous EPO action in adipocytes may contribute importantly to protection against obesity and energy homeostasis.

EPO and energy homeostasis in adipose tissue

Since the demonstration of that EPO overexpression in mice reduced body weight gain and white adipose tissue accumulation, improved glucose tolerance and reduced insulin resistance compared with control mice [58], and importantly, mice with EpoR restricted to hematopoietic cells resulting in increased body weight and white fat accumulation [6, 11], all these EPO activities extend EPO activity to energy metabolism and energy homeostasis especially in adipocytes.

Fat specific deletion of EpoR (generated by mating EpoR-floxed mice and aP2-Cre mice on a C57Bl6 background as a model system for diet induced obesity) preferentially increased body weight and fat mass, and by 30 weeks, the gene knockout mice have 65% greater fat mass with reduced oxygen consumption and total respiratory exchange ratio (RER) on normal chow [11]. On a high fat diet, these mice also show a significantly greater increase in fat mass, increased glucose intolerance and insulin resistance. Wild type mice treated with EPO show increased hematocrit and a significant decrease in body weight, while mice with fat knockout of EpoR show the significant increase in hematocrit but no significant reduction in body mass. Serial experimental evidences in these mice also demonstrated that specific EPO activity in fat is strongly associated with the regulation of mitochondrial biogenesis, cellular oxygen consumption and fatty acid metabolism by EPO in adipocytes [11, 46]. Although the indirect effect of EPO on whole body cannot be entirely excluded, the study of fat specific knock out of EpoR demonstrates for the first time that the loss of EPO/EpoR signaling in adipose tissue is sufficient to develop metabolic syndrome phenotype including obesity, glucose intolerance and insulin resistance, and supports the idea that EPO activity in fat may contribute to energy homeostasis. These findings indicate that endogenous and exogenous EPO/EpoR in adipocytes plays an important role in the regulation of body weight, fat mass accumulation and glucose metabolism and insulin sensitivity. Another interesting and important finding is that EPO may contribute to the development of a brown fat–like gene program in white adipose tissue. EPO was found to promote brown fat–like characteristics including increased mitochondrial content and uncoupled respiration in white adipocytes, enhanced brown fat associated gene expression. Functionally active BAT is inversely correlated with BMI [59, 60], and a higher level of BAT may be protective against obesity and have stimulated interest concerning the therapeutic potential of augmenting brown fat to combat obesity and its associated metabolic disease. Thus, increasing these brown fat enriched characteristics and factors by EPO may be a plausible strategy for the treatment of obesity and its associated disease. C57BL/6 mouse is used as a suitable model for diet induced diabetes [61-63]. In contrast to the increased susceptibility to diet induced obesity in mice with EpoR fat specific knockout on the C57BL/6 background, mice with fat specific knockout of EpoR in mice on a mixed background were reported to behave metabolically similarly to control

mice, although it was not demonstrated how control mice on this mixed background respond metabolically to EPO administration [64]. Nevertheless, the differences between these two studies underscore the complexities of genetic influences on obesity and EPO response.

EPO/EpoR in pancreatic beta cells

Emerging evidence has suggested that EPO contributes to glucose metabolism and insulin sensitivity that are closely associated with diabetes development. It is believed that insufficient functional pancreatic β-cell mass is one of pathogenic mechanisms of type 1 and 2 diabetes. Therefore, promoting β-cell growth and survival may be helpful for diabetes prevention and treatment. EPO, required for erythroid progenitor cell proliferation, survival and differentiation, has been demonstrated for the cytoprotective effects on nonerythroid cells. Although detailed expression regulation mechanism remains unknown, EpoR expression presence was demonstrated on pancreatic islets in human and rodents (Figure 1), which provides the possibility for pancreatic islet cell response to EPO [65]. The evidence that recombinant EPO treatment in culture or EPO overexpression in human islets protects islets from destruction and apoptosis confirmed the response of pancreatic islet cell to EPO [65, 66]. Further in vivo experiments demonstrated that EPO protects mice against streptozotocin (STZ) induced type 1 diabetes via reverse STZ-mediated β-cell destruction [67]. EPO also has protective effects against diabetes in db/db mouse model of type 2 diabetes [67]. Furthermore, the pancreatic islet specific knock out of EpoR demonstrated that EpoR is required for EPO mediated diabetes protection under STZ induced diabetes conditions but not under basal conditions, and this protection effect is mediated by EPO stimulated increase in β-cell mass and viability rather than the direct effects of EPO on β-cell function, suggesting that the pancreatic islet cell response to EPO resulted in promotion of β-cell growth and survival [7]. It is worth noting that JAK2, the immediate downstream kinase of EPO/EpoR signaling, is required for diabetes protection by EPO under induced diabetes conditions [7], indicating that pancreatic β-cells may use similar signaling pathway as that which is used in erythroid system to respond to EPO stimulation. Another report demonstrated that EPO may also protect pancreatic β-cells from apoptosis and increase survival of β-cells via PI3K-AKT pathway [68]. Interestingly, the pancreatic islet response to EPO was also demonstrated by promotion of gene expression associated with proliferation and angiogenesis such as *c-myc*, *c-kit* and *vegf* [7]. Collectively, pancreatic islet response to EPO suggests potential protection effect of EPO against diabetes via EPO mediated increase in β-cell mass including promoting proliferation, antiapoptosis and possibly increased angiogenesis. These observations strongly suggest that the nonhematopoietic effects of EPO may extend to encompass the regulation of fat and glucose metabolism, thereby implicating its signaling as a regulator of glucose tolerance and possibly insulin sensitivity.

EPO/EpoR in skeletal muscle

EpoR expression and regulation in myoblasts

We previously demonstrated that EPO stimulates myoblast proliferation and delays differentiation and contributes to myoblast survival and muscle repair from injury [36, 37, 69]. Consistently, the EpoR transgenic expression showed that EpoR expression follows the expression of basic-helix-loop-helix muscle transcription factors Myf5, a myoblast specific factor, but not MyoD and myogenin, required for muscle differentiation [36]. Importantly, only skeletal muscle myoblasts but not matured myotubes were found to show proliferative response to EPO stimulation (Figure 1). Further expression evidence demonstrated that EpoR is only expressed in muscle myoblasts and decreased with the skeletal muscle differentiation and no EPO response was detected in mature muscle fibers [12, 36]. EPO stimulation in myoblasts promoted EpoR expression and muscle specific transcription factors, such as Myf5 and MyoD [14, 36]. However, only Myf5 regulates EpoR expression in myoblasts. In addition, GATA factors including GATA3 and GATA4 and another basic-helix-loop-helix factor, TAL1 were found to be up-regulated by EPO stimulation in myoblasts and down-regulated with myoblast differentiation [14, 37]. The class III deacetylase, Sirt1, which is most homologous to yeast Sir2 and is a NAD+-dependent deacetylase, was observed to be increased by EPO stimulation in myoblast to regulate GATA4 and TAL1 expression, leading to inhibited muscle differentiation [14]. In addition, EpoR can be up-regulated by GATA3, GATA4 and TAL1 in myoblast, which explains partially the reason that these factors increase myoblast response to EPO and myoblast proliferation but inhibit myogenesis [14].

EPO function in muscle metabolism and muscle fiber type specification

Interestingly, ectopic EPO expression specifically in skeletal muscles resulted in reduced body weight and adipose tissue mass accompanied by improved fasting insulin levels and glucose tolerance in the high-fat fed mice [57]. The observation that increased

expression of genes related to lipid metabolism and thermogenesis, also increased muscular fat oxidation in EPO overexpressed mice suggest the important role of EPO in muscle metabolism [57]. Although the absence of EpoR in mature muscle fiber indicates that the EPO effect in muscle fiber may be indirect and probably related to an increased oxygen delivery in this tissue, however, another study suggests EPO increases submaximal endurance performance to a greater extent than what can be explained by increased oxygen delivery, suggesting EPO may stimulate related metabolic activity in muscle via other unknown manners [70]. In human, EPO treatment enhances skeletal muscle mitochondrial oxidative phosphorylation capacity and electron transport capacity [71]. In addition, overexpressed EPO during muscle development may contribute to muscle type specification via EPO changed metabolic activity with the development. Skeletal muscles of vertebrates contain two types of myofibers, slow twitch (type I) and fast twitch (type II), that differ in function, mitochondrial density, and metabolic properties. Slow twitch (ST) myofibers contain a high concentration of mitochondria and high oxidative capacity. In contrast, fast-twitch myofibers such as type IIB fibers, show low mitochondrial density and low oxidative metabolism [72]. The percentage of ST fibers is reduced in obese and type 2 diabetic patients, and within each fiber type, obese and type 2 diabetic patients have lower oxidative enzyme activity and a corresponding greater lipid content and smaller mitochondria in skeletal muscle [73-75]. Defective insulin signaling has been suggested to be associated with mitochondrial dysfunction [76, 77]. Furthermore, mice engineered with increased type I muscle fibers exhibit resistance to obesity and improved metabolic profiles. It was observed that skeletal muscles from mice with high EPO production in vivo exhibit an increase in the proportion of slow twitch myofibers and increased mitochondrial activity [12]. In rats, erythropoietin treatment increases significantly the specific activities of oxidative enzyme and induces a shift of muscle phenotype from fast glycolytic to slow oxidative [78]. The important metabolism sensors, AMPK and PGC-1α, which also are demonstrated to promote the slow and oxidative fiber specification [79, 80], were activated by EPO in the muscle [12]. In comparison, skeletal muscle from wild type mice and mice with erythropoietin activity restricted to erythroid tissue has fewer slow twitch myofibers and reduced mitochondrial activity compared to the mice with overexpressed EPO [12]. These observations suggest that EPO contributes to skeletal muscle fiber programming and metabolism, which may link EPO activity to the potential of anti-obesity and metabolism disorder.

EPO/EpoR in heart

EPO and heart protection

Although early studies suggested that EPO/EpoR activity is required for heart development especially embryonic heart since EPO-/- and EpoR-/- mice show heart development defects including ventricular hypoplasia, epicardium detachment and abnormalities in the vascular network and these defects were speculated to be related to ineffective cell proliferation and expansion of myocardium [9], however, later studies using the mice with EpoR expression restricted to erythroid system found that these mice do not display severe defects including epicardium detachment and abnormalities in the vascular network [81], suggesting severe anemia might be pertinent to the blockage of heart development and EPO/EpoR system may do not relate directly to epicardium or capillary development in the embryonic heart. It is worth noting that EpoR expression was detected in the developing heart including in pericardium, epicardium and endocardium. The essential factor for cardioprotection, GATA4 has been reported to positively regulate EpoR expression [82], however, the regulatory mechanisms of EpoR expression in cardiomyocytes in health and disease are still largely unknown yet.

These observations indicate that endogenous EPO activity may still contribute to cardiac function although not crucial to heart development. Later studies using adult animal model demonstrated that EPO can directly protect the ischemic and infarcted heart independent on increased hematocrit by preventing myocyte apoptosis and attenuating postinfarct deterioration [83-85]. EPO also provides direct protection during ischemia-reperfusion injury that associates with preservation of ATP levels in the ischemic myocardium [83].

Another important contribution of EPO in the cardiac protection is that EPO stimulates endothelial nitric oxide (NO) synthase (eNOS) activity to promote NO production to provide cardioprotection. Especially during hypoxia, low oxygen tension increases endothelial cell capacity to produce NO in response to EPO by induction of both EpoR and eNOS [45, 86-88]. EPO promoted eNOS activation is associated with PI3K-AKT signaling and contributes to cardiac mitochondrial biogenesis by enhancing PGC-1α, the important energy metabolism sensor [13]. Ectopic expression of EPO in mice increased circulating and vascular tissue NO level that prevented cardiovascular diseases such as hypertension and thromboembolism [88]. The cardioprotection role of EPO is also mediated by suppressed inflammatory response. In rodents with myocardial ischemia-reperfusion injury

and in the mice with chronic post-myocardial infarction associated heart failure, EPO pretreatment reduced inflammatory cell infiltration and fibrosis and the incidence of ventricular arrhythmia and decreased serum level of proinflammatory cytokines [86, 89, 90].

EPO/EpoR and neural system

EpoR expression in hypothalamus and the role of EPO in food intake

The hypothalamus is a neural organ responsible for regulating energy metabolism. Two populations of neurons in the arcuate nucleus of hypothalamus act in an opposing manner to control food intake, energy expenditure and glucose metabolism. Proopiomelanocortin (POMC)-expressing neurons sense the change in peripheral regulatory hormones, leptin and insulin, and respond by secreting α-melanocyte-stimulating hormone that stimulates melanocortin 3 and 4 receptors in target neurons to decrease food intake and increase energy expenditure [91, 92]. Activation of neuropeptide Y (NPY)/agouti-related protein (AGRP) neurons expressing orexigenic neuron peptides NPY and AGRP stimulates orexigens MCH and orexins expression in lateral hypothalamus target neurons to increase food intake and decrease energy expenditure [91-93]. An intriguing finding that EpoR expression is detected at a relative high level in hypothalamus supports the view that EPO may play a role in central regulation of energy homeostasis [6]. Furthermore, EPO treatment in WT-mice increased POMC mRNA by threefold and in vitro culture of hypothalamic neurons stimulated with EPO demonstrated that EPO can directly promote POMC expression, but not NPY, AGRP, pro-MCH and prepro-orexin [6]. Also the immunostaining of EpoR on POMC neurons in the hypothalamus provides further support for that endogenous EPO activity in regulation of food intake and energy metabolism homeostasis. In contrast, EpoR expression in the hypothalamus was absent in the mice with EpoR restricted in the erythroid system (Tg-mice) and significantly reduced in ob/ob-mice compared with control mice [6]. Consistently, the expression of POMC that regulates activity, food intake and energy homeostasis, decreased more than 50% in Tg-mice, suggesting that EPO regulates energy homeostasis at least through regulating its target, POMC expression, in the hypothalamus (Figure 1). Further experiments are required to investigate the detailed mechanism by which EPO regulates POMC expression and the role of EPO in the central regulation of energy homeostasis.

EPO and neuroprotection

Another important and well documented function of EPO is neuroprotective role (Figure 1). The protective function of EPO is mediated by blocking neuronal cell apoptosis, promoting cell survival and neurogenesis and regulating angiogenesis. EPO is neuroprotective against hypoxia, ischemic brain injury inflammation and glutamate toxicity [8, 94-98]. Neural cells from the mice with EpoR restricted in erythroid system (Tg-mice) proliferated more slowly and were less tolerant to hypoxia [99]. The Tg-mice also exhibited increased apoptosis in the developing brain, neural cells with increased sensitivity to hypoxia and decreased neural cell proliferation and increased sensitivity to glutamate toxicity in adult brain [50]. Furthermore, the mice with brain-specific deletion of EpoR showed defective neural progenitor cell migration to the peri-infarct cortex in response to ischemic stroke [50]. EPO administration in brain of rodents reduced injury and improved performance in the Morris water maze test [100, 101]. EPO was also neuroprotective against peripheral inflammatory pain in postnatal rats [102]. EPO neuroprotection may also result from the indirect effect of EPO on other cell type rather than neural cells such as endothelial cells. EPO may increases VEGF production and induced VEGFR2, resulting in enhanced angiogenesis [103]. EPO can also stimulate endothelial cells to increase eNOS activity to produce nitric oxide (NO), particularly at low pO_2 to provide neuroprotection function [104]. EPO is also protective in hypoxia-induced cell death in both embryonic and postnatal hippocampal neurons [8, 95].

EPO and energy metabolism regulation

EPO and cancer

Cancer-triggered systemic metabolic disease in the host, called cancer cachexia, leads to weight loss, muscular atrophy, adipose atrophy, and other systemic disorders. It is estimated that approximately 25% of all cancer patients eventually die of cachexia. The studies performed in animal cancer cachexia models suggested that in tumor-bearing rodents with reduced white adipose tissue, EPO administration partially preserved adipose tissue and increased lipoprotein lipase activity [105], suggesting that in animals with cachexia and markedly reduced fat stores, EPO increases fatty acid synthesis in adipocytes and provides a sparing effect and acts to normalize metabolic homeostasis under pathological state. However, some conflicting findings about the direct effect of EPO on cancer cells have been reported by various research groups, which restricted the clinical use of EPO in cancer patient therapy. For the past few dec-

ades, EPO has been used widely for treatment of cancer patients with anemia induced by chemotherapy. The EPO treatment in these clinical trials increased red blood cell level, hemoglobin concentration and decreased fatigue, leading to improved quality of life [106-108]. Controversially, EPO treatment for anemia in cancer patients has been challenged by some later studies. It was reported that EPO promotes tumor growth through regulating tumor angiogenesis such as in hepatocellular carcinomas, renal carcinoma and glioma [109-111]. A shorter survival and increased risk of mortality by EPO treatment were also demonstrated especially in solid cancers such as breast cancer and head and neck cancer [112-114] , where EPO treatment may facilitate tumor invasion and metastasis via modulating the tumor vasculature and increasing thrombin deposition [115, 116]. These conflicting clinical outcomes of EPO promote us to pay more attention to the EPO therapy in treatment of anemia in cancer patients.

The relationship of EPO and obesity/ diabetes

Diabetes, especially type 2 diabetes is a metabolic disorder related disease and is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency. Insulin is the principal hormone that regulates uptake and metabolism of glucose. Therefore, abnormal insulin secretion and improper insulin action are central and critical to the pathogenesis of diabetes. Numerous factors have been implicated in the insulin secretion and insulin signaling pathway regulation. Obesity and its associated metabolic syndrome including glucose intolerance and insulin resistance are also well-documented risk factors for type 2 diabetes. Obesity is tightly associated with energy metabolism including glucose and lipid metabolism. Understanding of the mechanism of the disrupt energy metabolism relating to obesity and abnormal metabolic processes can provide insight on the development of potential therapeutic anti-obesity strategies, which will also contribute to the protection against diabetes and metabolic syndrome.

Some early clinical trials have suggested the effects of EPO in metabolism since EPO was introduced into the clinic treatment of anemia associated with chronic renal failure and cancer in 1989. In addition, it was observed that EPO treatment improved glycemic control and insulin sensitivity in hemodialysis and diabetic patients [117-119]. Positive regulation of lipid metabolism was also accompanied together with EPO treatment in uremic patients with end-stage renal disease including improved plasma triglycerides and cholesterol levels [120]. Although some other studies suggested controversial observation that EPO treatment in hemodialysis patients and healthy human subjects did not improve lipid profile and glucose metabolism [121], significantly increased energy expenditure and augmented systemic and muscular oxygen consumption and delivery were observed [122, 123]. In addition, improvement in physical exercise capacity and muscle metabolic rate in predialytic uremic patients and hemodialysis patients has also been noted in some small clinical studies [124-127]. Improved metabolic parameters including fasting glucose level and insulin sensitivity were also observed in EPO treated diabetic and chronic renal disease patients [118, 120, 128, 129].

Since EPO and EpoR null mice die of severe anemia around embryonic day 13.5 [1, 130], the mice with endogenous EpoR expression restricted largely to hematopoietic and endothelial were made (the normal erythropoiesis was restored by transgene EpoR driven by erythroid-specific GATA promoter into the EpoR knockout background mice to rescue mice [81]. These mice (Tg-mice) exhibit increased body weight from the first week after birth and become obese due to increased fat mas accumulation [6]. These obese mice also show insulin resistance, decreased energy expenditure and glucose intolerance. In addition, overexpression of EPO either in the muscle or human platelet-derived growth factor B-chain promoter driven human EPO transgenic mice (tg6) show improved glucose tolerance and insulin sensitivity, and lower body weight and fat mass [57, 58]. EPO treatment in WT mice and obese (ob/ob) mice (genetically deficient in leptin) improves overall metabolic characteristics, reduces blood glucose levels, improves glucose tolerance and attenuates weight gain and fat mass accumulation [6, 58]. EPO treatment in diabetes (db/db) mice provides protection for cardiomypathy and insulin producing pancreatic β-cells via antiapoptotic and angiogenic effects in the islets [7, 131, 132]. EPO was also protective against diabetic europathy in the streptozotocin induced diabetic rat model [67]. These findings of EPO activity in animals provide more insights on potential EPO regulation in energy metabolism and homeostasis.

EPO regulates oxygen consumption and oxidative metabolism

About 95% of the protein content of the red blood cells is hemoglobin that binds oxygen cooperatively and transports oxygen from the lungs to the tissues. Induction of EPO production in response to low oxygen or ischemic stress increases mature red blood cell production [133]. Increasing red cell number increases the oxygen carrying capacity of blood to enhance oxygen delivery to the tissues. Hence, the primary function of EPO is to regulate oxygen delivery via the production of red blood cells and is facili-

tated by the hypoxia induction of EPO gene transcription resulting in sensitivity of EPO production to the local oxygen environment [133]. Beyond erythroid tissue, EPO also facilitates oxygen delivery to brain, heart and other non-hematopoietic tissues via stimulation of nitric oxide production by vascular endothelium. Increased oxygen supply will contribute to energy metabolism. The disrupted EPO activity in Tg-mice resulted in decreased energy expenditure and reduced total oxygen consumption and respiratory quotient [6]. In contrast, EPO treatment of wild type mice increased total physical activity and oxygen consumption. The mice with EpoR knock out specifically in fat tissue also exhibited decreased physical activity, Oxygen consumption, and respiratory exchange ratios [11]. These observations suggest the important role of EPO in energy homeostasis. The regulation of EPO in energy homeostasis may be critical in the development of obesity in Tg-mice and in suppressed fat mass accumulation in EPO-treated wild type mice. Another direct evidence for EPO in energy homeostasis is that EPO stimulates cellular oxygen consumption rate (OCR) beyond whole animal level. EPO increased basal OCR in cultured adipocytes and in skeletal myoblasts [11, 12]. The EPO-stimulated increase in OCR became even more evident under stress. For example, in the presence of oligomycin, which uncouples phosphorylation from mitochondrial respiration by blocking mitochondrial complex V, and with treatment of carbonyl cyanide p-trifluoromethoxy phenylhydrazone (FCCP), which uncouples oxidative phosphorylation from ATP synthesis and is used to assess maximal oxidative phosphorylation capacity, the OCR increased even more with EPO treatment in adipocytes and myoblasts [11, 12].

It is known that hypoxia decreases OCR, however, EPO treatment overcomes the hypoxia adverse effects on OCR and increases OCR in cultured mice adipocytes under basal condition and in the presence of oligomycin and FCCP under hypoxia [11]. In cultured human adipocyte, the same observation was also found [11]. These findings highlight a previously unrecognized role for EPO/EpoR activity in directly increasing cellular mitochondrial respiration and oxidative metabolism capacity beyond its effect of increased erythropoiesis and oxygen transport capacity, leading to increased oxygen utilization capacity and energy oxidative metabolism efficiency, which will contribute to prevention against obesity and metabolism disorder. Consistent with increased oxygen consumption rate, cultured adipocytes with EPO stimulation showed increased energy metabolism such as fatty acid oxidation. In contrast, disrupted EPO activity in fat tissue resulted in decreased fatty acid metabolism of mice adipocytes [11]. The increased fatty acid metabolism by EPO may limit the storage of excess lipid to protect against obesity and diabetes.

EPO promotes mitochondrial biogenesis and mitochondrial function

Increased mitochondrial biogenesis and related gene expression correlate with a reduction of diet induced obesity [134]. Mitochondrial dysfunction in adipose tissue is linked to obesity and type 2 diabetes in humans, as indicated by reduced oxidative phosphorylation capacity and reduced fatty acid oxidation in several tissues, including adipocytes [135, 136]. Interestingly, EPO treatment enhances muscle mitochondrial capacity as indicated by an upregulation of oxidative phosphorylation (OXPHOS) and electron transport capacity (ETS) in human skeletal muscle [71]. In mice, EPO also stimulates mitochondrial biogenesis in heart as indicated by increased mitochondrial density, mitochondrial mass and mitochondrial biogenesis gene expression including NRF-1, PGC-1α, and mitochondrial transcription factor-A (Tfam) [13, 137]. Increased EPO activity in mice also promotes mitochondrial activity as indicated by increased Citrate synthase (CS) activity in skeletal muscle and myoblasts, which may be mediated by increased PGC-1α and AMPK activity, the two important energy metabolism regulators [12]. The increased mitochondrial content by EPO stimulation indicated as increased mitochondrial DNA was also demonstrated in primary myoblasts and cultured myoblasts [12]. In addition, EPO contributes to cytoprotection during ischemic vascular injury through direct modulation of mitochondrial membrane potential in endothelial cells [138]. In adipocytes, EPO also increases mitochondrial density reflected by increased mitochondrial staining and mitochondrial DNA [11]. EPO also promotes mitochondrial biogenesis related factor level at gene level and protein level and increased CS activity in cultured adipocytes and fat tissue isolated from EPO treated mice [11]. In contrast, the loss of EPO activity in fat tissue results in decreased mitochondrial activity and density [11]. Functionally, EPO's effect on the mitochondria was reflected by increased energy metabolism efficiency such as increased fatty acid oxidation. In adipocytes, the increased metabolic activity by EPO is mediated by Sirt1, a NAD+ -dependent type III deacetylase, and peroxisome proliferator–activated receptor a (PPARα), an important regulator in lipid metabolism [139]. In human, EPO administration also stimulates resting energy expenditure and fat oxidation [140]. All of these observations provide evidence for the function of EPO in regulating mitochondrial activity and energy homeostasis in the multiple tis-

sues and highlight the new potential role of EPO in protection against metabolism related disease such as obesity, metabolism disorders and diabetes beyond its primary function in the erythroid system.

The crosstalk between EPO and energy sensors

EPO and PGC-1α

As we mentioned above, EPO activity is linked to some important energy sensors such as PGC -1α. The core function of PGC-1α is to stimulate mitochondrial biogenesis and oxidative metabolism to contribute to energy expenditure [141, 142]. PGC-1α is abundantly expressed in tissues with high energy demand, including the fat, heart, skeletal muscle, kidney and brain, and interacts with some other transcription factors to regulate the expression of a number of genes involved in diverse metabolic pathways such as fatty acid oxidation, glycolysis and gluconeogenesis. The change of the expression of these co-activators might strongly impact on whole-body energy homeostasis and on the pathogenesis of the metabolic syndrome [143, 144]. PGC-1α has also been demonstrated to be related to adipocyte differentiation, adaptive thermogenesis and muscle fiber development, which make the PGC-1α be involved in some diseases such as obesity, type 2 diabetes, and insulin-resistance [79, 145-148]. The function of EPO has been demonstrated to be linked to the PGC-1α activity in recent studies (Figure 2). EPO was found to increase PGC-1α expression in the cardiomyocytes to contribute to increased mitochondrial

biogenesis and cardioprotection [13, 137]. The increased PGC-1α by EPO possibly requires eNOS function because eNOS knock out abolished EPO increased cardiac PGC-1α expression [13]. In skeletal muscle and myoblasts, EPO also stimulated PGC-1α expression via increasing AMPK activity, an upstream regulator of PGC-1α expression and activity [144, 149-151], which may contribute to the EPO effect in regulating skeletal muscle fiber programming and metabolism [12]. In addition, in adipocytes, EPO increased PGC-1α expression and regulated PGC-1α activity via activating Sirt1 deacetylation activity to deacetylate PGC-1α, leading to the increased PGC-1α activity [11, 151]. These effects of EPO in cultured adipocyte and white fat tissue is believed to contribute to maintaining energy homeostasis and developing a brown fat–like gene program in white fat tissue [11]. The neural PGC-1α was markedly decreased after acute ischemic stroke stimulation, but EPO can preserve PGC-1α expression and PGC-1α + neurons especially combined with cyclosporine treatment, which contribute to the protecting brain from ischemic damage [152]. The various mechanisms by which EPO regulates PGC-1α expression and activity in multiple tissues promote us to speculate that EPO activity is linked to energy sensing system to sense the energy change and maintain energy homeostasis via regulating PGC-1α. EPO is linked to energy sensing system is also confirmed by the observation that EPO regulates NAD+/NADH level and Sirt1 activity, a direct regulator of PGC-1α activity and a master metabolic regulator [153].
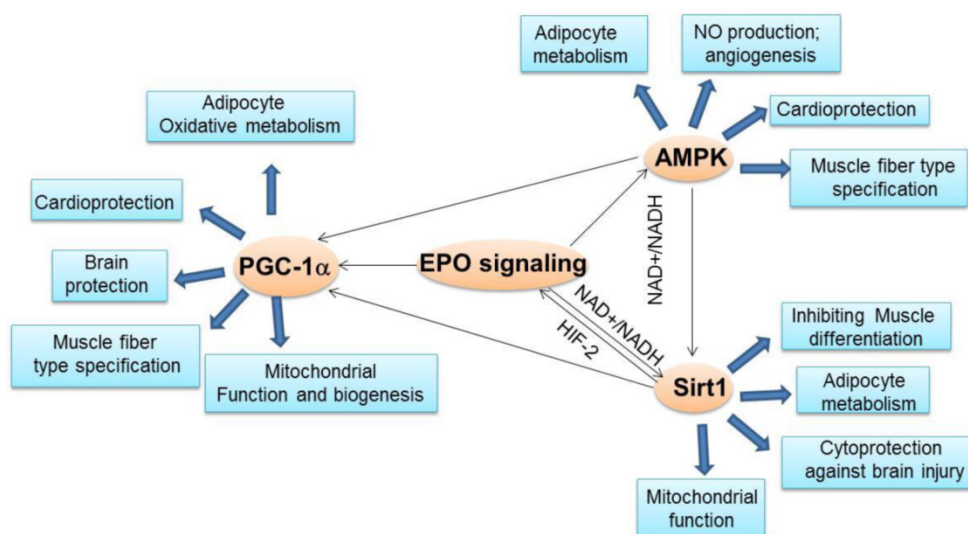

Figure 2. The crosstalk between EPO and important energy sensors. EPO regulated AMPK activity is involved in adipocyte energy metabolism, angiogenesis, cardioprotection and skeletal muscle fiber type specification. EPO production can be regulated by Sirt1 via HIF-2 under hypoxia condition. On the other hand, EPO modulates NAD+/NADH level and ratio to regulate Sirt1 activity, which contributes to energy metabolism in adipocytes, inhibition of skeletal muscle differentiation, and brain protection from injury and mitochondrial function. EPO regulated AMPK activity may regulate Sirt1 activity via modulating NAD+/NADH ratio. As the downstream target of Sirt1and AMPK, PGC-1α may also be directly regulated by EPO or via regulating Sirt1 and AMPK activity to promote adipocyte oxidative metabolism, cardioprotection, brain protection, mitochondrial biogenesis and function and muscle fiber type specification.

EPO and Sirt1

Sirt1 is one of the seven mammalian orthologs (sirtuins Sirt1-7) of the yeast protein silent information regulator 2 (Sirt2), a highly conserved NAD+-dependent protein deacetylases and/or ADP-ribosyltransferases [154-157]. Growing evidences in other model organisms have demonstrated that Sirt1 is also a crucial regulator of a variety of cellular processes, ranging from energy metabolism, and stress response, to tumorigenesis and aging. Sirt1 has a prominent role in metabolic tissues including liver, pancreatic islet, skeletal muscle, adipose tissues and brain, where it deacetylates a range of substrates, including P53, PGC-1α, LXR, Ucp2, NF-κB and FoxO1 proteins, which results in a pronounced effect on glucose homeostasis, insulin secretion, energy expenditure and homeostasis [151, 158-160]. Sirt1 regulates the activity of the nuclear receptor PPARγ and thus influences adipogenesis as well as fat storage in white adipose tissue [161]. Activating Sirt1 in mice contributes to protection against high-fat-induced obesity and metabolic disorders [162-164]. Sirt1 also promotes brown adipose tissue differentiation through repressing MyoD-mediated myogenic gene expression signature and stimulating PGC-1α-mediated mitochondrial gene expression [165]. Sirt1 is a vital regulator of pancreatic insulin secretion in response to the nutrient availability, which contributes to protection against obesity, glucose intolerance and insulin resistance [158, 166].

Sirt1 activity also appears to be an important player in the central regulation of nutrient sensing. Inhibition of Sirt1 activity in POMC neurons increases acetylation of FOXO1, resulting in increased POMC and decreased AgRP expressions, thereby decreasing food intake and body weight gain [167]. However, specific deletion of Sirt1 in POMC neurons in mice causes reduced energy expenditure, leading to hypersensitivity to diet-induced obesity [168]. Central administration of Sirt1 activator has shown promise in controlling of diet-induced obesity and diabetes [169].

In skeletal muscle, Sirt1-regulated activation of PGC-1α activates mitochondrial fatty-acid oxidation genes and upregulates GLUT4 to promote insulin sensitization [170]. Sirt1 may affect metabolic activity and energy homeostasis via its regulatory effect on mitochondrial function. The close relationship between Sirt1 and PGC-1α in multi tissues might provide some insights into the association between Sirt1 and mitochondrial function. Sirt1 can deacetylate PGC-1α at several lysine residues and thereby increase its ability to activate transcription of target genes involved in mitochondrial biogenesis. Indeed, feeding mice with resveratrol to activate Sirt1 upreg-

ulates the number of mitochondria in their muscle cells [163].

Interestingly, Sirt1 was demonstrated to transactivate EPO via activating HIF-2α in hypoxic hepatoma and human embryonic kidney cells [171]. On the other hand, EPO increases the expression and deacetylase activity of Sirt1 and its downstream target AKT and FoxO3a in the cerebral microvascular endothelial cells (ECs) to exert its cytoprotective ability against brain injury during oxygen-glucose deprivation [172]. The direct evidence for that EPO regulates Sirt1 activity is that EPO can modulate NAD+/NADH level, the regulator of Sirt1 activity [11, 14]. NAD+ or NADH levels are readout of cellular metabolic activity. NAD+ and NADH metabolism is also important in oxidative metabolism and energy homeostasis and has been linked to protection against dietary obesity and a therapeutic target for associated metabolic diseases [173-175]. Induced NAD+ level by pharmacological activation of NADH oxidation resolves diet induced obesity and related phenotypes in mice [176], in part analogous to the reduction in fat mass by EPO treatment in obese mice [6]. The accumulation of NADH within mitochondria further affects pyruvate metabolism, which contributes to metabolic acidosis. An increase in mitochondrial NADH can impair electron transport chain function. EPO treatment in a mouse model with severe sepsis can decrease NADH accumulation in skeletal muscle, which improves mitochondria oxidative phosphorylation and pyruvate metabolism [177]. Hypoxia can lead to an increase in intracellular NADH levels thereby decreasing the NAD+/NADH ratio by inhibiting NADH oxidation [178, 179]. Our study showed that EPO dramatically rescued the reduced NAD+/NADH ratio by hypoxia and decreased NADH level in adipocytes [11]. Modified NAD+ level and NAD+/NADH ratio by EPO were also observed in human and mice primary adipocytes in vitro and in white adipose tissue in vivo [11]. The increased NAD+ by EPO resulted in enhanced Sirt1 activity as shown by decreased PGC-1α acetylation in adipocytes, leading to the activation of PGC-1α [11]. Furthermore, Sirt1 knockdown attenuated the EPO-stimulated increase in brown fat associated and mitochondrial genes and in oxidative respiration and fatty acid oxidation, indicating Sirt1 mediates EPO activity in adipocyte metabolism [11]. EPO regulated Sirt1 activity is also illustrated by the effect of EPO in myoblasts where EPO increases NAD+ level and NAD+/NADH ratio to regulate Sirt1 activity [14]. Sirt1 has been found to negatively regulate muscle differentiation by deacetylating MyoD and forming a complex with the acetyltransferase PCAF and MyoD in a NAD+-dependent manner [180]. EPO was found to upregulate Sirt1 expression and increase

Sirt1 activity in myoblasts to regulate transcription factors GATA-4 and TAL1 and these factors together with Sirt1 coordinately regulate skeletal myoblast differentiation [14]. Given that the indispensable role of NAD+/NADH in cellular oxidation/reduction reactions and metabolic activity and the important role of Sirt1 in various cellular and pathophysiological processes, the regulation of EPO in NAD+/NADH and Sirt1 activity strongly suggest that EPO may exert its function in various processes such as sensing energy imbalance to change energy metabolic activity including lipid and glucose metabolism, which will contribute to protection from obesity, metabolism disorders and diabetes, regulating cell proliferation and differentiation and protecting from stresses (Figure 2). In addition, further experiments are required to clearly clarify the crosstalk among the EPO signaling, Sirt1, and PGC-1α factors.

EPO and AMPK

AMP-activated protein kinase (AMPK), a serine/threonine kinase, is an evolutionarily conserved energy metabolic sensor and an important regulator of energy homeostasis.

It has been demonstrated that AMPK agonists increased oxidative gene expression and protection against metabolic disease [181, 182]. AMPK activation induces fatty acid oxidation in liver and heart, inhibits hepatic lipogenesis and adipocytes differentiation, and stimulates glucose uptake and mitochondrial biogenesis to modulate energy balance in muscle [145, 149, 183]. AMPK can be activated to block body weight gain and increase fatty acid oxidation by inducing transcription regulators involved in energy homeostasis such as peroxisome proliferator-activated receptor alpha (PPAR-α) and peroxisome proliferator-activated receptor gamma (PPAR-γ) co-activator 1 (PGC-1α) [144, 149-151, 183, 184]. AMPK activator, metformin and thiazolidinediones have shown important therapeutic benefits in the treatment of type 2 diabetes and metabolic syndrome [150, 185, 186]. AMPK is also implicated in the appearance of brown features and increased mitochondrial activity in white adipose tissue via regulating or interacting with factors such as PRDM16, a master regulator of brown fat determination and uncoupled protein 1 (UCP1), a key regulator of brown fat thermogenesis [187-192]. In addition, AMPK also exerts its function in metabolic processes through interaction with Sirt1 [144, 160]. For example, Sirt1 directly deacetylates the AMPK protein kinase liver kinase B1 to increase the activity of AMPK to regulate hepatocyte lipid metabolism and improve systemic insulin sensitivity [193, 194]. Moreover, activation of AMPK by its synthetic activator, AICAR, increases cellular

NAD+/NADH ratio, resulting in activation of Sirt1 and its downstream target including PGC-1α and FOXO1 to contribute to energy homeostasis through increasing fatty acid oxidation and mitochondrial biogenesis in skeletal muscle [160, 195]. These observations suggest that AMPK and Sirt1 may coherently regulate metabolic processes and energy homeostasis (Figure 2). Intriguingly, AMPK activity has been involved in EPO effect in various biological processes (Figure 2). EPO administration in endothelial cells and mice increased phosphorylation and activity of AMPK, leading to increased eNOS activity and NO production and, ultimately angiogenesis [196]. EPO also exerts its cardioprotective role in an AMPK-dependent manner [197]. EPO regulated skeletal muscle fiber programming and metabolism may also be contributed by increased AMPK activity by EPO [12]. Our newly published data also revealed that EPO facilitates energy expenditure by regulating AMPK via CaMKK in white adipocytes (unpublished data). EPO alters cellular NAD+/NADH levels through regulating AMPK activity in adipocytes, which may lead to increased Sirt1 activity in adipocytes as we demonstrated in our published paper [11]. EPO mediated activation of AMPK also contributes to energy expenditure and reduction of hypoxia induced oxidative stress of adipocytes (unpublished data). Further experiments are required to investigate the important regulation role of EPO on AMPK activity in multiple tissues.

Since AMPK, Sirt1 and PGC-1α activity are tightly associated in regulating metabolic processes and metabolism diseases resistance and EPO is also involved in the regulation of these three important metabolism regulators, EPO may be part of the AMPK-Sirt1-PGC-1α pathway where EPO activates this energy sensing network or EPO regulates each energy sensor activity, respectively (Figure 2). However, further studies are required before the definitive role of EPO in this pathway is established. It would be of interest to determine the mechanism by which EPO activates AMPK phosphorylation through EPO downstream signal pathway directly or indirectly.

The protective role of EPO in metabolism related stresses

EPO and hypoxia

The protective role of EPO from stresses has been well-documented in multiple tissues such as hypoxia, reactive oxygen species (ROS), ischemia/reperfusion injury and inflammation. Some of the stresses are also associated with diseases including obesity, diabetes and neurodegenerative disease. For example, white adipose tissue is hypoxia in obese

condition and has reduced mitochondrial number and activity [198]. It is believed that mitochondria respiration and biogenesis are inhibited by hypoxia [199, 200]. This possibility is also supported by the reduced number of mitochondria in adipose tissue of obese people [198]. EPO improved mitochondrial function and number in obese white adipose tissue and EPO also modulated NAD+/NADH level and increased oxidative respiration under hypoxia in adipocytes [11]. These data suggested EPO can overcome the adverse effect induced by hypoxia in adipocytes, which contributes to EPO protection from obesity. The protective effect of EPO from hypoxia has also been revealed by many other studies. Under hypoxia conditions, the kidney will produce and secrete EPO to increase red blood cells. EPO also affects neuronal protection during hypoxic conditions. EPO provides protection to embryonic and postnatal hippocampal neurons from hypoxia-induced cell death [8, 95]. EPO can also prevent neuronal apoptosis in vitro in hypoxic neurons and in vivo in cerebral ischemia in rats [96].

EPO and reactive oxygen species (ROS)

Another stress related to disease is oxidative stress. Oxidative stress, defined as a pathological state characterized by increased reactive oxygen species (ROS) production or decreased ability to detoxify ROS, plays a causative role in tissue injury in many disease conditions, including cardiovascular disease, neurological disorder, cancers, and aging. Obesity can induce systemic oxidative stress and increased oxidative stress in accumulated fat and cause dysregulation of adipocytokines and development of metabolic syndrome [201]. Increased ROS in excessively accumulated fat also causes increased oxidative stress in blood and has deleterious effects on other organs including the liver, skeletal muscle and cardiovascular system. EPO was reported to reduce oxidative stress damage in heart disease to provide cardio protection [202, 203]. EPO also has antioxidant effect in thalassemic blood cells [204]. In end-stage renal failure patients undergoing continuous ambulatory peritoneal dialysis, EPO treatment significantly reduced ROS production in polymorphonuclear leukocytes, which may contribute to anti-inflammation effect [205]. It is also believed that increased oxidative stress in fat is important underlying cause of obesity-associated metabolic disorder [201]. Therefore, how to modulate the redox state and reduce ROS production in adipose tissue can be used as a potentially useful target to develop new therapies against obesity and its associated metabolism syndrome. In the diabetic condition, oxidative stress impairs glucose uptake in muscle and fat and decreases insulin secretion form pancreatic cells [206-209]. Both oxidative stress and hypoxia are known to occur in adipose tissue in response to diet induced obesity as well as in genetic models of obesity [210]. Insulin resistance has also been associated with the generation of ROS and hypoxia can induce ROS production in adipocytes [211]. Of noting, our unpublished data found that EPO inhibits ROS production and stimulates the expression of antioxidant enzyme in adipocytes. It is possible that the antioxidant stress effect of EPO mediates the protection effects of EPO against obesity and the improvement in insulin resistance in obese mice [6, 11]. It is now clear that the overproduction of ROS in diabetes is a direct consequence of hyperglycemia and that various types of cells including renal tubular cells can produce ROS under hyperglycemic conditions. Dang et.al revealed that EPO inhibited high glucose-induced ROS generation in renal tubular cell and protected the cells from apoptosis, suggesting EPO contributes to prevention of Diabetic kidney disease via suppressing ROS production [212].

EPO and its anti-inflammatory function

Recently, some studies have revealed another important role of EPO in anti-inflammatory effect. EPO treatment has been reported to stimulate anti-inflammatory signaling, which was suggested to contribute to its direct neuroprotective effect during cerebral ischemia [213]. The administration of EPO also inhibits the inflammatory response and delays the onset of an autoimmune disease of the CNS and multiple sclerosis [214-217]. In a rat model of renal injury and in liver ischemia-reperfusion injury, EPO was observed to show anti-inflammatory activity [218, 219], suggesting the potential for EPO therapy as an anti-inflammatory agent following injury. In cardiovascular system, EPO pretreatment can suppress the systemic inflammatory response in myocardial ischemia-reperfusion injury in rodents [86]. Moreover, in chemically induced colitis, EPO administration impaired the production of several macrophage-derived inflammatory mediators such as NO, TNF-α, and IL-6 both in vitro and in vivo and decreased the production of NF-κB-inducible immune mediators, thus limiting tissue damage and ameliorating disease severity [220].

Inflammation is also now realized to be tightly associated with metabolic stress. Typically, obesity is found to induce inflammation that is causally involved in the development of insulin resistance and glucose intolerance [221, 222]. In obese patients, a chronic inflammation occurs demonstrated by increased plasma levels of inflammatory cytokines [223], which will affect metabolic homeostasis over time. Inflammation is also observed in particular in

diabetic elderly patients [224]. Inflammation in pancreatic islets can reduce insulin secretion and stimulate β cell apoptosis, which plays a critical role in the progression of diabetes [225]. Emerging studies have suggested that EPO can regulate inflammatory cytokines to regulate inflammation. For instance, EPO treatment decreased IL-1β and TNF-α to attenuate the inflammatory response in injured rat brain and EPO stimulated neuroblastoma cells became resistant to TNF-α induced cell death and apoptosis [226, 227]. EPO treatment in mice mitigated the high fat diet induced inflammatory TNF-α and IL-6 production, toll like receptor 4 expression and NF-κB and JNK in the liver [228]. EPO also inhibits the activation of NF-κB, one of the central transcription factors to initiate and perpetuate inflammation, to increase the sensitivity of chemotherapeutics to promote tumor cells to undergo apoptosis [229]. In addition, EPO inhibits the induction of proinflammatory genes including TNF-α, IL-6 and inducible nitric oxide synthase (iNOS/NOS2) in activated macrophages via the blockage of nuclear factor NF-κB p65 activation by EPO in the gut [220].

Experimental and clinical data have clearly established that adipose tissue itself is an important site of inflammation during obesity. In the adipose tissue of obese mice, it was observed increased production of TNF-α and upregulated genes of inflammatory factors and increased accumulation of immune cells, which play important role in the development of insulin resistance [230-233]. Given that the important role of EPO in the adipocytes in the protection from obesity and the improvement from glucose intolerance and insulin resistance [6, 11, 58], it is reasonable to speculate that EPO may exert its anti-inflammatory function in the adipose tissue to contribute to the protection from obesity and metabolic disorder including glucose intolerance and insulin resistance. In supporting our view, Teng et al. observed increased expression of proinflammatory adipokines, Ccl1 and TNF-α but reduced IL-10 in the adipocytes from the mice with EpoR knock out in non-hematopoietic tissues that showed obese phenotype, glucose intolerance and insulin resistance, suggesting loss of EPO activity in non-hematopoietic tissue contributes to increased inflammation giving rise to insulin resistance [48]. A newly published paper also reported that EPO signaling can act as a novel regulator of white adipose tissue inflammation during diet-induced obesity. The anti-inflammatory effects of EPO were found to be driven by EPO mediated Stat3 activation to increase "anti-inflammatory" M2-like macrophages and reduce "pro-inflammatory" M1-like macrophages [234], suggesting that an anti-inflammatory effect of EPO in adipose tissue immune system may contribute to the protective role of EPO against obesity and associated metabolism disorders. Although the detection of EpoR in macrophages of atherosclerotic lesion and in bone marrow-derived macrophages provide further evidence for EPO effects in macrophages [235, 236], further experiments are still needed to reveal the EPO effect in anti-inflammation in adipose tissue and other tissues, which will highlight the therapeutic potential of EPO in many diseases via regulating inflammation beyond its primary function in hematopoietic system.

Conclusion

The primary physiological function of the EPO/EpoR system is to stimulate erythroid progenitor cell production to provide adequate red blood cells and oxygen delivery. However, emerging evidences revealed previously less realized roles of EPO/EpoR signaling beyond hematopoietic system. For example, EPO stimulated angiogenesis via endothelial cells in cardiovascular system contributes to cardioprotection to ischemic injury. EPO also provides neuroprotection during ischemic/hypoxic stress or disease via stress induced EPO production and EpoR expression in the brain to exert the antiapoptotic, oxygen delivery and anti-inflammatory effects. The protective function and the wound healing and repair role of EPO in skeletal muscle during ischemic/injury were also demonstrated. These pleiotropic activities of EPO in non-erythroid system strongly suggest therapeutic potential of EPO beyond its traditionally primary clinical application in hematopoietic system. More interestingly, recently published data confirm the tight link between EPO/EpoR signaling and energy metabolism and homeostasis. EPO administration in mice protects mice from diet induced obesity, promotes energy expenditure, reduces fat mass accumulation and improves glucose intolerance and insulin resistance. The loss of EPO activity in non-hematopoietic system in mice leads to glucose intolerant and insulin resistant with the development of obesity. EPO activity is also demonstrated to play a role in central regulation of appetite. The crosstalk between EPO and other important energy sensors including PGC-1α, Sirt1 and AMPK further provides a mechanism for EPO regulating energy homeostasis (Figure 2). Although these novel findings highlight the possible therapeutic potential of EPO in non-hematopoietic diseases, there is still a long way to go before EPO is really used in clinical trials for these diseases. Further studies are required to uncover the detailed mechanism by which EPO regulates central control of energy homeostasis and energy metabolism in multiple tissues.

Competing Interests

The authors have declared that no competing interest exists.

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