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Erythropoietin and Oxidative Stress

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Abstract

Unmitigated oxidative stress can lead to diminished cellular longevity, accelerated aging, and accumulated toxic effects for an organism. Current investigations further suggest the significant disadvantages that can occur with cellular oxidative stress that can lead to clinical disability in a number of disorders, such as myocardial infarction, dementia, stroke, and diabetes. New therapeutic strategies are therefore sought that can be directed toward ameliorating the toxic effects of oxidative stress. Here we discuss the exciting potential of the growth factor and cytokine erythropoietin for the treatment of diseases such as cardiac ischemia, vascular injury, neurodegeneration, and diabetes through the modulation of cellular oxidative stress. Erythropoietin controls a variety of signal transduction pathways during oxidative stress that can involve Janus-tyrosine kinase 2, protein kinase B, signal transducer and activator of transcription pathways, Wnt proteins, mammalian forkhead transcription factors, caspases, and nuclear factor κB. Yet, the biological effects of erythropoietin may not always be beneficial and may be poor tolerated in a number of clinical scenarios, necessitating further basic and clinical investigations that emphasize the elucidation of the signal transduction pathways controlled by erythropoietin to direct both successful and safe clinical care.

Keywords

Alzheimer’s disease; Akt; angiogenesis; apoptosis; cancer; cardiac; caspases; diabetes; endothelial; erythropoietin; forkhead; FoxO; GSK-3β; inflammation; mitochondria; NF-κB; renal; STATs; Wnt

OXIDATIVE STRESS

Initial work in pathways that can lead to oxidative stress by early investigators observed that increased metabolic rates could be detrimental to animals in an elevated oxygen environment. More current studies point to the potential aging mechanisms and accumulated toxic effects for an organism that are tied to oxidative stress (Maiese, *et al.*, 2008a). Oxygen consumption

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in organisms, or at least the rate of oxygen consumption in organisms, has intrigued several investigators. Pearl proposed that increased exposure to oxygen through an increased metabolic rate could lead to a shortened life span (Pearl, 1928). Subsequent work by multiple investigators has furthered this hypothesis by demonstrating that increased metabolic rates could be detrimental to animals in an elevated oxygen environment (Muller, *et al.*, 2007). When one moves to more current work, oxygen free radicals and mitochondrial DNA mutations have become associated with oxidative stress injury, aging mechanisms, and accumulated toxicity for an organism (Yui and Matsuura, 2006).

Oxygen free radicals can be generated in elevated quantities during the reduction of oxygen and subsequently lead to cell injury and apoptosis. Oxidative stress occurs as a result of the development of reactive oxygen species that consist of oxygen free radicals and other chemical entities. These agents can involve superoxide free radicals, hydrogen peroxide, singlet oxygen, nitric oxide (NO), and peroxynitrite (Chong, *et al.*, 2005e). Most species are produced at low levels during normal physiological conditions and are scavenged by endogenous antioxidant systems that include superoxide dismutase, glutathione peroxidase, catalase, and small molecule substances such as vitamins C and E. Other closely linked pathways to oxidative stress may be tempered by different vitamins, such as vitamin D_3 (Regulska, *et al.*, 2007) and the amide form of niacin or vitamin B_3, nicotinamide (Chlopicki, *et al.*, 2007, Chong, *et al.*, 2002d, Feng, *et al.*, 2006, Hara, *et al.*, 2007, Ieraci and Herrera, 2006, Lin, *et al.*, 2000, Maiese and Chong, 2003).

Throughout the body, cell survival and lifespan is tied to the presence of oxidative stress and the subsequent induction of apoptotic cell injury (Chong, *et al.*, 2006a, De Felice, *et al.*, 2007, Lin and Maiese, 2001). It has recently been shown that genes involved in the apoptotic process are replicated early during processes that involve cell replication and transcription, suggesting a much broader role for these genes than originally anticipated (Cohen, *et al.*, 2007). Apoptotic induced oxidative stress in conjunction with processes of mitochondrial dysfunction can contribute to a variety of disease states such as diabetes, ischemia, general cognitive loss, Alzheimer's disease, and trauma (Chong, *et al.*, 2005e, Chong, *et al.*, 2005f, Harris, *et al.*, 2007, Leuner, *et al.*, 2007, Okouchi, *et al.*, 2007). Oxidative stress can lead to apoptosis in a variety of cell types that involve neurons, endothelial cells (ECs), cardiomyocytes, and smooth muscle cells through multiple cellular pathways (Chong, *et al.*, 2004a, Chong, *et al.*, 2007b, Harris, *et al.*, 2007, Kang, *et al.*, 2003b, Karunakaran, *et al.*, 2007, Verdaguer, *et al.*, 2007).

Oxidative stress can impair mitochondrial permeability and function. Mitochondrial membrane transition pore permeability is increased (Chong, *et al.*, 2003a, Di Lisa, *et al.*, 2001, Kang, *et al.*, 2003b, Lin, *et al.*, 2000) and leads to a significant loss of mitochondrial NAD^+ stores and subsequent apoptotic cell injury (Chong, *et al.*, 2005g, Maiese and Chong, 2003). In addition, mitochondria are a significant source of superoxide radicals that are associated with oxidative stress (Chong, *et al.*, 2005e, Maiese and Chong, 2004). Blockade of the electron transfer chain at the flavin mononucleotide group of complex I or at the ubiquinone site of complex III results in the active generation of free radicals which can impair mitochondrial electron transport and enhance free radical production (Chong and Maiese, 2007b, Li, *et al.*, 2006a). Furthermore, mutations in the mitochondrial genome have been associated with the potential development of a host of disorders, such as hypertension, hypercholesterolemia, and hypomagnesemia (Li, *et al.*, 2004b, Wilson, *et al.*, 2004). Reactive oxygen species also may lead to the induction of acidosis-induced cellular toxicity and subsequent mitochondrial failure (Chong, *et al.*, 2005f). Disorders, such as hypoxia (Roberts and Chih, 1997), diabetes (Cardella, 2005, Kratzsch, *et al.*, 2006), and excessive free radical production (Ito, *et al.*, 1997, Vincent, *et al.*, 1999a, Vincent, *et al.*, 1999b) can result in the disturbance of intracellular pH.

Apoptotic cell death is a dynamic process that entails both early and late events. Membrane phosphatidylserine (PS) externalization is an early event during cell apoptosis (Maiese, *et al.*, 2000, Mari, *et al.*, 2004) and can become a signal for the phagocytosis of cells (Chong, *et al.*, 2005a, Li, *et al.*, 2006b, Lin and Maiese, 2001). As an example, externalization of membrane PS residues occur in neurons during anoxia (Maiese, 2001, Maiese and Boccone, 1995, Vincent and Maiese, 1999a), nitric oxide exposure (Chong, *et al.*, 2003f, Maiese, *et al.*, 1997), and during the administration of agents that induce the production of reactive oxygen species, such as 6-hydroxydopamine (Salinas, *et al.*, 2003). Membrane PS externalization on platelets also has been associated with clot formation in the vascular cell system (Leytin, *et al.*, 2006). The translocation of membrane PS residues from the inner cellular membrane to the outer surface is a necessary component under most conditions for the removal of apoptotic cells (Maiese, *et al.*, 2003, Maiese and Vincent, 2000a, Maiese and Vincent, 2000b). The loss of membrane phospholipid asymmetry leads to the externalization of membrane PS residues and assists microglia to target cells for phagocytosis (Chong, *et al.*, 2003d, Kang, *et al.*, 2003a, Kang, *et al.*, 2003b, Maiese and Chong, 2003, Mallat, *et al.*, 2005). This process occurs with the expression of the phosphatidylserine receptor (PSR) on microglia during oxidative stress (Li, *et al.*, 2006a, Li, *et al.*, 2006c), since blockade of PSR function in microglia prevents the activation of microglia (Chong, *et al.*, 2003b, Kang, *et al.*, 2003a).

In contrast to the early externalization of membrane PS residues, the cleavage of genomic DNA into fragments (Maiese, *et al.*, 1999, Maiese and Vincent, 2000a, Maiese and Vincent, 2000b) is considered to be a later event during apoptotic injury (Dombroski, *et al.*, 2000, Jessel, *et al.*, 2002, Kang, *et al.*, 2003b, Maiese and Vincent, 2000b). Endonucleases lead to DNA degradation and have been differentiated based on their ionic sensitivities to zinc (Torriglia, *et al.*, 1997), magnesium (Sun and Cohen, 1994), and calcium (Maiese, *et al.*, 1999), an important regulator that can independently impair cell survival. In the nervous system, three separate endonuclease activities are present. These include a constitutive acidic cation-independent endonuclease, a constitutive calcium/magnesium-dependent endonuclease, and an inducible magnesium dependent endonuclease (Chong, *et al.*, 2005f, Vincent and Maiese, 1999b, Vincent, *et al.*, 1999a). In the consideration of oxidative stress-induced pathways (Arcasoy, 2008, Maiese, *et al.*, 2008c), erythropoietin (EPO) offers a unique opportunity to prevent the exposure of membrane PS residues, inhibit the committed stages of genomic DNA destruction, and block cell injury.

EPO EXPRESSION, STRUCTURE, AND RECEPTOR ROLE IN CELLS AND TISSUES

EPO can be found in the breath of healthy individuals (Schumann, *et al.*, 2006), suggesting its broad availability in the body (Maiese, *et al.*, 2007a, Maiese, *et al.*, 2007c). In addition, it has been suggested that EPO may provide developmental cognitive support in humans with the recent observations that elevated EPO concentrations during infant maturation have been correlated with increased Mental Development Index scores (Bierer, *et al.*, 2006). The primary organs of EPO production and secretion are the kidney, liver, brain, and uterus. EPO production and secretion occurs foremost in the kidney (Fliser and Haller, 2007). The kidney peritubular interstitial cells are responsible for the production and secretion of EPO (Fisher, 2003). With the use of cDNA probes derived from the EPO gene, peritubular ECs, tubular epithelial cells, and nephron segments in the kidney also have been demonstrated to be vital cells for the production and secretion of EPO (Lacombe, *et al.*, 1991, Mujais, *et al.*, 1999). During periods of acute renal failure, EPO may provide assistance for the protection of nephrons (Sharples, *et al.*, 2005, Sharples and Yaqoob, 2006). Secondary sites of EPO production and secretion occur in the liver and the uterus (Chong, *et al.*, 2002a). Hepatocytes, hepatoma cells, and Kupffer cells of the liver can produce EPO (Fisher, 2003) and, in turn, EPO may provide a protective environment for these cells (Schmeding, *et al.*, 2007). In relation to the uterine production of

EPO, it is believed that the occurrence of neonatal anemia that can take place in the early weeks after birth may partly result from the loss of EPO production and secretion by placenta (Davis, *et al.*, 2003).

The Food and Drug Administration has approved EPO for the treatment of anemia. However, recent work has shown that EPO is not only required for erythropoiesis, but also functions in other organs and tissues, such as the brain, heart, and vascular system (Chong, *et al.*, 2003b, Chong, *et al.*, 2002b, Chong and Maiese, 2007a, Mikati, *et al.*, 2007, Moon, *et al.*, 2006, Um, *et al.*, 2007). It is the discovery of EPO and its receptor in the nervous and vascular systems that has resulted in great interest and enthusiasm for the potential clinical applications of EPO, such as in Alzheimer's disease, cardiac insufficiency (Assaraf, *et al.*, 2007, Palazzuoli, *et al.*, 2006), and cardiac transplantation (Gleissner, *et al.*, 2006, Mocini, *et al.*, 2007). In the nervous system, the major sites of EPO production and secretion are in the hippocampus, internal capsule, cortex, midbrain, cerebral ECs, and astrocytes (Digicaylioglu, *et al.*, 2004, Genc, *et al.*, 2004, Maiese, *et al.*, 2004, Maiese, *et al.*, 2005c). Further work has revealed several other organs as secretory tissues for EPO that include peripheral ECs (Anagnostou, *et al.*, 1994), myoblasts (Ogilvie, *et al.*, 2000), insulin-producing cells (Fenjves, *et al.*, 2003), and cardiac tissue (Fliser and Haller, 2007, Maiese, *et al.*, 2005c).

The EPO glycoprotein is 30.4 kDa with approximately half of its molecular weight derived from carbohydrates that can vary among species (Maiese, *et al.*, 2005c). EPO contains four glycosylated chains including three *N*-linked and one *O*-linked acidic oligosaccharide side chains. *N*-linked glycosylation sites occur at the positions 24, 38, and 83 of aspartyl residues, while the *O*-linked glycosylation site is at Serine^{126}. Three *N*-glycan chains of human EPO consist of the tetra-antennary structure with or without N-acetyllactosamine repeating units (Tsuda, *et al.*, 1988). The *O*-linked sugar chain is composed of Gal-GalNAc and sialic acids (Sasaki, *et al.*, 1987). The production and secretion of the mature EPO also relies upon the integrity of the *N*- and *O*-linked chains. The EPO gene is located on chromosome 7, exists as a single copy in a 5.4 kb region of the genomic DNA, and encodes a polypeptide chain containing 193 amino acids (Jacobs, *et al.*, 1985). During the production and secretion of EPO, a 166 amino acid peptide is initially generated following the cleavage of a 27 amino acid hydrophobic secretory leader at the amino-terminal (Imai, *et al.*, 1990). In addition, a carboxy-terminal arginine in position 166 is removed both in the mature human and recombinant human EPO (rhEPO) resulting in a circulatory mature protein of 165 amino acids (Chong, *et al.*, 2002a). The glycosylated chains are important for the biological activity of EPO and can protect EPO from oxygen radical degradation. The carbohydrate chains stabilize EPO (Toyoda, *et al.*, 2000) and the oligosaccharides in EPO may protect the protein from oxygen radical activity (Uchida, *et al.*, 1997). The N-glycosylated chains are believed to contribute to the thermal stability of EPO (Tsuda, *et al.*, 1988). In addition, the *N*- and *O*-linked chains may be necessary for the production and secretion of the mature EPO (Krantz, 1991). Replacement of asparagines 38 and 83 by glutamate or serine 126 by glycine can decrease the production and secretion of EPO (Dube, *et al.*, 1988). The presence of the carbohydrates also are important in the control of the metabolism of EPO, since EPO molecules with high sialic acid content can be easily cleared by the body through specific binding in the liver (Tsuda, *et al.*, 1990).

The biological activity of EPO also relies upon two disulfide bonds formed between cysteines at positions 7 and 160 and at positions 29 and 33 (Li, *et al.*, 2004a). The requirement of these disulfide bridges has been demonstrated by the evidence that reduction of these bonds results in the loss of the biologic activity of EPO. Alkylation of the sulfhydryl groups results in irreversible loss of the biological activity of EPO. Re-oxidization of EPO after reduction by guanidine restores eighty-five percent of the biological activity of EPO (Wang, *et al.*, 1985). Replacement of cysteine 33 with proline also reduces the biological function of EPO.

Following cloning of the EPO gene (Jacobs, *et al.*, 1985, Lin, *et al.*, 1985), the EPO receptor (EPOR) was found to be expressed in both normal and transformed erythroid cells (D'Andrea and Zon, 1990). The EPOR is part of the type 1 super-family of cytokine receptors and is activated *via* homodimerization (Bazan, 1990, Watowich, *et al.*, 1994). This receptor family shares a common domain structure consisting of an extracellular ligand-binding domain, a transmembrane domain, and an intracellular domain. The extracellular domain is necessary for the initial binding of EPO and the intracellular domain is responsible for the transduction of intracellular signaling (Mulcahy, 2001).

EPO controls bone marrow erythroid cell proliferation, differentiation, and survival through its binding to an erythroid progenitor cell surface EPOR. The EPOR also is expressed in numerous non-erythroid blood lines that include neurons, microglia, astrocytes, and in cerebral ECs (Anagnostou, *et al.*, 1994, Fliser and Haller, 2007, Genc, *et al.*, 2004, Maiese, *et al.*, 2004, Maiese, *et al.*, 2005c) as well as on myelin sheaths of radicular nerves in human peripheral nerves (Hassan, *et al.*, 2004), suggesting both a developmental and potential protective role for EPO in the central and peripheral nervous systems. The EPOR also is expressed in primary cerebral ECs (Chong, *et al.*, 2003a, Chong, *et al.*, 2003c) as well as in human umbilical veins, bovine adrenal capillaries, and rat brain capillaries (Anagnostou, *et al.*, 1994, Yamaji, *et al.*, 1996).

Production of EPO and the expression of its receptor are altered during development. Elevated expression of the EPOR occurs in early embryonic neuronal tissues at levels similar to that observed in the adult spleen and bone marrow (Liu, *et al.*, 1994). Yet, the level of endogenous EPOR expression is significantly reduced following the maturation of the brain (Liu, *et al.*, 1997). During gestation, EPO production is increased, but later becomes suppressed following birth to be regulated by the tissue oxygen supply (Chong, *et al.*, 2002c). A deficiency in tissue oxygen results in the production of EPO and an increase in the expression of the EPOR not only in peripheral organs (Fliser and Haller, 2007, Li, *et al.*, 2004a, Maiese, *et al.*, 2004, Maiese, *et al.*, 2005c), but also in the brain (Li, *et al.*, 2007a) that may be responsible for hypoxic tolerance in some species (Ravid, *et al.*, 2007). EPO secretion in the brain appears to be more sustained than in peripheral organs such as the kidney (Chikuma, *et al.*, 2000), suggesting that EPO production may originate in the brain and possibly cross the blood-brain barrier to reach the blood and peripheral organs (Li, *et al.*, 2004a). Work performed *in vivo* with subjects exposed to hypoxia also demonstrates an increase in expression of EPO and EPOR mRNA following reduced oxygenation (Marti, *et al.*, 1996). Furthermore, both primary neurons (Chikuma, *et al.*, 2000, Liu, *et al.*, 2006) and neuronal cell lines (Stolze, *et al.*, 2002) have been found to retain the capacity to express EPO in an oxygen-dependent manner. Although EPO is recognized as a critical modulator of erythropoiesis, a low concentration of red blood cells alone does not directly stimulate EPO production, but requires the presence of a diminished oxygen tension. Once a hypoxic stimulus is received, EPO is subsequently released into the peripheral blood circulation and upon arrival in the bone marrow, EPO binds to its receptor that is highly expressed on the surface of erythroid progenitor cells and leads to erythropoiesis (Broudy, *et al.*, 1991). This results in an elevation in the number of mature erythrocytes and the improvement of oxygen supply. EPO also functions to stimulate colony-forming erythroid cells to induce these cells to proliferate, mature into erythrocytes, and possibly assist with reticulocyte release to the blood (Sathyanarayana, *et al.*, 2007).

Hypoxia-inducible factor 1 (HIF-1) controls expression of EPO and EPOR during periods of reduced oxygen content. HIF-1 is essential for the production and secretion of EPO in response to hypoxia (Ikeda, 2005). At the transcriptional level, the hypoxia- dependent gene transcription of EPO and EPOR directly results from the activation of the HIF-1 pathway under hypoxic conditions. Gene transcription of EPO is mediated by the transcription enhancer located in the 3′-flanking region of the EPO gene that specifically binds to HIF-1 (Wang and

Semenza, 1995). HIF-1 is a basic helix-loop-helix heterodimeric transcription factor containing two subunits, HIF-1α and HIF-1β. HIF-1β is a constitutively expressed 91–94 kDa subunit that was characterized previously as aryl hydrocarbon receptor nuclear translocator (ARNT) (Hoffman, *et al.*, 1991). HIF-1α is a 120 kDa oxygen-labile subunit that is degraded through the ubiquitin-proteasome pathway under normoxic conditions (Huang, *et al.*, 1998). During hypoxia or conditions such as iron chelation that can mimic hypoxia, blocking HIF-1α association with von Hippel-Lindau protein impairs degradation of HIF-1α (Maxwell, *et al.*, 1999). HIF-1α subsequently translocates to the nucleus and heterodimerizes with HIF-1β to form a stable HIF-1 complex. The HIF complex then binds to the conserved sequence (5′ RCGTG3′) near the 5′ end of the hypoxia-responsive enhancer of the EPO gene to up-regulate EPO gene transcription (Bunn, *et al.*, 1998). Increased DNA binding activity of HIF-1 occurs in rat cortical neurons during oxidative stress, suggesting that HIF-1 may function as oxygen sensor regulating adaptive gene transcription and resulting in the production and secretion of the EPO protein during hypoxia in the nervous system (Maiese, *et al.*, 2004, Maiese, *et al.*, 2005c). It is important to note that each of the HIF family members HIF-1α, HIF-1β, and HIF-3α play important roles in regulating the expression of EPO and the EPOR to foster protection against hypoxic cell injury (Heidbreder, *et al.*, 2003).

Reduced oxygen content is not the only factor responsible for the expression of EPO and the EPOR. The production and secretion of EPO in female reproductive organs is estrogen-dependent. During the cyclic development of the uterine endometrium, 17β-estradiol can lead to a rapid and transient increase in EPO mRNA in the uterus (Yasuda, *et al.*, 1998), oviducts, and ovaries (Masuda, *et al.*, 2000). Hypoxic induced EPO mRNA expression in uterine tissue occurs only in the presence of 17β-estradiol. EPO mRNA expression by hypoxia in the uterus is less pronounced than the EPO expression that occurs in the kidney and the brain (Chikuma, *et al.*, 2000). Interestingly, a variety of cellular disturbances may lead to either increased or decreased EPO expression through the control of HIF, such as hypoglycemia, cadmium exposure, raised intracellular calcium, or intense neuronal depolarizations generated by mitochondrial reactive oxygen species (Chong, *et al.*, 2002c, Genc, *et al.*, 2004, Obara, *et al.*, 2003). Anemic stress, insulin release, and several cytokines, including insulin-like growth factor, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) (Nagai, *et al.*, 2001) also can lead to increased expression of EPO and the EPOR (Maiese, *et al.*, 2004, Maiese, *et al.*, 2005c).

EPO AND CELLULAR SIGNAL TRANSDUCTION

EPO cellular signal transduction requires the activation of the EPOR. Once EPO is bound to the EPOR, the EPOR activates Janus-tyrosine kinase 2 (Jak2) through phosphorylation. Jak2 is a member of a family of Janus-type proteintyrosine kinases including Jak1, Jak2, Jak3, and Tyk2 that are characterized by a kinase domain in the carboxyl portion, a kinase-like domain, and a large amino-terminal domain (Wilks, *et al.*, 1991). The amino-terminal domain of Jak2 is responsible for the binding of Jak2 with the β-subunit of the EPOR at a region proximal to the membrane that contains the Box 1 sequence (Zhao, *et al.*, 1995). EPO can prevent apoptotic injury through its reliance on Jak2 phosphorylation (Kawakami, *et al.*, 2001, Sharples, *et al.*, 2004), since loss of Jak2 activity reduces protection by EPO (Digicaylioglu, *et al.*, 2004, Lipton, 2007).

The signal transducer and activator of transcription (STAT) proteins are direct substrates of Janus kinases. Seven mammalian STAT genes encoding proteins exist and are considered to be latent DNA binding factors that can be activated by tyrosine phosphorylation (Reich, 2007). Activation of Janus kinases results in tyrosine phosphorylation and dimerization of STATs. Once activated, STATs translocate to the nucleus and bind to specific DNA sequences in the promoter regions of responsive genes to lead to gene transcription. Closely linked to

these transcription pathways are the mitogen-activated protein kinases that include the extracellular signal-related kinases (ERKs), the c-Jun-amino terminal kinases, and p38 MAP kinase that can oversee erythroid proliferation and differentiation (Nagata, *et al.*, 1998). However, in regards to cytoprotection, EPO has been shown to not only activate STAT 3 (Asaumi, *et al.*, 2007, Chong and Maiese, 2007a, Parsa, *et al.*, 2003), STAT 5 (Chong and Maiese, 2007a, Menon, *et al.*, 2006b, Moon, *et al.*, 2006, Um and Lodish, 2006, Wei, *et al.*, 2006), and ERK 1/2 (Bullard, *et al.*, 2005, Menon, *et al.*, 2006a), but also to employ these pathways for cell development and cell protection. EPO activates STAT3, STAT5, and ERK 1/2 in primary cerebral vascular cells, suggesting that EPO may require these cellular pathways to confer EC cytoprotection during oxidative stress (Chong and Maiese, 2007a). In addition, activation of STAT5 also can modulate EPO proliferation as well as protection against cellular apoptosis (Damen, *et al.*, 1995). In erythroleukemic cell lines, EPO-dependent cell survival is accompanied by sustained STAT5 DNA-binding activity. Stable expression of the truncated STAT5a has been shown to enhance STAT5-DNA binding activity and reduce the induction of apoptosis (Bittorf, *et al.*, 2000). In contrast, induction of apoptosis can be observed in cells that lack STAT5 ($STAT5a^{-/-}/5b^{-/}$) function (Socolovsky, *et al.*, 2001). For example, $STAT5a^{-/-}5b^{-/-}$ fetal liver erythroid progenitors show higher levels of apoptosis and are less responsive to the presence of EPO (Socolovsky, *et al.*, 1999).

Downstream from Janus kinases, STATS, and the ERKs are the apoptotic pathways of the caspase family. Caspases are composed of three domains including an N-terminal prodomain, a large subunit, and a small subunit (Earnshaw, *et al.*, 1999). As a result of their activation sequence, caspases are classified as either initiator caspases (also known as apical caspases) or effector caspases (Li, *et al.*, 2006a, Maiese, *et al.*, 2005a, Okouchi, *et al.*, 2007). An initiator caspase cleaves and subsequently activates an effector caspase. The apoptotic-associated caspases include initiator caspases, such as caspase 2, 8, 9, and 10, that activate downstream effector caspases, resulting in an amplification of cascade activity. The initiator caspases consist of long N-terminal prodomains that contain caspase recruitment domains (CARDs) in caspase 2 and caspase 9 or death effector domains (DEDs) in caspase 8 and caspase 10 (Hofmann, *et al.*, 1997). The effector caspases consist of caspase 3, 6, and 7 that function to directly cleave crucial cellular protein substrates to result in cell destruction. The caspases 1 and 3 have each been linked to the independent apoptotic pathways of genomic DNA cleavage and cellular membrane PS exposure (Chong, *et al.*, 2003a, Chong, *et al.*, 2003e, Takahashi, *et al.*, 1999). These caspases, in addition to caspase 8 and 9, are also tied to the direct activation and proliferation of microglia (Chong, *et al.*, 2003b, Kang, *et al.*, 2003a, Kang, *et al.*, 2003b). Caspase 1 is believed to be principally responsible for the externalization of membrane PS residues in several cell systems that can subsequently activate microglial phagocytosis (Maiese and Vincent, 2000b, Vanags, *et al.*, 1996). Furthermore, caspase 9 is activated through a process that involves the cytochrome c -apoptotic protease-activating factor-1 (Apaf-1) complex (Chong, *et al.*, 2004b, Li, *et al.*, 1997). In addition, caspase 8 serves as an upstream initiator of executioner caspases, such as caspase 3, and also leads to the mitochondrial release of cytochrome c (Engels, *et al.*, 2000, Stegh, *et al.*, 2002). Following caspase 8 and caspase 9 activation, caspase 3 directly leads to genomic DNA degradation.

The ability of EPO to prevent specific caspase 1 and caspase 3-like activities appears to play a significant role in its cellular protection (Chong, *et al.*, 2003b, Chong, *et al.*, 2002b, Chong, *et al.*, 2003e, Digicaylioglu, *et al.*, 2004, Li, *et al.*, 2007a, Okutan, *et al.*, 2007, Wu, *et al.*, 2007a). In regards to caspase 1, EPO prevents PS externalization primarily through the inhibition of caspase 1 -like activity and, to a lesser degree, through other caspases such as 3, 8, and 9 (Chong, *et al.*, 2003a, Chong, *et al.*, 2003b, Chong, *et al.*, 2002b, Chong, *et al.*, 2003e). EPO also can block genomic DNA degradation through the inhibition of cytochrome c and the subsequent inhibition of caspase 3 - like activity (Chong, *et al.*, 2003b). EPO prevents cellular apoptosis through parallel pathways that prevent the induction of Apaf-1, caspase 8,

and caspase 9 as well as by preserving mitochondrial membrane potential in conjunction with enhanced Bcl-x_L expression (Chong, *et al.*, 2003a, Chong, *et al.*, 2003b, Chong, *et al.*, 2003e, Sharples, *et al.*, 2004)

The ability of EPO to enhance cell survival during injury also directly relies upon the phosphatidylinositol 3-kinase (PI 3-K) pathway through the serine-threonine kinase protein kinase B (Akt). Phosphorylation of Akt in conjunction with EPO administration leads to its activation and protects against genomic DNA degradation and membrane PS exposure (Chong, *et al.*, 2003a, Chong, *et al.*, 2003b, Chong, *et al.*, 2003e). Up-regulation of Akt activity during multiple injury paradigms, such as vascular and cardiomyocyte ischemia (Miki, *et al.*, 2006, Parsa, *et al.*, 2003), free radical exposure (Chong, *et al.*, 2003b, Matsuzaki, *et al.*, 1999), matrix detachment (Rytomaa, *et al.*, 2000), neuronal axotomy (Namikawa, *et al.*, 2000), N-methyl-D-aspartate toxicity (Dzietko, *et al.*, 2004), hypoxia (Chong, *et al.*, 2002b, Zhang, *et al.*, 2007), β-amyloid toxicity (Chong, *et al.*, 2005d, Martin, *et al.*, 2001), DNA damage (Chong, *et al.*, 2004a, Chong, *et al.*, 2002b, Henry, *et al.*, 2001, Kang, *et al.*, 2003a), metabotropic ligand (Anjaneyulu, *et al.*, 2008, Maiese, *et al.*, 2005a) and receptor signaling (Chong, *et al.*, 2005a, Chong, *et al.*, 2006b, Maiese, *et al.*, 2005a), cell metabolic pathways (Chong, *et al.*, 2005g, Maiese and Chong, 2003), and oxidative stress (Chong, *et al.*, 2004a, Kang, *et al.*, 2003a, Kang, *et al.*, 2003b) increases cell survival. Akt also can directly control microglial activation through the prevention of Bcl-x_L degradation (Chong, *et al.*, 2004a) and the inhibition of caspase 1-, 3-, and 9-like activities (Chong, *et al.*, 2005a, Kang, *et al.*, 2003a, Kang, *et al.*, 2003b).

Akt also appears to be a vital component for EPO cytoprotection especially during inflammatory cell activation, since inhibition of Akt activity blocks cellular protection and anti-inflammatory mechanisms by EPO (Chong, *et al.*, 2003a, Chong, *et al.*, 2003b, Chong, *et al.*, 2003e). Activation of Akt is usually cytoprotective, such as during free radical exposure (Chong, *et al.*, 2003b, Matsuzaki, *et al.*, 1999), hyperglycemia (Anitha, *et al.*, 2006), endothelial cell hypoxia (Chong, *et al.*, 2002b), β-amyloid toxicity (Chong, *et al.*, 2007a, Chong, *et al.*, 2005d), cardiomyopathy (Kim, *et al.*, 2008), and oxidative stress (Chong, *et al.*, 2004a, Kang, *et al.*, 2003a, Kang, *et al.*, 2003b). EPO uses the PI 3-K/Akt pathway in a variety of experimental models of injury (Bahlmann, *et al.*, 2004, Chong, *et al.*, 2003b, Chong, *et al.*, 2002b, Chong, *et al.*, 2003e, Chong and Maiese, 2007a, Li, *et al.*, 2006b, Miki, *et al.*, 2006, Parsa, *et al.*, 2003, Sharples, *et al.*, 2004, Um, *et al.*, 2007, Um and Lodish, 2006, Wu, *et al.*, 2007b). These can involve transcription factor regulation (Chong and Maiese, 2007a), maintenance of $\Delta\Psi_m$, prevention of cytochrome c release (Chong, *et al.*, 2003a, Chong, *et al.*, 2003b, Chong, *et al.*, 2003e), and blockade of caspase activity (Chong, *et al.*, 2003a, Chong, *et al.*, 2003b, Chong, *et al.*, 2002b).

Several novel pathways that may mediate the ability of EPO to prevent cellular apoptosis are intimately tied to Akt. Akt is a primary mediator of phosphorylation of the mammalian forkhead transcription factors of the O class (FoxOs), FoxO1, FoxO3a, and FoxO4 (Chong, *et al.*, 2005b, Maiese, *et al.*, 2007b). More than 100 forkhead genes and 19 human subgroups that range from *FOXA* to *FOXS* are now known to exist since the initial discovery of the fly *Drosophila melanogaster gene forkhead* (Maiese, *et al.*, 2007b). The fork-head box (FOX) family of genes is characterized by a conserved forkhead domain commonly noted as a “forkhead box” or a “winged helix” as a result of the butterfly-like appearance on X-ray crystallography (Clark, *et al.*, 1993) and nuclear magnetic resonance (Jin, *et al.*, 1998). All Fox proteins contain the 100-amino acid winged helix domain, but it should be noted that not all winged helix domains are Fox proteins (Larson, *et al.*, 2007). FoxO proteins are expressed throughout the body and are found in the ovary, prostate, skeletal muscle, blood vessels, brain, heart, lung, liver, pancreas, spleen, thymus, and testis (Maiese, *et al.*, 2008b, Maiese, *et al.*, 2007b). Of the FoxOs, FoxO3a is one member that has emerged as a versatile target for a

number of disorders. Akt can prevent cellular apoptosis through the phosphorylation of FoxO proteins. Post-translational phosphorylation of FoxO proteins will maintain FoxO transcription factors in the cytoplasm by association with 14-3-3 proteins and prevent the transcription of proapoptotic target genes (Chong and Maiese, 2007a, Maiese, *et al.*, 2005c). In the absence of inhibitory Akt1 phosphorylation, FoxO3a is activate, can translocate to the nucleus, and controls a variety of functions that involve cell cycle progression, cell longevity, and apoptosis (Lehtinen, *et al.*, 2006, Li, *et al.*, 2006a, Maiese, *et al.*, 2007a). As a result, control of FoxO3a is considered to be a viable therapeutic target for agents such as metabotropic glutamate receptors (Chong, *et al.*, 2006b, Maiese, *et al.*, 2005a), neurotrophins (Zheng, *et al.*, 2002), and NAD^+ precursors (Chong, *et al.*, 2004c, Li, *et al.*, 2006a, Li, *et al.*, 2006b) to increase cell survival. In addition, FOXO3a interfaces with several pathways that regulate cellular lifespan and function to control neoplastic growth (Li, *et al.*, 2007b). EPO controls the phosphorylation and degradation of FOXO3a to retain it in the cytoplasm through binding to 14-3-3 protein and foster vascular cell protection during oxidative stress (Chong and Maiese, 2007a). Regulation of caspase 3 - like activity by EPO also has recently been linked to a unique regulatory mechanism that blocks the proteolytic degradation of phosphorylated forkhead transcription factors by caspase 3. Given that specific proapoptotic transcription factors, such as FoxO3a, have been shown to be a substrate for caspase 3-like proteases at the consensus sequence $DELD^{304}A$ (Charvet, *et al.*, 2003), studies have shown that blockade of caspase 3 - like activity prevents the destruction of the inactive phosphorylated FoxO3a during oxidative stress to increase cell survival (Chong and Maiese, 2007a).

Akt also is associated with proteins are derived from the *Drosophila Wingless* (*Wg*) and the mouse *Int-1* genes (Chong, *et al.*, 2007a, Chong, *et al.*, 2007c, Li, *et al.*, 2006c, Speese and Budnik, 2007). The Wnt proteins are secreted cysteine-rich glycosylated proteins that can control cell proliferation, differentiation, survival, and tumorigenesis (Chong and Maiese, 2004, Li, *et al.*, 2006c). More than eighty target genes of Wnt signaling pathways have been demonstrated in human, mouse, *Drosophila*, Xenopus, and zebrafish. These genes are present in several cellular populations, such as neurons, cardiomyocytes, endothelial cells, cancer cells, and pre-adipocytes (Maiese, 2008b). At least nineteen of twenty-four Wnt genes that express Wnt proteins have been identified in the human (Li, *et al.*, 2005, Li, *et al.*, 2006c, Maiese, *et al.*, 2008d).

Wnt proteins are generally divided into functional classes based on their ability to induce a secondary body axis in *Xenopus* embryos and to activate certain signaling cascades that consist of the Wnt1 class and the Wnt5a class (Maiese, 2008b, Maiese, *et al.*, 2008d). These involve intracellular signaling pathways are critical for Wnt signal transduction (Maiese, 2008a, Maiese, 2008b). One Wnt pathway involves intracellular calcium release and is termed the non-canonical or Wnt/calcium pathway consisting primarily of Wnt4, Wnt5a, and Wnt11. The non-canonical system functions through non-β-catenin-dependent pathways and also includes the planar cell polarity (PCP) pathway or the Wnt-calcium-dependent pathways (Li, *et al.*, 2005, Li, *et al.*, 2006c, Maiese, *et al.*, 2008d). A second pathway controls target gene transcription through β-catenin, generally referred to as the canonical pathway that involves Wnt1, Wnt3a, and Wnt8. Wnt signaling can prevent cell injury through a variety of mechanisms. Wnt prevents apoptosis through β-catenin/Tcf transcription mediated pathways (Chen, *et al.*, 2001) and also can protect cells against c-myc induced apoptosis through cyclooxygenase-2 and Wnt induced secreted protein (You, *et al.*, 2002). Wnt signaling also can inhibit apoptosis during oxidative stress (Chong and Maiese, 2004) and β-amyloid toxicity that may require modulation of glycogen synthase kinase-3β (GSK-3β) and β-catenin (Chong, *et al.*, 2007a).

Abnormalities in the Wnt signaling pathways, such as with transcription factor 7-like 2 gene, may impart increased risk for type 2 diabetes in some populations (Grant, *et al.*, 2006,

Lehman, *et al.*, 2007, Scott, *et al.*, 2006) as well as have increased association with obesity (Guo, *et al.*, 2006). Yet, intact Wnt family members may offer glucose tolerance and increased insulin sensitivity (Wright, *et al.*, 2007) as well as protect glomerular mesangial cells from elevated glucose induced apoptosis (Lin, *et al.*, 2006). These observations suggest a potential protective cellular mechanism for EPO through Wnt signaling to improve clinical cardiac function in diabetic patients (Silverberg, *et al.*, 2006) and decrease complications in woman with diabetic pregnancies (Teramo, *et al.*, 2004). Cell culture studies demonstrate that the Wnt1 protein is necessary and sufficient to impart cellular protection during elevated glucose exposure (Chong, *et al.*, 2007c). Administration of exogenous Wnt1 protein can significantly prevent apoptotic EC injury during elevated glucose exposure. EPO maintains the expression of Wnt1 during elevated glucose exposure and prevents loss of Wnt1 expression that would occur in the absence of EPO during elevated glucose. Blockade of Wnt1 with a Wnt1Ab also can neutralize the protective capacity of EPO, illustrating that Wnt1 is a critical component in the cytoprotection of EPO during elevated glucose exposure (Chong, *et al.*, 2007c).

In the Wnt pathway, modulation of glycogen synthase kinase-β (GSK-3β) and nuclear factor-κB (NF-κB) activity can affect cell survival during oxidative stress (Li, *et al.*, 2005, Maiese, 2008b, Maiese, *et al.*, 2008d). GSK-3β is considered to be a therapeutic target for some neurodegenerative disorders (Balaraman, *et al.*, 2006, Chong, *et al.*, 2005e, Nurmi, *et al.*, 2006, Qin, *et al.*, 2006) and also may to influence inflammatory cell survival (Chong, *et al.*, 2007b) and activation (Tanuma, *et al.*, 2006). During models of diabetes, inactivation of GSK-3β by small molecule inhibitors or RNA interference prevents toxicity from high concentrations of glucose and increases rat beta cell replication, suggesting a possible target of GSK-3β for pancreatic beta cell regeneration (Mussmann, *et al.*, 2007). Clinical applications for GSK-3β are attractive, especially in concert with EPO. For example, both the potential benefits of EPO to improve cardiovascular function in diabetic patients (Silverberg, *et al.*, 2006, Silverberg, *et al.*, 2001) and the positive effects of exercise to improve glycemic control during DM (Maiorana, *et al.*, 2002) appear to rely upon the inhibition of GSK-3β activity. EPO blocks GSK-3β activity (Chong, *et al.*, 2005e, Chong, *et al.*, 2007b, Rowe, *et al.*, 2007, Wu, *et al.*, 2007a) and combined with exercise may offer synergistic benefits, since physical exercise also has been shown to phosphorylate and inhibit GSK-3β activity (Howlett, *et al.*, 2006). Expression and cytoprotection of EPO is dependent, in part, upon Akt and NF-κB. The phosphorylation of IκB proteins by the IκB kinase (IKK) and their subsequent degradation lead to the release of NF-κB for its translocation to the nucleus to initiate gene transcription (Hayden and Ghosh, 2004). Dependent upon Akt controlled pathways, the transactivation domain of the p65 subunit of NF-κB is activated by IKK and the IKKα catalytic subunit to lead to the induction of protective anti-apoptotic pathways (Chong, *et al.*, 2005b). Increased expression of NF-κB during injury models can occur in inflammatory microglial cells (Chong, *et al.*, 2005d, Chong, *et al.*, 2007b, Guo and Bhat, 2006) and in neurons (Sanz, *et al.*, 2002). NF-κB represents a critical pathway that is responsible for the activation of inhibitors of apoptotic proteins (IAPs), the maintenance of Bcl-x_L expression, (Chen, *et al.*, 2000, Chong, *et al.*, 2005f), and protection against cell injury during oxidative stress (Chong, *et al.*, 2005d). NF-κB also is strongly associated with the cytopro-tection of trophic factors that includes EPO (Chong, *et al.*, 2005d, Nakata, *et al.*, 2004, Sae-Ung, *et al.*, 2005). NF-κB also plays a key role in the expression of EPO during HIF-1 induction. Akt can significantly increase NF-κB and HIF-1 activation resulting in the enhancement of EPO expression. Although NF-κB has not consistently been found to be beneficial in all cell systems (Esposito, *et al.*, 2006, Jacobsen, *et al.*, 2006) and may sometimes not be cytoprotective (Nurmi, *et al.*, 2006, Xu, *et al.*, 2005), EPO subsequently uses NF-κB to prevent apoptosis through the enhanced expression and translocation of NF-κB to the nucleus to elicit anti-apoptotic gene activation (Bittorf, *et al.*, 2001, Chong, *et al.*, 2005d, Li, *et al.*, 2006b, Spandou, *et al.*, 2006).

EPO AND CLINICAL ENTITIES

Cardiac and Vascular Integrity

The control of angiogenesis by EPO offers an important level of cytoprotection (Chong, *et al.*, 2002a, Walshe and D'Amore, 2008). In neonatal mesenteric microvascular ECs, EPO leads to vasculogenesis (Ashley, *et al.*, 2002). In clinical studies, EPO serum levels are significantly associated with the number and function of circulating endothelial progenitor cells and EPO can stimulate postnatal neovascularization by increasing endothelial progenitor cell mobilization from the bone marrow (Heeschen, *et al.*, 2003). Angiogenesis also has been observed in endothelial samples derived from human adult myocardial tissue following treatment with EPO (Jaquet, *et al.*, 2002). In addition, the uterine endometrium and the ovaries are dependent upon EPO for the induction of angiogenesis to compensate for lost vessels during the estrus cycle. EPO has been shown to be necessary to foster blood vessel formation in the endometrium in ovariectomized mice and to be required for the formation of a capillary network for the development of follicles and the corpora lutea (Yasuda, *et al.*, 1998). In models of cerebral ischemia, EPO promotes factors for angiogenesis such as Tie-2 and Angiopoietin-2 that may assist with the restoration of cerebral blood flow to pre-ischemic levels (Li, *et al.*, 2007a). EPO controlled angiogenesis also may play a significant role during renal inflammation and prevention of allograft rejection (Reinders, *et al.*, 2006). Yet, it is important to consider the inhibition of angiogenesis. Although EPO induced angiogenesis may impart beneficial effects to ischemic cells of the nervous and cardiovascular systems for nutrient and oxygen supply, other scenarios that involve ocular neovascularization may seek to block or limit angiogenesis by EPO to prevent disease progression (Zhang and Ma, 2007).

In the vascular system, EPO also offers direct preservation of EC integrity (Chong, *et al.*, 2002a, Chong, *et al.*, 2003a, Chong, *et al.*, 2002b). EPO has both a mitogenic and chemotactic effect that can lead to matrix metalloproteinase-2 production, cell proliferation, and vessel formation in EC lines (Maiese, *et al.*, 2004, Maiese, *et al.*, 2005c). In cultured human and bovine ECs, EPO stimulates EC proliferation and fosters the migration of ECs (Anagnostou, *et al.*, 1990). It is important to note that as a large molecule, EPO may maintain the establishment of EC communication and function that could become crucial in a number of scenarios, such as repair of the blood-brain barrier during injury (Martinez-Estrada, *et al.*, 2003). In addition, by assuring EC integrity, EPO prevents ischemic cardiac demise by reducing myocardial injury and cardiomyocyte apoptosis (Burger, *et al.*, 2006), lessening myocardial ischemia (Bullard, *et al.*, 2005), modulating cardiac remodeling (Miki, *et al.*, 2006, Toma, *et al.*, 2007), reducing ventricular dysfunction (Parsa, *et al.*, 2004, Parsa, *et al.*, 2003), and improving cardiac function (Gao, *et al.*, 2007, Westenbrink, *et al.*, 2007). Therefore, EPO plays a critical role in the vascular and renal systems with the maintenance of erythrocyte (Foller, *et al.*, 2007) and podocyte (Eto, *et al.*, 2007) integrity, regulates the survival of ECs (Chong, *et al.*, 2003a, Chong, *et al.*, 2002b), and may act as a powerful endogenous protectant during cardiac injury (Asaumi, *et al.*, 2007). EPO can protect against myocardial cell apoptosis and decrease infarct size, resulting in improved left ventricular contractility. In isolated rat heart preparations following ischemia/reperfusion experiments, beneficial effects of treatment with EPO have been shown to significantly improve post-ischemic recovery of left ventricular pressure (Moon, *et al.*, 2003, van der Meer, *et al.*, 2004a). EPO treatment also can prevent myocardial cell apoptosis and decrease infarct size, resulting in enhanced cardiac function and recovery (Parsa, *et al.*, 2004). At the onset of coronary artery occlusion, EPO administered also can significantly inhibit apoptosis in the central region of myocardial ischemia (Tramontano, *et al.*, 2003). Even in acute scenarios following coronary artery ligation, EPO leads to a decrease in apoptotic cells by fifty percent in the myocardium and significantly improves cardiac function (Moon, *et al.*, 2003, Parsa, *et al.*, 2003).

Although some cardiac injury experimental models do not consistently demonstrate a benefit with EPO (Olea, *et al.*, 2006), initial studies in patients with anemia or on chronic hemodialysis have suggested a direct cardiac benefit from EPO administration (Goldberg, *et al.*, 1992, Silverberg, *et al.*, 2001). Randomized control studies with EPO administration in patients with congestive heart failure or diabetes combined with congestive heart failure also demonstrate an improved cardiac output and a decrease in medical resource utilization (Maiese, *et al.*, 2005c, Silverberg, *et al.*, 2006). Subsequent work has demonstrated that EPO administration can improve exercise tolerance either during cardiac or renal insufficiency in patients with anemia and congestive heart failure (Mancini, *et al.*, 2003, Palazzuoli, *et al.*, 2006) and that this may be tied to improved pulmonary function (Wu, *et al.*, 2006). Other studies have shown that patients with acute myocardial infarction have increased plasma EPO levels within seven days of the cardiac insult, suggesting a possible protective response from the body (Ferrario, *et al.*, 2007). Serum levels of EPO also may function as a biomarker for cardiovascular injury (Fu and Van Eyk, 2006) as well as improve survival following out of hospital cardiac arrest (Cariou, *et al.*, 2008). A randomized, concealed, multicenter trail of 1460 patients who received 40,000 U of epoetin alfa up to a 3 week maximum following intensive care unit admission also demonstrated a reduced mortality in patients with trauma (Corwin, *et al.*, 2007).

Unfortunately, agents such as EPO may not be well tolerated by all individuals, especially those with comorbid conditions such as congestive heart failure and hypertension. Some studies suggest that elevated plasma levels of EPO independent of hemoglobin concentration can be associated with increased severity of disease in individuals with congestive heart failure (van der Meer, *et al.*, 2004b) and that EPO may contribute to vascular stenosis with intima hyperplasia (Reddy, *et al.*, 2007). Adverse effects during treatment with EPO are not uncommon, such as an increased incidence of thrombotic vascular effects (Corwin, *et al.*, 2007) or the use of EPO in cancer patients receiving chemotherapy that has been associated with nonfatal myocardial infarction, pyrexia, vomiting, shortness of breath, paresthesias, and upper respiratory tract infection (Henry, *et al.*, 2004). In addition, the use of EPO in patients with hypertension must proceed with caution, since both acute and long-term administration of EPO can significantly elevate mean arterial pressure (Kanbay, *et al.*, 2007).

Immune Function and the Nervous System

Given the impact that inflammatory cells may have upon the progression or resolution of degenerative insults throughout the body, it becomes essential to consider agents that can control inflammatory pathways (Chong, *et al.*, 2005a, Chong, *et al.*, 2004a, Kang, *et al.*, 2003b). Therefore, cytoprotective agents that are known to modulate inflammatory cell function may offer attractive therapeutic considerations (Chong, *et al.*, 2007b, Li, *et al.*, 2006b). EPO appears to fill such a need in regards to its role during periods of cellular inflammation. EPO can reduce cytokine gene expression in endothelial cells exposed to tumor necrosis factor (Avasarala and Konduru, 2005), prevent ulcer progression in cases of scleroderma (Ferri, *et al.*, 2007), and block primary microglial activation and proliferation during oxidative stress (Chong, *et al.*, 2003b, Chong, *et al.*, 2005d). Furthermore, EPO can block microglial cell activation and proliferation to prevent phagocytosis of injured cells through pathways that involve cellular membrane PS exposure, protein kinase B (Chong, *et al.*, 2004a), and the regulation of caspases (Chong, *et al.*, 2003a, Chong, *et al.*, 2003b, Wu, *et al.*, 2007a). EPO can directly inhibit several pro-inflammatory cytokines, such as IL-6, TNF-α, and monocyte chemoattractant protein 1 (Li, *et al.*, 2004a, Maiese, *et al.*, 2005c), as well as reduce leukocyte inflammation (Contaldo, *et al.*, 2007). In addition, EPO may foster the preservation of microglial cells for neuronal and vascular restructuring by preventing apoptotic injury in microglia (Li, *et al.*, 2006b, Vairano, *et al.*, 2002). In regards to the capacity of EPO to maintain microglial cellular integrity, EPO retains its capacity to prevent early apoptotic injury with membrane PS externalization as well as later stages of apoptotic injury involving

DNA fragmentation in microglia (Li, *et al.*, 2006b) similar to other cell systems of neurovascular origin (Chong, *et al.*, 2003b, Chong, *et al.*, 2002b, Chong, *et al.*, 2005d, Parsa, *et al.*, 2003, Sharples, *et al.*, 2004).

As a robust cytoprotectant, EPO can enhance the survival of cells during several types of injury models in the nervous system (Lykissas, *et al.*, 2007, Maiese, *et al.*, 2004, Maiese, *et al.*, 2005c). In cells that involve the brain or the retina, EPO can prevent injury from hypoxic ischemia (Chong, *et al.*, 2003b, Chong, *et al.*, 2002b, Liu, *et al.*, 2006, Meloni, *et al.*, 2006, Wei, *et al.*, 2006, Yu, *et al.*, 2005), excitotoxicity (Montero, *et al.*, 2007, Yamasaki, *et al.*, 2005), infection (Kaiser, *et al.*, 2006), free radical exposure (Chong, *et al.*, 2003a, Chong, *et al.*, 2003e, Yamasaki, *et al.*, 2005), staurosporine (Pregi, *et al.*, 2006), and dopaminergic cell injury (Demers, *et al.*, 2005, McLeod, *et al.*, 2006). In addition, administration of EPO also represents a viable option for the prevention of retinal cell injury during glaucoma (Tsai, *et al.*, 2007). Systemic application of EPO also can improve functional outcome and reduce cell loss during spinal cord injury (King, *et al.*, 2007, Okutan, *et al.*, 2007), traumatic cerebral edema (Verdonck, *et al.*, 2007), cortical trauma (Cherian, *et al.*, 2007), and epileptic activity (Mikati, *et al.*, 2007, Nadam, *et al.*, 2007).

EPO also may improve cognition, such as memory loss or psychiatric illness. In particular, Alzheimer's disease has become a prime consideration for the applications of EPO. Alzheimer's disease leads to a progressive deterioration of cognitive function with memory loss and injury to hippocampal neurons. The generation of extracellular plaques of amyloid-β (Aβ) peptide aggregates composed of a 39-42 amino acid peptide are considered to be one of the pathological mechanisms that may promote the development of Alzheimer's disease (Chong, *et al.*, 2005f). Accumulation of Aβ accumulation can lead to apoptotic injury with chromatin condensation, DNA fragmentation, and cellular membrane PS exposure (Chong, *et al.*, 2005c, Chong, *et al.*, 2005f). Aβ also can release reactive oxygen species and lead to toxicity in neurons. In addition, Aβ can not only precipitate a significant inflammatory response with microglial activation and the secretion of TNF-α (Bornemann, *et al.*, 2001), but also Aβ can elicit the neuronal expression of inducible nitric oxide synthase, peroxinitrite production, and neuronal apoptosis during an acute inflammatory response (Chong, *et al.*, 2005e, Combs, *et al.*, 2001). Furthermore, Aβ may lead to the induction of caspase mediated pathways (Nakagawa, *et al.*, 2000, Troy, *et al.*, 2001) that work in concert with oxidative stress (Tamagno, *et al.*, 2003). As a result, therapeutic strategies that address the toxicity of Aβ as a result of oxidative stress may foster novel developments for the treatment of Alzheimer's disease. EPO appears to be both necessary and sufficient to protect neurons from Aβ toxicity. Application of a blocking antibody of EPO, which can bind to EPO and block its biological activities in cells (Koshimura, *et al.*, 1999), can otherwise negate the protective effects of EPO to increase neuronal hippocampal cell survival and prevent apoptotic injury during Aβ exposure (Chong, *et al.*, 2005d).

Enhanced survival by EPO also extends to afford protection of the neurovascular unit during cerebral vascular disease (Demers, *et al.*, 2005, Dzietko, *et al.*, 2004, Maiese, *et al.*, 2004, Wei, *et al.*, 2006). In addition, EPO can protect sensitive hippocampal neurons from both focal and global ischemic brain injury (Keogh, *et al.*, 2007, Wei, *et al.*, 2006, Yu, *et al.*, 2005, Zhang, *et al.*, 2006). Systemic administration of EPO also represents a viable option for several other disorders. EPO administration for retinal cell injury can protect retinal ganglion cells from apoptosis (Grimm, *et al.*, 2002), EPO can improve functional outcome and reduce lipid peroxidation during spinal cord injury (Kaptanoglu, *et al.*, 2004), and EPO can maintain autoregulation of cerebral blood flow, reverse basilar artery vasoconstriction, and enhance neuronal survival and functional recovery following subarachnoid hemorrhage (Olsen, 2003).

However, it should be noted that the biological effects with EPO administration may not always be entirely beneficial during cytoprotective therapy. Irrespective of the problems associated with EPO abuse and gene doping (Baoutina, *et al.*, 2007, Diamanti-Kandarakis, *et al.*, 2005, Segura, *et al.*, 2007), EPO has been correlated with the alteration of red cell membrane properties leading to a cognitive decrement in rodent animal models (Li, *et al.*, 2004a, Maiese, *et al.*, 2004, Maiese, *et al.*, 2005c). In addition, development of potentially detrimental side-effects during EPO therapy, such as for cerebral ischemia with increased metabolic rate and blood viscosity (Frietsch, *et al.*, 2007), could severely limit or halt the use of EPO for neurovascular diseases. As a result, alternate strategies have been suggested. New investigations are studying the role of targeted bioavailability for EPO such as in bone marrow stromal cells genetically engineered to secrete EPO (Eliopoulos, *et al.*, 2006) and controlled release of EPO from encapsulated cells (Orive, *et al.*, 2005, Ponce, *et al.*, 2006). The passage of EPO entry into the central nervous system continues to attract significant interest (Doolittle, *et al.*, 2007) as well as does the use of novel intranasal routes for EPO administration (Yu, *et al.*, 2005). Other avenues of study are directed to the development of derivations of EPO to reduce erythropoietic activity and the potential associated vascular complications (Montero, *et al.*, 2007). Yet, these lines of investigation are not without limitations, since chemical derivatives of EPO can become absent of clinical efficacy (Maiese, *et al.*, 2004, Maiese, *et al.*, 2005c) as well as possibly loose the ability to promote sustainable cytoprotective effects, such as neurogenesis (Gonzalez, *et al.*, 2007) and angiogenesis (Li, *et al.*, 2007a, Reinders, *et al.*, 2006, Slevin, *et al.*, 2006, Zhang and Ma, 2007).

Metabolic Disease

Clinical work indicates that EPO has a significant role during diabetes mellitus (DM). DM is a significant health concern for both young and older populations (Maiese, *et al.*, 2007a, Maiese, *et al.*, 2007c). Approximately 16 million individuals in the United States and more than 165 million individuals worldwide suffer from DM. By the year 2030, it is predicted that more than 360 million individuals will be afflicted with DM and its debilitating conditions (Wild, *et al.*, 2004). Type 2 DM represents at least 80 percent of all diabetics and is dramatically increasing in incidence as a result of changes in human behavior and increased body mass index (Laakso, 2001). Type 1 insulin-dependent DM is present in 5–10 percent of all diabetics (Maiese, *et al.*, 2007c), but is increasing in adolescent minority groups (Dabelea, *et al.*, 2007). Furthermore, the incidence of undiagnosed diabetes, impaired glucose tolerance, and fluctuations in serum glucose in the young raises additional concerns (Jacobson, *et al.*, 2007). Both type 1 and type 2 DM represent important health concerns whether they begin early or later in life (Maiese, *et al.*, 2007a), since each can result in long-term complications throughout the body (Daneman, 2006). In regards to the vascular and nervous systems, patients with DM can develop severe neurological and vascular disease (Donahoe, *et al.*, 2007, Singh, *et al.*, 2008) that can lead to an increased risk for cognitive decline especially from vascular disease (Chong, *et al.*, 2005e, Li, *et al.*, 2006a Schnaider Beeri, *et al.*, 2004). Disease of the nervous system can become the most debilitating complications for DM and affect sensitive cognitive regions of the brain, such as the hippocampus that modulates memory function, resulting in significant functional impairment and dementia (Awad, *et al.*, 2004, Gerozissis, 2003). DM also has been found to increase the risk for vascular dementia in elderly subjects (Schnaider Beeri, *et al.*, 2004, Xu, *et al.*, 2004). DM also may affect the course of Alzheimer's disease. Although some studies have found that diabetic patients may have less neuritic plaques and neurofibrillary tangles than non-diabetic patients (Beeri, *et al.*, 2005), contrasting work suggests a modest adjusted relative risk of Alzheimer's disease in patients with diabetes as compared with those without diabetes to be 1.3 (Luchsinger, *et al.*, 2001).

Both insulin resistance and the complications of DM are closely linked to the occurrence of cellular oxidative stress (Maiese, 2008a, Maiese, *et al.*, 2007a, Maiese, *et al.*, 2007c). In patients

with DM, elevated levels of ceruloplasmin are suggestive of increased reactive oxygen species (Memisogullari and Bakan, 2004) and acute glucose fluctuations may promote oxidative stress (Monnier, *et al.*, 2006). Hyperglycemia can lead to increased production of reactive oxygen species in endothelial cells, liver and pancreatic β-cells (Ceriello, *et al.*, 1996, Ihara, *et al.*, 1999, Ling, *et al.*, 2003, Yano, *et al.*, 2004). Prolonged duration of hyperglycemia is not necessary to lead to oxidative stress injury, since even short periods of hyperglycemia, generate reactive oxygen species, such as in vascular cells (Yano, *et al.*, 2004). Clinical correlates support these experimental studies to show that acute glucose swings in addition to chronic hyperglycemia can trigger oxidative stress mechanisms during type 2 DM, illustrating the importance for therapeutic interventions during acute and sustained hyperglycemic episodes (Monnier, *et al.*, 2006).

Since administration of antioxidants during elevated glucose concentrations can block free radical production and prevent the production of advanced glycation endproducts known to produce reactive oxygen species during DM (Giardino, *et al.*, 1996), EPO may offer an attractive alternative therapy to maintain proper cellular metabolism and mitochondrial membrane potential during DM. In clinical studies with DM, plasma EPO is often low in diabetic patients with anemia (Mojiminiyi, *et al.*, 2006) or without anemia (Symeonidis, *et al.*, 2006). Furthermore, the failure of these individuals to produce EPO in response to a declining hemoglobin level suggests an impaired EPO response in diabetic patients (Thomas, *et al.*, 2005). Yet, increased EPO secretion during diabetic pregnancies may represent the body's attempt at endogenous protection against the complications of DM (Teramo, *et al.*, 2004). Similar to the potential protective role of insulin (Duarte, *et al.*, 2006), EPO administration has been shown both in diabetics as well as non-diabetics with severe, resistant congestive heart failure to decrease fatigue, increase left ventricular ejection fraction, and significantly decrease the number of hospitalization days (Silverberg, *et al.*, 2006).

Cell culture studies with vascular cells exposed to elevated glucose also have elucidated a strong cytoprotective effect of EPO (Maiese, *et al.*, 2004). Administration of EPO can significantly improve EC survival in a 1.0 ng/ml range (Chong, *et al.*, 2007c). EPO administration in patients also can significantly increase plasma levels of EPO well above this range of 1.0 ng/ml that has been associated with potential EPO cellular protection in patients with cardiac or renal disease (Mason-Garcia, *et al.*, 1990, Namiuchi, *et al.*, 2005), suggesting that the effects of EPO observed during *in vitro* studies may parallel the cellular processes altered by EPO in patients with DM (Bierer, *et al.*, 2006). Furthermore, EPO can block apoptotic DNA degradation in ECs during elevated glucose similar to other models of oxidative stress in cardiac and vascular cell models (Avasarala and Konduru, 2005, Chong, *et al.*, 2003a, Chong, *et al.*, 2002b, Chong and Maiese, 2007a, Moon, *et al.*, 2006). The preservation of cellular energy reserves is dependent upon the maintenance of mitochondrial integrity during DM (Newsholme, *et al.*, 2007). Cytoprotection by EPO also is closely related to the maintenance of mitochondrial membrane potential ($\Delta\Psi_m$). Loss of $\Delta\Psi_m$ through the opening of the mitochondrial permeability transition pore represents a significant determinant for cell injury and the subsequent induction of apoptosis (Leuner, *et al.*, 2007, Maiese and Chong, 2004). EPO has the capacity to prevent the depolarization of the mitochondrial membrane that also affects the release of cytochrome c (Chong, *et al.*, 2002b, Chong, *et al.*, 2003e, Miki, *et al.*, 2006).

Cancer

The possible induction or progression of cancer represents a significant concern with EPO administration (Kokhaei, *et al.*, 2007, Maiese, *et al.*, 2005b). Not only has both EPO and its receptor been demonstrated in tumor specimens, but under some conditions EPO expression has been suggested to block tumor cell apoptosis through Akt (Maiese, *et al.*, 2008a, Maiese,

et al., 2008c), enhance tumor progression, increase metastatic disease, (Lai and Grandis, 2006), and negate the effects of radiotherapy by assisting with tumor angiogenesis (Ceelen, *et al.*, 2007). In studies of patients with head and neck cancer, EPO decreased disease progression-free survival and overall survival (Henke, *et al.*, 2003). Similar results were reported in trials with metastatic breast cancer (Leyland-Jones, *et al.*, 2005) and the expression of the EPOR in tumors appeared to suggest a worse prognosis (Henke, *et al.*, 2006). Yet, the potential risk of EPO administration to either initiate tumor growth or lead to tumor progression is not entirely understood. In regards to the possible tumor promoting ability of EPO (Rades, *et al*., 2007), a number of competing factors must be considered that include the possible benefits of EPO administration in patients with cancer that involve the synergistic effects of EPO with chemotherapeutic modalities (Ning, *et al.*, 2005, Sigounas, *et al.*, 2004) and the treatment of cancer-related anemia. New large scale prospective trials are necessary that can more clearly examine the attributes and contraindications for EPO.

CONCLUSIONS

EPO is a unique agent in many ways offering potential clinical treatment for a diverse range of disorders that can range from anemia to the restoration of cardiovascular and cognitive function. Yet, treatment applications for EPO are not without controversy especially in regards to the potential of this growth factor to promote tumorigenesis. Future cell and animal investigations that parallel new clinical trials are surely warranted with a strong emphasis upon the elucidation of the signal transduction pathways controlled by EPO to direct both successful and safe clinical care.

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References

Anagnostou A, Lee ES, Kessimian N, Levinson R, Steiner M. Erythropoietin has a mitogenic and positive chemotactic effect on endothelial cells. Proc Natl Acad Sci USA 1990;87:5978–82. [PubMed: 2165612]

Anagnostou A, Liu Z, Steiner M, Chin K, Lee ES, Kessimian N, Noguchi CT. Erythropoietin receptor mRNA expression in human endothelial cells. Proc Natl Acad Sci USA 1994;91:3974–8. [PubMed: 8171022]

Anitha M, Gondha C, Sutliff R, Parsadanian A, Mwangi S, Sitaraman SV, Srinivasan S. GDNF rescues hyperglycemia-induced diabetic enteric neuropathy through activation of the PI3K/Akt pathway. J Clin Invest 2006;116:344–56. [PubMed: 16453021]

Anjaneyulu M, Berent-Spillson A, Russell JW. Metabotropic glutamate receptors (mGluRs) and diabetic neuropathy. Curr Drug Targets 2008;9:85–93. [PubMed: 18220716]

Arcasoy MO. The non-haematopoietic biological effects of erythropoietin. Br J Haematol 2008;141:14–31. [PubMed: 18324962]

Asaumi Y, Kagaya Y, Takeda M, Yamaguchi N, Tada H, Ito K, Ohta J, Shiroto T, Shirato K, Minegishi N, Shimokawa H. Protective role of endogenous erythropoietin system in nonhematopoietic cells against pressure overload-induced left ventricular dysfunction in mice. Circulation 2007;115:2022–32. [PubMed: 17404160]

Ashley RA, Dubuque SH, Dvorak B, Woodward SS, Williams SK, Kling PJ. Erythropoietin stimulates vasculogenesis in neonatal rat mesenteric microvascular endothelial cells. Pediatr Res 2002;51:472–8. [PubMed: 11919332]

Assaraf MI, Diaz Z, Liberman A, Miller WH Jr, Arvanitakis Z, Li Y, Bennett DA, Schipper HM. Brain erythropoietin receptor expression in Alzheimer disease and mild cognitive impairment. J Neuropathol Exp Neurol 2007;66:389–98. [PubMed: 17483696]

Avasarala JR, Konduru SS. Recombinant erythropoietin down-regulates IL-6 and CXCR4 genes in TNF-alpha-treated primary cultures of human microvascular endothelial cells: implications for multiple sclerosis. J Mol Neurosci 2005;25:183–9. [PubMed: 15784966]

Awad N, Gagnon M, Messier C. The relationship between impaired glucose tolerance, type 2 diabetes, and cognitive function. J Clin Exp Neuropsychol 2004;26:1044–80. [PubMed: 15590460]

Bahlmann FH, Song R, Boehm SM, Mengel M, von Wasielewski R, Lindschau C, Kirsch T, de Groot K, Laudeley R, Niemczyk E, Guler F, Menne J, Haller H, Fliser D. Low-dose therapy with the long-acting erythropoietin analogue darbepoetin alpha persistently activates endothelial Akt and attenuates progressive organ failure. Circulation 2004;110:1006–12. [PubMed: 15302785]

Balaraman Y, Limaye AR, Levey AI, Srinivasan S. Glycogen synthase kinase 3beta and Alzheimer's disease: pathophysiological and therapeutic significance. Cell Mol Life Sci 2006;63:1226–35. [PubMed: 16568235]

Baoutina A, Alexander IE, Rasko JE, Emslie KR. Potential use of gene transfer in athletic performance enhancement. Mol Ther 2007;15:1751–66. [PubMed: 17680029]

Bazan JF. Structural design and molecular evolution of a cytokine receptor superfamily. Proc Natl Acad Sci USA 1990;87:6934–8. [PubMed: 2169613]

Beeri MS, Silverman JM, Davis KL, Marin D, Grossman HZ, Schmeidler J, Purohit DP, Perl DP, Davidson M, Mohs RC, Haroutunian V. Type 2 diabetes is negatively associated with Alzheimer's disease neuropathology. J Gerontol A Biol Sci Med Sci 2005;60:471–5. [PubMed: 15933386]

Bierer R, Peceny MC, Hartenberger CH, Ohls RK. Erythropoietin concentrations and neurodevelopmental outcome in preterm infants. Pediatrics 2006;118:e635–40. [PubMed: 16908620]

Bittorf T, Buchse T, Sasse T, Jaster R, Brock J. Activation of the transcription factor NF-kappaB by the erythropoietin receptor: structural requirements and biological significance. Cell Signal 2001;13:673–81. [PubMed: 11495725]

Bittorf T, Sasse T, Wright M, Jaster R, Otte L, Schneider-Mergener J, Brock J. cDNA cloning and functional analysis of a truncated STAT5a protein from autonomously growing FDCP-1 cells. Cell Signal 2000;12:721–30. [PubMed: 11152957]

Bornemann KD, Wiederhold KH, Pauli C, Ermini F, Stalder M, Schnell L, Sommer B, Jucker M, Staufenbiel M. Abeta-induced inflammatory processes in microglia cells of APP23 transgenic mice. Am J Pathol 2001;158:63–73. [PubMed: 11141480]

Broudy VC, Lin N, Brice M, Nakamoto B, Papayannopoulou T. Erythropoietin receptor characteristics on primary human erythroid cells. Blood 1991;77:2583–90. [PubMed: 1646044]

Bullard AJ, Govewalla P, Yellon DM. Erythropoietin protects the myocardium against reperfusion injury *in vitro* and *in vivo*. Basic Res Cardiol 2005;100:397–403. [PubMed: 15944807]

Bunn HF, Gu J, Huang LE, Park JW, Zhu H. Erythropoietin: a model system for studying oxygen-dependent gene regulation. J Exp Biol 1998;201:1197–201. [PubMed: 9510530]

Burger D, Lei M, Geoghegan-Morphet N, Lu X, Xenocostas A, Feng Q. Erythropoietin protects cardiomyocytes from apoptosis *via* up-regulation of endothelial nitric oxide synthase. Cardiovasc Res 2006;72:51–9. [PubMed: 16904088]

Cardella F. Insulin therapy during diabetic ketoacidosis in children. Acta Biomed 2005;76(Suppl 3):49–54. [PubMed: 16915797]

Cariou A, Claessens YE, Pene F, Marx JS, Spaulding C, Hababou C, Casadevall N, Mira JP, Carli P, Hermine O. Early high-dose erythropoietin therapy and hypothermia after out-of-hospital cardiac arrest: a matched control study. Resuscitation 2008;76:397–404. [PubMed: 18037223]

Ceelen W, Boterberg T, Smeets P, Van Damme N, Demetter P, Zwaenepoel O, Cesteleyn L, Houtmeyers P, Peeters M, Pattyn P. Recombinant human erythropoietin alpha modulates the effects of radiotherapy on colorectal cancer microvessels. Br J Cancer 2007;96:692–700. [PubMed: 17299396]

Ceriello A, dello Russo P, Amstad P, Cerutti P. High glucose induces antioxidant enzymes in human endothelial cells in culture. Evidence linking hyperglycemia and oxidative stress. Diabetes 1996;45:471–7. [PubMed: 8603769]

Charvet C, Alberti I, Luciano F, Jacquel A, Bernard A, Auberger P, Deckert M. Proteolytic regulation of Forkhead transcription factor FOXO3a by caspase-3-like proteases. Oncogene 2003;22:4557–68. [PubMed: 12881712]

Chen C, Edelstein LC, Gelinas C. The Rel/NF-kappaB family directly activates expression of the apoptosis inhibitor Bcl-x(L). Mol Cell Biol 2000;20:2687–95. [PubMed: 10733571]

Chen S, Guttridge DC, You Z, Zhang Z, Fribley A, Mayo MW, Kitajewski J, Wang CY. Wnt-1 signaling inhibits apoptosis by activating beta-catenin/T cell factor-mediated transcription. J Cell Biol 2001;152:87–96. [PubMed: 11149923]

Cherian L, Goodman JC, Robertson C. Neuroprotection with erythropoietin administration following controlled cortical impact injury in rats. J Pharmacol Exp Ther 2007;322:789–94. [PubMed: 17470644]

Chikuma M, Masuda S, Kobayashi T, Nagao M, Sasaki R. Tissue-specific regulation of erythropoietin production in the murine kidney, brain, and uterus. Am J Physiol Endocrinol Metab 2000;279:E1242–8. [PubMed: 11093910]

Chlopicki S, Swies J, Mogielnicki A, Buczko W, Bartus M, Lomnicka M, Adamus J, Gebicki J. 1-Methylnicotinamide (MNA), a primary metabolite of nicotinamide, exerts anti-thrombotic activity mediated by a cyclooxygenase-2/prostacyclin pathway. Br J Pharmacol 2007;152:230–9. [PubMed: 17641676]

Chong ZZ, Kang J, Li F, Maiese K. mGluRI Targets Microglial Activation and Selectively Prevents Neuronal Cell Engulfment Through Akt and Caspase Dependent Pathways. Curr Neurovasc Res 2005a;2:197–211. [PubMed: 16181114]

Chong ZZ, Kang JQ, Maiese K. Akt1 drives endothelial cell membrane asymmetry and microglial activation through Bcl-x(L) and caspase 1, 3, and 9. Exp Cell Res 2004a;296:196–207. [PubMed: 15149850]

Chong ZZ, Kang JQ, Maiese K. Angiogenesis and plasticity: role of erythropoietin in vascular systems. J Hematother Stem Cell Res 2002a;11:863–71. [PubMed: 12590701]

Chong ZZ, Kang JQ, Maiese K. Apaf-1, Bcl-xL, Cytochrome c, and Caspase-9 Form the Critical Elements for Cerebral Vascular Protection by Erythropoietin. J Cereb Blood Flow Metab 2003a;23:320–30. [PubMed: 12621307]

Chong ZZ, Kang JQ, Maiese K. Erythropoietin fosters both intrinsic and extrinsic neuronal protection through modulation of microglia, Akt1, Bad, and caspase-mediated pathways. Br J Pharmacol 2003b; 138:1107–1118. [PubMed: 12684267]

Chong ZZ, Kang JQ, Maiese K. Erythropoietin is a novel vascular protectant through activation of Akt1 and mitochondrial modulation of cysteine proteases. Circulation 2002b;106:2973–9. [PubMed: 12460881]

Chong ZZ, Kang JQ, Maiese K. Erythropoietin: cytoprotection in vascular and neuronal cells. Curr Drug Targets Cardiovasc Haematol Disord 2003c;3:141–54. [PubMed: 12769640]

Chong ZZ, Kang JQ, Maiese K. Essential cellular regulatory elements of oxidative stress in early and late phases of apoptosis in the central nervous system. Antioxid Redox Signal 2004b;6:277–87. [PubMed: 15025929]

Chong ZZ, Kang JQ, Maiese K. Hematopoietic factor erythropoietin fosters neuroprotection through novel signal transduction cascades. J Cereb Blood Flow Metab 2002c;22:503–14. [PubMed: 11973422]

Chong ZZ, Kang JQ, Maiese K. Metabotropic glutamate receptors promote neuronal and vascular plasticity through novel intracellular pathways. Histol Histopathol 2003d;18:173–89. [PubMed: 12507297]

Chong ZZ, Li F, Maiese K. Activating Akt and the brain's resources to drive cellular survival and prevent inflammatory injury. Histol Histopathol 2005b;20:299–315. [PubMed: 15578447]

Chong ZZ, Li F, Maiese K. Attempted cell cycle induction in post-mitotic neurons occurs in early and late apoptotic programs through Rb, E2F1, and caspase 3. Curr Neurovasc Res 2006a;3:25–39. [PubMed: 16472123]

Chong ZZ, Li F, Maiese K. Cellular demise and inflammatory microglial activation during beta-amyloid toxicity are governed by Wnt1 and canonical signaling pathways. Cell Signal 2007a;19:1150–62. [PubMed: 17289346]

Chong ZZ, Li F, Maiese K. Employing new cellular therapeutic targets for Alzheimer's disease: a change for the better? Curr Neurovasc Res 2005c;2:55–72. [PubMed: 16181100]

Chong ZZ, Li F, Maiese K. Erythropoietin requires NF-kappaB and its nuclear translocation to prevent early and late apoptotic neuronal injury during beta-amyloid toxicity. Curr Neurovasc Res 2005d; 2:387–99. [PubMed: 16375720]

Chong ZZ, Li F, Maiese K. Group I metabotropic receptor neuro-protection requires Akt and its substrates that govern FOXO3a, bim, and beta-catenin during oxidative stress. Curr Neurovasc Res 2006b; 3:107–17. [PubMed: 16719794]

Chong ZZ, Li F, Maiese K. Oxidative stress in the brain: Novel cellular targets that govern survival during neurodegenerative disease. Prog Neurobiol 2005e;75:207–46. [PubMed: 15882775]

Chong ZZ, Li F, Maiese K. Stress in the brain: novel cellular mechanisms of injury linked to Alzheimer's disease. Brain Res Brain Res Rev 2005f;49:1–21. [PubMed: 15960984]

Chong ZZ, Li F, Maiese K. The pro-survival pathways of mTOR and protein kinase B target glycogen synthase kinase-3beta and nuclear factor-kappaB to foster endogenous microglial cell protection. Int J Mol Med 2007b;19:263–72. [PubMed: 17203200]

Chong ZZ, Lin SH, Kang JQ, Maiese K. Erythropoietin prevents early and late neuronal demise through modulation of Akt1 and induction of caspase 1, 3, and 8. J Neurosci Res 2003e;71:659–69. [PubMed: 12584724]

Chong ZZ, Lin SH, Kang JQ, Maiese K. The tyrosine phosphatase SHP2 modulates MAP kinase p38 and caspase 1 and 3 to foster neuronal survival. Cell Mol Neurobiol 2003f;23:561–78. [PubMed: 14514016]

Chong ZZ, Lin SH, Li F, Maiese K. The sirtuin inhibitor nicotinamide enhances neuronal cell survival during acute anoxic injury through Akt, Bad, PARP, and mitochondrial associated "anti-apoptotic" pathways. Curr Neurovasc Res 2005g;2:271–85. [PubMed: 16181120]

Chong ZZ, Lin SH, Maiese K. Nicotinamide modulates mitochondrial membrane potential and cysteine protease activity during cerebral vascular endothelial cell injury. J Vasc Res 2002d;39:131–47. [PubMed: 12011585]

Chong ZZ, Lin SH, Maiese K. The NAD+ precursor nicotinamide governs neuronal survival during oxidative stress through protein kinase B coupled to FOXO3a and mitochondrial membrane potential. J Cereb Blood Flow Metab 2004c;24:728–43. [PubMed: 15241181]

Chong ZZ, Maiese K. Erythropoietin involves the phosphatidylinositol 3-kinase pathway, 14-3-3 protein and FOXO3a nuclear trafficking to preserve endothelial cell integrity. Br J Pharmacol 2007a; 150:839–50. [PubMed: 17339844]

Chong ZZ, Maiese K. Targeting WNT, protein kinase B, and mitochondrial membrane integrity to foster cellular survival in the nervous system. Histol Histopathol 2004;19:495–504. [PubMed: 15024710]

Chong ZZ, Maiese K. The Src homology 2 domain tyrosine phosphatases SHP-1 and SHP-2: diversified control of cell growth, inflammation, and injury. Histol Histopathol 2007b;22:1251–67. [PubMed: 17647198]

Chong ZZ, Shang YC, Maiese K. Vascular injury during elevated glucose can be mitigated by erythropoietin and Wnt signaling. Curr Neurovasc Res 2007c;4:194–204. [PubMed: 17691973]

Clark KL, Halay ED, Lai E, Burley SK. Co-crystal structure of the HNF-3/fork head DNA-recognition motif resembles histone H5. Nature 1993;364:412–20. [PubMed: 8332212]

Cohen SM, Cordeiro-Stone M, Kaufman DG. Early replication and the apoptotic pathway. J Cell Physiol 2007;213:434–9. [PubMed: 17520690]

Combs CK, Karlo JC, Kao SC, Landreth GE. beta-Amyloid stimulation of microglia and monocytes results in TNFalpha-dependent expression of inducible nitric oxide synthase and neuronal apoptosis. J Neurosci 2001;21:1179–88. [PubMed: 11160388]

Contaldo C, Meier C, Elsherbiny A, Harder Y, Trentz O, Menger MD, Wanner GA. Human recombinant erythropoietin protects the striated muscle microcirculation of the dorsal skinfold from postischemic injury in mice. Am J Physiol Heart Circ Physiol 2007;293:H274–83. [PubMed: 17337594]

Corwin HL, Gettinger A, Fabian TC, May A, Pearl RG, Heard S, An R, Bowers PJ, Burton P, Klausner MA, Corwin MJ. Efficacy and safety of epoetin alfa in critically ill patients. N Engl J Med 2007;357:965–76. [PubMed: 17804841]

D'Andrea AD, Zon LI. Erythropoietin receptor. Subunit structure and activation. J Clin Invest 1990;86:681–7. [PubMed: 2168441]

Dabelea D, Bell RA, D'Agostino RB Jr, Imperatore G, Johansen JM, Linder B, Liu LL, Loots B, Marcovina S, Mayer-Davis EJ, Pettitt DJ, Waitzfelder B. Incidence of diabetes in youth in the United States. JAMA 2007;297:2716–24. [PubMed: 17595272]

Damen JE, Wakao H, Miyajima A, Krosl J, Humphries RK, Cutler RL, Krystal G. Tyrosine 343 in the erythropoietin receptor positively regulates erythropoietin-induced cell proliferation and Stat5 activation. EMBO J 1995;14:5557–68. [PubMed: 8521813]

Daneman D. Type 1 diabetes. Lancet 2006;367:847–58. [PubMed: 16530579]

Davis LE, Widness JA, Brace RA. Renal and placental secretion of erythropoietin during anemia or hypoxia in the ovine fetus. Am J Obstet Gynecol 2003;189:1764–70. [PubMed: 14710111]

De Felice FG, Velasco PT, Lambert MP, Viola K, Fernandez SJ, Ferreira ST, Klein WL. Abeta oligomers induce neuronal oxidative stress through an N-methyl-D-aspartate receptor-dependent mechanism that is blocked by the Alzheimer drug memantine. J Biol Chem 2007;282:11590–601. [PubMed: 17308309]

Demers EJ, McPherson RJ, Juul SE. Erythropoietin protects dopa-minergic neurons and improves neurobehavioral outcomes in juvenile rats after neonatal hypoxia-ischemia. Pediatr Res 2005;58:297–301. [PubMed: 16055937]

Di Lisa F, Menabo R, Canton M, Barile M, Bernardi P. Opening of the mitochondrial permeability transition pore causes depletion of mitochondrial and cytosolic NAD+ and is a causative event in the death of myocytes in postischemic reperfusion of the heart. J Biol Chem 2001;276:2571–5. [PubMed: 11073947]

Diamanti-Kandarakis E, Konstantinopoulos PA, Papailiou J, Kandarakis SA, Andreopoulos A, Sykiotis GP. Erythropoietin abuse and erythropoietin gene doping: detection strategies in the genomic era. Sports Med 2005;35:831–40. [PubMed: 16180943]

Digicaylioglu M, Garden G, Timberlake S, Fletcher L, Lipton SA. Acute neuroprotective synergy of erythropoietin and insulin-like growth factor I. Proc Natl Acad Sci USA 2004;101:9855–60. [PubMed: 15210945]

Dombroski D, Balasubramanian K, Schroit AJ. Phosphatidylserine expression on cell surfaces promotes antibody- dependent aggregation and thrombosis in beta2-glycoprotein I-immune mice. J Autoimmun 2000;14:221–9. [PubMed: 10756084]

Donahoe SM, Stewart GC, McCabe CH, Mohanavelu S, Murphy SA, Cannon CP, Antman EM. Diabetes and mortality following acute coronary syndromes. JAMA 2007;298:765–75. [PubMed: 17699010]

Doolittle ND, Peereboom DM, Christoforidis GA, Hall WA, Palmieri D, Brock PR, Campbell KC, Dickey DT, Muldoon LL, O'Neill BP, Peterson DR, Pollock B, Soussain C, Smith Q, Tyson RM, Neuwelt EA. Delivery of chemotherapy and antibodies across the blood-brain barrier and the role of chemoprotection, in primary and metastatic brain tumors: report of the Eleventh Annual Blood-Brain Barrier Consortium meeting. J Neurooncol 2007;81:81–91. [PubMed: 16858513]

Duarte AI, Proenca T, Oliveira CR, Santos MS, Rego AC. Insulin restores metabolic function in cultured cortical neurons subjected to oxidative stress. Diabetes 2006;55:2863–70. [PubMed: 17003354]

Dube S, Fisher JW, Powell JS. Glycosylation at specific sites of erythropoietin is essential for biosynthesis, secretion, and biological function. J Biol Chem 1988;263:17516–21. [PubMed: 3182860]

Dzietko M, Felderhoff-Mueser U, Sifringer M, Krutz B, Bittigau P, Thor F, Heumann R, Buhrer C, Ikonomidou C, Hansen HH. Erythropoietin protects the developing brain against N-methyl-D-aspartate receptor antagonist neurotoxicity. Neurobiol Dis 2004;15:177–87. [PubMed: 15006687]

Earnshaw WC, Martins LM, Kaufmann SH. Mammalian caspases: structure, activation, substrates, and functions during apoptosis. Annu Rev Biochem 1999;68:383–424. [PubMed: 10872455]

Eliopoulos N, Gagnon RF, Francois M, Galipeau J. Erythropoietin delivery by genetically engineered bone marrow stromal cells for correction of anemia in mice with chronic renal failure. J Am Soc Nephrol 2006;17:1576–84. [PubMed: 16672321]

Engels IH, Stepczynska A, Stroh C, Lauber K, Berg C, Schwenzer R, Wajant H, Janicke RU, Porter AG, Belka C, Gregor M, Schulze-Osthoff K, Wesselborg S. Caspase-8/FLICE functions as an executioner caspase in anticancer drug-induced apoptosis. Oncogene 2000;19:4563–73. [PubMed: 11030145]

Esposito G, De Filippis D, Maiuri MC, De Stefano D, Carnuccio R, Iuvone T. Cannabidiol inhibits inducible nitric oxide synthase protein expression and nitric oxide production in beta-amyloid stimulated PC12 neurons through p38 MAP kinase and NF-kappaB involvement. Neurosci Lett 2006;399:91–5. [PubMed: 16490313]

Eto N, Wada T, Inagi R, Takano H, Shimizu A, Kato H, Kurihara H, Kawachi H, Shankland SJ, Fujita T, Nangaku M. Podocyte protection by darbepoetin: preservation of the cytoskeleton and nephrin expression. Kidney Int 2007;72:455–63. [PubMed: 17457371]

Feng Y, Paul IA, LeBlanc MH. Nicotinamide reduces hypoxic ischemic brain injury in the newborn rat. Brain Res Bull 2006;69:117–22. [PubMed: 16533659]

Fenjves ES, Ochoa MS, Cabrera O, Mendez AJ, Kenyon NS, Inverardi L, Ricordi C. Human, nonhuman primate, and rat pancreatic islets express erythropoietin receptors. Transplantation 2003;75:1356–60. [PubMed: 12717230]

Ferrario M, Massa M, Rosti V, Campanelli R, Ferlini M, Marinoni B, De Ferrari GM, Meli V, De Amici M, Repetto A, Verri A, Bramucci E, Tavazzi L. Early haemoglobin-independent increase of plasma erythropoietin levels in patients with acute myocardial infarction. Eur Heart J 2007;28:1805–13. [PubMed: 17412728]

Ferri C, Giuggioli D, Sebastiani M, Colaci M. Treatment of severe scleroderma skin ulcers with recombinant human erythropoietin. Clin Exp Dermatol 2007;32:287–90. [PubMed: 17397351]

Fisher JW. Erythropoietin: physiology and pharmacology update. Exp Biol Med (Maywood) 2003;228:1–14. [PubMed: 12524467]

Fliser D, Haller H. Erythropoietin and treatment of non-anemic conditions--cardiovascular protection. Semin Hematol 2007;44:212–7. [PubMed: 17631185]

Foller M, Kasinathan RS, Koka S, Huber SM, Schuler B, Vogel J, Gassmann M, Lang F. Enhanced susceptibility to suicidal death of erythrocytes from transgenic mice overexpressing erythropoietin. Am J Physiol Regul Integr Comp Physiol 2007;293:R1127–34. [PubMed: 17567717]

Frietsch T, Maurer MH, Vogel J, Gassmann M, Kuschinsky W, Waschke KF. Reduced cerebral blood flow but elevated cerebral glucose metabolic rate in erythropoietin overexpressing transgenic mice with excessive erythrocytosis. J Cereb Blood Flow Metab 2007;27:469–76. [PubMed: 16804549]

Fu Q, Van Eyk JE. Proteomics and heart disease: identifying biomarkers of clinical utility. Expert Rev Proteomics 2006;3:237–49. [PubMed: 16608436]

Gao E, Boucher M, Chuprun JK, Zhou RH, Eckhart AD, Koch WJ. Darbepoetin alfa, a long-acting erythropoietin analog, offers novel and delayed cardioprotection for the ischemic heart. Am J Physiol Heart Circ Physiol 2007;293:H60–8. [PubMed: 17384131]

Genc S, Koroglu TF, Genc K. Erythropoietin as a novel neuroprotectant. Restor Neurol Neurosci 2004;22:105–19. [PubMed: 15272145]

Gerozissis K. Brain insulin: regulation, mechanisms of action and functions. Cell Mol Neurobiol 2003;23:1–25. [PubMed: 12701881]

Giardino I, Edelstein D, Brownlee M. BCL-2 expression or anti-oxidants prevent hyperglycemia-induced formation of intracellular advanced glycation endproducts in bovine endothelial cells. J Clin Invest 1996;97:1422–8. [PubMed: 8617874]

Gleissner CA, Klingenberg R, Staritz P, Koch A, Ehlermann P, Wiggen-hauser A, Dengler TJ. Role of erythropoietin in anemia after heart transplantation. Int J Cardiol 2006;112:341–7. [PubMed: 16309765]

Goldberg N, Lundin AP, Delano B, Friedman EA, Stein RA. Changes in left ventricular size, wall thickness, and function in anemic patients treated with recombinant human erythropoietin. Am Heart J 1992;124:424–7. [PubMed: 1386184]

Gonzalez FF, McQuillen P, Mu D, Chang Y, Wendland M, Vexler Z, Ferriero DM. Erythropoietin enhances long–term neuroprotec–tion and neurogenesis in neonatal stroke. Dev Neurosci 2007;29:321–30. [PubMed: 17762200]

Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, Helgason A, Stefansson H, Emilsson V, Helgadottir A, Styrkarsdottir U, Magnusson KP, Walters GB, Palsdottir E, Jonsdottir T, Gudmundsdottir T, Gylfason A, Saemundsdottir J, Wilensky RL, Reilly MP, Rader DJ, Bagger Y, Christiansen C, Gudnason V, Sigurdsson G, Thorsteinsdottir U, Gulcher JR, Kong

A, Stefansson K. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat Genet 2006;38:320–3. [PubMed: 16415884]

Grimm C, Wenzel A, Groszer M, Mayser H, Seeliger M, Samardzija M, Bauer C, Gassmann M, Reme CE. HIF-1-induced erythropoietin in the hypoxic retina protects against light-induced retinal degeneration. Nat Med 2002;8:718–24. [PubMed: 12068288]

Guo G, Bhat NR. Hypoxia/reoxygenation differentially modulates NF-kappaB activation and iNOS expression in astrocytes and microglia. Antioxid Redox Signal 2006;8:911–8. [PubMed: 16771681]

Guo YF, Xiong DH, Shen H, Zhao LJ, Xiao P, Guo Y, Wang W, Yang TL, Recker RR, Deng HW. Polymorphisms of the low-density lipoprotein receptor-related protein 5 (LRP5) gene are associated with obesity phenotypes in a large family-based association study. J Med Genet 2006;43:798–803. [PubMed: 16723389]

Hara N, Yamada K, Shibata T, Osago H, Hashimoto T, Tsuchiya M. Elevation of cellular NAD levels by nicotinic acid and involvement of nicotinic acid phosphoribosyltransferase in human cells. J Biol Chem 2007;282:24574–82. [PubMed: 17604275]

Harris SE, Fox H, Wright AF, Hayward C, Starr JM, Whalley LJ, Deary IJ. A genetic association analysis of cognitive ability and cognitive ageing using 325 markers for 109 genes associated with oxidative stress or cognition. BMC Genet 2007;8:43. [PubMed: 17601350]

Hassan K, Gross B, Simri W, Rubinchik I, Cohen H, Jacobi J, Shasha SM, Kristal B. The presence of erythropoietin receptors in the human peripheral nervous system. Clin Nephrol 2004;61:127–9. [PubMed: 14989632]

Hayden MS, Ghosh S. Signaling to NF-kappaB. Genes Dev 2004;18:2195–224. [PubMed: 15371334]

Heeschen C, Aicher A, Lehmann R, Fichtlscherer S, Vasa M, Urbich C, Mildner-Rihm C, Martin H, Zeiher AM, Dimmeler S. Erythropoietin is a potent physiologic stimulus for endothelial progenitor cell mobilization. Blood 2003;102:1340–6. [PubMed: 12702503]

Heidbreder M, Frohlich F, Johren O, Dendorfer A, Qadri F, Dominiak P. Hypoxia rapidly activates HIF-3alpha mRNA expression. FASEB J 2003;17:1541–3. [PubMed: 12824304]

Henke M, Laszig R, Rube C, Schafer U, Haase KD, Schilcher B, Mose S, Beer KT, Burger U, Dougherty C, Frommhold H. Erythropoietin to treat head and neck cancer patients with anaemia undergoing radiotherapy: randomised, double-blind, placebo-controlled trial. Lancet 2003;362:1255–60. [PubMed: 14575968]

Henke M, Mattern D, Pepe M, Bezay C, Weissenberger C, Werner M, Pajonk F. Do erythropoietin receptors on cancer cells explain unexpected clinical findings? J Clin Oncol 2006;24:4708–13. [PubMed: 17028293]

Henry DH, Bowers P, Romano MT, Provenzano R. Epoetin alfa. Clinical evolution of a pleiotropic cytokine. Arch Intern Med 2004;164:262–76. [PubMed: 14769622]

Henry MK, Lynch JT, Eapen AK, Quelle FW. DNA damage-induced cell-cycle arrest of hematopoietic cells is overridden by activation of the PI-3 kinase/Akt signaling pathway. Blood 2001;98:834–41. [PubMed: 11468186]

Hoffman EC, Reyes H, Chu FF, Sander F, Conley LH, Brooks BA, Hankinson O. Cloning of a factor required for activity of the Ah (dioxin) receptor. Science 1991;252:954–8. [PubMed: 1852076]

Hofmann K, Bucher P, Tschopp J. The CARD domain: a new apoptotic signalling motif. Trends Biochem Sci 1997;22:155–6. [PubMed: 9175472]

Howlett KF, Sakamoto K, Yu H, Goodyear LJ, Hargreaves M. Insulin-stimulated insulin receptor substrate-2-associated phosphatidy-linositol 3-kinase activity is enhanced in human skeletal muscle after exercise. Metabolism 2006;55:1046–52. [PubMed: 16839840]

Huang LE, Gu J, Schau M, Bunn HF. Regulation of hypoxia-inducible factor 1alpha is mediated by an O2- dependent degradation domain *via* the ubiquitin-proteasome pathway. Proc Natl Acad Sci USA 1998;95:7987–92. [PubMed: 9653127]

Ieraci A, Herrera DG. Nicotinamide protects against ethanol-induced apoptotic neurodegeneration in the developing mouse brain. PLoS Med 2006;3:e101. [PubMed: 16478293]

Ihara Y, Toyokuni S, Uchida K, Odaka H, Tanaka T, Ikeda H, Hiai H, Seino Y, Yamada Y. Hyperglycemia causes oxidative stress in pancreatic beta-cells of GK rats, a model of type 2 diabetes. Diabetes 1999;48:927–32. [PubMed: 10102716]

Ikeda E. Cellular response to tissue hypoxia and its involvement in disease progression. Pathol Int 2005;55:603–10. [PubMed: 16185289]

Imai N, Kawamura A, Higuchi M, Oheda M, Orita T, Kawaguchi T, Ochi N. Physicochemical and biological comparison of recombinant human erythropoietin with human urinary erythropoietin. J Biochem (Tokyo) 1990;107:352–9. [PubMed: 2341370]

Ito N, Bartunek J, Spitzer KW, Lorell BH. Effects of the nitric oxide donor sodium nitroprusside on intracellular pH and contraction in hypertrophied myocytes. Circulation 1997;95:2303–11. [PubMed: 9142009]

Jacobs K, Shoemaker C, Rudersdorf R, Neill SD, Kaufman RJ, Mufson A, Seehra J, Jones SS, Hewick R, Fritsch EF, Kawakita M, Shimizu T, Miyake T. Isolation and characterization of genomic and cDNA clones of human erythropoietin. Nature 1985;313:806–10. [PubMed: 3838366]

Jacobsen EA, Ananieva O, Brown ML, Chang Y. Growth, differentiation, and malignant transformation of pre-B cells mediated by inducible activation of v-Abl oncogene. J Immunol 2006;176:6831–8. [PubMed: 16709843]

Jacobson AM, Musen G, Ryan CM, Silvers N, Cleary P, Waberski B, Burwood A, Weinger K, Bayless M, Dahms W, Harth J. Long-term effect of diabetes and its treatment on cognitive function. N Engl J Med 2007;356:1842–52. [PubMed: 17476010]

Jaquet K, Krause K, Tawakol-Khodai M, Geidel S, Kuck KH. Erythropoietin and VEGF exhibit equal angiogenic potential. Microvasc Res 2002;64:326–33. [PubMed: 12204656]

Jessel R, Haertel S, Socaciu C, Tykhonova S, Diehl HA. Kinetics of apoptotic markers in exogeneously induced apoptosis of EL4 cells. J Cell Mol Med 2002;6:82–92. [PubMed: 12003671]

Jin C, Marsden I, Chen X, Liao X. Sequence specific collective motions in a winged helix DNA binding domain detected by 15N relaxation NMR. Biochemistry 1998;37:6179–87. [PubMed: 9558357]

Kaiser K, Texier A, Ferrandiz J, Buguet A, Meiller A, Latour C, Peyron F, Cespuglio R, Picot S. Recombinant human erythropoietin prevents the death of mice during cerebral malaria. J Infect Dis 2006;193:987–95. [PubMed: 16518761]

Kanbay M, Akcay A, Delibasi T, Uz B, Kaya A, Koca C, Turgut F, Bavbek N, Uz E, Duranay M, Yigitoglu R. Comparison of effects of darbepoetin alfa and epoetin alfa on serum endothelin level and blood pressure. Adv Ther 2007;24:346–52. [PubMed: 17565925]

Kang JQ, Chong ZZ, Maiese K. Akt1 protects against inflammatory microglial activation through maintenance of membrane asymmetry and modulation of cysteine protease activity. J Neurosci Res 2003a;74:37–51. [PubMed: 13130504]

Kang JQ, Chong ZZ, Maiese K. Critical role for Akt1 in the modulation of apoptotic phosphatidylserine exposure and microglial activation. Mol Pharmacol 2003b;64:557–69. [PubMed: 12920191]

Kaptanoglu E, Solaroglu I, Okutan O, Surucu HS, Akbiyik F, Beskonakli E. Erythropoietin exerts neuroprotection after acute spinal cord injury in rats: effect on lipid peroxidation and early ultrastructural findings. Neurosurg Rev 2004;27:113–20. [PubMed: 12920606]

Karunakaran S, Diwakar L, Saeed U, Agarwal V, Ramakrishnan S, Iyengar S, Ravindranath V. Activation of apoptosis signal regulating kinase 1 (ASK1) and translocation of death-associated protein, Daxx, in substantia nigra pars compacta in a mouse model of Parkinson's disease: protection by alphalipoic acid. FASEB J 2007;21:2226–36. [PubMed: 17369508]

Kawakami M, Sekiguchi M, Sato K, Kozaki S, Takahashi M. Erythropoietin receptor-mediated inhibition of exocytotic glutamate release confers neuroprotection during chemical ischemia. J Biol Chem 2001;276:39469–75. [PubMed: 11504731]

Keogh CL, Yu SP, Wei L. The effect of recombinant human erythropoietin on neurovasculature repair after focal ischemic stroke in neonatal rats. J Pharmacol Exp Ther 2007;322:521–8. [PubMed: 17494864]

Kim KH, Oudit GY, Backx PH. Erythropoietin protects against doxorubicin-induced cardiomyopathy *via* a phosphatidylinositol 3-kinase-dependent pathway. J Pharmacol Exp Ther 2008;324:160–9. [PubMed: 17928571]

King VR, Averill SA, Hewazy D, Priestley JV, Torup L, Michael-Titus AT. Erythropoietin and carbamylated erythropoietin are neuro-protective following spinal cord hemisection in the rat. Eur J Neurosci 2007;26:90–100. [PubMed: 17614942]

Kokhaei P, Abdalla AO, Hansson L, Mikaelsson E, Kubbies M, Haselbeck A, Jernberg-Wiklund H, Mellstedt H, Osterborg A. Expression of erythropoietin receptor and *in vitro* functional effects of epoetins in B-cell malignancies. Clin Cancer Res 2007;13:3536–44. [PubMed: 17575216]

Koshimura K, Murakami Y, Sohmiya M, Tanaka J, Kato Y. Effects of erythropoietin on neuronal activity. J Neurochem 1999;72:2565–72. [PubMed: 10349868]

Krantz SB. Erythropoietin. Blood 1991;77:419–34. [PubMed: 1991159]

Kratzsch J, Knerr I, Galler A, Kapellen T, Raile K, Korner A, Thiery J, Dotsch J, Kiess W. Metabolic decompensation in children with type 1 diabetes mellitus associated with increased serum levels of the soluble leptin receptor. Eur J Endocrinol 2006;155:609–14. [PubMed: 16990661]

Laakso M. Cardiovascular disease in type 2 diabetes: challenge for treatment and prevention. J Intern Med 2001;249:225–35. [PubMed: 11285042]

Lacombe C, Da Silva JL, Bruneval P, Casadevall N, Camilleri JP, Bariety J, Tambourin P, Varet B. Erythropoietin: sites of synthesis and regulation of secretion. Am J Kidney Dis 1991;18:14–9. [PubMed: 1928074]

Lai SY, Grandis JR. Understanding the presence and function of erythropoietin receptors on cancer cells. J Clin Oncol 2006;24:4675–6. [PubMed: 17028292]

Larson ET, Eilers B, Menon S, Reiter D, Ortmann A, Young MJ, Lawrence CM. A winged-helix protein from Sulfolobus turreted icosahedral virus points toward stabilizing disulfide bonds in the intracellular proteins of a hyperthermophilic virus. Virology 2007;368:249–61. [PubMed: 17669459]

Lehman DM, Hunt KJ, Leach RJ, Hamlington J, Arya R, Abboud HE, Duggirala R, Blangero J, Goring HH, Stern MP. Haplotypes of Transcription Factor 7-Like 2 (TCF7L2) gene and its upstream region are associated with type 2 diabetes and age of onset in Mexican Americans. Diabetes 2007;56:389–93. [PubMed: 17259383]

Lehtinen MK, Yuan Z, Boag PR, Yang Y, Villen J, Becker EB, Di-Bacco S, de la Iglesia N, Gygi S, Blackwell TK, Bonni A. A conserved MST-FOXO signaling pathway mediates oxidative-stress responses and extends life span. Cell 2006;125:987–1001. [PubMed: 16751106]

Leuner K, Hauptmann S, Abdel-Kader R, Scherping I, Keil U, Strosznajder JB, Eckert A, Muller WE. Mitochondrial dysfunction: the first domino in brain aging and Alzheimer's disease? Antioxid Redox Signal 2007;9:1659–75. [PubMed: 17867931]

Leyland-Jones B, Semiglazov V, Pawlicki M, Pienkowski T, Tjulandin S, Manikhas G, Makhson A, Roth A, Dodwell D, Baselga J, Biakhov M, Valuckas K, Voznyi E, Liu X, Vercammen E. Maintaining normal hemoglobin levels with epoetin alfa in mainly nonanemic patients with metastatic breast cancer receiving first-line chemotherapy: a survival study. J Clin Oncol 2005;23:5960–72. [PubMed: 16087945]

Leytin V, Allen DJ, Mykhaylov S, Lyubimov E, Freedman J. Thrombin-triggered platelet apoptosis. J Thromb Haemost 2006;4:2656–63. [PubMed: 16961585]

Li F, Chong ZZ, Maiese K. Cell life versus cell longevity: the mysteries surrounding the nad(+) precursor nicotinamide. Curr Med Chem 2006a;13:883–95. [PubMed: 16611073]

Li F, Chong ZZ, Maiese K. Erythropoietin on a tightrope: balancing neuronal and vascular protection between intrinsic and extrinsic pathways. Neurosignals 2004a;13:265–89. [PubMed: 15627815]

Li F, Chong ZZ, Maiese K. Microglial integrity is maintained by erythropoietin through integration of Akt and its substrates of glycogen synthase kinase-3beta, beta-catenin, and nuclear factor-kappaB. Curr Neurovasc Res 2006b;3:187–201. [PubMed: 16918383]

Li F, Chong ZZ, Maiese K. Navigating novel mechanisms of cellular plasticity with the NAD+ precursor and nutrient nicotinamide. Front Biosci 2004b;9:2500–2520. [PubMed: 15353303]

Li F, Chong ZZ, Maiese K. Vital elements of the wnt-frizzled signaling pathway in the nervous system. Curr Neurovasc Res 2005;2:331–40. [PubMed: 16181124]

Li F, Chong ZZ, Maiese K. Winding through the WNT pathway during cellular development and demise. Histol Histopathol 2006c;21:103–24. [PubMed: 16267791]

Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 1997;91:479–89. [PubMed: 9390557]

Li Y, Lu Z, Keogh CL, Yu SP, Wei L. Erythropoietin-induced neurovascular protection, angiogenesis, and cerebral blood flow restoration after focal ischemia in mice. J Cereb Blood Flow Metab 2007a; 27:1043–54. [PubMed: 17077815]

Li Y, Wang Z, Kong D, Murthy S, Dou QP, Sheng S, Reddy GP, Sarkar FH. Regulation of FOXO3a/beta-catenin/GSK-3beta signaling by 3,3′-diindolylmethane contributes to inhibition of cell proliferation and induction of apoptosis in prostate cancer cells. J Biol Chem 2007b;282:21542–50. [PubMed: 17522055]

Lin CL, Wang JY, Huang YT, Kuo YH, Surendran K, Wang FS. Wnt/beta-catenin signaling modulates survival of high glucose-stressed mesangial cells. J Am Soc Nephrol 2006;17:2812–20. [PubMed: 16943306]

Lin FK, Suggs S, Lin CH, Browne JK, Smalling R, Egrie JC, Chen KK, Fox GM, Martin F, Stabinsky Z, Badrawi SM, Lai P-H, Goldwasser E. Cloning and expression of the human erythropoietin gene. Proc Natl Acad Sci USA 1985;82:7580–4. [PubMed: 3865178]

Lin SH, Maiese K. The metabotropic glutamate receptor system protects against ischemic free radical programmed cell death in rat brain endothelial cells. J Cereb Blood Flow Metab 2001;21:262–75. [PubMed: 11295881]

Lin SH, Vincent A, Shaw T, Maynard KI, Maiese K. Prevention of nitric oxide-induced neuronal injury through the modulation of independent pathways of programmed cell death. J Cereb Blood Flow Metab 2000;20:1380–91. [PubMed: 10994860]

Ling PR, Mueller C, Smith RJ, Bistrian BR. Hyperglycemia induced by glucose infusion causes hepatic oxidative stress and systemic inflammation, but not STAT3 or MAP kinase activation in liver in rats. Metabolism 2003;52:868–74. [PubMed: 12870163]

Lipton SA. Pathologically activated therapeutics for neuroprotection. Nat Rev Neurosci 2007;8:803–8. [PubMed: 17882256]

Liu C, Shen K, Liu Z, Noguchi CT. Regulated human erythropoietin receptor expression in mouse brain. J Biol Chem 1997;272:32395–400. [PubMed: 9405448]

Liu R, Suzuki A, Guo Z, Mizuno Y, Urabe T. Intrinsic and extrinsic erythropoietin enhances neuroprotection against ischemia and reper-fusion injury *in vitro*. J Neurochem 2006;96:1101–10. [PubMed: 16417583]

Liu ZY, Chin K, Noguchi CT. Tissue specific expression of human erythropoietin receptor in transgenic mice. Dev Biol 1994;166:159–69. [PubMed: 7958443]

Luchsinger JA, Tang MX, Stern Y, Shea S, Mayeux R. Diabetes mellitus and risk of Alzheimer's disease and dementia with stroke in a multiethnic cohort. Am J Epidemiol 2001;154:635–41. [PubMed: 11581097]

Lykissas MG, Korompilias AV, Vekris MD, Mitsionis GI, Sakellariou E, Beris AE. The role of erythropoietin in central and peripheral nerve injury. Clin Neurol Neurosurg 2007;109:639–44. [PubMed: 17624659]

Maiese K. Diabetic stress: new triumphs and challenges to maintain vascular longevity. Expert Rev Cardiovasc Ther 2008a;6:281–4. [PubMed: 18327989]

Maiese K. The dynamics of cellular injury: transformation into neuronal and vascular protection. Histol Histopathol 2001;16:633–44. [PubMed: 11332719]

Maiese K. Triple play: Promoting neurovascular longevity with nicotinamide, Wnt, and erythropoietin in diabetes mellitus. Biomed Pharmacother. 2008b10.1016/j.biopha.2008.01.009

Maiese K, Ahmad I, TenBroeke M, Gallant J. Metabotropic glutamate receptor subtypes independently modulate neuronal intracellular calcium. J Neurosci Res 1999;55:472–485. [PubMed: 10723057]

Maiese K, Boccone L. Neuroprotection by peptide growth factors against anoxia and nitric oxide toxicity requires modulation of protein kinase C. J Cereb Blood Flow Metab 1995;15:440–9. [PubMed: 7714002]

Maiese K, Chong ZZ. Insights into oxidative stress and potential novel therapeutic targets for Alzheimer disease. Restor Neurol Neurosci 2004;22:87–104. [PubMed: 15272144]

Maiese K, Chong ZZ. Nicotinamide: necessary nutrient emerges as a novel cytoprotectant for the brain. Trends Pharmacol Sci 2003;24:228–32. [PubMed: 12767721]

Maiese, K.; Chong, ZZ.; Kang, J. Transformation into treatment: Novel therapeutics that begin within the cell. In: Maiese, K., editor. Neuronal and Vascular Plasticity: Elucidating Basic Cellular

Mechanisms for Future Therapeutic Discovery. Kluwer Academic Publishers; Norwell, MA: 2003. p. 1-26.

Maiese K, Chong ZZ, Li F. Driving cellular plasticity and survival through the signal transduction pathways of metabotropic glutamate receptors. Curr Neurovasc Res 2005a;2:425–46. [PubMed: 16375723]

Maiese K, Chong ZZ, Li F, Shang YC. Erythropoietin: Elucidating new cellular targets that broaden therapeutic strategies. Prog Neurobiol. 2008a10.1016/j.pneurobio.2008.02.002

Maiese K, Chong ZZ, Shang YC. Mechanisitic insights into diabetes mellitus and oxidative stress. Curr Med Chem 2007a;14:1689–1699.

Maiese K, Chong ZZ, Shang YC. OutFOXOing disease and disability: the therapeutic potential of targeting FoxO proteins. Trends Mol Med. 2008b10.1016/j.molmed.2008.03.002

Maiese K, Chong ZZ, Shang YC. Raves and risks for erythropoietin. Cytokine Growth Factor Rev 2008c; 19:145–155. [PubMed: 18299246]

Maiese K, Chong ZZ, Shang YC. Sly as a FOXO": New paths with Forkhead signaling in the brain. Curr Neurovasc Res 2007b;4:295–302. [PubMed: 18045156]

Maiese K, Li F, Chong ZZ. Erythropoietin and cancer. JAMA 2005b;293:1858–1859.

Maiese K, Li F, Chong ZZ. Erythropoietin in the brain: can the promise to protect be fulfilled? Trends Pharmacol Sci 2004;25:577–583. [PubMed: 15491780]

Maiese K, Li F, Chong ZZ. New avenues of exploration for erythropoietin. JAMA 2005c;293:90–5. [PubMed: 15632341]

Maiese K, Li F, Chong ZZ, Shang Y. The Wnt signaling pathway: Aging gracefully as a protectionist? Pharmacol Ther. 2008d10.1016/j.pharmthera.2008.01.004

Maiese K, Morhan SD, Chong ZZ. Oxidative stress biology and cell injury during type 1 and type 2 diabetes mellitus. Curr Neurovasc Res 2007c;4:63–71. [PubMed: 17311546]

Maiese K, TenBroeke M, Kue I. Neuroprotection of lubeluzole is mediated through the signal transduction pathways of nitric oxide. J Neurochem 1997;68:710–4. [PubMed: 9003060]

Maiese K, Vincent A, Lin SH, Shaw T. Group I and Group III metabotropic glutamate receptor subtypes provide enhanced neuroprotection. J Neurosci Res 2000;62:257–272. [PubMed: 11020218]

Maiese K, Vincent AM. Critical temporal modulation of neuronal programmed cell injury. Cell Mol Neurobiol 2000a;20:383–400. [PubMed: 10789835]

Maiese K, Vincent AM. Membrane asymmetry and DNA degradation: functionally distinct determinants of neuronal programmed cell death. J Neurosci Res 2000b;59:568–80. [PubMed: 10679797]

Maiorana A, O'Driscoll G, Goodman C, Taylor R, Green D. Combined aerobic and resistance exercise improves glycemic control and fitness in type 2 diabetes. Diabetes Res Clin Pract 2002;56:115–23. [PubMed: 11891019]

Mallat M, Marin-Teva JL, Cheret C. Phagocytosis in the developing CNS: more than clearing the corpses. Curr Opin Neurobiol 2005;15:101–7. [PubMed: 15721751]

Mancini DM, Katz SD, Lang CC, LaManca J, Hudaihed A, Androne AS. Effect of erythropoietin on exercise capacity in patients with moderate to severe chronic heart failure. Circulation 2003;107:294–9. [PubMed: 12538431]

Mari C, Karabiyikoglu M, Goris ML, Tait JF, Yenari MA, Blankenberg FG. Detection of focal hypoxic-ischemic injury and neuronal stress in a rodent model of unilateral MCA occlusion/reperfusion using radiolabeled annexin V. Eur J Nucl Med Mol Imaging 2004;31:733–9. [PubMed: 14985868]

Marti HH, Wenger RH, Rivas LA, Straumann U, Digicaylioglu M, Henn V, Yonekawa Y, Bauer C, Gassmann M. Erythropoietin gene expression in human, monkey and murine brain. Eur J Neurosci 1996;8:666–76. [PubMed: 9081618]

Martin D, Salinas M, Lopez-Valdaliso R, Serrano E, Recuero M, Cuadrado A. Effect of the Alzheimer amyloid fragment Abeta(25–35) on Akt/PKB kinase and survival of PC12 cells. J Neurochem 2001;78:1000–8. [PubMed: 11553674]

Martinez-Estrada OM, Rodriguez-Millan E, Gonzalez-De Vicente E, Reina M, Vilaro S, Fabre M. Erythropoietin protects the *in vitro* blood-brain barrier against VEGF-induced permeability. Eur J Neurosci 2003;18:2538–44. [PubMed: 14622154]

Mason-Garcia M, Beckman BS, Brookins JW, Powell JS, Lanham W, Blaisdell S, Keay L, Li SC, Fisher JW. Development of a new radioimmunoassay for erythropoietin using recombinant erythropoietin. Kidney Int 1990;38:969–75. [PubMed: 2266682]

Masuda S, Kobayashi T, Chikuma M, Nagao M, Sasaki R. The oviduct produces erythropoietin in an estrogen- and oxygen- dependent manner. Am J Physiol Endocrinol Metab 2000;278:E1038–44. [PubMed: 10827006]

Matsuzaki H, Tamatani M, Mitsuda N, Namikawa K, Kiyama H, Miyake S, Tohyama M. Activation of Akt kinase inhibits apoptosis and changes in Bcl-2 and Bax expression induced by nitric oxide in primary hippocampal neurons. J Neurochem 1999;73:2037–46. [PubMed: 10537063]

Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wykoff CC, Pugh CW, Maher ER, Ratcliffe PJ. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 1999;399:271–5. [PubMed: 10353251]

McLeod M, Hong M, Mukhida K, Sadi D, Ulalia R, Mendez I. Erythropoietin and GDNF enhance ventral mesencephalic fiber outgrowth and capillary proliferation following neural transplantation in a rodent model of Parkinson's disease. Eur J Neurosci 2006;24:361–70. [PubMed: 16903847]

Meloni BP, Tilbrook PA, Boulos S, Arthur PG, Knuckey NW. Erythropoietin preconditioning in neuronal cultures: signaling, protection from *in vitro* ischemia, and proteomic analysis. J Neurosci Res 2006;83:584–93. [PubMed: 16435392]

Memisogullari R, Bakan E. Levels of ceruloplasmin, transferrin, and lipid peroxidation in the serum of patients with Type 2 diabetes mellitus. J Diabetes Complications 2004;18:193–7. [PubMed: 15207835]

Menon MP, Fang J, Wojchowski DM. Core erythropoietin receptor signals for late erythroblast development. Blood 2006a;107:2662–72. [PubMed: 16332976]

Menon MP, Karur V, Bogacheva O, Bogachev O, Cuetara B, Wojchowski DM. Signals for stress erythropoiesis are integrated *via* an erythropoietin receptor-phosphotyrosine-343-Stat5 axis. J Clin Invest 2006b;116:683–94. [PubMed: 16511603]

Mikati MA, Hokayem JA, Sabban ME. Effects of a single dose of erythropoietin on subsequent seizure susceptibility in rats exposed to acute hypoxia at p10. Epilepsia 2007;48:175–81. [PubMed: 17241225]

Miki T, Miura T, Yano T, Takahashi A, Sakamoto J, Tanno M, Kobayashi H, Ikeda Y, Nishihara M, Naitoh K, Ohori K, Shimamoto K. Alteration in erythropoietin-induced cardioprotective signaling by postinfarct ventricular remodeling. J Pharmacol Exp Ther 2006;317:68–75. [PubMed: 16377761]

Mocini D, Leone T, Tubaro M, Santini M, Penco M. Structure, production and function of erythropoietin: implications for therapeutical use in cardiovascular disease. Curr Med Chem 2007;14:2278–87. [PubMed: 17896976]

Mojiminiyi OA, Abdella NA, Zaki MY, El Gebely SA, Mohamedi HM, Aldhahi WA. Prevalence and associations of low plasma erythropoietin in patients with Type 2 diabetes mellitus. Diabet Med 2006;23:839–44. [PubMed: 16911620]

Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, Colette C. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. JAMA 2006;295:1681–7. [PubMed: 16609090]

Montero M, Poulsen FR, Noraberg J, Kirkeby A, van Beek J, Leist M, Zimmer J. Comparison of neuroprotective effects of erythropoietin (EPO) and carbamylerythropoietin (CEPO) against ischemia-like oxygen-glucose deprivation (OGD) and NMDA excitotoxicity in mouse hippocampal slice cultures. Exp Neurol 2007;204:106–17. [PubMed: 17157835]

Moon C, Krawczyk M, Ahn D, Ahmet I, Paik D, Lakatta EG, Talan MI. Erythropoietin reduces myocardial infarction and left ventricular functional decline after coronary artery ligation in rats. Proc Natl Acad Sci USA 2003;100:11612–7. [PubMed: 14500913]

Moon C, Krawczyk M, Paik D, Coleman T, Brines M, Juhaszova M, Sollott SJ, Lakatta EG, Talan MI. Erythropoietin, modified to not stimulate red blood cell production, retains its cardioprotective properties. J Pharmacol Exp Ther 2006;316:999–1005. [PubMed: 16306273]

Mujais SK, Beru N, Pullman TN, Goldwasser E. Erythropoietin is produced by tubular cells of the rat kidney. Cell Biochem Biophys 1999;30:153–66. [PubMed: 10099826]

Mulcahy L. The erythropoietin receptor. Semin Oncol 2001;28:19–23. [PubMed: 11395848]

Muller FL, Lustgarten MS, Jang Y, Richardson A, Van Remmen H. Trends in oxidative aging theories. Free Radic Biol Med 2007;43:477–503. [PubMed: 17640558]

Mussmann R, Geese M, Harder F, Kegel S, Andag U, Lomow A, Burk U, Onichtchouk D, Dohrmann C, Austen M. Inhibition of GSK3 promotes replication and survival of pancreatic beta cells. J Biol Chem 2007;282:12030–7. [PubMed: 17242403]

Nadam J, Navarro F, Sanchez P, Moulin C, Georges B, Laglaine A, Pequignot JM, Morales A, Ryvlin P, Bezin L. Neuroprotective effects of erythropoietin in the rat hippocampus after pilocarpine-induced status epilepticus. Neurobiol Dis 2007;25:412–26. [PubMed: 17166730]

Nagai A, Nakagawa E, Choi HB, Hatori K, Kobayashi S, Kim SU. Erythropoietin and erythropoietin receptors in human CNS neurons, astrocytes, microglia, and oligodendrocytes grown in culture. J Neuropathol Exp Neurol 2001;60:386–92. [PubMed: 11305874]

Nagata Y, Takahashi N, Davis RJ, Todokoro K. Activation of p38 MAP kinase and JNK but not ERK is required for erythropoietin-induced erythroid differentiation. Blood 1998;92:1859–69. [PubMed: 9731042]

Nakagawa T, Zhu H, Morishima N, Li E, Xu J, Yankner BA, Yuan J. Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta. Nature 2000;403:98–103. [PubMed: 10638761]

Nakata S, Matsumura I, Tanaka H, Ezoe S, Satoh Y, Ishikawa J, Era T, Kanakura Y. NF-kappaB family proteins participate in multiple steps of hematopoiesis through elimination of reactive oxygen species. J Biol Chem 2004;279:55578–86. [PubMed: 15485843]

Namikawa K, Honma M, Abe K, Takeda M, Mansur K, Obata T, Miwa A, Okado H, Kiyama H. Akt/protein kinase B prevents injury-induced motoneuron death and accelerates axonal regeneration. J Neurosci 2000;20:2875–86. [PubMed: 10751440]

Namiuchi S, Kagaya Y, Ohta J, Shiba N, Sugi M, Oikawa M, Kunii H, Yamao H, Komatsu N, Yui M, Tada H, Sakuma M, Watanabe J, Ichihara T, Shirato K. High serum erythropoietin level is associated with smaller infarct size in patients with acute myocardial infarction who undergo successful primary percutaneous coronary intervention. J Am Coll Cardiol 2005;45:1406–12. [PubMed: 15862410]

Newsholme P, Haber EP, Hirabara SM, Rebelato EL, Procopio J, Morgan D, Oliveira-Emilio HC, Carpinelli AR, Curi R. Diabetes associated cell stress and dysfunction: role of mitochondrial and non-mitochondrial ROS production and activity. J Physiol 2007;583:9–24. [PubMed: 17584843]

Ning S, Hartley C, Molineux G, Knox SJ. Darbepoietin alfa potentiates the efficacy of radiation therapy in mice with corrected or uncorrected anemia. Cancer Res 2005;65:284–90. [PubMed: 15665305]

Nurmi A, Goldsteins G, Narvainen J, Pihlaja R, Ahtoniemi T, Grohn O, Koistinaho J. Antioxidant pyrrolidine dithiocarbamate activates Akt-GSK signaling and is neuroprotective in neonatal hypoxia-ischemia. Free Radic Biol Med 2006;40:1776–84. [PubMed: 16678015]

Obara N, Imagawa S, Nakano Y, Suzuki N, Yamamoto M, Nagasawa T. Suppression of erythropoietin gene expression by cadmium depends on inhibition of HIF-1, not stimulation of GATA-2. Arch Toxicol 2003;77:267–73. [PubMed: 12734640]

Ogilvie M, Yu X, Nicolas-Metral V, Pulido SM, Liu C, Ruegg UT, Noguchi CT. Erythropoietin stimulates proliferation and interferes with differentiation of myoblasts. J Biol Chem 2000;275:39754–61. [PubMed: 10995753]

Okouchi M, Ekshyyan O, Maracine M, Aw TY. Neuronal apoptosis in neurodegeneration. Antioxid Redox Signal 2007;9:1059–96. [PubMed: 17571960]

Okutan O, Solaroglu I, Beskonakli E, Taskin Y. Recombinant human erythropoietin decreases myeloperoxidase and caspase-3 activity and improves early functional results after spinal cord injury in rats. J Clin Neurosci 2007;14:364–8. [PubMed: 17236773]

Olea FD, Vera Janavel G, De Lorenzi A, Cuniberti L, Yannarelli G, Cabeza Meckert P, Cearras M, Laguens R, Crottogini A. High-dose erythropoietin has no long-term protective effects in sheep with reperfused myocardial infarction. J Cardiovasc Pharmacol 2006;47:736–41. [PubMed: 16810073]

Olsen NV. Central nervous system frontiers for the use of erythropoietin. Clin Infect Dis 2003;37(Suppl 4):S323–30. [PubMed: 14582001]

Orive G, De Castro M, Ponce S, Hernandez RM, Gascon AR, Bosch M, Alberch J, Pedraz JL. Long-term expression of erythropoietin from myoblasts immobilized in biocompatible and neovascularized microcapsules. Mol Ther 2005;12:283–9. [PubMed: 15935736]

Palazzuoli A, Silverberg D, Iovine F, Capobianco S, Giannotti G, Calabro A, Campagna SM, Nuti R. Erythropoietin improves anemia exercise tolerance and renal function and reduces B-type natriuretic peptide and hospitalization in patients with heart failure and anemia. Am Heart J 2006;152:1096, e9–15. [PubMed: 17161060]

Parsa CJ, Kim J, Riel RU, Pascal LS, Thompson RB, Petrofski JA, Matsumoto A, Stamler JS, Koch WJ. Cardioprotective effects of erythropoietin in the reperfused ischemic heart: a potential role for cardiac fibroblasts. J Biol Chem 2004;279:20655–62. [PubMed: 15020586]

Parsa CJ, Matsumoto A, Kim J, Riel RU, Pascal LS, Walton GB, Thompson RB, Petrofski JA, Annex BH, Stamler JS, Koch WJ. A novel protective effect of erythropoietin in the infarcted heart. J Clin Invest 2003;112:999–1007. [PubMed: 14523037]

Pearl, R. The rate of living. University of London Press; London: 1928.

Ponce S, Orive G, Hernandez RM, Gascon AR, Canals JM, Munoz MT, Pedraz JL. *In vivo* evaluation of EPO-secreting cells immobilized in different alginate-PLL microcapsules. J Control Release 2006;116:28–34. [PubMed: 17081643]

Pregi N, Vittori D, Perez G, Leiros CP, Nesse A. Effect of erythropoietin on staurosporine-induced apoptosis and differentiation of SH-SY5Y neuroblastoma cells. Biochim Biophys Acta 2006;1763:238–46. [PubMed: 16500719]

Qin W, Peng Y, Ksiezak-Reding H, Ho L, Stetka B, Lovati E, Pasinetti GM. Inhibition of cyclooxygenase as potential novel therapeutic strategy in N141I presenilin-2 familial Alzheimer's disease. Mol Psychiatry 2006;11:172–81. [PubMed: 16331303]

Rades D, Golke H, Schild SE, Kilic E. The impact of tumor expression of erythropoietin receptors and erythropoietin on clinical outcome of esophageal cancer patients treated with chemoradiation. Int J Radiat Oncol Biol Phys. 2007[Epub ahead of print]

Ravid O, Shams I, Ben Califa N, Nevo E, Avivi A, Neumann D. An extracellular region of the erythropoietin receptor of the subterranean blind mole rat Spalax enhances receptor maturation. Proc Natl Acad Sci USA 2007;104:14360–5. [PubMed: 17724331]

Reddy MK, Vasir JK, Hegde GV, Joshi SS, Labhasetwar V. Erythropoietin induces excessive neointima formation: a study in a rat carotid artery model of vascular injury. J Cardiovasc Pharmacol Ther 2007;12:237–47. [PubMed: 17875952]

Regulska M, Leskiewicz M, Budziszewska B, Kutner A, Jantas D, Basta-Kaim A, Kubera M, Jaworska-Feil L, Lason W. Inhibitory effects of 1,25-dihydroxyvitamin D(3) and its low-calcemic analogues on staurosporine-induced apoptosis. Pharmacol Rep 2007;59:393–401. [PubMed: 17901567]

Reich NC. STAT dynamics. Cytokine Growth Factor Rev 2007;18:511–8. [PubMed: 17683973]

Reinders ME, Rabelink TJ, Briscoe DM. Angiogenesis and endothelial cell repair in renal disease and allograft rejection. J Am Soc Nephrol 2006;17:932–42. [PubMed: 16481411]

Roberts E Jr, Chih CP. The influence of age of pH regulation in hippocampal slices before, during, and after anoxia. J Cereb Blood Flow Metab 1997;17:560–6. [PubMed: 9183294]

Rowe MK, Wiest C, Chuang DM. GSK-3 is a viable potential target for therapeutic intervention in bipolar disorder. Neurosci Biobehav Rev 2007;31:920–31. [PubMed: 17499358]

Rytomaa M, Lehmann K, Downward J. Matrix detachment induces caspase-dependent cytochrome c release from mitochondria: inhibition by PKB/Akt but not Raf signalling. Oncogene 2000;19:4461–8. [PubMed: 11002418]

Sae-Ung N, Matsushima T, Choi I, Abe Y, Winichagoon P, Fucharoen S, Nawata H, Muta K. Role of NF-kappa B in regulation of apoptosis of erythroid progenitor cells. Eur J Haematol 2005;74:315–23. [PubMed: 15777344]

Salinas M, Diaz R, Abraham NG, Ruiz de Galarreta CM, Cuadrado A. Nerve growth factor protects against 6-hydroxydopamine-induced oxidative stress by increasing expression of heme oxygenase-1 in a phosphatidylinositol 3-kinase-dependent manner. J Biol Chem 2003;278:13898–904. [PubMed: 12578834]

Sanz O, Acarin L, Gonzalez B, Castellano B. NF-kappaB and IkappaBalpha expression following traumatic brain injury to the immature rat brain. J Neurosci Res 2002;67:772–80. [PubMed: 11891791]

Sasaki Y, Sasaki Y, Kanno K, Hidaka H. Disorganization by calcium antagonists of actin microfilament in aortic smooth muscle cells. Am J Physiol 1987;253:C71–8. [PubMed: 3605329]

Sathyanarayana P, Menon MP, Bogacheva O, Bogachev O, Niss K, Kapelle WS, Houde E, Fang J, Wojchowski DM. Erythropoietin modulation of podocalyxin and a proposed erythroblast niche. Blood 2007;110:509–18. [PubMed: 17403918]

Schmeding M, Neumann UP, Boas-Knoop S, Spinelli A, Neuhaus P. Erythropoietin reduces ischemia-reperfusion injury in the rat liver. Eur Surg Res 2007;39:189–97. [PubMed: 17377393]

Schnaider Beeri M, Goldbourt U, Silverman JM, Noy S, Schmeidler J, Ravona-Springer R, Sverdlick A, Davidson M. Diabetes mellitus in midlife and the risk of dementia three decades later. Neurology 2004;63:1902–7. [PubMed: 15557509]

Schumann C, Triantafilou K, Krueger S, Hombach V, Triantafilou M, Becher G, Lepper PM. Detection of erythropoietin in exhaled breath condensate of nonhypoxic subjects using a multiplex bead array. Mediators Inflamm 2006;2006:18061. [PubMed: 17392570]

Scott LJ, Bonnycastle LL, Willer CJ, Sprau AG, Jackson AU, Narisu N, Duren WL, Chines PS, Stringham HM, Erdos MR, Valle TT, Tuomilehto J, Bergman RN, Mohlke KL, Collins FS, Boehnke M. Association of transcription factor 7-like 2 (TCF7L2) variants with type 2 diabetes in a Finnish sample. Diabetes 2006;55:2649–53. [PubMed: 16936217]

Segura J, Pascual JA, Gutierrez-Gallego R. Procedures for monitoring recombinant erythropoietin and analogues in doping control. Anal Bioanal Chem 2007;388:1521–9. [PubMed: 17516052]

Sharples EJ, Patel N, Brown P, Stewart K, Mota-Philipe H, Sheaff M, Kieswich J, Allen D, Harwood S, Raftery M, Thiemermann C, Yaqoob MM. Erythropoietin protects the kidney against the injury and dysfunction caused by ischemia-reperfusion. J Am Soc Nephrol 2004;15:2115–24. [PubMed: 15284297]

Sharples EJ, Thiemermann C, Yaqoob MM. Mechanisms of disease: Cell death in acute renal failure and emerging evidence for a protective role of erythropoietin. Nat Clin Pract Nephrol 2005;1:87–97. [PubMed: 16932374]

Sharples EJ, Yaqoob MM. Erythropoietin in experimental acute renal failure. Nephron Exp Nephrol 2006;104:e83–8. [PubMed: 16837817]

Sigounas G, Sallah S, Sigounas VY. Erythropoietin modulates the anticancer activity of chemotherapeutic drugs in a murine lung cancer model. Cancer Lett 2004;214:171–9. [PubMed: 15363543]

Silverberg DS, Wexler D, Iaina A, Schwartz D. The interaction between heart failure and other heart diseases, renal failure, and anemia. Semin Nephrol 2006;26:296–306. [PubMed: 16949468]

Silverberg DS, Wexler D, Sheps D, Blum M, Keren G, Baruch R, Schwartz D, Yachnin T, Steinbruch S, Shapira I, Laniado S, Iaina A. The effect of correction of mild anemia in severe, resistant congestive heart failure using subcutaneous erythropoietin and intravenous iron: a randomized controlled study. J Am Coll Cardiol 2001;37:1775–80. [PubMed: 11401110]

Singh DK, Winocour P, Farrington K. Mechanisms of disease: the hypoxic tubular hypothesis of diabetic nephropathy. Nat Clin Pract Nephrol 2008;4:216–26. [PubMed: 18268525]

Slevin M, Kumar P, Gaffney J, Kumar S, Krupinski J. Can angiogenesis be exploited to improve stroke outcome? Mechanisms and therapeutic potential. Clin Sci (Lond) 2006;111:171–83. [PubMed: 16901264]

Socolovsky M, Fallon AE, Wang S, Brugnara C, Lodish HF. Fetal anemia and apoptosis of red cell progenitors in Stat5a−/−5b−/− mice: a direct role for Stat5 in Bcl-X(L) induction. Cell 1999;98:181–91. [PubMed: 10428030]

Socolovsky M, Nam H, Fleming MD, Haase VH, Brugnara C, Lodish HF. Ineffective erythropoiesis in Stat5a(−/−)5b(−/−) mice due to decreased survival of early erythroblasts. Blood 2001;98:3261–73. [PubMed: 11719363]

Spandou E, Tsouchnikas I, Karkavelas G, Dounousi E, Simeonidou C, Guiba-Tziampiri O, Tsakiris D. Erythropoietin attenuates renal injury in experimental acute renal failure ischaemic/reperfusion model. Nephrol Dial Transplant 2006;21:330–6. [PubMed: 16221709]

Speese SD, Budnik V. Wnts: up-and-coming at the synapse. Trends Neurosci 2007;30:268–75. [PubMed: 17467065]

Stegh AH, Barnhart BC, Volkland J, Algeciras-Schimnich A, Ke N, Reed JC, Peter ME. Inactivation of caspase-8 on mitochondria of Bcl-xL-expressing MCF7-Fas cells: role for the bifunctional apoptosis regulator protein. J Biol Chem 2002;277:4351–60. [PubMed: 11733517]

Stolze I, Berchner-Pfannschmidt U, Freitag P, Wotzlaw C, Rossler J, Frede S, Acker H, Fandrey J. Hypoxia-inducible erythropoietin gene expression in human neuroblastoma cells. Blood 2002;100:2623–8. [PubMed: 12239177]

Sun XM, Cohen GM. Mg(2+)-dependent cleavage of DNA into kilobase pair fragments is responsible for the initial degradation of DNA in apoptosis. J Biol Chem 1994;269:14857–60. [PubMed: 8195114]

Symeonidis A, Kouraklis-Symeonidis A, Psiroyiannis A, Leotsinidis M, Kyriazopoulou V, Vassilakos P, Vagenakis A, Zoumbos N. Inappropriately low erythropoietin response for the degree of anemia in patients with noninsulin-dependent diabetes mellitus. Ann Hematol 2006;85:79–85. [PubMed: 16132904]

Takahashi H, Nakamura S, Asano K, Kinouchi M, Ishida-Yamamoto A, Iizuka H. Fas antigen modulates ultraviolet B-induced apoptosis of SVHK cells: sequential activation of caspases 8, 3, and 1 in the apoptotic process. Exp Cell Res 1999;249:291–8. [PubMed: 10366428]

Tamagno E, Robino G, Obbili A, Bardini P, Aragno M, Parola M, Danni O. H2O2 and 4-hydroxynonenal mediate amyloid beta-induced neuronal apoptosis by activating JNKs and p38MAPK. Exp Neurol 2003;180:144–55. [PubMed: 12684028]

Tanuma N, Sakuma H, Sasaki A, Matsumoto Y. Chemokine expression by astrocytes plays a role in microglia/macrophage activation and subsequent neurodegeneration in secondary progressive multiple sclerosis. Acta Neuropathol (Berl) 2006;112:195–204. [PubMed: 16733654]

Teramo K, Kari MA, Eronen M, Markkanen H, Hiilesmaa V. High amniotic fluid erythropoietin levels are associated with an increased frequency of fetal and neonatal morbidity in type 1 diabetic pregnancies. Diabetologia 2004;47:1695–703. [PubMed: 15502930]

Thomas MC, Cooper ME, Tsalamandris C, MacIsaac R, Jerums G. Anemia with impaired erythropoietin response in diabetic patients. Arch Intern Med 2005;165:466–9. [PubMed: 15738380]

Toma C, Letts DP, Tanabe M, Gorcsan J 3rd, Counihan PJ. Positive effect of darbepoetin on peri-infarction remodeling in a porcine model of myocardial ischemia-reperfusion. J Mol Cell Cardiol 2007;43:130–6. [PubMed: 17597149]

Torriglia A, Chaudun E, Courtois Y, Counis MF. On the use of Zn2+ to discriminate endonucleases activated during apoptosis. Biochimie 1997;79:435–8. [PubMed: 9352093]

Toyoda T, Itai T, Arakawa T, Aoki KH, Yamaguchi H. Stabilization of human recombinant erythropoietin through interactions with the highly branched N-glycans. J Biochem (Tokyo) 2000;128:731–7. [PubMed: 11056384]

Tramontano AF, Muniyappa R, Black AD, Blendea MC, Cohen I, Deng L, Sowers JR, Cutaia MV, El-Sherif N. Erythropoietin protects cardiac myocytes from hypoxia-induced apoptosis through an Akt-dependent pathway. Biochem Biophys Res Commun 2003;308:990–4. [PubMed: 12927817]

Troy CM, Rabacchi SA, Xu Z, Maroney AC, Connors TJ, Shelanski ML, Greene LA. beta-Amyloid-induced neuronal apoptosis requires c-Jun N-terminal kinase activation. J Neurochem 2001;77:157–64. [PubMed: 11279271]

Tsai JC, Song BJ, Wu L, Forbes M. Erythropoietin: a candidate neuroprotective agent in the treatment of glaucoma. J Glaucoma 2007;16:567–71. [PubMed: 17873720]

Tsuda E, Goto M, Murakami A, Akai K, Ueda M, Kawanishi G, Takahashi N, Sasaki R, Chiba H, Ishihara H, Mori M, Tejima S, Endo S, Arata Y. Comparative structural study of N-linked oligosaccharides of urinary and recombinant erythropoietins. Biochemistry 1988;27:5646–54. [PubMed: 3179269]

Tsuda E, Kawanishi G, Ueda M, Masuda S, Sasaki R. The role of carbohydrate in recombinant human erythropoietin. Eur J Biochem 1990;188:405–11. [PubMed: 2156701]

Uchida E, Morimoto K, Kawasaki N, Izaki Y, Abdu Said A, Hayakawa T. Effect of active oxygen radicals on protein and carbohydrate moieties of recombinant human erythropoietin. Free Radic Res 1997;27:311–23. [PubMed: 9350435]

Um M, Gross AW, Lodish HF. A “classical” homodimeric erythropoietin receptor is essential for the antiapoptotic effects of erythropoietin on differentiated neuroblastoma SH-SY5Y and pheochromocytoma PC-12 cells. Cell Signal 2007;19:634–45. [PubMed: 17045782]

Um M, Lodish HF. Antiapoptotic Effects of Erythropoietin in Differentiated Neuroblastoma SH-SY5Y Cells Require Activation of Both the STAT5 and AKT Signaling Pathways. J Biol Chem 2006;281:5648–56. [PubMed: 16407271]

Vairano M, Dello Russo C, Pozzoli G, Battaglia A, Scambia G, Tringali G, Aloe-Spiriti MA, Preziosi P, Navarra P. Erythropoietin exerts anti-apoptotic effects on rat microglial cells *in vitro*. Eur J Neurosci 2002;16:584–92. [PubMed: 12270034]

van der Meer P, Lipsic E, Henning RH, de Boer RA, Suurmeijer AJ, van Veldhuisen DJ, van Gilst WH. Erythropoietin improves left ventricular function and coronary flow in an experimental model of ischemia-reperfusion injury. Eur J Heart Fail 2004a;6:853–9. [PubMed: 15556046]

van der Meer P, Voors AA, Lipsic E, Smilde TD, van Gilst WH, van Veldhuisen DJ. Prognostic value of plasma erythropoietin on mortality in patients with chronic heart failure. J Am Coll Cardiol 2004b;44:63–7. [PubMed: 15234408]

Vanags DM, Porn-Ares MI, Coppola S, Burgess DH, Orrenius S. Protease involvement in fodrin cleavage and phosphatidylserine exposure in apoptosis. J Biol Chem 1996;271:31075–85. [PubMed: 8940103]

Verdaguer E, Susana Gde A, Clemens A, Pallas M, Camins A. Implication of the transcription factor E2F-1 in the modulation of neuronal apoptosis. Biomed Pharmacother 2007;61:390–9. [PubMed: 17178208]

Verdonck O, Lahrech H, Francony G, Carle O, Farion R, Van de Looij Y, Remy C, Segebarth C, Payen JF. Erythropoietin protects from post-traumatic edema in the rat brain. J Cereb Blood Flow Metab 2007;27:1369–76. [PubMed: 17264861]

Vincent AM, Maiese K. Direct temporal analysis of apoptosis induction in living adherent neurons. J Histochem Cytochem 1999a;47:661–72. [PubMed: 10219058]

Vincent AM, Maiese K. Nitric oxide induction of neuronal endo-nuclease activity in programmed cell death. Exp Cell Res 1999b;246:290–300. [PubMed: 9925743]

Vincent AM, TenBroeke M, Maiese K. Metabotropic glutamate receptors prevent programmed cell death through the modulation of neuronal endonuclease activity and intracellular pH. Exp Neurol 1999a; 155:79–94. [PubMed: 9918707]

Vincent AM, TenBroeke M, Maiese K. Neuronal intracellular pH directly mediates nitric oxide-induced programmed cell death. J Neurobiol 1999b;40:171–84. [PubMed: 10413448]

Walshe TE, D’Amore PA. The role of hypoxia in vascular injury and repair. Annu Rev Pathol 2008;3:615–43. [PubMed: 18039132]

Wang FF, Kung CK, Goldwasser E. Some chemical properties of human erythropoietin. Endocrinology 1985;116:2286–92. [PubMed: 3996312]

Wang GL, Semenza GL. Purification and characterization of hypoxia-inducible factor 1. J Biol Chem 1995;270:1230–7. [PubMed: 7836384]

Watowich SS, Hilton DJ, Lodish HF. Activation and inhibition of erythropoietin receptor function: role of receptor dimerization. Mol Cell Biol 1994;14:3535–49. [PubMed: 8196600]

Wei L, Han BH, Li Y, Keogh CL, Holtzman DM, Yu SP. Cell death mechanism and protective effect of erythropoietin after focal ischemia in the whisker-barrel cortex of neonatal rats. J Pharmacol Exp Ther 2006;317:109–16. [PubMed: 16357210]

Westenbrink BD, Lipsic E, van der Meer P, van der Harst P, Oeseburg H, Du Marchie Sarvaas GJ, Koster J, Voors AA, van Veldhuisen DJ, van Gilst WH, Schoemaker RG. Erythropoietin improves cardiac function through endothelial progenitor cell and vascular endothelial growth factor mediated neovascularization. Eur Heart J 2007;28:2018–27. [PubMed: 17576662]

Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047–53. [PubMed: 15111519]

Wilks AF, Harpur AG, Kurban RR, Ralph SJ, Zurcher G, Ziemiecki A. Two novel protein-tyrosine kinases, each with a second phosphotransferase-related catalytic domain, define a new class of protein kinase. Mol Cell Biol 1991;11:205765.

Wilson FH, Hariri A, Farhi A, Zhao H, Petersen KF, Toka HR, Nelson-Williams C, Raja KM, Kashgarian M, Shulman GI, Scheinman SJ, Lifton RP. A cluster of metabolic defects caused by mutation in a mitochondrial tRNA. Science 2004;306:1190–4. [PubMed: 15498972]

Wright WS, Longo KA, Dolinsky VW, Gerin I, Kang S, Bennett CN, Chiang SH, Prestwich TC, Gress C, Burant CF, Susulic VS, Macdougald OA. Wnt10b Inhibits Obesity in ob/ob and Agouti Mice. Diabetes 2007;56:295–303. [PubMed: 17259372]

Wu H, Ren B, Zhu J, Dong G, Xu B, Wang C, Zheng X, Jing H. Pretreatment with recombined human erythropoietin attenuates ischemia-reperfusion-induced lung injury in rats. Eur J Cardiothorac Surg 2006;29:902–7. [PubMed: 16675226]

Wu Y, Shang Y, Sun S, Liang H, Liu R. Erythropoietin prevents PC12 cells from 1-methyl-4-phenylpyridinium ion-induced apoptosis *via* the Akt/GSK-3beta/caspase-3 mediated signaling pathway. Apoptosis 2007a;12:1365–75. [PubMed: 17508273]

Wu Y, Shang Y, Sun S, Liu R. Antioxidant effect of erythropoietin on 1-methyl-4-phenylpyridinium-induced neurotoxicity in PC12 cells. Eur J Pharmacol 2007b;564:47–56. [PubMed: 17362920]

Xu B, Dong GH, Liu H, Wang YQ, Wu HW, Jing H. Recombinant human erythropoietin pretreatment attenuates myocardial infarct size: a possible mechanism involves heat shock Protein 70 and attenuation of nuclear factor-kappaB. Ann Clin Lab Sci 2005;35:161–8. [PubMed: 15943180]

Xu WL, Qiu CX, Wahlin A, Winblad B, Fratiglioni L. Diabetes mellitus and risk of dementia in the Kungsholmen project: a 6-year follow-up study. Neurology 2004;63:1181–6. [PubMed: 15477535]

Yamaji R, Okada T, Moriya M, Naito M, Tsuruo T, Miyatake K, Nakano Y. Brain capillary endothelial cells express two forms of erythropoietin receptor mRNA. Eur J Biochem 1996;239:494–500. [PubMed: 8706759]

Yamasaki M, Mishima HK, Yamashita H, Kashiwagi K, Murata K, Minamoto A, Inaba T. Neuroprotective effects of erythropoietin on glutamate and nitric oxide toxicity in primary cultured retinal ganglion cells. Brain Res 2005;1050:15–26. [PubMed: 15979589]

Yano M, Hasegawa G, Ishii M, Yamasaki M, Fukui M, Nakamura N, Yoshikawa T. Short-term exposure of high glucose concentration induces generation of reactive oxygen species in endothelial cells: implication for the oxidative stress associated with postprandial hyperglycemia. Redox Rep 2004;9:111–6. [PubMed: 15231066]

Yasuda Y, Masuda S, Chikuma M, Inoue K, Nagao M, Sasaki R. Estrogen-dependent production of erythropoietin in uterus and its implication in uterine angiogenesis. J Biol Chem 1998;273:25381–7. [PubMed: 9738005]

You Z, Saims D, Chen S, Zhang Z, Guttridge DC, Guan KL, MacDougald OA, Brown AM, Evan G, Kitajewski J, Wang CY. Wnt signaling promotes oncogenic transformation by inhibiting c-Myc-induced apoptosis. J Cell Biol 2002;157:429–40. [PubMed: 11980918]

Yu YP, Xu QQ, Zhang Q, Zhang WP, Zhang LH, Wei EQ. Intranasal recombinant human erythropoietin protects rats against focal cerebral ischemia. Neurosci Lett 2005;387:5–10. [PubMed: 16054296]

Yui R, Matsuura ET. Detection of deletions flanked by short direct repeats in mitochondrial DNA of aging Drosophila. Mutat Res 2006;594:155–61. [PubMed: 16289600]

Zhang F, Signore AP, Zhou Z, Wang S, Cao G, Chen J. Erythropoietin protects CA1 neurons against global cerebral ischemia in rat: potential signaling mechanisms. J Neurosci Res 2006;83:1241–51. [PubMed: 16511866]

Zhang SX, Ma JX. Ocular neovascularization: Implication of endogenous angiogenic inhibitors and potential therapy. Prog Retin Eye Res 2007;26:1–37. [PubMed: 17074526]

Zhang Y, Park TS, Gidday JM. Hypoxic preconditioning protects human brain endothelium from ischemic apoptosis by Akt-dependent survivin activation. Am J Physiol Heart Circ Physiol 2007;292:H2573–81. [PubMed: 17337592]

Zhao Y, Wagner F, Frank SJ, Kraft AS. The amino-terminal portion of the JAK2 protein kinase is necessary for binding and phosphorylation of the granulocyte-macrophage colony- stimulating factor receptor beta c chain. J Biol Chem 1995;270:13814–8. [PubMed: 7775438]

Zheng WH, Kar S, Quirion R. FKHRL1 and its homologs are new targets of nerve growth factor Trk receptor signaling. J Neurochem 2002;80:1049–61. [PubMed: 11953455]