Published in final edited form as:

Biomed Pharmacother. 2008 ; 62(4): 218–232.

TRIPLE PLAY: PROMOTING NEUROVASCULAR LONGEVITY WITH NICOTINAMIDE, WNT, AND ERYTHROPOIETIN IN DIABETES MELLITUS

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Summary

Oxidative stress is a principal pathway for the dysfunction and ultimate destruction of cells in the neuronal and vascular systems for several disease entities, non promoting the ravages of oxidative stress to any less of a degree than diabetes mellitus. Diabetes mellitus is increasing in incidence as a result of changes in human behavior that relate to diet and daily exercise and is predicted to affect almost 400 million individuals worldwide in another two decades. Furthermore, both type 1 and type 2 diabetes mellitus can lead to significant disability in the nervous and cardiovascular systems, such as cognitive loss and cardiac insufficiency. As a result, innovative strategies that directly target oxidative stress to preserve neuronal and vascular longevity could offer viable therapeutic options to diabetic patients in addition to more conventional treatments that are designed to control serum glucose levels. Here we discuss the novel application of nicotinamide, Wnt signaling, and erythropoietin that modulate cellular oxidative stress and offer significant promise for the prevention of diabetic complications in the nervous and vascular systems. Essential to this process is the precise focus upon diverse as well as common cellular pathways governed by nicotinamide, Wnt signaling, and erythropoietin to outline not only the potential benefits, but also the challenges and possible detriments of these therapies. In this way, new avenues of investigation can hopefully bypass toxic complications, or at the very least, avoid contraindications that may limit care and offer both safe and robust clinical treatment for patients.

Keywords

endothelial; neurodegeneration; oxidative stress

1. Introduction

Diabetes mellitus (DM) is a significant health concern in the clinical population [1]. The disease is present in at least 16 million individuals in the United States and more than 165 million individuals worldwide [2]. Furthermore, by the year 2030, it is predicted that more than 360

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million individuals will be afflicted with DM [3]. At least 80 percent of all diabetics have type 2 DM that is increasing in incidence as a result of changes in human behavior that relate to diet and daily exercise [4]. Although type 1 insulin-dependent diabetes mellitus accounts for only 5–10 percent of all diabetics [5], it is increasing in adolescent minority groups [6]. Of potentially greater concern is the incidence of undiagnosed diabetes that consists of impaired glucose tolerance and fluctuations in serum glucose that can increase the risk for the development of DM [7]. Individuals with impaired glucose tolerance have a greater than two times the risk for the development of diabetic complications than individuals with normal glucose tolerance [8].

Both acute and long-term occurrence of type 1 and type 2 DM can result in complications of the neuronal and vascular systems. For example, DM can impair vascular integrity and alter cardiac output [9] that eventually diminishes the capacity of sensitive cognitive regions of the brain leading to functional impairment and dementia [10–12]. 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 [13]. DM also has been found to increase the risk for vascular dementia in elderly subjects [12,14] as well as potentially alter 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 [15], 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 [16]. Furthermore, costs to care for cognitive impairments resulting from diabetes that can mimic Alzheimer's disease can approach \$100 billion a year [17–19]

2. The role of oxidative stress in DM

Closely tied to the development of insulin resistance and the complications of DM in the nervous and vascular systems is the presence of cellular oxidative stress and the release of reactive oxygen species [5]. Oxidative stress occurs as a result of the development of reactive oxygen species that consist of oxygen free radicals and other chemical entities. Oxygen consumption in organisms, or at least the rate of oxygen consumption in organisms, has intrigued a host of investigators and may have had some of its original origins with the work of Pearl. Pearl proposed that increased exposure to oxygen through an increased metabolic rate could lead to a shortened life span [20]. 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 [21]. 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 [22].

Oxidative stress represents a significant mechanism for the destruction of cells that can involve apoptotic neuronal and vascular cell injury [23–25]. In fact, 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 [26]. 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 [10,27–30]. 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 [28,31–35].

Membrane phosphatidylserine (PS) externalization is an early event during cell apoptosis [36,37] and can become a signal for the phagocytosis of cells [25,38,39]. The loss of membrane phospholipid asymmetry leads to the externalization of membrane PS residues and assists

microglia to target cells for phagocytosis [33,40–43]. This process occurs with the expression of the phosphatidylserine receptor (PSR) on microglia during oxidative stress [11,44], since blockade of PSR function in microglia prevents the activation of microglia [41,45]. As an example, externalization of membrane PS residues occur in neurons during anoxia [46–48], nitric oxide exposure [49,50], and during the administration of agents that induce the production of reactive oxygen species, such as 6-hydroxydopamine [51]. Membrane PS externalization on platelets also has been associated with clot formation in the vascular system [52].

The cleavage of genomic DNA into fragments [53–55] is considered to be a later event during apoptotic injury [56]. Several enzymes responsible for DNA degradation have been differentiated and include the acidic, cation independent endonuclease (DNase II), cyclophilins, and the 97 kDa magnesium - dependent endonuclease [10,57]. Three separate endonuclease activities are present in neurons that include a constitutive acidic cation-independent endonuclease, a constitutive calcium/magnesium-dependent endonuclease, and an inducible magnesium dependent endonuclease [58,59].

During oxidative stress, mitochondrial membrane transition pore permeability also is increased [33,60–62], a significant loss of mitochondrial NAD⁺ stores occurs, and further generation of superoxide radicals leads to cell injury [42,63]. In addition, mitochondria are a significant source of superoxide radicals that are associated with oxidative stress [10,17]. 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 [11,57]. Furthermore, mutations in the mitochondrial genome have been associated with the potential development of a host of disorders, such as hypertension, hypercholesterolemia, and hypomagnesemia [64,65]. Reactive oxygen species also may lead to the induction of acidosis-induced cellular toxicity and subsequent mitochondrial failure [27]. Disorders, such as hypoxia [66], diabetes [67,68], and excessive free radical production [59,69,70] can result in the disturbance of intracellular pH.

In disorders such as DM, elevated levels of ceruloplasmin have been suggested to represent increased reactive oxygen species [71] and acute glucose fluctuations have been described as a potential source of oxidative stress [72]. Elevated serum glucose also has been shown to lead to increased production of reactive oxygen species in ECs, but prolonged duration of hyperglycemia is not necessary to lead to oxidative stress injury, since even short periods of hyperglycemia can generate reactive oxygen species in vascular cells [73]. Recent 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 [72].

The maintenance of cellular energy reserves and mitochondrial integrity also becomes a significant factor in DM [74]. During DM, fatty acid accumulation leads to both the generation of reactive oxygen species and mitochondrial DNA damage [75]. A decrease in the levels of mitochondrial proteins and mitochondrial DNA in adipocytes has been correlated with the development of type 2 DM [76]. In addition, insulin resistance in the elderly has been linked to fat accumulation and reduction in mitochondrial oxidative and phosphorylation activity [77,78].

3. Innovative strategies for neurovascular protection during DM

Possible pathways that may decrease neuronal and vascular longevity during DM are broad in scope and involve multiple precipitating factors. Yet, oxidative stress-induced cellular

signaling is believed to be one significant factor responsible for cell injury that initially is set in motion following hyperglycemia. For example, studies have shown that administration of insulin or insulin growth factor at concentrations that were insufficient to reverse hyperglycemia could nevertheless reduce oxidative stress injury to cells and maintain mitochondrial inner membrane potential [1,5]. As a result, innovative strategies that directly target the reduction of oxidative stress toxicity to neuronal and vascular cells could offer viable therapeutic options to patients with DM in addition to more conventional treatments that are targeted to control serum glucose levels.

4. Nicotinamide, a precursor for the coenzyme β -nicotinamide adenine dinucleotide (NAD⁺)

As the amide form of niacin or vitamin B₃, nicotinamide plays a critical role in cellular metabolism and can offer significant neuronal and vascular cell protection during a wide range of disorders that include DM. Nicotinamide is the precursor for the coenzyme β -nicotinamide adenine dinucleotide (NAD⁺) and is essential for the synthesis of nicotinamide adenine dinucleotide phosphate (NADP⁺) [42,64]. Nicotinamide and nicotinic acid can be obtained either through synthesis in the body, such as in the liver, or through a dietary source that is rapidly absorbed through the gastrointestinal epithelium. Once nicotinamide is obtained in the body, it is utilized to synthesize NAD⁺ [11].

In clinical studies for DM, oral nicotinamide protects β -cell function, prevents clinical disease in islet-cell antibody-positive first-degree relatives of type-1 DM [79], and in combination therapy with insulin can reduce HbA_{1c} levels [80]. Potentially relevant to diabetic patients with renal failure, nicotinamide also has been shown to reduce intestinal absorption of phosphate and prevent the development of hyperphosphatemia and progressive renal dysfunction [81]. In animal and cell culture studies, nicotinamide also can maintain normal fasting blood glucose in animals with streptozotocin-induced diabetes [82,83], reduce peripheral nerve injury during elevated glucose [84], lead to the remission of type 1 DM in mice with acetyl-L-carnitine [85], and can inhibit oxidative stress pathways that lead to apoptosis [63,86–89].

Nicotinamide can have protean endocrine effects in the body [90] and derive its protective capacity through a number of cellular pathways. In addition to the neuroprotective attributes of nicotinamide [91–93], one potential pathway to consider for the protective capacity of nicotinamide in DM involves the maintenance of vascular integrity [11,42,64]. For example, nicotinamide can protect the function of the blood brain barrier [94,95], influence arteriolar dilatation and blood flow [96], potentially lead to decreased atherosclerotic plaque through inhibition of poly(ADP-ribose) polymerase [97], and promote platelet production through megakaryocyte maturation [98]. Nicotinamide also can maintain EC viability during reactive oxygen species exposure [42,87,99,100]. Nicotinamide is believed to be responsible for the preservation of cerebral [101] and endocardial [102,103] ECs during models of oxidative stress [102,103]. Interestingly, during periods such as ischemia and oxidative stress, acidosis-induced cellular toxicity may ensue [27] and lead to subsequent mitochondrial failure [104]. Yet, nicotinamide cannot prevent cellular injury during intracellular acidification paradigms [62].

An alternative mechanism for nicotinamide may require the maintenance of the mitochondrial membrane potential ($\Delta\Psi_m$) to protect cells from injury (Figure 1). Nicotinamide can preserve mitochondrial NAD-linked respiration and block the depolarization of the mitochondrial membrane [62,87]. Interestingly, nicotinamide appears to act directly at the level of mitochondrial membrane pore formation to prevent cytochrome c release [62,87].

Nicotinamide also can prevent inflammatory cell demise through the maintenance of membrane asymmetry, activation of protein kinase B (Akt), and the inhibition of cytokine

release [11,42,64]. Nicotinamide blocks membrane PS externalization during a variety of insults that involve anoxia, free radical exposure, and oxygen-glucose deprivation [62,87,99]. Nicotinamide regulates membrane PS exposure and microglial activation through activation of Akt, a central pathway for cytoprotection [92]. Phosphorylation of Akt leads to its activation and protects cells against genomic DNA degradation and membrane PS exposure [45,61,105]. Up-regulation of Akt activity during multiple injury paradigms, such as vascular and cardiomyocyte ischemia [106,107], free radical exposure [45,108], N-methyl-D-aspartate toxicity [109], hypoxia [110,111], β -amyloid toxicity [112–114], DNA damage [31,41,110,115], metabotropic receptor signaling [38,116,117], cell metabolic pathways [42,63], and oxidative stress [31,33,41] increases cell survival. Cytoprotection through Akt also can involve control of inflammatory cell activation [33,41,61], transcription factor regulation [118], maintenance of mitochondrial membrane potential ($\Delta\Psi_m$), prevention of cytochrome c release [45,61,105], and blockade of caspase activity [45,61,110].

In addition to targeting the activity of membrane PS exposure and microglial activation, nicotinamide inhibits several pro-inflammatory cytokines, such as interleukin-1 β , interleukin-6, interleukin-8, tissue factor, and tumor necrosis factor- α (TNF- α) [119–122]. Nicotinamide also can alter major histocompatibility complexes [123], inhibit intracellular adhesion molecule expression [124], and modulate the production of TNF in vascular cells [123] that may be responsible for the ability of nicotinamide to reduce demyelination in models of multiple sclerosis [125]. However, translation of these experimental studies to clinical efficacy appears to require further work, since some studies show that oral nicotinamide administration following endotoxin challenge in healthy volunteers did not demonstrate a significant effect upon serum cytokine levels [126].

Other studies suggest that nicotinamide also may utilize pathways of the mammalian forkhead transcription factor family that oversees processes that can involve cell metabolism, hormone modulation, and apoptosis [1,127,128]. The first member of this family was the *Drosophila melanogaster* gene *Fork head*. Since this time, greater than 100 forkhead genes and 19 human subgroups are known to exist that extend from FOXA to FOXS [128]. The forkhead 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 [129] and nuclear magnetic resonance [130]. 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 [131].

Of the forkhead transcription factors, FOXO3a is one member that exemplifies the ability to function as a versatile component during normal physiology as well as during disorders such as DM [128]. In relation to the designation of human Fox proteins, all letters are capitalized, otherwise only the initial letter is listed as uppercase. FOXO3a appears to be involved in several pathways responsible for cell metabolism, DM onset, and diabetic complications [57,117,132,133]. A clinical study of 734 individuals that examined all exons of the FOXO genes FOXO1a, FOXO3a, and FOXO4 found one promoter single nucleotide polymorphism in the 5' flanking region of FOXO3a that displayed a significant association with body mass index such that the highest body mass index was present in individuals homozygous for this allele [134]. Although other studies have reported that haplotype analyses of FOXO1a rather than FOXO3a in individuals is associated with higher HbA_{1c} levels to suggest evidence of at least an association with disorders of glucose intolerance, FOXO3a haplotypes also have been associated with an increased risk for stroke [135]. In addition, the human immunodeficiency virus (HIV) -1 accessory protein Vpr has been reported to contribute to insulin resistance in HIV patients by interfering with Foxo3a signaling with protein 14-3-3 [136].

In regards to experimental work for DM, administration of a high-fat diet in animals that lead to hyperinsulinemic insulin-resistant obesity was associated with an increased expression of Foxo3a [137]. Some studies have suggested that Foxo3a may be beneficial during elevated glucose exposure and DM. For example, interferon-gamma driven expression of tryptophan catabolism by cytotoxic T lymphocyte antigen 4 may activate Foxo3a to protect dendritic cells from injury in nonobese diabetic mice [138]. Yet, the role of forkhead transcription factors can vary among different cell types and tissues. For example, mice overexpressing FOXO1 in skeletal muscle suffer from reduced skeletal muscle mass and poor glycemic control [139]. Additional investigations have linked diabetic nephropathy to Foxo3a by demonstrating that phosphorylation of Foxo3a increases in rat and mouse renal cortical tissues two weeks after the induction of diabetes by streptozotocin [140]. Furthermore, enteric neurons can be protected from hyperglycemia by glial cell line-derived neurotrophic factor that can affect Akt signaling and prevent Foxo3a activation and nuclear translocation [141]. Interestingly, the ability of Akt to also inhibit pyruvate dehydrogenase kinase-4 expression, a protein that conserves gluconeogenic substrates during DM, requires the inhibition of Foxo3a activity [142].

As a result, FOXO3a has emerged as an important target for DM. Akt can phosphorylate FOXO3a and inhibit its activity to sequester FOXO3a in the cytoplasm by association with 14-3-3 proteins [118,136,143–145]. 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 [1,11,146]. Control of FOXO3a is considered to be a viable therapeutic target for agents such as metabotropic glutamate receptors [116], neurotrophins [147], and cytokines such as erythropoietin (EPO) [118] to increase cell survival. Given the potential treatment advantages of nicotinamide in DM, it should be of interest that nicotinamide may be cytoprotective through two separate mechanisms of post-translational modification of Foxo3a. Nicotinamide can not only maintain phosphorylation of Foxo3a and inhibit its activity, but also preserve the integrity of the Foxo3a protein [92] to block Foxo3a proteolysis that can yield potentially pro-apoptotic amino-terminal (Nt) fragments [148].

5. Cysteine-rich glycosylated Wnt proteins

Wnt proteins are secreted cysteine-rich glycosylated proteins that can be dependent upon Akt signaling and oversee embryonic cell proliferation, differentiation, survival, and death [44, 149–151]. More than eighty target genes of Wnt signaling pathways have been demonstrated in human, mouse, *Drosophila*, *Xenopus*, and zebrafish. This representation encompasses several cellular populations, such as neurons, cardiomyocytes, endothelial cells, cancer cells, and pre-adipocytes [152,153]. In addition, at least nineteen of twenty-four Wnt genes that express Wnt proteins have been identified in the human.

In general, all Wnt signaling pathways are initiated by interaction of Wnt proteins with Frizzled (FZD) receptors and the binding of the Wnt protein to the FZD transmembrane receptor in the presence of the co-receptor LRP-5/6 [154] (Figure 1). Once Wnt protein binds to the FZD transmembrane receptor and the co-receptor LRP-5/6, this is followed by recruitment of dishevelled, a cytoplasmic multifunctional phosphoprotein [153,155,156]. The Wnt-Frizzled transduction pathway plays a significant role in the control of the pattern of the body axis as well as the development and maturation of the central nervous system [157,158], cardiovascular system [159–162], and the limbs [163]. During embryological development, alternations of the Wnt-FZD pathway can lead to abnormal morphogenesis in animal models [158,164,165] and congenital defects in humans [166–168]. In mature tissues, the Wnt-FZD pathway is involved in the self-renewal of pluripotent embryonic stem cells [169], bone formation [170], and may be responsible for the maintenance of many normal tissues [171–174] as well as cellular senescence [175]. Other studies have revealed that dysfunction of the

Wnt-FZD pathway can lead to neurodegenerative disorders, such as Alzheimer's disease [149,176–179] and heart failure [44,180–182].

Wnt signaling can prevent cell injury through β -catenin/Tcf transcription mediated pathways [183] and against c-myc induced apoptosis through cyclooxygenase-2 and Wnt induced secreted protein [184]. However, more recent work has linked Wnt cytoprotection in neuronal and vascular cells with more unconventional pathways of Wnt that involve Akt. For example, neuronal cell differentiation that is dependent upon Wnt signaling and trophic factor induction is blocked during the repression of Akt activity [185] and Wnt differentiation of cardiomyocytes does not proceed without Akt activation [186]. Soluble secreted FZD-related proteins, which can modulate Wnt signaling, also employ Akt for cardiac tissue repair [187]. Reduction in tissue injury through Wnt signaling during pressure overload cardiac hypertrophy is linked to Akt activation [182] and the benefits of cardiac ischemic preconditioning appear to rely upon Akt [181]. In the neuronal system, Wnt over-expression can independently increase the phosphorylation and the activation of Akt to promote neuronal protection [149]. Inhibition of the phosphatidylinositol 3-kinase (PI 3-K) pathway or gene silencing of Akt expression prevents Wnt from blocking apoptotic injury and microglial activation [149].

Abnormalities in the Wnt signaling pathways, such as with transcription factor 7-like 2 gene, may impart increased risk for type 2 DM in some populations [188–190] as well as have increased association with obesity [191]. Additional work has described the expression of Wnt5b in adipose tissue, the pancreas, and the liver in diabetic patients, suggesting a potential regulation of adipose cell function [192]. Clinical observations in patients with coronary artery disease and the combined metabolic syndrome with hypertension, hyperlipidemia, and DM have observed impaired Wnt signaling through a missense mutation in LRP-6 [193]. Experimental studies in mice that develop hyperglycemia through a high fat diet also demonstrate increased expression of some Wnt family members, such as Wnt3a and Wnt7a [194]. Yet, intact Wnt family members may offer glucose tolerance and increased insulin sensitivity [195] as well as protect glomerular mesangial cells from elevated glucose induced apoptosis [196]. Animals that over-expressed Wnt10b and were placed on a high-fat diet had a reduction in bodyweight, hyperinsulinemia, triglyceride plasma levels, and improved glucose homeostasis [197].

These clinical and experimental investigations for the Wnt pathway suggest a potential protective cellular mechanism for Wnt during DM. Recent *in vitro* studies demonstrate that the Wnt1 protein is necessary and sufficient to provide cellular protection during elevated glucose exposure [150]. Administration of exogenous Wnt1 protein can significantly prevent apoptotic EC injury during elevated glucose exposure. Interestingly, this protection by Wnt1 can be regulated by the growth factor and cytokine EPO [132,133,198]. Through the Wnt pathway, EPO may offer an attractive therapy to maintain proper cellular metabolism and mitochondrial membrane potential ($\Delta\Psi_m$) during conditions of oxidative stress and DM. In cell culture and animal studies, EPO is cytoprotective during elevated glucose [150] and has the capacity to prevent the depolarization of the mitochondrial membrane that also affects the release of cytochrome c [105,106,110]. With the Wnt pathway, EPO maintains the expression of Wnt1 during elevated glucose exposure and prevents loss of Wnt1 expression that would normally occur in the absence of EPO during elevated glucose. In addition, blockade of Wnt1 with a Wnt1 antibody can neutralize the protective capacity of EPO, illustrating that Wnt1 is a critical component in the cytoprotection of EPO during elevated glucose exposure [150].

Interestingly, Wnt also can protect cells during oxidative stress [152] and other toxic injuries such as β -amyloid toxicity [152] through the modulation of glycogen synthase kinase-3 β (GSK-3 β) and β -catenin [149]. Inhibition of GSK-3 β activity can increase cell survival during oxidative stress and, as a result, GSK-3 β is considered to be a therapeutic target for some

neurodegenerative disorders [10,179,199,200]. GSK-3 β also may influence inflammatory cell survival [32] and activation [201]. In regards to metabolic disease, inactivation of GSK-3 β 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-3 β for pancreatic beta cell regeneration [202]. Clinical applications for Wnt that involve GSK-3 β are attractive [203], especially in concert with EPO. For example, both the potential benefits of EPO to improve cardiovascular function in diabetic patients [204,205] and the positive effects of exercise to improve glycemic control during DM [206] appear to rely upon the inhibition of GSK-3 β activity. EPO blocks GSK-3 β activity [39,150,207] and combined with exercise may offer synergistic benefits, since physical exercise also has been shown to phosphorylate and inhibit GSK-3 β activity [208].

6. Erythropoietin, a cytokine and growth factor

EPO is a 30.4 kDa glycoprotein with approximately fifty-percent of its molecular weight derived from carbohydrates [133]. As a growth factor and cytokine, EPO is considered to be ubiquitous in the body [1,5], since it can be detected in the breath of healthy individuals [209]. EPO also 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 [210]. Although EPO is currently approved for the treatment of anemia, the role of EPO has become far more reaching beyond the need for erythropoiesis in other organs and tissues, such as the brain, heart, and vascular system [45,110,118,211–213]. It is the discovery of EPO and the EPO receptor (EPOR) in the nervous and vascular systems that has resulted in a heightened level of interest and enthusiasm for the potential clinical applications of EPO, such as in Alzheimer's disease, cardiac insufficiency [214,215], and cardiac transplantation [216,217]. In the nervous system, the major sites of EPO production and secretion are in the hippocampus, internal capsule, cortex, midbrain, cerebral ECs, and astrocytes [218,219]. Further work has revealed several other organs as secretory tissues for EPO that include peripheral ECs [220], myoblasts [221], insulin-producing cells [222], and cardiac tissue [133,223].

As a strong cytoprotectant against oxidative, stress EPO can enhance the survival of a number of cells in the nervous system [132,133,224]. In cells that involve the brain or the retina, EPO can prevent injury from hypoxic ischemia [45,110,225–227], excitotoxicity [228,229], infection [230], free radical exposure [61,105,229], amyloid exposure [112], staurosporine [231], and dopaminergic cell injury [232]. In addition, administration of EPO also represents a viable option for the prevention of retinal cell injury during glutamate toxicity [233] and glaucoma [234]. Systemic application of EPO also can improve functional outcome and reduce cell loss during spinal cord injury [235,236], traumatic cerebral edema [237], cortical trauma [238], and epileptic activity [211,239]. In direct relation to the potential protective cerebral effects of EPO, enhanced survival by EPO also extends to afford protection of the neurovascular unit during cerebral vascular disease [17,240]. In addition, EPO can protect sensitive hippocampal neurons from both focal and global ischemic brain injury [227,241]. 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 [242], EPO can improve functional outcome and reduce lipid peroxidation during spinal cord injury [243], and EPO can maintain autoregulation of cerebral blood flow, reverse basilar artery vasoconstriction, and enhance neuronal survival and functional recovery following subarachnoid hemorrhage [244].

EPO also plays a significant role in the cardiovascular system [132,133] and in the renal system [245] to limit injury from oxidative stress that ultimately can affect the function of the nervous system. For example, in patients with anemia, EPO administration can increase left ventricular

ejection fraction and stroke volume [246]. More recent studies have shown that patients with acute myocardial infarction have increased plasma EPO levels within seven days of a cardiac insult, suggesting a possible protective response from the body [247]. In addition, EPO administration in patients with anemia and congestive heart failure can improve exercise tolerance, renal function, and left ventricular systolic function [215,248]. Tightly integrated with cardiac performance, pulmonary function also is believed to be enhanced during EPO administration, especially in the setting of ischemic reperfusion injury of the lung [249]. Serum levels of EPO also may function as a biomarker for cardiovascular injury [250]. Work from experimental studies illustrates that EPO plays a critical role in the vascular and renal systems with the maintenance of erythrocyte [251] and podocyte [252] integrity, regulates the survival of ECs [61,110], and may act as a powerful endogenous protectant during cardiac injury [253].

In light of the fact that antioxidants during elevated glucose concentrations can block free radical production and prevent the production of advanced glycation end-products known to produce reactive oxygen species and oxidative stress during DM [254], EPO may offer an attractive alternative therapy to maintain proper cellular metabolism and mitochondrial membrane potential ($\Delta\Psi_m$) during DM (Figure 1). In clinical studies with DM, plasma EPO is often low in diabetic patients with anemia [255] or without anemia [256]. Furthermore, the failure of these individuals to produce EPO in response to a declining hemoglobin level suggests an impaired EPO response in diabetic patients [257]. Yet, increased EPO secretion during diabetic pregnancies may represent the body's attempt at endogenous protection against the complications of DM [258]. Similar to the potential protective role of insulin [259], 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 [205]. In studies that examine the toxic effects of elevated glucose upon vascular cells, EPO is protective and prevents early apoptotic membrane PS exposure and late DNA degradation at concentrations that are clinically relevant [110] to cellular protection in patients with cardiac or renal disease [260, 261].

Also relevant to cellular metabolism and DM management, cellular protection by EPO is closely tied to Akt and the maintenance of mitochondrial membrane potential ($\Delta\Psi_m$) to prevent cell injury and the subsequent induction of the apoptotic cascades [110,118,211]. Similar to nicotinamide, EPO may require other substrates of the Akt pathway, such as the forkhead transcription factor FOXO3a to prevent cell injury. FOXO3a interfaces with several pathways that regulate cellular lifespan [146] and function to control neoplastic growth [262]. In relation to cell survival, 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 [118].

Cytoprotection by EPO also is mediated through the activation of nuclear factor- κ B (NF- κ B) tied to Akt. NF- κ B proteins are composed of several homo- and heterodimer proteins that can bind to common DNA elements. It is the phosphorylation of I κ B proteins by the I κ B kinase (IKK) and their subsequent degradation that lead to the release of NF- κ B for its translocation to the nucleus to initiate gene transcription [263]. Dependent upon Akt controlled pathways, the transactivation domain of the p65 subunit of NF- κ B is activated by IKK and the IKK κ catalytic subunit to lead to the induction of protective anti-apoptotic pathways [264]. Increased expression of NF- κ B during injury can occur in cells, such as in inflammatory microglial cells [32,112,265] and in neurons [266]. NF- κ B represents a critical pathway that is responsible for the activation of inhibitors of apoptotic proteins (IAPs), the maintenance of Bcl-x_L expression, [27,267], and protection against cell injury during oxidative stress [112]. EPO employs NF-

κ B to prevent apoptosis through the enhanced expression and translocation of NF- κ B to the nucleus to elicit anti-apoptotic gene activation [39,112,268,269].

7. Controversies and challenges for clinical applications

Application of innovative agents and pathways can offer great promise to extend neuronal and vascular cell longevity during conditions such as DM, but treatments that depend upon nicotinamide, Wnt signaling, or EPO may stray from perfection for some patients and ultimately lead to disease progression or other consequences. For example, nicotinamide has been reported to have diverse biological roles that include cellular lifespan reduction. Prolonged exposure to nicotinamide in some studies can lead to impaired β -cell function and reduction in cell growth [270,271]. Nicotinamide also may inhibit P450 and hepatic metabolism [272] and play a role in the progression of Parkinson's disease if cellular compartmentation is abruptly changed [273]. Under other conditions, nicotinamide has been described as an agent that limits cell growth and promotes cell injury. Nicotinamide in the presence of transforming growth factor β -1 can block hepatic cell proliferation and lead to apoptosis with caspase 3 activation [274]. During moderate temperature hyperthermia or carbogen breathing, nicotinamide also can result in enhanced solid tumor radiosensitivity and assist with tumor load reduction [275]. In addition, nicotinamide offers cellular protection in millimole concentrations against oxidative stress, but in relation to cell longevity, lower concentrations of nicotinamide can function as an inhibitor of sirtuins that are necessary for the promotion of increased lifespan in yeast and metazoans [11,276,277]. Interestingly, it has been postulated that sirtuins may prevent nicotinamide from assisting with DNA repair by altering the accessibility of DNA damaged sites for repair enzymes [278]. Given the intimate and inverse relationship with nicotinamide and cell longevity, alternative approaches for the protection of neuronal and vascular cells during DM may be required that may involve the tight modulation of intracellular nicotinamide accumulation.

If one examines the Wnt pathway, Wnt signaling can either facilitate or prevent apoptosis depending upon the environmental stimuli. For example, Wnt proteins can enhance apoptosis within rhombomeres 3 and 5 in the developing hindbrain and in limb buds during vertebrate limb development to control growth of the hindbrain and limbs [279–281]. Wnt signaling also has been closely linked to tumorigenesis for a number of years [44,282]. Furthermore, in studies that involve DM, neuronal disorders, or vascular disease, it is not consistently clear whether mutations in genes of the Wnt pathway or alterations in protein expression of the Wnt pathway components during these disorders confer protective or detrimental effects.

With annual sale revenues in the United States for EPO reported to approach 9 billion dollars [283], adverse effects or lack of efficacy during treatment with EPO are also becoming increasingly evident. Some cardiac injury experimental models do not consistently demonstrate a benefit with EPO [284] and elevated plasma levels of EPO independent of hemoglobin concentration can be associated with increased severity of disease in individuals with congestive heart failure [285] or contribute to vascular stenosis with intima hyperplasia [286]. Other adverse conditions associated with EPO can include increased incidence of thrombotic vascular effects, elevation in mean arterial pressure, and increased metabolic rate and blood viscosity [133,287]. The potential progression of cancer has been another significant concern raised with EPO administration [288,289]. 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 [290], enhance tumor progression, increase metastatic disease, [291], decrease survival in cancer patients [292], and negate the effects of radiotherapy by assisting with tumor angiogenesis [293]. In consideration of the possible tumor promoting ability of EPO [294], 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 [295,296], potential protection against chemotherapy tissue injury [297], and the treatment of cancer-related anemia.

For innovative therapeutic strategies to effectively and safely work against the complications of DM and address effective approaches to extend neuronal and vascular cell longevity, future studies that involve basic and clinical research must carefully and systematically address both the potential benefits and the detriments of new therapies. Critical to this process is the targeted focus upon intricate and often common cellular pathways governed by potential strategies, such as nicotinamide, Wnt signaling, and EPO to overcome the present challenges and controversies of existing or developing therapies. As a result, enthusiasm for new therapeutic agents to preserve neuronal and vascular longevity during debilitating conditions such as DM will continue to grow at an exponential pace and avoid clinical complications to offer patients the best available care.

Acknowledgements

This work was supported by the following grants (KM): American Diabetes Association, American Heart Association (National), Bugher Foundation Award, Janssen Neuroscience Award, LEARN Foundation Award, MI Life Sciences Challenge Award, Nelson Foundation Award, NIH NIEHS (P30 ES06639), and NIH NINDS/NIA.

References

1. Maiese K, Chong ZZ, Shang YC. Mechanistic insights into diabetes mellitus and oxidative stress. *Curr Med Chem* 2007;14:1689–1699. [PubMed: 17584073]
2. Quinn L. Type 2 diabetes: epidemiology, pathophysiology, and diagnosis. *Nurs Clin North Am* 2001;36(2):175–92. v. [PubMed: 11382558]
3. 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(5):1047–53. [PubMed: 15111519]
4. Laakso M. Cardiovascular disease in type 2 diabetes: challenge for treatment and prevention. *J Intern Med* 2001;249(3):225–35. [PubMed: 11285042]
5. Maiese K, Morhan SD, Chong ZZ. Oxidative stress biology and cell injury during type 1 and type 2 diabetes mellitus. *Curr Neurovasc Res* 2007;4(1):63–71. [PubMed: 17311546]
6. Dabelea D, Bell RA, D'Agostino RB Jr, Imperatore G, Johansen JM, Linder B, et al. Incidence of diabetes in youth in the United States. *Jama* 2007;297(24):2716–24. [PubMed: 17595272]
7. Jacobson AM, Musen G, Ryan CM, Silvers N, Cleary P, Waberski B, et al. Long-term effect of diabetes and its treatment on cognitive function. *N Engl J Med* 2007;356(18):1842–52. [PubMed: 17476010]
8. Harris MI, Eastman RC. Early detection of undiagnosed diabetes mellitus: a US perspective. *Diabetes Metab Res Rev* 2000;16(4):230–6. [PubMed: 10934451]
9. Donahoe SM, Stewart GC, McCabe CH, Mohanavelu S, Murphy SA, Cannon CP, et al. Diabetes and mortality following acute coronary syndromes. *JAMA* 2007;298(7):765–75. [PubMed: 17699010]
10. Chong ZZ, Li F, Maiese K. Oxidative stress in the brain: Novel cellular targets that govern survival during neurodegenerative disease. *Prog Neurobiol* 2005;75(3):207–46. [PubMed: 15882775]
11. Li F, Chong ZZ, Maiese K. Cell Life Versus Cell Longevity: The Mysteries Surrounding the NAD (+) Precursor Nicotinamide. *Curr Med Chem* 2006;13(8):883–95. [PubMed: 16611073]
12. Schnaider Beeri M, Goldbourt U, Silverman JM, Noy S, Schmeidler J, Ravona-Springer R, et al. Diabetes mellitus in midlife and the risk of dementia three decades later. *Neurology* 2004;63(10):1902–7. [PubMed: 15557509]
13. Awad N, Gagnon M, Messier C. The relationship between impaired glucose tolerance, type 2 diabetes, and cognitive function. *J Clin Exp Neuropsychol* 2004;26(8):1044–80. [PubMed: 15590460]
14. 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(7):1181–6. [PubMed: 15477535]

15. Beeri MS, Silverman JM, Davis KL, Marin D, Grossman HZ, Schmeidler J, et al. Type 2 diabetes is negatively associated with Alzheimer's disease neuropathology. *J Gerontol A Biol Sci Med Sci* 2005;60(4):471–5. [PubMed: 15933386]
16. 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(7):635–41. [PubMed: 11581097]
17. Maiese K, Chong ZZ. Insights into oxidative stress and potential novel therapeutic targets for Alzheimer disease. *Restor Neurol Neurosci* 2004;22(2):87–104. [PubMed: 15272144]
18. McCormick WC, Hardy J, Kukull WA, Bowen JD, Teri L, Zitzer S, et al. Healthcare utilization and costs in managed care patients with Alzheimer's disease during the last few years of life. *J Am Geriatr Soc* 2001;49(9):1156–60. [PubMed: 11559373]
19. Mendiondo MS, Kryscio RJ, Schmitt FA. Models of progression in AD: predicting disability and costs. *Neurology* 2001;57(6):943–4. [PubMed: 11571313]
20. Pearl, R. The rate of living. University of London Press; London: 1928.
21. Muller FL, Lustgarten MS, Jang Y, Richardson A, Van Remmen H. Trends in oxidative aging theories. *Free Radic Biol Med* 2007;43(4):477–503. [PubMed: 17640558]
22. Yui R, Matsuura ET. Detection of deletions flanked by short direct repeats in mitochondrial DNA of aging *Drosophila*. *Mutat Res* 2006;594(1–2):155–61. [PubMed: 16289600]
23. 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* 2006;3(1):25–39. [PubMed: 16472123]
24. De Felice FG, Velasco PT, Lambert MP, Viola K, Fernandez SJ, Ferreira ST, et al. 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(15):11590–601. [PubMed: 17308309]
25. 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(3):262–75. [PubMed: 11295881]
26. Cohen SM, Cordeiro-Stone M, Kaufman DG. Early replication and the apoptotic pathway. *J Cell Physiol* 2007;213(2):434–9. [PubMed: 17520690]
27. 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* 2005;49(1):1–21. [PubMed: 15960984]
28. Harris SE, Fox H, Wright AF, Hayward C, Starr JM, Whalley LJ, et al. 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]
29. Leuner K, Hauptmann S, Abdel-Kader R, Scherping I, Keil U, Strosznajder JB, et al. Mitochondrial dysfunction: the first domino in brain aging and Alzheimer's disease? *Antioxid Redox Signal* 2007;9(10):1659–75. [PubMed: 17867931]
30. Okouchi M, Ekshyyan O, Maracine M, Aw TY. Neuronal apoptosis in neurodegeneration. *Antioxid Redox Signal* 2007;9(8):1059–96. [PubMed: 17571960]
31. 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* 2004;296(2):196–207. [PubMed: 15149850]
32. 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* 2007;19(2):263–72. [PubMed: 17203200]
33. Kang JQ, Chong ZZ, Maiese K. Critical role for Akt1 in the modulation of apoptotic phosphatidylserine exposure and microglial activation. *Mol Pharmacol* 2003;64(3):557–69. [PubMed: 12920191]
34. Karunakaran S, Diwakar L, Saeed U, Agarwal V, Ramakrishnan S, Iyengar S, et al. 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 alpha-lipoic acid. *Faseb J* 2007;21(9):2226–36. [PubMed: 17369508]

35. 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(7):390–9. [PubMed: 17178208]
36. 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(2):257–272. [PubMed: 11020218]
37. 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(5):733–9. [PubMed: 14985868]
38. 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* 2005;2(3):197–211. [PubMed: 16181114]
39. 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* 2006;3(3):187–201. [PubMed: 16918383]
40. Chong ZZ, Kang JQ, Maiese K. Metabotropic glutamate receptors promote neuronal and vascular plasticity through novel intracellular pathways. *Histol Histopathol* 2003;18(1):173–89. [PubMed: 12507297]
41. 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* 2003;74(1):37–51. [PubMed: 13130504]
42. Maiese K, Chong ZZ. Nicotinamide: necessary nutrient emerges as a novel cytoprotectant for the brain. *Trends Pharmacol Sci* 2003;24(5):228–32. [PubMed: 12767721]
43. Mallat M, Marin-Teva JL, Cheret C. Phagocytosis in the developing CNS: more than clearing the corpses. *Curr Opin Neurobiol* 2005;15(1):101–7. [PubMed: 15721751]
44. Li F, Chong ZZ, Maiese K. Winding through the WNT pathway during cellular development and demise. *Histol Histopathol* 2006;21(1):103–24. [PubMed: 16267791]
45. 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* 2003;138(6):1107–1118. [PubMed: 12684267]
46. Maiese K. The dynamics of cellular injury: transformation into neuronal and vascular protection. *Histol Histopathol* 2001;16(2):633–44. [PubMed: 11332719]
47. 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(3):440–9. [PubMed: 7714002]
48. Vincent AM, Maiese K. Direct temporal analysis of apoptosis induction in living adherent neurons. *J Histochem Cytochem* 1999;47(5):661–72. [PubMed: 10219058]
49. 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* 2003;23(4–5):561–78. [PubMed: 14514016]
50. Maiese K, TenBroeke M, Kue I. Neuroprotection of lubeluzole is mediated through the signal transduction pathways of nitric oxide. *J Neurochem* 1997;68(2):710–4. [PubMed: 9003060]
51. 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(16):13898–904. [PubMed: 12578834]
52. Leytin V, Allen DJ, Mykhaylov S, Lyubimov E, Freedman J. Thrombin-triggered platelet apoptosis. *J Thromb Haemost* 2006;4(12):2656–63. [PubMed: 16961585]
53. 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]
54. Maiese K, Vincent AM. Critical temporal modulation of neuronal programmed cell injury. *Cell Mol Neurobiol* 2000;20(3):383–400. [PubMed: 10789835]

55. Maiese K, Vincent AM. Membrane asymmetry and DNA degradation: functionally distinct determinants of neuronal programmed cell death. *J Neurosci Res* 2000;59(4):568–80. [PubMed: 10679797]
56. 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* 2004;6(2):277–87. [PubMed: 15025929]
57. 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* 2007;22(11):1251–67. [PubMed: 17647198]
58. Vincent AM, Maiese K. Nitric oxide induction of neuronal endonuclease activity in programmed cell death. *Exp Cell Res* 1999;246(2):290–300. [PubMed: 9925743]
59. 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* 1999;155(1):79–94. [PubMed: 9918707]
60. 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(4):2571–5. [PubMed: 11073947]
61. 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* 2003;23(3):320–30. [PubMed: 12621307]
62. 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(9):1380–91. [PubMed: 10994860]
63. Chong ZZ, Lin SH, Li F, Maiese K. The sirutin inhibitor nicotinamide enhances neuronal cell survival during acute anoxic injury through Akt, Bad, PARP, and mitochondrial associated “anti-apoptotic” pathways. *Curr Neurovasc Res* 2005;2(4):271–85. [PubMed: 16181120]
64. Li F, Chong ZZ, Maiese K. Navigating novel mechanisms of cellular plasticity with the NAD⁺ precursor and nutrient nicotinamide. *Front Biosci* 2004;9:2500–2520. [PubMed: 15353303]
65. Wilson FH, Hariri A, Farhi A, Zhao H, Petersen KF, Toka HR, et al. A cluster of metabolic defects caused by mutation in a mitochondrial tRNA. *Science* 2004;306(5699):1190–4. [PubMed: 15498972]
66. 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(5):560–6. [PubMed: 9183294]
67. Cardella F. Insulin therapy during diabetic ketoacidosis in children. *Acta Biomed* 2005;76(Suppl 3):49–54. [PubMed: 16915797]
68. Kratzsch J, Knerr I, Galler A, Kapellen T, Raile K, Korner A, et al. Metabolic decompensation in children with type 1 diabetes mellitus associated with increased serum levels of the soluble leptin receptor. *Eur J Endocrinol* 2006;155(4):609–14. [PubMed: 16990661]
69. 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(9):2303–11. [PubMed: 9142009]
70. Vincent AM, TenBroeke M, Maiese K. Neuronal intracellular pH directly mediates nitric oxide-induced programmed cell death. *J Neurobiol* 1999;40(2):171–84. [PubMed: 10413448]
71. 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(4):193–7. [PubMed: 15207835]
72. Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, et al. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *JAMA* 2006;295(14):1681–7. [PubMed: 16609090]
73. Yano M, Hasegawa G, Ishii M, Yamasaki M, Fukui M, Nakamura N, et al. 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(2):111–6. [PubMed: 15231066]

74. Newsholme P, Haber EP, Hirabara SM, Rebelato EL, Procopio J, Morgan D, et al. Diabetes associated cell stress and dysfunction: role of mitochondrial and non-mitochondrial ROS production and activity. *J Physiol* 2007;583(Pt 1):9–24. [PubMed: 17584843]
75. Rachek LI, Thornley NP, Grishko VI, LeDoux SP, Wilson GL. Protection of INS-1 cells from free fatty acid-induced apoptosis by targeting hOGG1 to mitochondria. *Diabetes* 2006;55(4):1022–8. [PubMed: 16567524]
76. Choo HJ, Kim JH, Kwon OB, Lee CS, Mun JY, Han SS, et al. Mitochondria are impaired in the adipocytes of type 2 diabetic mice. *Diabetologia* 2006;49(4):784–91. [PubMed: 16501941]
77. Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, et al. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science* 2003;300(5622):1140–2. [PubMed: 12750520]
78. Pospisilik JA, Knauf C, Joza N, Benit P, Orthofer M, Cani PD, et al. Targeted deletion of AIF decreases mitochondrial oxidative phosphorylation and protects from obesity and diabetes. *Cell* 2007;131(3):476–91. [PubMed: 17981116]
79. Olmos PR, Hodgson MI, Maiz A, Manrique M, De Valdes MD, Foncea R, et al. Nicotinamide protected first-phase insulin response (FPIR) and prevented clinical disease in first-degree relatives of type-1 diabetics. *Diabetes Res Clin Pract.* 2005
80. Crino A, Schiaffini R, Ciampalini P, Suraci MC, Manfrini S, Visalli N, et al. A two year observational study of nicotinamide and intensive insulin therapy in patients with recent onset type 1 diabetes mellitus. *J Pediatr Endocrinol Metab* 2005;18(8):749–54. [PubMed: 16200840]
81. Eto N, Miyata Y, Ohno H, Yamashita T. Nicotinamide prevents the development of hyperphosphataemia by suppressing intestinal sodium-dependent phosphate transporter in rats with adenine-induced renal failure. *Nephrol Dial Transplant* 2005;20(7):1378–84. [PubMed: 15870221]
82. Reddy S, Bibby NJ, Wu D, Swinney C, Barrow G, Elliott RB. A combined casein-free-nicotinamide diet prevents diabetes in the NOD mouse with minimum insulinitis. *Diabetes Res Clin Pract* 1995;29(2):83–92. [PubMed: 8591703]
83. Hu Y, Wang Y, Wang L, Zhang H, Zhao B, Zhang A, et al. Effects of nicotinamide on prevention and treatment of streptozotocin-induced diabetes mellitus in rats. *Chin Med J (Engl)* 1996;109(11):819–22. [PubMed: 9275363]
84. Stevens MJ, Li F, Drel VR, Abatan OI, Kim H, Burnett D, et al. Nicotinamide reverses neurological and neurovascular deficits in streptozotocin diabetic rats. *J Pharmacol Exp Ther* 2007;320(1):458–64. [PubMed: 17021258]
85. Cresto JC, Fabiano de Bruno LE, Cao GF, Pastorale CF, Confalonieri N, del Carmen Camberos M, et al. The association of acetyl-L-carnitine and nicotinamide remits the experimental diabetes in mice by multiple low-dose streptozotocin. *Pancreas* 2006;33(4):403–11. [PubMed: 17079947]
86. Chlopicki S, Swies J, Mogielnicki A, Buczek W, Bartus M, Lomnicka M, et al. 1-Methylnicotinamide (MNA), a primary metabolite of nicotinamide, exerts anti-thrombotic activity mediated by a cyclooxygenase-2/prostacyclin pathway. *Br J Pharmacol* 2007;152(2):230–9. [PubMed: 17641676]
87. Chong ZZ, Lin SH, Maiese K. Nicotinamide Modulates Mitochondrial Membrane Potential and Cysteine Protease Activity during Cerebral Vascular Endothelial Cell Injury. *J Vasc Res* 2002;39(2):131–47. [PubMed: 12011585]
88. 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(34):24574–82. [PubMed: 17604275]
89. Ieraci A, Herrera DG. Nicotinamide Protects against Ethanol-Induced Apoptotic Neurodegeneration in the Developing Mouse Brain. *PLoS Med* 2006;3(4):e101. [PubMed: 16478293]
90. Aoyagi S, Archer TK. Nicotinamide uncouples hormone-dependent chromatin remodeling from transcription complex assembly. *Mol Cell Biol* 2008;28(1):30–9. [PubMed: 17954562]
91. Anderson DW, Bradbury KA, Schneider JS. Neuroprotection in Parkinson models varies with toxin administration protocol. *Eur J Neurosci* 2006;24(11):3174–82. [PubMed: 17156378]
92. 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* 2004;24(7):728–43. [PubMed: 15241181]

93. Feng Y, Paul IA, LeBlanc MH. Nicotinamide reduces hypoxic ischemic brain injury in the newborn rat. *Brain Res Bull* 2006;69(2):117–22. [PubMed: 16533659]
94. Hoane MR, Gilbert DR, Holland MA, Pierce JL. Nicotinamide reduces acute cortical neuronal death and edema in the traumatically injured brain. *Neurosci Lett* 2006;408(1):35–9. [PubMed: 16987607]
95. Hoane MR, Kaplan SA, Ellis AL. The effects of nicotinamide on apoptosis and blood-brain barrier breakdown following traumatic brain injury. *Brain Res* 2006;1125(1):185–93. [PubMed: 17109832]
96. Giulumian AD, Meszaros LG, Fuchs LC. Endothelin-1-induced contraction of mesenteric small arteries is mediated by ryanodine receptor Ca²⁺ channels and cyclic ADP-ribose. *J Cardiovasc Pharmacol* 2000;36(6):758–63. [PubMed: 11117376]
97. Oumouna-Benachour K, Hans CP, Suzuki Y, Naura A, Datta R, Belmadani S, et al. Poly(ADP-ribose) polymerase inhibition reduces atherosclerotic plaque size and promotes factors of plaque stability in apolipoprotein E-deficient mice: effects on macrophage recruitment, nuclear factor-kappaB nuclear translocation, and foam cell death. *Circulation* 2007;115(18):2442–50. [PubMed: 17438151]
98. Giammona LM, Fuhrken PG, Papoutsakis ET, Miller WM. Nicotinamide (vitamin B3) increases the polyploidisation and proplatelet formation of cultured primary human megakaryocytes. *Br J Haematol* 2006;135(4):554–66. [PubMed: 17054670]
99. Lin SH, Chong ZZ, Maiese K. Nicotinamide: A Nutritional Supplement that Provides Protection Against Neuronal and Vascular Injury. *J Med Food* 2001;4(1):27–38. [PubMed: 12639285]
100. Autor AP, Bonham AC, Thies RL. Toxicity of oxygen radicals in cultured pulmonary endothelial cells. *J Toxicol Environ Health* 1984;13(2–3):387–95. [PubMed: 6234402]
101. Sadanaga-Akiyoshi F, Yao H, Tanuma S, Nakahara T, Hong JS, Ibayashi S, et al. Nicotinamide attenuates focal ischemic brain injury in rats: with special reference to changes in nicotinamide and NAD⁺ levels in ischemic core and penumbra. *Neurochem Res* 2003;28(8):1227–34. [PubMed: 12834263]
102. Bowes J, Piper J, Thiernemann C. Inhibitors of the activity of poly (ADP-ribose) synthetase reduce the cell death caused by hydrogen peroxide in human cardiac myoblasts. *Br J Pharmacol* 1998;124(8):1760–6. [PubMed: 9756394]
103. Cox MJ, Sood HS, Hunt MJ, Chandler D, Henegar JR, Aru GM, et al. Apoptosis in the left ventricle of chronic volume overload causes endocardial endothelial dysfunction in rats. *Am J Physiol Heart Circ Physiol* 2002;282(4):H1197–205. [PubMed: 11893552]
104. Sensi SL, Jeng JM. Rethinking the excitotoxic ionic milieu: the emerging role of zn(2+) in ischemic neuronal injury. *Curr Mol Med* 2004;4(2):87–111. [PubMed: 15032707]
105. 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* 2003;71(5):659–69. [PubMed: 12584724]
106. Miki T, Miura T, Yano T, Takahashi A, Sakamoto J, Tanno M, et al. Alteration in erythropoietin-induced cardioprotective signaling by postinfarct ventricular remodeling. *J Pharmacol Exp Ther* 2006;317(1):68–75. [PubMed: 16377761]
107. Parsa CJ, Matsumoto A, Kim J, Riel RU, Pascal LS, Walton GB, et al. A novel protective effect of erythropoietin in the infarcted heart. *J Clin Invest* 2003;112(7):999–1007. [PubMed: 14523037]
108. Matsuzaki H, Tamatani M, Mitsuda N, Namikawa K, Kiyama H, Miyake S, et al. 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(5):2037–46. [PubMed: 10537063]
109. Dzielko M, Felderhoff-Mueser U, Siffringer M, Krutz B, Bittigau P, Thor F, et al. Erythropoietin protects the developing brain against N-methyl-D-aspartate receptor antagonist neurotoxicity. *Neurobiol Dis* 2004;15(2):177–87. [PubMed: 15006687]
110. Chong ZZ, Kang JQ, Maiese K. Erythropoietin is a novel vascular protectant through activation of Akt1 and mitochondrial modulation of cysteine proteases. *Circulation* 2002;106(23):2973–9. [PubMed: 12460881]
111. 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(6):H2573–81. [PubMed: 17337592]

112. 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* 2005;2(5):387–99. [PubMed: 16375720]
113. Du B, Ohmichi M, Takahashi K, Kawagoe J, Ohshima C, Igarashi H, et al. Both estrogen and raloxifene protect against beta-amyloid-induced neurotoxicity in estrogen receptor alpha-transfected PC12 cells by activation of telomerase activity via Akt cascade. *J Endocrinol* 2004;183(3):605–15. [PubMed: 15590986]
114. Nakagami Y, Nishimura S, Murasugi T, Kubo T, Kaneko I, Meguro M, et al. A novel compound RS-0466 reverses beta-amyloid-induced cytotoxicity through the Akt signaling pathway in vitro. *Eur J Pharmacol* 2002;457(1):11–7. [PubMed: 12460638]
115. 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(3):834–41. [PubMed: 11468186]
116. Chong ZZ, Li F, Maiese K. Group I Metabotropic Receptor Neuroprotection Requires Akt and Its Substrates that Govern FOXO3a, Bim, and beta-Catenin During Oxidative Stress. *Curr Neurovasc Res* 2006;3(2):107–17. [PubMed: 16719794]
117. Maiese K, Chong ZZ, Li F. Driving cellular plasticity and survival through the signal transduction pathways of metabotropic glutamate receptors. *Curr Neurovasc Res* 2005;2(5):425–46. [PubMed: 16375723]
118. 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* 2007;150(7):839–50. [PubMed: 17339844]
119. Reddy S, Young M, Ginn S. Immunoexpression of interleukin-1beta in pancreatic islets of NOD mice during cyclophosphamide-accelerated diabetes: co-localization in macrophages and endocrine cells and its attenuation with oral nicotinamide. *Histochem J* 2001;33(6):317–27. [PubMed: 11758808]
120. Chen CF, Wang D, Hwang CP, Liu HW, Wei J, Lee RP, et al. The protective effect of niacinamide on ischemia-reperfusion-induced liver injury. *J Biomed Sci* 2001;8(6):446–52. [PubMed: 11702007]
121. Moberg L, Olsson A, Berne C, Felldin M, Foss A, Kallen R, et al. Nicotinamide inhibits tissue factor expression in isolated human pancreatic islets: implications for clinical islet transplantation. *Transplantation* 2003;76(9):1285–8. [PubMed: 14627904]
122. Ungerstedt JS, Blomback M, Soderstrom T. Nicotinamide is a potent inhibitor of proinflammatory cytokines. *Clin Exp Immunol* 2003;131(1):48–52. [PubMed: 12519385]
123. Fukuzawa M, Satoh J, Muto G, Muto Y, Nishimura S, Miyaguchi S, et al. Inhibitory effect of nicotinamide on in vitro and in vivo production of tumor necrosis factor-alpha. *Immunol Lett* 1997;59(1):7–11. [PubMed: 9334851]
124. Hiromatsu Y, Sato M, Yamada K, Nonaka K. Inhibitory effects of nicotinamide on recombinant human interferon- gamma-induced intercellular adhesion molecule-1 (ICAM-1) and HLA-DR antigen expression on cultured human endothelial cells. *Immunol Lett* 1992;31(1):35–9. [PubMed: 1347751]
125. Kaneko S, Wang J, Kaneko M, Yiu G, Hurrell JM, Chitnis T, et al. Protecting axonal degeneration by increasing nicotinamide adenine dinucleotide levels in experimental autoimmune encephalomyelitis models. *J Neurosci* 2006;26(38):9794–804. [PubMed: 16988050]
126. Soop A, Albert J, Weitzberg E, Bengtsson A, Nilsson CG, Sollevi A. Nicotinamide does not influence cytokines or exhaled NO in human experimental endotoxaemia. *Clin Exp Immunol* 2004;135(1):114–8. [PubMed: 14678271]
127. Cuesta I, Zaret KS, Santisteban P. The forkhead factor FoxE1 binds to the thyroperoxidase promoter during thyroid cell differentiation and modifies compacted chromatin structure. *Mol Cell Biol* 2007;27(20):7302–14. [PubMed: 17709379]
128. Maiese K, Chong ZZ, Shang YC. Sly as a FOXO”: New paths with Forkhead signaling in the brain. *Curr Neurovasc Res* 2007;4(4):295–302. [PubMed: 18045156]
129. 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(6436):412–20. [PubMed: 8332212]

130. 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(17):6179–87. [PubMed: 9558357]
131. Larson ET, Eilers B, Menon S, Reiter D, Ortmann A, Young MJ, et al. A winged-helix protein from *Sulfolobus turreted* icosahedral virus points toward stabilizing disulfide bonds in the intracellular proteins of a hyperthermophilic virus. *Virology*. 2007
132. Maiese K, Li F, Chong ZZ. Erythropoietin in the brain: can the promise to protect be fulfilled? *Trends Pharmacol Sci* 2004;25(11):577–583. [PubMed: 15491780]
133. Maiese K, Li F, Chong ZZ. New avenues of exploration for erythropoietin. *JAMA* 2005;293(1):90–5. [PubMed: 15632341]
134. Kim JR, Jung HS, Bae SW, Kim JH, Park BL, Choi YH, et al. Polymorphisms in FOXO gene family and association analysis with BMI. *Obesity (Silver Spring)* 2006;14(2):188–93. [PubMed: 16571842]
135. Kuningas M, Magi R, Westendorp RG, Slagboom PE, Remm M, van Heemst D. Haplotypes in the human Foxo1a and Foxo3a genes; impact on disease and mortality at old age. *Eur J Hum Genet* 2007;15(3):294–301. [PubMed: 17245409]
136. Kino T, De Martino MU, Charmandari E, Ichijo T, Outas T, Chrousos GP. HIV-1 accessory protein Vpr inhibits the effect of insulin on the Foxo subfamily of forkhead transcription factors by interfering with their binding to 14-3-3 proteins: potential clinical implications regarding the insulin resistance of HIV-1-infected patients. *Diabetes* 2005;54(1):23–31. [PubMed: 15616007]
137. Relling DP, Esberg LB, Fang CX, Johnson WT, Murphy EJ, Carlson EC, et al. High-fat diet-induced juvenile obesity leads to cardiomyocyte dysfunction and upregulation of Foxo3a transcription factor independent of lipotoxicity and apoptosis. *J Hypertens* 2006;24(3):549–61. [PubMed: 16467659]
138. Fallarino F, Bianchi R, Orabona C, Vacca C, Belladonna ML, Fioretti MC, et al. CTLA-4-Ig activates forkhead transcription factors and protects dendritic cells from oxidative stress in nonobese diabetic mice. *J Exp Med* 2004;200(8):1051–62. [PubMed: 15492127]
139. Kamei Y, Miura S, Suzuki M, Kai Y, Mizukami J, Taniguchi T, et al. Skeletal muscle FOXO1 (FKHR) transgenic mice have less skeletal muscle mass, down-regulated Type I (slow twitch/red muscle) fiber genes, and impaired glycemic control. *J Biol Chem* 2004;279(39):41114–23. [PubMed: 15272020]
140. Kato M, Yuan H, Xu ZG, Lanting L, Li SL, Wang M, et al. Role of the Akt/FoxO3a pathway in TGF-beta1-mediated mesangial cell dysfunction: a novel mechanism related to diabetic kidney disease. *J Am Soc Nephrol* 2006;17(12):3325–35. [PubMed: 17082237]
141. Anitha M, Gondha C, Sutliff R, Parsadianian A, Mwangi S, Sitaraman SV, et al. GDNF rescues hyperglycemia-induced diabetic enteric neuropathy through activation of the PI3K/Akt pathway. *J Clin Invest* 2006;116(2):344–56. [PubMed: 16453021]
142. Kwon HS, Huang B, Unterman TG, Harris RA. Protein kinase B-alpha inhibits human pyruvate dehydrogenase kinase-4 gene induction by dexamethasone through inactivation of FOXO transcription factors. *Diabetes* 2004;53(4):899–910. [PubMed: 15047604]
143. Brunet A, Kanai F, Stehn J, Xu J, Sarbassova D, Frangioni JV, et al. 14-3-3 transits to the nucleus and participates in dynamic nucleocytoplasmic transport. *J Cell Biol* 2002;156(5):817–28. [PubMed: 11864996]
144. Dong S, Kang S, Gu TL, Kardar S, Fu H, Lonial S, et al. 14-3-3 integrates prosurvival signals mediated by the AKT and MAPK pathways in ZNF198-FGFR1-transformed hematopoietic cells. *Blood* 2007;110(1):360–9. [PubMed: 17389761]
145. Munoz-Fontela C, Marcos-Villar L, Gallego P, Arroyo J, Da Costa M, Pomeranz KM, et al. Latent protein LANA2 from Kaposi's sarcoma-associated herpesvirus interacts with 14-3-3 proteins and inhibits FOXO3a transcription factor. *J Virol* 2007;81(3):1511–6. [PubMed: 17108038]
146. Lehtinen MK, Yuan Z, Boag PR, Yang Y, Villen J, Becker EB, et al. A conserved MST-FOXO signaling pathway mediates oxidative-stress responses and extends life span. *Cell* 2006;125(5):987–1001. [PubMed: 16751106]
147. Zheng WH, Kar S, Quirion R. FKHRL1 and its homologs are new targets of nerve growth factor Trk receptor signaling. *J Neurochem* 2002;80(6):1049–61. [PubMed: 11953455]

148. Charvet C, Alberti I, Luciano F, Jacquel A, Bernard A, Auberger P, et al. Proteolytic regulation of Forkhead transcription factor FOXO3a by caspase-3-like proteases. *Oncogene* 2003;22(29):4557–68. [PubMed: 12881712]
149. 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* 2007;19(6):1150–62. [PubMed: 17289346]
150. Chong ZZ, Shang YC, Maiese K. Vascular injury during elevated glucose can be mitigated by erythropoietin and Wnt signaling. *Curr Neurovasc Res* 2007;4(3):194–204. [PubMed: 17691973]
151. Speese SD, Budnik V. Wnts: up-and-coming at the synapse. *Trends Neurosci* 2007;30(6):268–75. [PubMed: 17467065]
152. 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(2):495–504. [PubMed: 15024710]
153. Li F, Chong ZZ, Maiese K. Vital elements of the wnt-frizzled signaling pathway in the nervous system. *Curr Neurovasc Res* 2005;2(4):331–40. [PubMed: 16181124]
154. Mao J, Wang J, Liu B, Pan W, Farr GH 3rd, Flynn C, et al. Low-density lipoprotein receptor-related protein-5 binds to Axin and regulates the canonical Wnt signaling pathway. *Mol Cell* 2001;7(4):801–9. [PubMed: 11336703]
155. Patapoutian A, Reichardt LF. Roles of Wnt proteins in neural development and maintenance. *Curr Opin Neurobiol* 2000;10(3):392–9. [PubMed: 10851180]
156. Salinas PC. Wnt factors in axonal remodelling and synaptogenesis. *Biochem Soc Symp* 1999;65:101–9. [PubMed: 10320935]
157. Augustine K, Liu ET, Sadler TW. Antisense attenuation of Wnt-1 and Wnt-3a expression in whole embryo culture reveals roles for these genes in craniofacial, spinal cord, and cardiac morphogenesis. *Dev Genet* 1993;14(6):500–20. [PubMed: 8111977]
158. Ikeya M, Lee SM, Johnson JE, McMahon AP, Takada S. Wnt signalling required for expansion of neural crest and CNS progenitors. *Nature* 1997;389(6654):966–70. [PubMed: 9353119]
159. Marvin MJ, Di Rocco G, Gardiner A, Bush SM, Lassar AB. Inhibition of Wnt activity induces heart formation from posterior mesoderm. *Genes Dev* 2001;15(3):316–27. [PubMed: 11159912]
160. Naito AT, Shiojima I, Akazawa H, Hidaka K, Morisaki T, Kikuchi A, et al. Developmental stage-specific biphasic roles of Wnt/beta-catenin signaling in cardiomyogenesis and hematopoiesis. *Proc Natl Acad Sci U S A* 2006;103(52):19812–7. [PubMed: 17170140]
161. Palpant NJ, Yasuda S, MacDougald O, Metzger JM. Non-canonical Wnt signaling enhances differentiation of Sca1+/c-kit+ adipose-derived murine stromal vascular cells into spontaneously beating cardiac myocytes. *J Mol Cell Cardiol* 2007;43(3):362–70. [PubMed: 17706246]
162. Singh AM, Li FQ, Hamazaki T, Kasahara H, Takemaru K, Terada N. Chibby, an antagonist of the Wnt/beta-catenin pathway, facilitates cardiomyocyte differentiation of murine embryonic stem cells. *Circulation* 2007;115(5):617–26. [PubMed: 17261658]
163. Kengaku M, Twombly V, Tabin C. Expression of Wnt and Frizzled genes during chick limb bud development. *Cold Spring Harb Symp Quant Biol* 1997;62:421–9. [PubMed: 9598377]
164. Stark K, Vainio S, Vassileva G, McMahon AP. Epithelial transformation of metanephric mesenchyme in the developing kidney regulated by Wnt-4. *Nature* 1994;372(6507):679–83. [PubMed: 7990960]
165. Liu P, Wakamiya M, Shea MJ, Albrecht U, Behringer RR, Bradley A. Requirement for Wnt3 in vertebrate axis formation. *Nat Genet* 1999;22(4):361–5. [PubMed: 10431240]
166. Jordan BK, Mohammed M, Ching ST, Delot E, Chen XN, Dewing P, et al. Up-regulation of WNT-4 signaling and dosage-sensitive sex reversal in humans. *Am J Hum Genet* 2001;68(5):1102–9. [PubMed: 11283799]
167. Rodova M, Islam MR, Maser RL, Calvet JP. The polycystic kidney disease-1 promoter is a target of the beta-catenin/T-cell factor pathway. *J Biol Chem* 2002;277(33):29577–83. [PubMed: 12048202]
168. Niemann S, Zhao C, Pascu F, Stahl U, Aulepp U, Niswander L, et al. Homozygous WNT3 mutation causes tetra-amelia in a large consanguineous family. *Am J Hum Genet* 2004;74(3):558–63. [PubMed: 14872406]

169. Bakre MM, Hoi A, Mong JC, Koh YY, Wong KY, Stanton LW. Generation of multipotential mesendodermal progenitors from mouse embryonic stem cells via sustained Wnt pathway activation. *J Biol Chem* 2007;282(43):31703–12. [PubMed: 17711862]
170. Canalis E, Giustina A, Bilezikian JP. Mechanisms of anabolic therapies for osteoporosis. *N Engl J Med* 2007;357(9):905–16. [PubMed: 17761594]
171. Ross SE, Hemati N, Longo KA, Bennett CN, Lucas PC, Erickson RL, et al. Inhibition of adipogenesis by Wnt signaling. *Science* 2000;289(5481):950–3. [PubMed: 10937998]
172. Willert K, Brown JD, Danenberg E, Duncan AW, Weissman IL, Reya T, et al. Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature* 2003;423(6938):448–52. [PubMed: 12717451]
173. Reya T, Duncan AW, Ailles L, Domen J, Scherer DC, Willert K, et al. A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature* 2003;423(6938):409–14. [PubMed: 12717450]
174. He XC, Zhang J, Tong WG, Tawfik O, Ross J, Scoville DH, et al. BMP signaling inhibits intestinal stem cell self-renewal through suppression of Wnt-beta-catenin signaling. *Nat Genet* 2004;36(10):1117–21. [PubMed: 15378062]
175. Liu H, Fergusson MM, Castilho RM, Liu J, Cao L, Chen J, et al. Augmented Wnt signaling in a mammalian model of accelerated aging. *Science* 2007;317(5839):803–6. [PubMed: 17690294]
176. Soriano S, Kang DE, Fu M, Pestell R, Chevallier N, Zheng H, et al. Presenilin 1 negatively regulates beta-catenin/T cell factor/lymphoid enhancer factor-1 signaling independently of beta-amyloid precursor protein and notch processing. *J Cell Biol* 2001;152(4):785–94. [PubMed: 11266469]
177. Marambaud P, Shioi J, Serban G, Georgakopoulos A, Sarner S, Nagy V, et al. A presenilin-1/gamma-secretase cleavage releases the E-cadherin intracellular domain and regulates disassembly of adherens junctions. *Embo J* 2002;21(8):1948–56. [PubMed: 11953314]
178. Morin PJ, Medina M, Semenov M, Brown AM, Kosik KS. Wnt-1 expression in PC12 cells induces exon 15 deletion and expression of L-APP. *Neurobiol Dis* 2004;16(1):59–67. [PubMed: 15207262]
179. 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(11):1226–35. [PubMed: 16568235]
180. Barandon L, Couffignal T, Ezan J, Dufourcq P, Costet P, Alzieu P, et al. Reduction of infarct size and prevention of cardiac rupture in transgenic mice overexpressing FrzA. *Circulation* 2003;108(18):2282–9. [PubMed: 14581414]
181. Barandon L, Dufourcq P, Costet P, Moreau C, Allieres C, Daret D, et al. Involvement of FrzA/sFRP-1 and the Wnt/frizzled pathway in ischemic preconditioning. *Circ Res* 2005;96(12):1299–306. [PubMed: 15920021]
182. van de Schans VA, van den Borne SW, Strzelecka AE, Janssen BJ, van der Velden JL, Langen RC, et al. Interruption of Wnt signaling attenuates the onset of pressure overload-induced cardiac hypertrophy. *Hypertension* 2007;49(3):473–80. [PubMed: 17210832]
183. Chen S, Guttridge DC, You Z, Zhang Z, Fribley A, Mayo MW, et al. Wnt-1 signaling inhibits apoptosis by activating beta-catenin/T cell factor-mediated transcription. *J Cell Biol* 2001;152(1):87–96. [PubMed: 11149923]
184. You Z, Saims D, Chen S, Zhang Z, Guttridge DC, Guan KL, et al. Wnt signaling promotes oncogenic transformation by inhibiting c-Myc-induced apoptosis. *J Cell Biol* 2002;157(3):429–40. [PubMed: 11980918]
185. Fukumoto S, Hsieh CM, Maemura K, Layne MD, Yet SF, Lee KH, et al. Akt participation in the Wnt signaling pathway through Dishevelled. *J Biol Chem* 2001;276(20):17479–83. [PubMed: 11278246]
186. Naito AT, Akazawa H, Takano H, Minamino T, Nagai T, Aburatani H, et al. Phosphatidylinositol 3-kinase-Akt pathway plays a critical role in early cardiomyogenesis by regulating canonical Wnt signaling. *Circ Res* 2005;97(2):144–51. [PubMed: 15994435]
187. Mirotsov M, Zhang Z, Deb A, Zhang L, Gneccchi M, Noisieux N, et al. Secreted frizzled related protein 2 (Sfrp2) is the key Akt-mesenchymal stem cell-released paracrine factor mediating myocardial survival and repair. *Proc Natl Acad Sci U S A* 2007;104(5):1643–8. [PubMed: 17251350]

188. Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, et al. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet* 2006;38(3):320–3. [PubMed: 16415884]
189. Lehman DM, Hunt KJ, Leach RJ, Hamlington J, Arya R, Abboud HE, et al. 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(2):389–93. [PubMed: 17259383]
190. Scott LJ, Bonnycastle LL, Willer CJ, Sprau AG, Jackson AU, Narisu N, et al. Association of transcription factor 7-like 2 (TCF7L2) variants with type 2 diabetes in a Finnish sample. *Diabetes* 2006;55(9):2649–53. [PubMed: 16936217]
191. Guo YF, Xiong DH, Shen H, Zhao LJ, Xiao P, Guo Y, et al. 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(10):798–803. [PubMed: 16723389]
192. Kanazawa A, Tsukada S, Sekine A, Tsunoda T, Takahashi A, Kashiwagi A, et al. Association of the gene encoding wingless-type mammary tumor virus integration-site family member 5B (WNT5B) with type 2 diabetes. *Am J Hum Genet* 2004;75(5):832–43. [PubMed: 15386214]
193. Mani A, Radhakrishnan J, Wang H, Mani A, Mani MA, Nelson-Williams C, et al. LRP6 mutation in a family with early coronary disease and metabolic risk factors. *Science* 2007;315(5816):1278–82. [PubMed: 17332414]
194. Al-Aly Z, Shao JS, Lai CF, Huang E, Cai J, Behrmann A, et al. Aortic Msx2-Wnt calcification cascade is regulated by TNF-alpha-dependent signals in diabetic Ldlr-/- mice. *Arterioscler Thromb Vasc Biol* 2007;27(12):2589–96. [PubMed: 17932314]
195. Wright WS, Longo KA, Dolinsky VW, Gerin I, Kang S, Bennett CN, et al. Wnt10b Inhibits Obesity in ob/ob and Agouti Mice. *Diabetes* 2007;56(2):295–303. [PubMed: 17259372]
196. 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(10):2812–20. [PubMed: 16943306]
197. Aslanidi G, Kroutov V, Philipsberg G, Lamb K, Campbell-Thompson M, Walter GA, et al. Ectopic expression of Wnt10b decreases adiposity and improves glucose homeostasis in obese rats. *Am J Physiol Endocrinol Metab* 2007;293(3):E726–36. [PubMed: 17578883]
198. Nangaku M, Fliser D. Erythropoiesis-stimulating agents: past and future. *Kidney Int Suppl* 2007;(107):S1–3. [PubMed: 17943137]
199. Nurmi A, Goldsteins G, Narvainen J, Pihlaja R, Ahtoniemi T, Grohn O, et al. Antioxidant pyrrolidine dithiocarbamate activates Akt-GSK signaling and is neuroprotective in neonatal hypoxia-ischemia. *Free Radic Biol Med* 2006;40(10):1776–84. [PubMed: 16678015]
200. Qin W, Peng Y, Ksiezak-Reding H, Ho L, Stetka B, Lovati E, et al. Inhibition of cyclooxygenase as potential novel therapeutic strategy in N141I presenilin-2 familial Alzheimer's disease. *Mol Psychiatry* 2006;11(2):172–81. [PubMed: 16331303]
201. 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(2):195–204. [PubMed: 16733654]
202. Musmann R, Geese M, Harder F, Kegel S, Andag U, Lomow A, et al. Inhibition of glycogen synthase kinase (GSK) 3 promotes replication and survival of pancreatic beta cells. *J Biol Chem*. 2007
203. Rowe MK, Wiest C, Chuang DM. GSK-3 is a viable potential target for therapeutic intervention in bipolar disorder. *Neurosci Biobehav Rev* 2007;31(6):920–31. [PubMed: 17499358]
204. Silverberg DS, Wexler D, Sheps D, Blum M, Keren G, Baruch R, et al. 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(7):1775–80. [PubMed: 11401110]
205. 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(4):296–306. [PubMed: 16949468]

206. 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(2):115–23. [PubMed: 11891019]
207. 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-3 β /caspase-3 mediated signaling pathway. *Apoptosis* 2007;12(8):1365–75. [PubMed: 17508273]
208. Howlett KF, Sakamoto K, Yu H, Goodyear LJ, Hargreaves M. Insulin-stimulated insulin receptor substrate-2-associated phosphatidylinositol 3-kinase activity is enhanced in human skeletal muscle after exercise. *Metabolism* 2006;55(8):1046–52. [PubMed: 16839840]
209. Schumann C, Triantafilou K, Krueger S, Hombach V, Triantafilou M, Becher G, et al. Detection of erythropoietin in exhaled breath condensate of nonhypoxic subjects using a multiplex bead array. *Mediators Inflamm* 2006;2006(5):18061. [PubMed: 17392570]
210. Bierer R, Peceny MC, Hartenberger CH, Ohls RK. Erythropoietin concentrations and neurodevelopmental outcome in preterm infants. *Pediatrics* 2006;118(3):e635–40. [PubMed: 16908620]
211. 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(1):175–81. [PubMed: 17241225]
212. Moon C, Krawczyk M, Paik D, Coleman T, Brines M, Juhaszova M, et al. Erythropoietin, modified to not stimulate red blood cell production, retains its cardioprotective properties. *J Pharmacol Exp Ther* 2006;316(3):999–1005. [PubMed: 16306273]
213. 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(3):634–45. [PubMed: 17045782]
214. Assaraf MI, Diaz Z, Liberman A, Miller WH Jr, Arvanitakis Z, Li Y, et al. Brain erythropoietin receptor expression in Alzheimer disease and mild cognitive impairment. *J Neuropathol Exp Neurol* 2007;66(5):389–98. [PubMed: 17483696]
215. Palazzuoli A, Silverberg D, Iovine F, Capobianco S, Giannotti G, Calabro A, et al. 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(6):1096 e9–15. [PubMed: 17161060]
216. Gleissner CA, Klingenberg R, Staritz P, Koch A, Ehlermann P, Wiggerhauser A, et al. Role of erythropoietin in anemia after heart transplantation. *Int J Cardiol* 2006;112(3):341–7. [PubMed: 16309765]
217. 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(21):2278–87. [PubMed: 17896976]
218. Chong ZZ, Kang JQ, Maiese K. Angiogenesis and plasticity: role of erythropoietin in vascular systems. *J Hematother Stem Cell Res* 2002;11(6):863–71. [PubMed: 12590701]
219. Li F, Chong ZZ, Maiese K. Erythropoietin on a Tightrope: Balancing Neuronal and Vascular Protection between Intrinsic and Extrinsic Pathways. *Neurosignals* 2004;13(6):265–89. [PubMed: 15627815]
220. Anagnostou A, Liu Z, Steiner M, Chin K, Lee ES, Kessimian N, et al. Erythropoietin receptor mRNA expression in human endothelial cells. *Proc Natl Acad Sci U S A* 1994;91(9):3974–8. [PubMed: 8171022]
221. Ogilvie M, Yu X, Nicolas-Metral V, Pulido SM, Liu C, Ruegg UT, et al. Erythropoietin stimulates proliferation and interferes with differentiation of myoblasts. *J Biol Chem* 2000;275(50):39754–61. [PubMed: 10995753]
222. Fenjves ES, Ochoa MS, Cabrera O, Mendez AJ, Kenyon NS, Inverardi L, et al. Human, nonhuman primate, and rat pancreatic islets express erythropoietin receptors. *Transplantation* 2003;75(8):1356–60. [PubMed: 12717230]
223. Fliser D, Haller H. Erythropoietin and treatment of non-anemic conditions--cardiovascular protection. *Semin Hematol* 2007;44(3):212–7. [PubMed: 17631185]

224. 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(8):639–44. [PubMed: 17624659]
225. Liu R, Suzuki A, Guo Z, Mizuno Y, Urabe T. Intrinsic and extrinsic erythropoietin enhances neuroprotection against ischemia and reperfusion injury in vitro. *J Neurochem* 2006;96(4):1101–10. [PubMed: 16417583]
226. 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(4):584–93. [PubMed: 16435392]
227. 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(1):5–10. [PubMed: 16054296]
228. Montero M, Poulsen FR, Norberg J, Kirkeby A, van Beek J, Leist M, et al. 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(1):106–17. [PubMed: 17157835]
229. Yamasaki M, Mishima HK, Yamashita H, Kashiwagi K, Murata K, Minamoto A, et al. Neuroprotective effects of erythropoietin on glutamate and nitric oxide toxicity in primary cultured retinal ganglion cells. *Brain Res* 2005;1050(1–2):15–26. [PubMed: 15979589]
230. Kaiser K, Texier A, Ferrandiz J, Buguet A, Meiller A, Latour C, et al. Recombinant human erythropoietin prevents the death of mice during cerebral malaria. *J Infect Dis* 2006;193(7):987–95. [PubMed: 16518761]
231. 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(2):238–46. [PubMed: 16500719]
232. 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(2):361–70. [PubMed: 16903847]
233. Zhong Y, Yao H, Deng L, Cheng Y, Zhou X. Promotion of neurite outgrowth and protective effect of erythropoietin on the retinal neurons of rats. *Graefes Arch Clin Exp Ophthalmol* 2007;245(12):1859–1867. [PubMed: 17828550]
234. Tsai JC, Song BJ, Wu L, Forbes M. Erythropoietin: a candidate neuroprotective agent in the treatment of glaucoma. *J Glaucoma* 2007;16(6):567–71. [PubMed: 17873720]
235. King VR, Averill SA, Hewazy D, Priestley JV, Torup L, Michael-Titus AT. Erythropoietin and carbamylated erythropoietin are neuroprotective following spinal cord hemisection in the rat. *Eur J Neurosci* 2007;26(1):90–100. [PubMed: 17614942]
236. 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(4):364–8. [PubMed: 17236773]
237. Verdonck O, Lahrech H, Francony G, Carle O, Farion R, Van de Looij Y, et al. Erythropoietin protects from post-traumatic edema in the rat brain. *J Cereb Blood Flow Metab* 2007;27(7):1369–76. [PubMed: 17264861]
238. Cherian L, Goodman JC, Robertson C. Neuroprotection with erythropoietin administration following controlled cortical impact injury in rats. *J Pharmacol Exp Ther* 2007;322(2):789–94. [PubMed: 17470644]
239. Nadam J, Navarro F, Sanchez P, Moulin C, Georges B, Laglaine A, et al. Neuroprotective effects of erythropoietin in the rat hippocampus after pilocarpine-induced status epilepticus. *Neurobiol Dis* 2007;25(2):412–26. [PubMed: 17166730]
240. 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(2):521–8. [PubMed: 17494864]
241. 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(7):1241–51. [PubMed: 16511866]

242. Grimm C, Wenzel A, Groszer M, Mayser H, Seeliger M, Samardzija M, et al. HIF-1-induced erythropoietin in the hypoxic retina protects against light-induced retinal degeneration. *Nat Med* 2002;8(7):718–24. [PubMed: 12068288]
243. 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(2):113–20. [PubMed: 12920606]
244. Olsen NV. Central nervous system frontiers for the use of erythropoietin. *Clin Infect Dis* 2003;37 (Suppl 4):S323–30. [PubMed: 14582001]
245. Sharples EJ, Yaqoob MM. Erythropoietin in experimental acute renal failure. *Nephron Exp Nephrol* 2006;104(3):e83–8. [PubMed: 16837817]
246. 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(2):424–7. [PubMed: 1386184]
247. Ferrario M, Massa M, Rosti V, Campanelli R, Ferlini M, Marinoni B, et al. Early haemoglobin-independent increase of plasma erythropoietin levels in patients with acute myocardial infarction. *Eur Heart J* 2007;28(15):1805–13. [PubMed: 17412728]
248. Palazzuoli A, Silverberg DS, Iovine F, Calabro A, Campagna MS, Gallotta M, et al. Effects of beta-erythropoietin treatment on left ventricular remodeling, systolic function, and B-type natriuretic peptide levels in patients with the cardiorenal anemia syndrome. *Am Heart J* 2007;154(4):645 e9–15. [PubMed: 17892986]
249. Wu H, Ren B, Zhu J, Dong G, Xu B, Wang C, et al. Pretreatment with recombined human erythropoietin attenuates ischemia-reperfusion-induced lung injury in rats. *Eur J Cardiothorac Surg* 2006;29(6):902–7. [PubMed: 16675226]
250. Fu Q, Van Eyk JE. Proteomics and heart disease: identifying biomarkers of clinical utility. *Expert Rev Proteomics* 2006;3(2):237–49. [PubMed: 16608436]
251. Foller M, Kasinathan RS, Koka S, Huber SM, Schuler B, Vogel J, et al. Enhanced susceptibility to suicidal death of erythrocytes from transgenic mice overexpressing erythropoietin. *Am J Physiol Regul Integr Comp Physiol* 2007;293(3):R1127–34. [PubMed: 17567717]
252. Eto N, Wada T, Inagi R, Takano H, Shimizu A, Kato H, et al. Podocyte protection by darbepoetin: preservation of the cytoskeleton and nephrin expression. *Kidney Int* 2007;72(4):455–63. [PubMed: 17457371]
253. Asaumi Y, Kagaya Y, Takeda M, Yamaguchi N, Tada H, Ito K, et al. Protective role of endogenous erythropoietin system in nonhematopoietic cells against pressure overload-induced left ventricular dysfunction in mice. *Circulation* 2007;115(15):2022–32. [PubMed: 17404160]
254. Giardino I, Edelstein D, Brownlee M. BCL-2 expression or antioxidants prevent hyperglycemia-induced formation of intracellular advanced glycation endproducts in bovine endothelial cells. *J Clin Invest* 1996;97(6):1422–8. [PubMed: 8617874]
255. 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(8):839–44. [PubMed: 16911620]
256. Symeonidis A, Kouraklis-Symeonidis A, Psiroyiannis A, Leotsinidis M, Kyriazopoulou V, Vassilakos P, et al. Inappropriately low erythropoietin response for the degree of anemia in patients with noninsulin-dependent diabetes mellitus. *Ann Hematol* 2006;85(2):79–85. [PubMed: 16132904]
257. Thomas MC, Cooper ME, Tsalamandris C, MacIsaac R, Jerums G. Anemia with impaired erythropoietin response in diabetic patients. *Arch Intern Med* 2005;165(4):466–9. [PubMed: 15738380]
258. 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(10):1695–703. [PubMed: 15502930]
259. 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(10):2863–70. [PubMed: 17003354]

260. Mason-Garcia M, Beckman BS, Brookins JW, Powell JS, Lanham W, Blaisdell S, et al. Development of a new radioimmunoassay for erythropoietin using recombinant erythropoietin. *Kidney Int* 1990;38(5):969–75. [PubMed: 2266682]
261. Namiuchi S, Kagaya Y, Ohta J, Shiba N, Sugi M, Oikawa M, et al. 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(9):1406–12. [PubMed: 15862410]
262. Li Y, Wang Z, Kong D, Murthy S, Dou QP, Sheng S, et al. 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* 2007;282(29):21542–50. [PubMed: 17522055]
263. Hayden MS, Ghosh S. Signaling to NF-kappaB. *Genes Dev* 2004;18(18):2195–224. [PubMed: 15371334]
264. Chong ZZ, Li F, Maiese K. Activating Akt and the brain's resources to drive cellular survival and prevent inflammatory injury. *Histol Histopathol* 2005;20(1):299–315. [PubMed: 15578447]
265. Guo G, Bhat NR. Hypoxia/Reoxygenation Differentially Modulates NF-kappaB Activation and iNOS Expression in Astrocytes and Microglia. *Antioxid Redox Signal* 2006;8(5–6):911–8. [PubMed: 16771681]
266. 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(6):772–80. [PubMed: 11891791]
267. 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(8):2687–95. [PubMed: 10733571]
268. 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(9):673–81. [PubMed: 11495725]
269. Spandou E, Tsouchnikas I, Karkavelas G, Dounousi E, Simeonidou C, Guiba-Tziampiri O, et al. Erythropoietin attenuates renal injury in experimental acute renal failure ischaemic/reperfusion model. *Nephrol Dial Transplant* 2006;21(2):330–6. [PubMed: 16221709]
270. Liu HK, Green BD, Platt PR, McClenaghan NH, McCluskey JT. Effects of long-term exposure to nicotinamide and sodium butyrate on growth, viability, and the function of clonal insulin secreting cells. *Endocr Res* 2004;30(1):61–8. [PubMed: 15098920]
271. Reddy S, Salari-Lak N, Sandler S. Long-term effects of nicotinamide-induced inhibition of poly (adenosine diphosphate-ribose) polymerase activity in rat pancreatic islets exposed to interleukin-1 beta. *Endocrinology* 1995;136(5):1907–12. [PubMed: 7720637]
272. Gaudineau C, Auclair K. Inhibition of human P450 enzymes by nicotinic acid and nicotinamide. *Biochem Biophys Res Commun* 2004;317(3):950–6. [PubMed: 15081432]
273. Williams AC, Cartwright LS, Ramsden DB. Parkinson's disease: the first common neurological disease due to auto-intoxication? *Qjm* 2005;98(3):215–26. [PubMed: 15728403]
274. Traister A, Breitman I, Bar-Lev E, Zvibel I, Harel A, Halpern Z, et al. Nicotinamide induces apoptosis and reduces collagen I and pro-inflammatory cytokines expression in rat hepatic stellate cells. *Scand J Gastroenterol* 2005;40(10):1226–34. [PubMed: 16165703]
275. Griffin RJ, Ogawa A, Williams BW, Song CW. Hyperthermic enhancement of tumor radiosensitization strategies. *Immunol Invest* 2005;34(3):343–59. [PubMed: 16136785]
276. Porcu M, Chiarugi A. The emerging therapeutic potential of sirtuin-interacting drugs: from cell death to lifespan extension. *Trends Pharmacol Sci* 2005;26(2):94–103. [PubMed: 15681027]
277. Saunders LR, Verdin E. Sirtuins: critical regulators at the crossroads between cancer and aging. *Oncogene* 2007;26(37):5489–504. [PubMed: 17694089]
278. Kruszewski M, Szumiel I. Sirtuins (histone deacetylases III) in the cellular response to DNA damage-Facts and hypotheses. *DNA Repair (Amst)*. 2005
279. Ellies DL, Church V, Francis-West P, Lumsden A. The WNT antagonist cSFRP2 modulates programmed cell death in the developing hindbrain. *Development* 2000;127(24):5285–95. [PubMed: 11076751]

280. Grotewold L, Ruther U. Bmp, Fgf and Wnt signalling in programmed cell death and chondrogenesis during vertebrate limb development: the role of Dickkopf-1. *Int J Dev Biol* 2002;46(7):943–7. [PubMed: 12455632]
281. Grotewold L, Ruther U. The Wnt antagonist Dickkopf-1 is regulated by Bmp signaling and c-Jun and modulates programmed cell death. *Embo J* 2002;21(5):966–75. [PubMed: 11867524]
282. Emami KH, Corey E. When prostate cancer meets bone: control by wnts. *Cancer Lett* 2007;253(2):170–9. [PubMed: 17462819]
283. Donohue JM, Cevasco M, Rosenthal MB. A decade of direct-to-consumer advertising of prescription drugs. *N Engl J Med* 2007;357(7):673–81. [PubMed: 17699817]
284. Olea FD, Vera Janavel G, De Lorenzi A, Cuniberti L, Yannarelli G, Cabeza Meckert P, et al. High-dose erythropoietin has no long-term protective effects in sheep with reperfused myocardial infarction. *J Cardiovasc Pharmacol* 2006;47(6):736–41. [PubMed: 16810073]
285. 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* 2004;44(1):63–7. [PubMed: 15234408]
286. 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(3):237–47. [PubMed: 17875952]
287. Corwin HL, Gettinger A, Fabian TC, May A, Pearl RG, Heard S, et al. Efficacy and safety of epoetin alfa in critically ill patients. *N Engl J Med* 2007;357(10):965–76. [PubMed: 17804841]
288. Kokhaei P, Abdalla AO, Hansson L, Mikaelsson E, Kubbies M, Haselbeck A, et al. Expression of erythropoietin receptor and in vitro functional effects of epoetins in B-cell malignancies. *Clin Cancer Res* 2007;13(12):3536–44. [PubMed: 17575216]
289. Maiese K, Li F, Chong ZZ. Erythropoietin and cancer. *JAMA* 2005;293(15):1858–1859. [PubMed: 15840858]
290. Hardee ME, Rabbani ZN, Arcasoy MO, Kirkpatrick JP, Vujaskovic Z, Dewhirst MW, et al. Erythropoietin inhibits apoptosis in breast cancer cells via an Akt-dependent pathway without modulating in vivo chemosensitivity. *Mol Cancer Ther* 2006;5(2):356–61. [PubMed: 16505109]
291. Lai SY, Grandis JR. Understanding the presence and function of erythropoietin receptors on cancer cells. *J Clin Oncol* 2006;24(29):4675–6. [PubMed: 17028292]
292. Leyland-Jones B, Semiglazov V, Pawlicki M, Pienkowski T, Tjulandin S, Manikhas G, et al. 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(25):5960–72. [PubMed: 16087945]
293. Ceelen W, Boterberg T, Smeets P, Van Damme N, Demetter P, Zwaenepoel O, et al. Recombinant human erythropoietin alpha modulates the effects of radiotherapy on colorectal cancer microvessels. *Br J Cancer* 2007;96(5):692–700. [PubMed: 17299396]
294. 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
295. 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(1):284–90. [PubMed: 15665305]
296. Sigounas G, Sallah S, Sigounas VY. Erythropoietin modulates the anticancer activity of chemotherapeutic drugs in a murine lung cancer model. *Cancer Lett* 2004;214(2):171–9. [PubMed: 15363543]
297. Joyeux-Faure M. Cellular protection by erythropoietin: new therapeutic implications? *J Pharmacol Exp Ther* 2007;323(3):759–62. [PubMed: 17717190]

Figure 1. Nicotinamide, Wnt signaling, and erythropoietin employ diverse as well as common pathways to foster cellular longevity

As illustrated in cell schematic with nucleus, erythropoietin (EPO) and the EPO receptor (EPOR) can increase cellular longevity through protein kinase B (Akt), the forkhead transcription factor family member FOXO3a, glycogen synthase kinase-3 β (GSK-3 β), nuclear factor- κ B (NF- κ B), and Bcl-x_L. Similar to EPO, nicotinamide modulates the activity of FOXO3a along with 14-3-3 protein and can maintain cellular integrity and prevent inflammatory activation of microglia that ultimately can lead to apoptosis through the maintenance of mitochondrial membrane potential ($\Delta\Psi_m$), the release of cytochrome c, and the prevention of caspase activation. Wnt signaling begins with Frizzled (FZD) receptors resulting in the activation of Dishevelled followed by the inhibition of glycogen synthase kinase (GSK-3 β). The suppressed GSK-3 β along with other Wnt signaling complexes prevents phosphorylation (p) of β -catenin and leads to the accumulation of β -catenin. β -catenin enters into cellular nucleus and contributes to the formation of Lef/Tcf lymphocyte enhancer factor/T cell factor (Lef/Tcf) and β -catenin complex that may cooperate with factors activated by other signaling pathways resulting in cellular proliferation, differentiation, survival and apoptosis through the induction of target nuclear gene transcription. Interconnected pathways with Wnt, nicotinamide, and EPO involve I κ B kinase (IKK), I κ B, inhibitors of apoptotic protein (IAPs), GSK-3 β , NF- κ B, mitochondrial membrane potential ($\Delta\Psi_m$), and cytochrome c. Ultimately these pathways converge upon early apoptotic injury with phosphatidylserine (PS) exposure and later apoptotic DNA degradation.