Novel Directions for Diabetes Mellitus Drug Discovery

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Abstract

Introduction—Diabetes mellitus impacts almost 200 million individuals worldwide and leads to debilitating complications. New avenues of drug discovery must target the underlying cellular processes of oxidative stress, apoptosis, autophagy, and inflammation that can mediate multi-system pathology during diabetes mellitus.

Areas Covered—We examine novel directions for drug discovery that involve the β-nicotinamide adenine dinucleotide (NAD$^+$) precursor nicotinamide, the cytokine erythropoietin, the NAD$^+$-dependent protein histone deacetylase SIRT1, the serine/threonine-protein kinase mammalian target of rapamycin (mTOR), and the *wingless* pathway. Implications for the targeting of these pathways that oversee gluconeogenic genes, insulin signaling and resistance, fatty acid beta-oxidation, inflammation, and cellular survival are presented.

Expert Opinion—Nicotinamide, erythropoietin, and the downstram pathways of SIRT1, mTOR, forkhead transcription factors, and *wingless* signaling offer exciting prospects for novel directions of drug discovery for the treatment of metabolic disorders. Future investigations must dissect the complex relationship and fine modulation of these pathways for the successful translation of robust reparative and regenerative strategies against diabetes mellitus and the complications of this disorder.

Keywords

Akt; apoptosis; autophagy; β-cell; Beclin 1; β-catenin; CCN family; diabetes mellitus; erythropoietin; forkhead transcription factors; FoxO; FRAP1; glycogen synthase kinase-3β (GSK-3β); insulin; mammalian target of rapamycin (mTOR); nicotinamide; nicotinamide adenine dinucleotide (NAD$^+$); oxidative stress; peroxisome proliferators activated receptor (PPAR); peroxisome proliferators-activated receptor-γ coactivator (PGC); phosphoinositide; 3–kinase (PI 3-K); poly (ADP-ribose) polymerase-1 (PARP-1); programmed cell death (PCD); protein tyrosine phosphatase; SIRT1; sirtuin; wingless; Wnt1 inducible signaling pathway protein 1 (WISP1)

1. Introduction

The incidence of obesity in the population throughout the world is increasing at an alarming rate that ultimately leads to metabolic disease and diabetes mellitus (DM) [1]. Recent studies have shown that the duration of obese-years rather than body mass index (BMI) translates into a strong risk for developing diabetes mellitus [2]. Increased weight gain also leads to other disorders that may be a result of metabolic disease, such as coronary artery calcifications and the loss of cognition [3, 4]. Yet, despite significant improvements over the

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prior thirty years in the management and care of individuals who suffer from DM, life expectancy in highly developed countries such as the United States and the United Kingdom continues to fall behind other developed countries such as Japan. This in part may be a result of the growing level of obesity in countries such as the United States that is not occurring at the same pace in other countries, but also signals the critical need for further understanding of the cellular pathways that may foster complications in debilitating disorders such as DM and the necessity to develop new directions to prevent and limit the course of this disease.

In the United States alone, DM has reached proportions of the population affecting almost 26 million individuals in all age groups. An additional 7 million individuals are estimated to be undiagnosed with DM [3]. In younger individuals, impaired glucose tolerance is of significant concern, since those with impaired glucose tolerance have a greater than twice the risk for the development of diabetic complications than individuals with normal glucose tolerance. Worldwide, more than 165 million individuals are afflicted with DM and this number could reach close to 400 million individuals by the year of 2030.

DM is defined by two categories that are either insulin dependent or non-insulin dependent. Type 2 non-insulin dependent DM is the most prominent and occurs in approximately eighty percent of all diabetics in individuals greater than 40 years of age. Type 2 DM represents a progressive deterioration of glucose tolerance with early β-cell compensation through cell hyperplasia followed by a decrease in β-cell mass [5]. Insulin resistance or defective insulin action occurs when physiological levels of insulin result in a subnormal physiologic response. Although insulin resistance forms the basis for the development of Type 2 DM, elevated serum glucose levels also occur from impairment in insulin secretion. Poor insulin secretion may be a result of defective β-cell function, chronic exposure to free fatty acids and hyperglycemia, or the absence of inhibitory feedback through plasma glucagon levels. Type 1 insulin dependent DM is present in a much smaller group of individuals in approximately 5–10 percent of diabetic patients [1, 3, 4]. Type 1 DM is an autoimmune disorder with the presence of alleles of the Human leukocyte antigen (HLA) class II genes within the major histocompatibility complex (MHC). Inflammatory infiltration of the islets of Langerhans and the selective destruction of β-cells in the pancreas that leads to insulin loss are considered to be significant components of Type 1 DM. Almost 90 percent of individuals with Type 1 DM have increased titers of autoantibodies (Type 1A DM). The remaining ten percent of Type 1 DM individuals do not have serum autoantibodies. These individuals are considered to have maturity-onset diabetes of the young (MODY) that can be a result of β-cell dysfunction with autosomal-dominant inheritance (Type IB DM). Some individuals with Type 1 DM also may suffer from insulin resistance that is found in Type 2 DM. In addition, approximately 10 percent of individuals with Type 2 DM may have elevated serum autoantibodies similar to Type 1 DM.

2. Diabetes Mellitus, Oxidative Stress, and Cell Injury

Given the systemic manifestations of metabolic disorders, DM can lead to complications in multiple areas of the body. DM can result in hepatic dysfunction, mood disorders, cognitive loss, renal disease, platelet disorders, sympathetic nervous system dysfunction, and cardiac injury [1, 3]. Each of these complications of DM can be a manifestation of oxidative stress.

Oxidative stress occurs following the production of reactive oxygen species (ROS) that are formed through superoxide free radicals, hydrogen peroxide, singlet oxygen, nitric oxide (NO), and peroxynitrite [5–7]. ROS can be maintained at non-toxic levels by antioxidant systems in the body that include catalase, superoxide dismutase, glutathione peroxidase, and vitamins C, D, E, and K. However, excessive production of ROS or deficits in the endogenous antioxidant system can lead to oxidative stress and cell death through DNA

degradation, mitochondrial dysfunction, and protein misfolding. Oxidative stress can result in mitochondrial injury, nutritional impairment, and DNA damage in diabetic patients [1, 3].

Oxidative stress during DM can lead to cell injury through pathways of programmed cell death (PCD) that involve either autophagy or apoptosis [8]. Autophagy leads to the destruction of organelles and the preservation of cytoskeleton structures, allowing cells to recycle cytoplasmic components. Autophagy consists of three different categories known as microautophagy, macroautophagy, and chaperone-mediated autophagy. Macroautophagy represents the degradation of cytoplasmic material and the sequestration of the cytoplasmic protein and organelles into autophagosomes. Autophagosomes fuse with lysosomes for degradation and can regenerate for other cellular processes. Microautophagy consists of the sequestration of cytoplasmic components by invagination of the lysosomal membrane. Vesicles that are formed are transferred to the lumen of the lysosomes for digestion. In chaperone mediated autophagy, the cytoplasmic component is delivered by cytosolic chaperones to the receptors on the lysosomal membranes for translocation across lysosomal membranes into the lumen. During oxidative stress, autophagy can lead to cardiac injury, astrocytic cell death, endothelial cell loss, dopaminergic cell injury, and spinal cord injury [9, 10]. Yet, some studies support a protective role for the induction of autophagy during hypoxia-ischemia in the brain, in models of Huntington's disease, and prion neurotoxicity [11, 12]. During DM, autophagy may be necessary to remove misfolded proteins and eliminate non-functioning mitochondria in β-cells to prevent β-cell dysfunction and the onset of DM [13]. Exercise in mice has been shown to initiate autophagy and regulate glucose homeostasis [14]. However, the generation of advanced glycation end products (AGEs) during DM also may lead to autophagy that contributes to vascular smooth muscle proliferation, atherosclerosis [15], and potential cardiomyopathy [16]. The elevation of free fatty acids in cell models of DM suggest that fatty acids may be necessary to activate autophagy in beta cells through endoplasmic reticulum stress [17]. During endoplasmic reticulum stress and elevated low-density lipoproteins, autophagy and oxidative stress has been associated with the progression of diabetic retinopathy in animal and human tissue[18]. In other scenarios, autophagy may not have a significant influence upon cell death [8,19].

Apoptosis also is considered important for tissue re-modeling especially during development, but apoptosis also can lead to cell demise during DM [1]. Apoptosis consists of two distinct components that involve genomic DNA degradation and the loss of plasma membrane lipid asymmetry. The cleavage of genomic DNA into fragments during apoptosis occurs during later stages once a cell has been committed to die. In contrast, the loss of asymmetry of membrane phosphatidylserine (PS) distribution is an early reversible feature of apoptosis. Both membrane PS exposure and genomic DNA degradation are considered to be the outcomes of nuclease and protease activation that occurs during apoptosis [6, 20]. During DM, apoptosis can lead to vascular complications, impair endothelial cell survival, destroy immune mediated cells, affect cardiomyocyte function, inhibit wound repair, and injure neurons [6, 9, 20]. It is important to note that the pathways of autophagy and apoptosis also may intersect to control cell survival. Induction of autophagy may delay the onset of apoptotic cell injury [21]. Under other circumstances, apoptosis may lead to the inhibition of autophagy during cell death [22]. Cell demise also can be a result of the combined effects of autophagy and apoptosis [23].

3. Novel Considerations for Drug Discovery

Nicotinamide

Given that the pathways of oxidative stress, apoptosis, and autophagy can be significant contributors to the pathogenesis and the subsequent complications of DM, development of strategies that can modulate these pathways may prove fruitful for both the prevention and

treatment of DM. Nicotinamide is one agent that has become highly relevant for the treatment of metabolic disorders (Figure 1). Nicotinamide is the amide form of vitamin B_3 (niacin) and is generated through the conversion of nicotinic acid in the liver or through the hydrolysis of NAD^+. After nicotinamide is obtained in the body, it becomes the precursor for the coenzyme β-nicotinamide adenine dinucleotide (NAD^+) [24]. Nicotinamide also is essential for the synthesis of nicotinamide adenine dinucleotide phosphate ($NADP^+$).

Nicotinamide affects both early apoptotic changes with membrane PS exposure and late apoptotic injury with DNA degradation. Nicotinamide prevents membrane PS exposure in endovascular cells that can prevent cardiovascular injury and potentially block thrombotic disease [25]. Nicotinamide can control the activation of inflammatory cells as well as early PS membrane exposure in neurons during oxidative stress [26, 27]. During the later stages of apoptotic DNA degradation, nicotinamide can limit traumatic brain injury, reduce cortical damage during ischemic and ketamine injury, control cytokine release during sepsis, and prevent apoptotic cell injury during oxidative stress [25–29]. Interestingly during tumorigenesis and unchecked cellular proliferation, nicotinamide may block cell growth and prevent tumor progression through the induction of apoptotic pathways [28]. Nicotinamide also may promote autophagy induction that can affect mitochondrial membrane potential and lead to mitochondrial fragmentation [30]. Under other conditions, nicotinamide may alter apoptosis rather than autophagy during oxidative stress [31, 32].

Nicotinamide may maintain cellular energy homeostasis during DM through mitochondrial pathways [24, 33]. During DM, lipid toxicity and oxidative stress can lead to mitochondrial damage and impaired pancreatic β-cell function [34]. Preservation of mitochondrial integrity may improve immune and cardiac function during DM. Nicotinamide can maintain mitochondrial membrane polarization during oxidative stress and prevent the release of cytochrome c [25, 27, 29]. Nicotinamide can control mitochondrial function and prevent induction of the apoptotic cascade through a number of pathways that involve Bad, modulation of caspase activity, reduction in Bax expression, and control of forkhead transcription factors [25–29].

In clinical studies, oral nicotinamide administration protects β-cell function and prevents clinical disease in islet-cell antibody-positive first-degree relatives of Type 1 DM [35]. Nicotinamide administration in patients with recent onset Type 1 DM and intensive insulin therapy can significantly reduced HbA_{1c} levels [36]. Nicotinamide also has been shown to reduce intestinal absorption of phosphate and prevent the development of hyperphosphatemia and progressive renal dysfunction [37]. In non-clinical studies, nicotinamide can prevent peripheral nerve injury during elevated glucose [38], result in the remission of Type 1 DM in mice in combination therapy with acetyl-l-carnitine [39], and improve glucose utilization and prevent excessive lactate production in ischemic animal models [40]. During models of diabetic neuropathy in rats, nicotinamide treatment can reduce poly (ADP-ribose) polymerase-1 (PARP-1) activity to partially restore vital NAD^+ and ATP levels [41]. Nicotinamide also has been reported to reduce the production of ROS and enhance regulatory T cell immune function in animal models of DM [42]. However, some reports suggest that nicotinamide may have reduced protective function in older animal subjects [43] and excessive nicotinamide levels may promote progression of Type 2 DM [44]. Nicotinamide also may reduce embryonic stem cell proliferation, but assist with the production of insulin secreting cells [45]. Since increased levels of nicotinamide also can limit cytoprotective sirtuin activity that may be detrimental to cell survival [27, 46, 47], determination of the degree of nicotinamide exposure may be a significant component for effective cellular protection during DM. These observations fall in line with the need to determine effective and safe concentrations of nicotinamide, since prior clinical trails that examined nicotinamide application to prevent Type I DM onset did not identify an effective concentration [48].

Erythropoietin

Similar to nicotinamide, erythropoietin (EPO) is a novel agent that also can modulate a number of components in the apoptotic cascade to potentially prevent the devastating effects of DM [49] (Figure 1). EPO is a 30.4 kDa protein with four glycosylated chains that include three *N*-linked and one *O*-linked acidic oligosaccharide side chains [50, 51]. The function and integrity of EPO depend upon the *N*- and *O*-linked chains. Biological activity of EPO is dependent upon the two disulfide bonds formed between cysteine[7] and cysteine[160] as well as between cysteine[29] and cysteine[33]. Alkylation of the sulfhydryl groups results in the loss of the biological activity of EPO. EPO is produced in multiple organs of the body that include the brain, liver, and uterus. The expression of EPO can be regulated by hypoxia as well as other stimuli. Hypoxia-dependent expression of EPO and its receptor, the EPO receptor (EPOR), are modulated through hypoxia-inducible factor 1 (HIF-1). Gene transcription of EPO and EPOR are tied to the activation of HIF-1 [50, 51]. In addition to hypoxia, free radical exposure can alter EPO and EPOR expression in vascular cells and in neurons to result in increased HIF-1 expression and subsequent increase in EPO expression. EPO and the EPOR are present in renal tubular cells during high glucose-induced oxidative stress [52]. Anemic stress, insulin release, and several cytokines, including insulin-like growth factor, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) also can result in increased expression of EPO and the EPOR [50, 51]. Additional changes in the cellular environment, such as hypoglycemia, cadmium exposure, raised intracellular calcium, or strong neuronal depolarizations also can alter the expression of EPO.

EPO can block cell injury during DM through reduction in cellular oxidative stress and limiting apoptotic cell injury. EPO can limit the generation of ROS, may prevent oxidative stress at high altitudes, and is cytoprotective against oxidative stress during a variety of insults such as hypoxia, TNF-α, glutamate toxicity, and amyloid [50, 51]. EPO can preserve cellular survival in neurons, vascular cells, and inflammatory cells during oxidative stress exposure. In regards to preventing apoptotic cell injury, EPO blocks mitochondrial depolarization and the release of cytochrome c. EPO can control mitochondrial signaling through Bad, Bax, Puma. EPO also blocks Apaf-1 activation and prevents the early activation of several caspases such as caspase 1, caspase 3, and caspase 9 that can lead to both early apoptotic membrane PS exposure and late genomic DNA degradation [50–55]. Recent work suggests that EPO may control caspase activation through downstream pathways of the proline rich Akt substrate 40 kDa (PRAS40), an inhibitor of the mammalian target of rapamaycin (mTOR) pathway [56, 57].

In clinical studies, EPO plasma levels may be depressed in diabetic patients with anemia or without anemia. Reports that patients with DM cannot produce EPO in response to low hemoglobin levels indicate that an impaired EPO response during DM may be present. Interestingly, increased EPO secretion during diabetic pregnancies occurs and may represent an endogenous protection response against the complications of DM. For example, EPO can promote neurite outgrowth that may prevent cognitive decline [50, 51]. Treatment with EPO has been shown in individuals with DM with severe congestive heart failure to decrease fatigue, increase left ventricular ejection fraction, and lessen hospitalization duration [58]. In addition, EPO can serve to reverse the complications of anemia during DM [59].

During studies with elevated glucose and experimental models of diabetes, EPO can reduce ROS generation and prevent high glucose-induced renal cell apoptosis [52], promote wound repair in diabetic mice [60], and maintain vascular cell integrity in cellular models of DM through pathways that involve the sirtuin SIRT1 [53]. Intravitreal EPO treatment in diabetic rat retinas has been shown to modulate the expression of pro-apoptotic genes and limit inflammation [61]. EPO also may have a role in the prevention of diabetic neuropathy [62]. EPO in streptozotocin-induced and db/db mouse models of Type 1 and Type 2 DM also can

prevent apoptotic pancreatic β-cell loss through aβ-cell specific EPOR [63]. Yet, the protective effects of EPO may not always require a direct association with the EPOR. Some studies suggest that although mRNA for the EPOR is present in numerous tissues, the presence of the EPOR protein in the tissue samples examined may be functionally low in non-hematopoeitc cell types [64]. Prevention of the complications of DM may be closely linked to the effects of EPO on vascular cells. EPO can block vascular injury through activation of wingless pathways, by limiting apoptotic forkhead subcellular trafficking to the nucleus, and through the modulation of downstream β-catenin and glycogen synthase kinase-3β (GSK-3β) pathways [65–67].

Sirtuins

Both nicotinamide and EPO are closely linked to the metabolic pathways of the sirtuin SIRT1 that can influence cellular metabolism during DM. Sirtuins are class III NAD+-dependent protein histone deacetylases that are the mammalian homologues of Sir2 of the yeast silent information regulator-2 (Sir2) [6]. Of the seven mammalian homologues of Sir2, SIRT1 is present in the brain, heart, liver, pancreas, skeletal muscle, spleen, and adipose tissues. During DM, SIRT1 is protective against cellular injury. For example, SIRT1 activation prevents endothelial senescence during hyperglycemia, blocks oxidative stress injury in cardiomyocytes, limits atherosclerotic lesions during elevated lipid states, and prevents endothelial cell apoptosis during experimental diabetes [7, 68].

Activation of SIRT1 can increase lifespan in higher organisms such as Drosophila and protect cells from oxidative stress [27, 46]. Loss of SIRT1 is associated with insulin resistance. Gene deletion or inhibition of SIRT1 impairs insulin signaling by interfering with insulin stimulated insulin receptor phosphorylation and glycogen synthase [69]. In contrast, over-expression of SIRT1 decreases hepatic steatosis and improves insulin sensitivity that leads to improved glucose homeostasis [70]. SIRT1 can increase insulin signaling in insulin-sensitive organs through protein kinase B (Akt) and phosphotidylinositide 3-kinase (PI 3-K) [71]. SIRT1 also can stimulate glucose-dependent insulin secretion from pancreatic β cells by repressing the uncoupling protein (UCP) gene UCP2 [72]. In addition, SIRT1 controls insulin sensitivity through tyrosine phosphatase1B (PTP1B). PTP1B deficiency leads to improved insulin sensitivity and glycemic control. SIRT1 over-expression or SIRT1 activation can reduce both PTP1B mRNA and protein levels during insulin-resistance. However, an increase in PTP1B expression prevents SIRT1 mediated glucose uptake and insulin receptor phosphorylation in response to insulin stimulation [69]. SIRT1 also may improve insulin sensitivity through the regulation of fat mobilization, gluconeogenesis, and inflammation [6, 7, 68].

SIRT1 can regulate food intake, cellular metabolism, and lipid homeostasis (Figure 2). SIRT1 is expressed in anorexigenic proopiomelanocortin (POMC) neurons and orexigenic agouti-related peptide (AgRP) neurons in the arcuate nucleus of the hypothalamus that control food intake [73]. Over-expression of SIRT1 in theses regions of the hypothalamus prevents the forkhead transcription factor FoxO1 from promoting hyperphagia and body weight gain [73]. In contrast, absence of SIRT1 in POMC neurons leads to obesity due to reduced energy expenditure. During the regulation of hepatic glucose output and lipid homeostasis, SIRT1 controls peroxisome proliferators-activated receptor-γ coactivator (PGC)-1α via deacetylation in the liver to transcribe gluconeogenic genes and increase hepatic glucose output. As a transcriptional coactivator, PGC-1α interacts with transcription factors to activate transcription and increase the expression of genes that regulate mitochondrial functions and fatty acid oxidation. Increased PGC-1α activity may function to protect against some metabolic diseases and improve mitochondrial biogenesis. SIRT1 also controls the ability of PGC-1α to repress glycolytic genes in response to fasting and pyruvate [74]. SIRT1 modulates fatty acid metabolism and cellular stress through peroxisome

proliferators activated receptor-α (PPAR-α). Liver-specific SIRT1 knockout mice develop hepatic steatosis, hepatic inflammation, and endoplasmic reticulum stress when challenged with a high fat diet since hepatocyte-specific deletion of SIRT1 impairs PPAR-α signaling and decreases fatty acid beta-oxidation [75]. In addition, SIRT1 requires PPAR-α to protect cardiac cells from hypertrophy, metabolic dysfunction, and inflammation [76].

SIRT1 is one pathway that allows EPO to prevent cell injury during DM. During vascular cell protection, EPO increases endogenous SIRT1 activity and promotes the subcellular trafficking of SIRT1 to the nucleus [53]. SIRT1 also can increase Akt activity that is considered to be a principle pathway for cellular proliferation and survival, for the cytoprotective capacity of EPO and nicotinamide, and for mediating insulin signaling [24, 50, 53, 77–79]. SIRT1 can block the activity of forkhead transcription factors such as FoxO1, FoxO3a, and FoxO4. SIRT1 activates Akt1 to control the phosphorylation and subcellular trafficking of the forkhead transcription factor FoxO3a [53]. Mammalian forkhead transcription factors of the O class (FoxO1, FoxO3, FoxO4, and FoxO6), such as FoxO3a also have been tied to the cellular pathways of EPO as well as nicotinamide and are intimately involved with cellular metabolism, insulin sensitivity, and oxidative stress (Figure 2) [80, 81]. Phosphorylation of FoxOs leads to the retention of these transcription factors in the cytoplasm and the subsequent inhibition of their transcriptional activity. SIRT1 relies upon the Akt pathway for cytoprotection and the subsequent phosphorylation of target genes such as FoxOs [53, 71]. However, SIRT1 may control a fine balance over FoxO activity since acetylation of FoxOs also can limit their transcriptional activity. SIRT1 can lead to the deacetylation of FoxOs to increase the activity of these transcription factors [82, 83]. Yet, the deacetylation of FoxOs may ultimately lead to degradation of these transcription factors [84]. SIRT1 also may rely upon the regulation of FoxO transcription factors to protect against inflammation, to promote β-cell function during DM, and to maintain endothelial survival during oxidative stress [7,83]. In addition, an increase in FoxO3a and SIRT1 activity can occur in the heart during exercise [85], suggesting that beneficial physical activity for the cardiovascular system and for patients with DM may be mediated through SIRT1 and FoxO proteins. FoxOs also exert a positive feedback mechanism regulating SIRT1 expression. FoxO1 can directly bind to the SIRT1 promoter region containing a cluster of five putative FoxO1 core binding repeat motifs (IRS-1) and a forkhead-like consensus-binding site (FKHD-L). This results in FoxO1 modulating SIRT1 transcription and leads to an increase in the expression of SIRT1 [86]. FoxO3a also can regulate the expression of SIRT1 by binding to two p53 binding sites within the SIRT1 promoter to foster SIRT1 transcription during acute nutrient withdrawal [80, 81].

Unlike EPO, a more complex relationship appears to exist between nicotinamide and SIRT1 in regards to cell survival and longevity. Nicotinamide can block sirtuin activity by intercepting an ADP-ribosyl-enzyme-acetyl peptide intermediate with the regeneration of NAD^+ (transglycosidation). As a result, reduction in nicotinamide levels during nicotinamidase expression can increase SIRT1 activity and foster cellular protection [27, 46]. Although nicotinamide provides cellular protection in millimole concentrations against early apoptotic membrane exposure and late DNA degradation, physiological concentrations of nicotinamide noncompetitively inhibit sirtuins such as SIRT1, indicating that nicotinamide is a physiologically relevant regulator of sirtuin activity. In addition, sirtuins may regulate nicotinamide by preventing nicotinamide from assisting with DNA repair by altering the accessibility of DNA damaged sites for repair enzymes [87].

Serine/threonine-protein kinase mTOR

Metabolic signaling that is mediated through Akt not only relies upon pathways with nicotinamide, EPO, and SIRT1, but also controls metabolic cell pathways through the serine/threonine protein kinase mTOR that has other alternative names including

mechanistic target of rapamycin and FK506-binding protein 12-rapamycin complex-associated protein 1 (FRAP1) [88] (Figure 2). As part of the PI 3-K related kinase family that is activated through the PI 3-K and Akt, mTOR is a 289-kDa serine/threonine protein kinase that can control transcription, cytoskeleton organization, cellular survival, and cellular metabolism [9, 89]. mTOR signaling is dependent upon the protein complexes mTOR Complex 1 (mTORC1) or mTOR Complex 2 (mTORC2) that each contain mTOR. p70 ribosomal S6 kinase (p70S6K) and eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4EBP1) are downstream targets of mTORC1 that are relevant in DM pathways. Phosphorylation of p70S6K promotes mRNA biogenesis, translation of ribosomal proteins, and cell growth. In contrast, phosphorylation of 4EBP1 results in its inactivation. Hypophosphorylated 4EBP1 is active and binds competitively with eukaryotic translation initiation factor 4 gamma (eIF4G) to eukaryotic translation initiation factor 4 epsilon (eIF4E) that regulate translation initiation by interacting with the 5′-mRNA cap structure. The phosphorylation of 4EBP1 by mTORC1 results in its dissociation from eIF4E allowing eIF4G to interact with eIF4E and promote protein translation.

The signaling pathways of mTOR can be closely tied to pathways associated with cell protection and longevity, such as those with EPO and SIRT1. EPO uses mTOR signaling for the neuronal differentiation of post-mortem neural precursors [90]. Retinal progenitor cells are resistant to hypoxia when exposed to EPO that leads to mTOR activation [91]. EPO regulates bone homeostasis with osteoblastogenesis and osteoclastogenesis through mTOR activation [92]. EPO can activate mTOR to block apoptotic cell death during oxidative stress in inflammatory cells [93]. In cellular models of Alzheimer's disease, amyloid degeneration of microglia is limited by EPO through combined activation of PI 3-K and mTOR pathways [55]. In regards to SIRT1 and mTOR, loss of SIRT1 expression leads to hepatic glucose overproduction, hyperglycemia, products of oxidative stress, and inhibition of the gene encoding Rictor that lead to impaired TORC2 and Akt signaling [94]. Under some conditions, SIRT1 and mTOR may have an inverse relationship. For example, SIRT1 attenuates hepatic steatosis, ameliorates insulin resistance, and restores glucose homeostasis primarily through the inhibition of mTORC1 [70]. SIRT1 also may promote neuronal growth through pathways that inhibit mTOR signaling [95]. With nicotinamide, mTOR activity can be increased that may be dependent upon blockade of SIRT1 activity [96]. During high glucose exposure to mesangial cells, loss of SIRT1 activity, such as with the application of nicotinamide, is necessary for mTOR to arrest mesangial cell senescence [97].

During metabolic disorders, mTOR signaling may be necessary for maintenance of insulin function. Loss of mTOR signaling with inhibition of p70S6K can result in hypoinsulinemia, glucose intolerance, insulin insensitivity to glucose secretion, and a decrease in pancreatic β-cell size [9, 89]. Conversely, activation of p70S6K and inhibition of 4EBP1 in pancreatic β-cells in murine models of DM results in improved insulin secretion and resistance to β-cell streptozotocin toxicity and obesity [98]. However, mTOR inhibition with rapamycin application leads to insulin resistance, reduces β-cell function and mass, limits insulin secretion, and results in DM [99]. Although inhibition of mTOR reduces food intake and prevents fat-diet induced obesity in mice, loss of mTOR activity also attenuates glucose uptake and metabolism in skeletal muscle through the prevention of insulin generated Akt activation and alteration in the translocation of glucose transporters to the plasma membrane [100]. Other studies support a role for components of mTORC1 and mTORC2 in insulin signaling. Loss of the mTORC1 substrate p70S6K activity with combined loss of the mTORC2 substrate Akt2 results in defects in both insulin action and β-cell function, suggesting that both mTOR components are required to maintain insulin signaling and prevent DM [101].

Wingless Signaling

Cytoprotective signaling through mTOR during metabolic disease has recently been associated with the *wingless* pathway. The *wingless* pathway consists of the Wnt family of cysteine-rich glycosylated proteins that control stem cell development, angiogenesis, tumorigenesis, and metabolism [102, 103]. Genetic variations in Wnt signaling pathways, such as with transcription factor 7-like 2 gene, may impart an increased risk for Type 2 DM in some populations [104]. Intronic variants of the low-density lipoprotein receptor-related protein 5 (LRP5) gene, a member of the Wnt signaling pathway, are also markedly associated with obesity [105]. Impaired Wnt signaling through a missense mutation in LRP6 has been reported in patients with coronary artery disease and the combined metabolic syndrome with hypertension, hyperlipidemia, and DM [106].

Wnt family members can provide improved glucose tolerance, increased insulin sensitivity, weight control, protect glomerular mesangial cells from apoptotic cell injury during elevated glucose, and prevent vascular cell injury in models of DM (Figure 2) [102]. Wnt signaling requires mTOR to prevent cell injury during models of cardiac ischemia, oxidative stress, and during amyloid exposure [102, 107]. Interestingly, a significant crosstalk between mTOR and Wnt exists to promote insulin signaling and cellular proliferation [108].

As a component of the Wnt signaling pathway, Wnt1 inducible signaling pathway protein 1 (WISP1) also is cytoprotective and may represent a new target for metabolic disorders. WISP1 is a member of the CCN family of proteins and is known as CCN4. The CCN family of proteins is termed by the first three members of the family that include Cysteine-rich protein 61, Connective tissue growth factor, and Nephroblastoma over-expressed gene and consists of six secreted extracellular matrix associated proteins. WISP1 initially was shown to prevent p53 mediated DNA damage and apoptosis in kidney fibroblasts [109]. A reparative and regenerative role for WISP1 may exist since WISP1 has been shown to have elevated expression during cardiac ischemia, neuronal exposure to oxidative stress, lung epithelial damage, and cellular repair of fractured bone [19, 109–111]. Subsequent work has demonstrated that WISP1 can initiate cardiac remodeling after myocardial infarction, stimulate lung tissue repair, promote cardiomyocyte proliferation, and foster vascular smooth muscle growth during cytokine exposure. WISP1 also can prevent cell death during bone fractures and block oxygen-glucose deprivation injury in primary neuronal cells [19, 110, 111]. In relation to DM, WISP1 expression has been shown to be up-regulated during pancreatic regeneration in rats following partial pancreatectomy [112], suggesting that pancreatic and β-cell regeneration may require WISP1 expression similar to the role mTOR has recently been shown to play during β-cell proliferation [101].

4. Conclusion

DM impacts a significant proportion of the world's population with a large number of individuals that continue to remain undiagnosed and without treatment. In addition, incidence in young individuals with DM continues to grow. The complications of DM involve multiple systems of the body and can rapidly lead to disability as well as death. New avenues of discovery that can address the complications of DM and the underlying cellular processes of oxidative stress, apoptosis, and autophagy may foster the development of new therapeutic strategies for DM. Novel considerations for drug discovery for the treatment of metabolic disorders include nicotinamide, EPO, and the targeted pathways of SIRT1, mTOR, and *wingless* signaling. These pathways have an intricate relationship that requires a fine modulation to achieve desired biological and clinical outcomes that can be both reparative and regenerative against the multi-systemic complications of DM.

5. Expert Opinion

Our understanding of the biological processes that lead to DM and the complications of this disorder have grown remarkably over the prior decades. Yet despite these advances, the incidence of DM continues to grow worldwide especially in young adults. The increased complications that are observed with DM in both a young and an aging population can negatively affect life expectancy. Recent studies also point to new concerns. Although intensive therapy to control plasma glucose levels may promote long-term benefits, studies note that intensive glycemic control in patients with Type 2 DM may not improve renal outcomes or prevent the development of renal disease [113]. In addition, current therapies for plasma glucose control are not without risk and may lead to hypoglycemia, weight gain, or increased mortality [114].

As a result, development of novel strategies for DM and its complications are highly warranted and met with enthusiasm. Yet, development of new strategies will require thorough analysis of appropriate clinical applications and the elucidation of cellular pathways that can foster reparative and regenerative mechanisms but lack detrimental clinical outcomes. For example, recent work has suggested that in some injury models nicotinamide may offer greater benefit for cytoprotection in young rather than aged subjects [43]. Temporal administration with nicotinamide also may affect expected clinical outcome since prolonged administration of nicotinamide may lead to β-cell dysfunction and limit P450 and hepatic metabolism [24]. Nicotinamide treatment during DM also requires further investigations that can assess effective treatment concentrations. Although other NAD$^+$ precursors similar to nicotinamide, such as nicotinamide riboside, can increase NAD$^+$ levels and activate SIRT1 resulting in protection against high-fat diet-induced metabolic abnormalities [115], NAD$^+$ precursors such as nicotinamide also can lead to cellular energy depletion that is detrimental to cell survival. Nicotinamide is a substrate for PARP-1 and maintains the integrity of PARP-1 to prevent its cleavage. At elevated concentrations, nicotinamide can promote PARP-1 activation that can deplete NAD$^+$ stores, lower ATP production, and lead to cell death [87]. During inflammatory disorders that activate TNFβ-1, nicotinamide also can block hepatic cell proliferation and lead to apoptosis with caspase 3 activation [24]. In addition, increased levels of nicotinamide may negate the benefits of SIRT1 during DM thereby limiting treatment efficacy [27, 46, 47]. Similar to nicotinamide, EPO administration also requires careful consideration in regards to duration of administration and the co-morbidity factors present in patients with DM. For example, increased expression of EPO may lead to progressive hepatic cell ischemic injury as well as the development of thrombotic and hypertensive complications in patients with DM [50, 51]. EPO also has been associated with tumorigenesis that may complicate administration of EPO [116–118]. In addition, EPO may be contraindicated during severe hypertension that can occur in diabetic patients since EPO may raise mean arterial blood pressure [51, 119, 120].

Vital to moderating potential adverse effects and maximizing clinical efficacy is the further investigation of the novel pathways of SIRT1, mTOR, and *wingless* that can mediate the cytoprotective pathways of nicotinamide and EPO. Although offering potential efficacy for the treatment of DM and the complications of this disorder, the pathways of SIRT1, mTOR and *wingless* are cellular proliferative and can have the potential to lead to unchecked cell growth. Under some conditions, loss of the protein Deleted in Breast Cancer 1 (DBC1), a negative regulator of SIRT1, can result in excessive cell growth, inhibition of apoptosis, and increased risk for tumorigenesis [121]. Activation of mTOR signaling may prevent insulin resistance during diabetes mellitus, but long-term mTOR activity may worsen conditions such as diabetic retinopathy and diabetic cardiac disease given the ability of mTOR to promote angiogenesis [10, 89]. In addition, mTOR activation can lead to tumorigenesis and epilepsy [122, 123] as well as impairment in neuronal stem cell maturation [124] and

dyskinesias [125]. In regards to *wingless* signaling, pathways such as Wnt and WISP1 may promote vascular smooth muscle growth that has the potential to lead to vascular complications in diabetic patients already at risk for adverse vascular events [126].

In consideration of cell repair and the potential for cell regeneration during DM, new studies also must address the complex relationship between apoptosis and autophagy during DM. For example, cytoprotection against some toxins in the nervous system such as methamphatamine requires the induction of "anti-apoptotic" pathways that block autophagy [10, 89], suggesting that under some conditions cellular protection may require the inhibition of both apoptosis and autophagy. However, under other conditions, cytoprotection may require the promotion of autophagy but the inhibition of apoptosis to limit apoptotic cell death [21]. Furthermore, other scenarios suggest that autophagy may have a limited role in controlling cell death during DM even though pathways that are responsible for cell protection such as PI 3-K and Akt may be necessary for modulating autophagy [19, 23]. Over the next several years, future investigations that can address a number of parameters that include clinical appropriateness for new therapeutic strategies for a diabetic population that suffers from multiple disease complications, duration and temporal administration of treatments that can ultimately impact multiple cellular pathways, and the further dissection of the complex and intricate cellular pathways that determine biological outcome will be crucial for the successful translation of novel strategies for DM and its complications into realistic and effective treatments.

Acknowledgments

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Highlights

- Oxidative stress during diabetes mellitus can lead to cell injury through the pathways of programmed cell death that involve either autophagy or apoptosis

- Nicotinamide can maintain cellular energy homeostasis during diabetes mellitus but may have an inverse relationship with the sirtuin SIRT1

- The cytokine erythropoietin can offer cellular protection during diabetes mellitus by controlling inflammation and preventing the induction of apoptotic mitochondrial pathways

- The sirtuin SIRT1 controls insulin sensitivity, food intake, and lipid homeostasis through pathways that involve UCP, PTP1B, PGC-1α, and PPAR-α signaling

- Forkhead transcription factors (FoxOs) are intimately tied to nicotinamide, erythropoietin, and SIRT1 to inflammation, β-cell function, and cellular survival during diabetes mellitus

- Wingless (Wnt) family members can promote improved glucose tolerance, increased insulin sensitivity, and protection in neuronal, vascular, and renal cells during diabetes mellitus
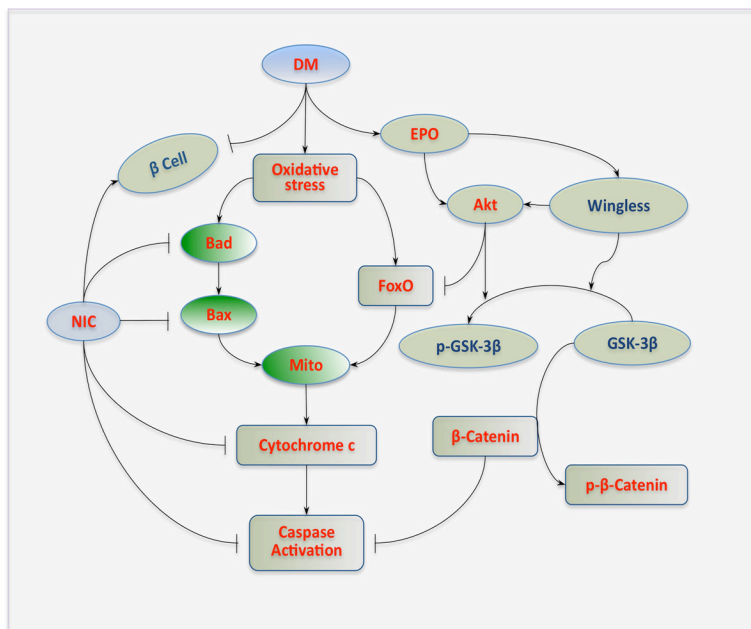
Figure 1. Nicotinamide, erythropoietin, and *wingless* modulation of diabetes mellitus
During diabetes mellitus (DM), elevated glucose leads to oxidative stress and results in the activation of "pro-apoptotic" proteins Bad, Bax, and forkhead transcription factors (FoxOs). Activation of these pathways subsequently impairs mitochondrial (Mito) membrane potential to result in cytochrome c release and caspase activation. Nicotinamide (NIC) prevents cell injury by maintaining mitochondrial membrane potential and modulating Bad, Bax, and FoxO to prevent cytochrome c release from mitochondria and block caspase activation. NIC also may be beneficial during DM through the protection of β-cells and promoting insulin secretion. The cytokine EPO is another therapeutic target that can limit cell injury during DM. EPO can promote the activation of protein kinase B (Akt) that inhibits the activity of FoxO and glycogen synthase kinase-3β (GSK-3β) by phosphorylating (p) these entities. EPO maintains the integrity of *wingless* pathways, such as Wnt1, during elevated glucose and relies upon Wnt1 to inactivate GSK-3β through phosphorylation (p) that ultimately protects the "anti-apoptotic" properties of β-catenin and prevents its degradation.
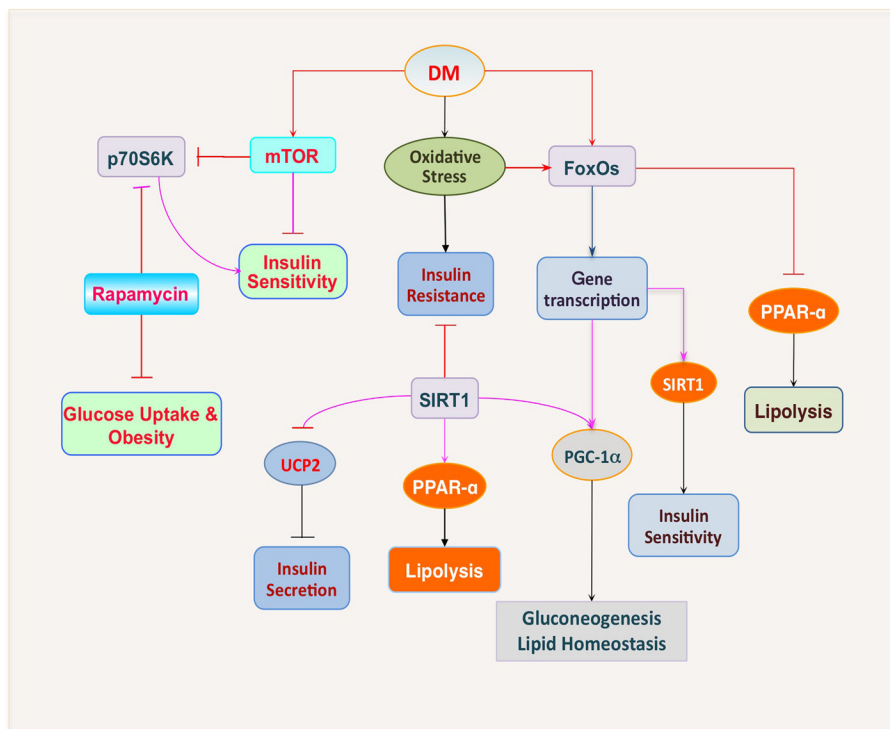
Figure 2. The intimate relationship among SIRT1, mTOR, and FoxOs during diabetes mellitus
Following the induction of oxidative stress during diabetes mellitus (DM), insulin resistance can ensue. Activation of SIRT1 can alter insulin sensitivity and increase the secretion of insulin by repressing the mitochondrial uncoupling protein 2 (UCP2), promote lypolysis by mediating peroxisome proliferators-activated receptor-γ (PPAR-α), and increase gluconeogenesis and lipid homeostasis by regulating proliferators-activated receptor-γ coactivator (PGC)-1α. In addition, increased activity of forkhead transcription factors (FoxOs) can increase gene transcription of PGC-1α and also SIRT1 to regulate insulin sensitivity and glucose metabolism. Yet, FoxOs can have an inverse relationship with SIRT1 and may prevent lipolysis through inhibition of PPAR-α. During DM, the activity of the serine/threonine protein kinase mammalian target of rapamaycin (mTOR) may be blocked. Activation of mTOR and its downstream pathways of p70S6K promote the secretion of insulin and increase insulin sensitivity. If mTOR activity is inhibited, such as during rapamycin application, insulin sensitivity and glucose uptake are reduced although obesity may be averted.
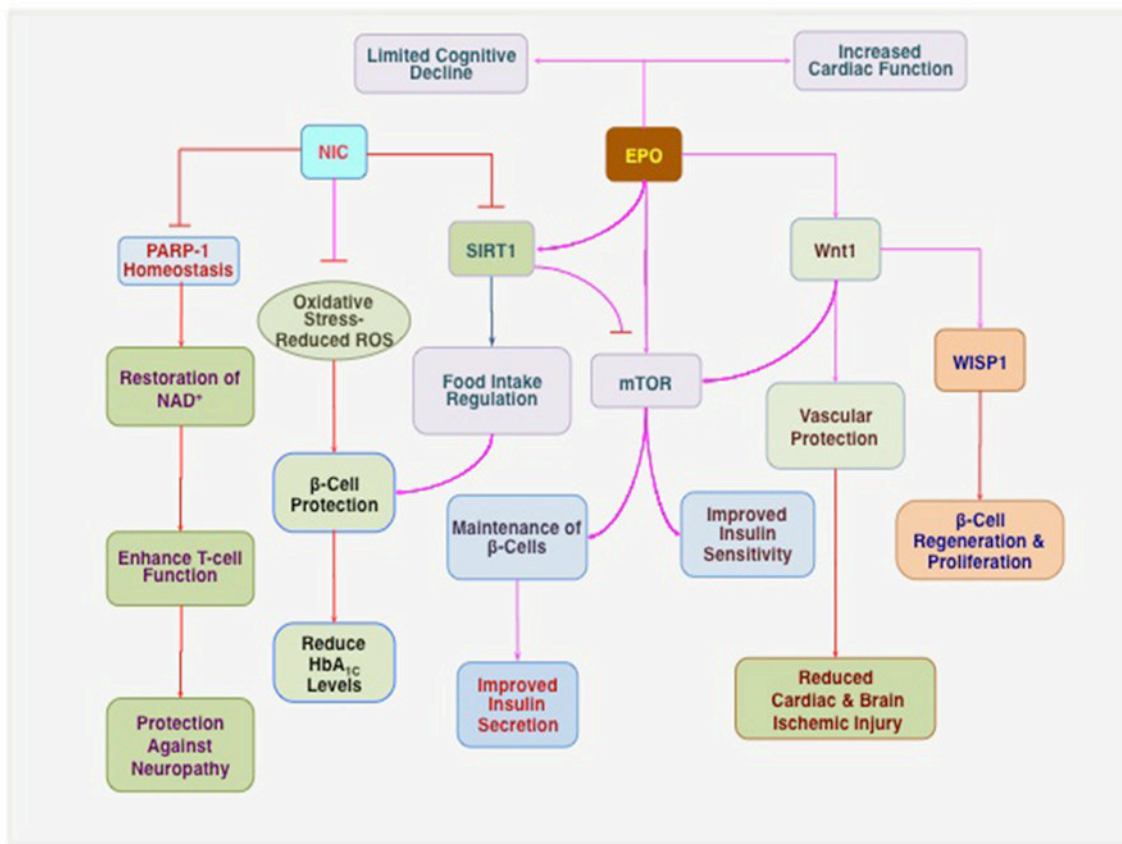
Figure 3. Novel clinical pathways for drug development during diabetes mellitus
Nicotinamide (NIC) can maintain poly (ADP-ribose) polymerase-1 (PARP-1) homeostasis, restore NAD$^+$ and ATP levels, enhance T-cell immune function, and lead to the prevention of diabetic neuropathy. NIC can limit reactive oxygen species (ROS) generation and oxidative stress during diabetes mellitus (DM) to assist with β-cell protection and also reduce HbA$_{1C}$ levels. NIC can function as a feedback mechanism to inhibit SIRT1 and activate the mammalian target of rapamycin (mTOR) to promote insulin sensitivity and maintain β-cell function. However, high concentrations of NIC may be detrimental to cell survival by limiting the activity of SIRT1 that is necessary for food intake regulation and cellular metabolism. Erythropoietin (EPO) can limit cognitive decline, promote neurite outgrowth, and increase cardiac function during DM that may function through the ability of EPO to modulate SIRT1 activity and foster cellular protection. EPO also directly or through Wnt1 increases mTOR activity to maintain β-cell survival and insulin sensitivity. Following Wnt1 activation, Wnt1 can promote vascular protection and reduce complications of DM, such as cardiac and brain ischemic injury. Wnt1 also governs Wnt1 inducible signaling pathway protein 1 (WISP1) that may assist with β-cell regeneration and proliferation.